GENOME-WIDE TRANSCRIPTIONAL PROFILING OF *NEISSERIA MENINGITIDIS* IN *EX VIVO* MODELS OF SEPTICAEMIA

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A thesis presented for the degree of

Doctor of Philosophy of Imperial College London

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February 2009

Declaration

I declare that all work presented in this thesis is my own and that no material has been previously submitted and approved for the award of a degree by this or any other University. I certify that all material in this thesis which is not my own work has been identified and appropriately acknowledged.

London,

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Abstract

Neisseria meningitidis is a Gram negative bacterium that commonly colonizes the nasopharyngeal mucosa of 10% of the population at any given time. Infrequently it invades to cause life threatening septicaemia and/or meningitis. I have used microarray technology to study the repertoire of meningococcal gene expression in the course of prolonged incubation in human plasma and blood, with the aim of identifying genes that are progressively up-regulated during sepsis, especially those encoding surface antigens. I hypothesize that any surface exposed gene products that are expressed at a higher level in the bloodstream than on the mucosal surface, where immune selection pressure drives antigenic diversity, should be less variable, and so, attractive as cross-protective vaccine candidates.

In my thesis research I explored experimental methods for modelling meningococcal bacteraemia *ex vivo*. I characterised the transcriptional response of a serogroup B strain of *N. meningitidis* during prolonged incubation (up to 4 hours) in fresh frozen non-bactericidal plasma from two individuals. Subsequently the meningococcal transcriptome was examined during incubation in fresh, anticoagulated non-bactericidal whole human blood for different periods up to 4 hours, using microarrays and real-time PCR technology.

The cellular localisation of proteins encoded by genes confirmed to be significantly differentially expressed in plasma and/or blood, both by microarray technology and by real-time PCR, was explored using bioinformatic resources, and those predicted (or established) to be surface-exposed were brought forward for further evaluation. Amongst these, I selected genes up-regulated in bacteria incubated in plasma and/or

blood, but down-regulated (according to data previously obtained) for organisms isolated from an experimental system modelling the nasopharyngeal carriage state, for examination of sequence diversity. Using serogroup B meningococcal strains of established (multi locus sequence type) diversity; I have used direct sequencing to address the starting hypothesis that these are comparatively invariant genes.

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Abbreviations

aa	amino acid
ABC	ATP-binding cassette
bp	base pair
cDNA	complementary or copy DNA
CFU	colony forming units
C^T	threshold cycle
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleoside triphosphates
DTT	dithiothreitol
ET	electrophoretic type
FBS	fetal bovine serum
FDR	false discovery rate
FFP	fresh frozen plasma
fH	factor H
GC	gonococcal media
gDNA	genomic DNA
hI	heat inactivated
i.p.	intraperitoneal
kb	kilobase
kDa	kilo Dalton
KOH	Potassium hydroxide
Loess	locally weighted polynomial regression

log	logarithmic
LOS	lipooligosaccharide
LPS	lipopolysaccharide
NHS	normal human serum
MenB	*Neisseria meningitidis* serogroup B
MH	Muller-Hinton
mRNA	messenger RNA
nt	nucleotide
OD	optical density
OM	outer membrane
OMP	outer membrane protein
OMV	outer membrane vesicle
ORF	open reading frame
PCR	polymerase chain reaction
PEG	polyethylene glycol
RNA	ribonucleic acid
SBA	serum bactericidal assay
SDS	Sodium dodecyl sulphate
STM	Site-directed transposon mutagenesis
TAE	Tris acetate EDTA buffer
TIGR	The institute for genomic research
TFRC	TIGR functional role category
V	volt
WBA	whole blood assay
wt	wild type

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Acknowledgements

I would like to thank my supervisor Prof. Simon Kroll for giving me the opportunity to work on this project and for invaluable help along the way.
I would also like to especially thank Dr. Ming-Shi Li for helpful discussions regarding all aspects of the project and for advice regarding experimental work.

I would also like to thank other people in the MIDG group that have helped me during my PhD project. First, thanks to Dr. Clíona O'Dwyer for assistance in the laboratory, as well as for reading and commenting on this thesis. Thanks to Dr. Paul Langford for commenting and giving input on important aspects of the project. I would also like to thank Dr. Sunita Sinha, especially for coming in early mornings to buddy me in the CL3. Thanks to Dr. Janine Bossé and Dr. Victoria Wright for reading and commenting on this thesis. Lastly, thanks to the rest of the people in the MIDG group and people at the Department of Paediatrics.

I wish to thank the George John and Shelagh Livanos trust for sponsoring my PhD studentship and Sanofi Pasteur (France) for providing further funds for the project.

I am grateful to my parents, Jan and Jill, for their support and encouragement over the years. Finally, I would like to thank Mattias for his love and encouragement, which made this work possible.

Chapter 1

General Introduction

1.1 The genus Neisseria

The genus Neisseria consists of Gram-negative proteobacteria that all colonise mucosa. Hosts range from humans (ten species including the only pathogenic species *Neisseria meningitidis* and *Neisseria gonorrhoeae* and the commensal *Neisseria lactamica*) to animals (five species) (Dent, 1982; Barrett & Sneath, 1994). All human Neisserial species colonise the nasopharynx except *N. gonorrhoeae*, which resides in the urogenital tract. Disease caused by meningococci or gonococci results in very different symptoms, even though the two species are closely related. Infection by *N. gonorrhoeae* leads to local inflammation of the urogenital tract (gonorrhoea), but rarely leads to systematic spread (disseminated disease). In contrast *N. meningitidis*, usually an asymptomatic coloniser of the nasopharyngeal mucosa, occasionally causes invasive disease, which although infrequent, may result in fatal septicaemia or meningitis, or both.

1.2 *N. meningitidis*

N. meningitidis is an aerobic Gram-negative diplococcus. The vast majority of strains isolated from cases of invasive disease are encapsulated, whereas strains isolated from asymptomatic carriers are commonly unencapsulated (Claus *et al.*, 2002; Caugant *et al.*, 2007). Humans are the only natural host for meningococci. The host-specificity

is linked to meningococcal surface structures, such as pili, which interact exclusively with human surface receptors for binding and invasion (Rudel *et al.*, 1995; Källström *et al.*, 1997). In addition, meningococcal iron-uptake systems are mainly able to scavenge iron from human iron transport proteins (Schryvers & Gonzalez, 1989).

1.2.1 Classification

Traditionally *N. meningitidis* strains have been classified according to antigenic differences in their surface structures. Serological tests divide strains into serogroups (polysaccharide capsule), serotypes (PorB, class 2 or 3 outer membrane proteins (OMPs)), serosubtypes (PorA, class 1 OMPs) and immunotypes (lipo-oligosaccharide (LOS)) (Frasch *et al.*, 1985). Thirteen serogroups are recognised (A, B, C, D, 29E, H, I, K, L, W135, X, Y and Z) based on the chemical composition of the polysaccharide capsule. The majority of cases of invasive disease are associated with the five serogroups A, B, C, W135 and Y. There are 21 serotypes characterised by variants in surface epitopes in class 2 or 3 (PorB) OMPs, of which two (2 and 15) have been particularly associated with invasive disease (Tzeng & Stephens, 2000). Strains can be further divided into serosubtypes based on antigenic variation in PorA. However, this protein is not expressed in all strains (Newcombe *et al.*, 1998). Meningococci can be classified into thirteen immunotypes (L1–L13) defined by heterogeneity in LOS structures. Three of these (L3, 7, 9) are particularly associated with invasive disease (Achtman *et al.*, 1992; Scholten *et al.*, 1994; Tzeng & Stephens, 2000). While serological methods based on surface structures have proved valuable in, for example, the delineation of outbreaks, they have some disadvantages. Recognition is limited to serological variants included in the panel of monoclonal antibodies used, and between 20 and 60% of serogroup B and C strains are non-typeable for PorB (Sacchi *et al.*, 1998). Both the serotype and serosubtype can be more reliably deduced from the nucleotide sequence of the genes. Of more fundamental importance, meningococcal outer membrane structures are under immune selection by the host, and subject to high frequency phase and antigenic variation. They are therefore of limited use for studies of the evolution of the meningococcal population.

For studies of meningococcal population structure and epidemiology, molecular typing methods such as Multi Locus Enzyme Electrophoresis (MLEE) and Multi Locus Sequence Typing (MLST) are more suitable. MLEE is based on the electrophoretic mobility of metabolic enzymes on a starch gel (Selander *et al.*, 1986). Each strain is assigned an electrophoretic subtypes (ET) profile. The method has successfully been employed to identify meningococcal hyperinvasive lineages (Kertesz *et al.*, 1998). However, MLEE gives only an indirect measure of the genotype and differences in sequence that do not give rise to structural changes will not be detected. Additionally, the technique is highly labour intensive and standardisation between laboratories is difficult. Fortunately, MLST overcomes the problems associated with MLEE. MLST is based on the sequencing of internal fragments of seven housekeeping genes, followed by assignment of each sequence to a specific allele (Maiden *et al.*, 1998). MLST has enough resolution and discrimination to be used in epidemiological surveillance. A large collection of *N. meningitidis* MLST data is stored in a public database that can be assessed and interrogated through a webpage interface (`http://pubmlst.org/neisseria`) (Jolley *et al.*, 2004).

1.2.2 Genomics

The full sequencing of several Neisserial genomes has opened up the possibility of performing comparative genomics. To date, the genome sequences of seven *N. meningitidis* strains have been published; Z2491 (serogroup A), MC58 (serogroup B), FAM18 (serogroup C) and 053442 (serogroup C), and three carriage strains (serogroup W135, 29E and non-typeable) (Tettelin *et al.*, 2000; Parkhill *et al.*, 2000; Bentley *et al.*, 2007; Peng *et al.*, 2008; Schoen *et al.*, 2008). The genomes of strains MC58, FAM18 and Z2491 differ by approximately 10%, with the major structural differences found in so-called islands of horizontally transferred (IHT) DNA. The genome sequences of the commensal *N. lactamica* strain ST-640 (`http://www.sanger.ac.uk/Projects/N_lactamica/`), as well as the pathogenic *N. gonorroheae* strain FA1090 are also available for comparison (Lewis *et al.*, 2003). Sequencing of additional Neisserial strains continually adds additional genes to the Neisserial gene bank, indicative of an open

pan-genome, a common characteristic of organisms that readily take up foreign DNA (Medini *et al.*, 2005). Genome comparisons of *N. meningitidis* isolates have shown that genes encoding meningococcal virulence factors can be found both in disease and carriage associated isolates (Schoen *et al.*, 2008). Genomotyping methods, based on hybridisation to microarrays, have been used to compare the genetic material of non-sequenced Neisserial strains to any of the sequence strains (Stabler *et al.*, 2005; Hotopp *et al.*, 2006; Snyder & Saunders, 2006; Schoen *et al.*, 2008).

1.2.3 Epidemiology

1.2.3.1 Invasive disease

The annual incidence of meningococcal disease in non-epidemic settings is between 0.5 and 10 per 100,000 persons, but can under epidemics in the so called "meningitis belt" in sub-Saharan Africa reach incidences of 1,000 per 100,000. World-wide, serogroups A, B and C have been responsible for 90% of cases of meningococcal disease, with serogroup A causing the most cases in Africa.

Pandemics, until now mainly caused by *N. meningitidis* serogroup A, occur in irregular cycles every 5–12 years in the sub-Saharan area from Senegal to Ethiopia. The epidemics usually last for 2–3 years, with 75% of disease affecting those under the age of 15. The case rate peaks at the end of the dry season and wanes during the rainy season (Greenwood *et al.*, 1984). Between 1998 and 2003 most serogroup A disease in Africa was caused by sequence types ST-5 and ST-7 (Nicolas *et al.*, 2005). Meningococcal disease caused by serogroup W135 has been relatively uncommon, but major outbreaks of serogroup W135 meningococcal disease have occurred, for example among Hajj pilgrims in 2000 (Taha *et al.*, 2000) and in Burkina Faso in 2002 (Teyssou & Rouzic, 2007). Other serogroups have the potential to emerge as significant problems; for example serogroup X in Niger (Djibo *et al.*, 2003) and Ghana (Gagneux *et al.*, 2002).

In the developed world serogroups B and C are responsible for most disease. In Europe, serogroups B and C are responsible for 90% of cases, while in the United

States they account for around 70% of disease, with *N. meningitidis* serogroup Y accounting for a large proportion of the remaining cases (Rosenstein *et al.*, 1999). A relatively small number of sequence types are associated with serogroup B and C invasive disease, so called hyperinvasive lineages. A large European survey conducted between 2000–2002 identified around 1,000 discrete STs among disease isolates, but only ten STs were predominant and accounted for half of the cases. The five most common clonal complexes were ST-8, ST-11, ST-32, ST-41/44 and ST-269 (Brehony *et al.*, 2007). In Europe, the clonal complexes ST-32, ST-41/44 and ST-269 are generally associated with serogroup B, whereas ST-11 and ST-8 are largely associated with serogroup C. During the same period ST-11, ST-41/44 and ST-269 were the most common clonal complexes in the UK, but the proportion of ST-11 strains has since decreased following the introduction of a national vaccination programme against serogroup C disease (in 1999) (Gray *et al.*, 2006; Brehony *et al.*, 2007). Today, most of the meningococcal disease in the UK is caused by serogroup B (87% in 2003/2004), although the total number of cases has decreased since the late 1990s. The most common serogroup B strain, B:4:P1.4 phenotype, was responsible for 17% of the disease in 2003/2004 (Gray *et al.*, 2006).

Serogroup B and C meningococci are less frequently associated with epidemics than serogroup A. Epidemics (otherwise termed hyperendemics) due to *N. meningitidis* serogroup B (MenB) have occurred in, for example Brazil (Costa *et al.*, 1991; Sacchi *et al.*, 1992), Cuba (Sierra *et al.*, 1991) and Norway (Bøvre *et al.*, 1977; Bøvre & Gedde-Dahl, 1980) and recently in New Zealand (Wilson *et al.*, 1995). Epidemics with serogroup B have been long-lived, persisting for 5 to 10 years or longer. In the New Zealand epidemic, starting in 1991, the incidence peaked in 1997 at 16.9 per 100,000 and stayed high with an average of 14 per 100,000 (Baker *et al.*, 2001) until the nation-wide introduction of a tailor-made vaccine in 2004 that reduced disease to previous endemic levels (Oster *et al.*, 2005). An increase in serogroup C meningococcal disease in the United Kingdom during the 1990s prompted the introduction of a new vaccine. The increase in disease was due to a strain of the C:2a phenotype

belonging to the hypervirulent lineage ST-11 clonal complex associated with invasive disease and rarely recovered from asymptomatic carriers. Strains belonging to this hyperinvasive lineage have been involved in several outbreaks and are associated with increased mortality (Swartley *et al.*, 1997; Kertesz *et al.*, 1998; Kriz *et al.*, 1999; Pérez-Trallero *et al.*, 2002). Introduction of routine vaccination with glycoconjugate vaccines raised concerns about a possible increase in disease caused by other serogroups not covered by vaccine, such as serogroup B strains. Capsular switching, as the result of replacement of the *siaD* gene through transformation and recombination, is known to occur in *N. meningitidis*, and this would be a mechanism for the bacteria to escape the host's immune response (Swartley *et al.*, 1997). In epidemiological data of meningococcal disease (until season 2003/2004), no evidence has been seen for an increase of serogroup B cases caused by B:2a in the UK, or indeed by any other serogroup B strains (Trotter *et al.*, 2006b). However, in the winter of 2008/2009 an increase in the number of cases of invasive MenB disease has been observed in the UK (Health protection agency, 2009).

1.2.3.2 Asymptomatic carriage

The carriage rate of *N. meningitidis* in the population is around 10% at any given time, although rates exceeding 40% have been recorded in, for example, military recruits in high-density living (Andersen *et al.*, 1998). The meningococcal carriage rate increases through childhood and peaks in adolescence. In young children below the age of five, colonisation of nasopharyngeal mucosa by commensal Neisseria species, such as *N. lactamica*, is six times more common than carriage by *N. meningitidis* (Gold *et al.*, 1978; Cartwright *et al.*, 1987; Bennett *et al.*, 2005). Colonisation with *N. lactamica* is thought to offer cross-protective immunity to *N. meningitidis* (Oliver *et al.*, 2002; Bennett *et al.*, 2005). Introduction of vaccines aimed at prevention of invasive disease can also affect carriage. After the introduction of the conjugate polysaccharide C vaccine in the UK, there was a marked decrease in carriage of *N. meningitidis* serogroup C strains expressing high levels of capsule, particularly strains of the hyperinvasive ST-11 type, both in vaccinated and unvaccinated individuals.

No significant decrease was detected in carriage of serogroup C strains expressing low levels of the polysaccharide capsule (Maiden *et al.*, 2008).

1.2.4 Meningococcal disease

The annual global burden of meningococcal disease in non-epidemic settings is approximately 500,000 cases with overall mortality of about 10%. The highest incidence of invasive disease is in children of less than four years, with a second peak in adolescents (Stephens *et al.*, 2007). In Europe and the USA, the highest incidence is in infants below one year of age, and the case fatality is also higher in this age group. Meningococcal disease incidence follows a seasonal pattern, with the highest numbers of cases recorded during late winter. Higher incidence of disease is also correlated with low humidity, poor living conditions, smoking, viral infections and close contact with infected individuals, such as family members. Invasive disease with *N. meningitidis* is most commonly manifested as septicaemia and/or meningitis (Brandtzaeg & van Deuren, 2002). In its mildest form, almost certainly underdiagnosed, disease is characterised by a transient bacteremic illness that resolves spontaneously in 1 to 2 days. In its most serious form there is fulminant septicaemia associated with multisystem involvement (affecting 5–15% of patients) and a high mortality. The case fatality of meningococcal disease in industrialised countries is 5–15% (up to 40% for septicaemia), but higher in developing countries with less advanced healthcare (Goldacre *et al.*, 2003). Of the survivors, between 10–20% are left with permanent disability, such as loss of limbs, deafness or mental retardation (Danzig, 2004). Clinical signs of meningococcal disease include fever and altered mental status. Patients often complain of severe headache, limb pain, nausea, vomiting and photophobia. A characteristic of fulminant meningococcal sepsis, in cases with or without meningitis, is the development of skin haemorrhages (petechiae, purpura) (van Deuren *et al.*, 2000). Where bacteria invade the cerebrospinal fluid (CSF) from the bloodstream by traversing the blood brain barrier (BBB), the result is meningitis.

1.2.5 Meningococcal pathogenesis

In most individuals in whom the respiratory tract epithelium (nasopharyngeal and tonsillar mucosa) is colonised with *N. meningitidis*, the condition is entirely asymptomatic. However, for reasons poorly understood *N. meningitidis* occasionally invades the mucosa and enters the bloodstream to cause life threatening septicaemia and/or meningitis. This process consists of multiple stages. First the host must be exposed to the meningococcus. This is then followed by invasion of the nasopharyngeal epithelium, survival and proliferation in the bloodstream to cause bacteraemia and finally the crossing of the BBB (from the blood stream or directly from the nasopharynx) to the CSF by some bacteria, resulting in meningitis. Important meningococcal virulence factors at different stages of disease include the polysaccharide capsule, LOS and pili.

Meningococci are spread between humans in aerosol droplets or by direct contact with respiratory secretions from asymptomatic carriers. The polysaccharide capsule is thought to offer protection to *N. meningitidis* during transmission (Berger & Döring, 1969). Colonisation of the nasopharyngeal mucosa by *N. meningitidis* is generally a transient event, but can in some individuals result in systemic meningococcal disease. Bacteria initially attach to form micro-colonies on the mucosal surface, interacting exclusively with non-ciliated cells (Stephens *et al.*, 1983; Merz & So, 2000). Studies using cultured epithelial cells or nasopharyngeal explants suggest that the initial adhesion of meningococci to host cells is mediated through interactions between meningococcal type IV pili (Tfp) and the host receptor CD46 (Källström *et al.*, 1997; Nassif *et al.*, 1997; Merz & So, 2000). However, piliated meningococci can interact with erythrocytes, lacking detectable CD46, suggesting involvement of additional host cell receptors. Pilin can be modified by O-glycosylation mediated by proteins PglA–D (Power *et al.*, 2000), but it is unclear if glycosylation is important for host cell binding or other virulent properties (Virji *et al.*, 1993b; Marceau *et al.*, 1998). Pilin mediated binding of the meningococcus to CD46 has been shown to induce intracellular signalling in the host cell, triggering an increase in free calcium in the cytosol

that regulates cellular processes (Källström *et al.*, 1998). After initial adherence, the meningococcus down-regulates pilus and the polysaccharide capsule and this is followed by closer adherence to the host cells (Pujol *et al.*, 1997). Down-regulation of the capsule is thought to make the meningococcus more adherent (Stephens *et al.*, 1993), and this is supported by the observation that the capsule is generally found to be expressed at lower levels or to be completely absent in colonising strains (Caugant *et al.*, 2007). Absence of the polysaccharide capsule may either be due to phase-variation of key genes, or to the presence of a capsule null locus (cnl). Retraction of pilus is also essential for intimate adhesion to host cells. *pilT* mutants, unable to retract pilus, are incapable of intimate adherence to host cells (Pujol *et al.*, 1999). Down-regulation of pili and capsule facilitates the interaction between meningococcal OMPs and host cells. Sialylated LOS has also been shown to inhibit meningococcal binding through OMPs such as opacity proteins (de Vries *et al.*, 1996). The phase variable opacity proteins (Opa and Opc) contribute to both the adhesion and invasion of host cells (Virji *et al.*, 1993a). Receptors for opacity proteins on the host cells, facilitating adhesion and invasion, are thought to be CD66 family receptors and heparan sulphate proteoglycans (Virji *et al.*, 1996; de Vries *et al.*, 1998). However, it is worth noting that the attachment through opacity proteins may not be essential. Pujol *et al.* (1997) presented some evidence for this by showing that a $\Delta opa/\Delta opc$ strain could adhere and form intimate adhesion to a polarized monolayer, *in vitro*, suggesting that the attachment may be mediated through some other surface protein. Some evidence also suggests a role for LOS in adhesion to host cells (Porat *et al.*, 1995; Song *et al.*, 2000).

Only a small proportion of individuals infected with *N. meningitidis* go on to develop invasive disease. Initial invasion is thought to occur by invasion of host cells by meningococci on the mucosal surface through endocytosis and crossing the epithelium to the blood stream (Merz & So, 2000). Specific risk factors for invasive disease are not entirely clear but are likely to be a combination of environmental factors as well as host and bacterial genetics. Invasive meningococcal disease has been correlated

with certain age groups (young children and teenagers) and immunocompromised individuals (although not HIV), as well as factors that disturb the mechanical barrier of the nasopharyngeal epithelium such as smoking and viral illnesses (Trotter *et al.*, 2006a). There is also some genetic predisposition, such as relatively rare deficiencies in components of the complement pathways as well as more common polymorphisms in genes of the immune system (discussed in Section 1.2.5.1). Invasiveness is also associated with the infecting strain, some meningococcal strains being more commonly associated with disease than others (Salit *et al.*, 1984; Caugant *et al.*, 2007). Bacterial factors that function in invasion include IgA1 protease, which is only found in pathogenic Neisseria species (Mulks & Plaut, 1978). The protease has been found to be expressed at higher levels in invasive isolates of meningococci than non-invasive isolates (Vitovski *et al.*, 1999). The IgA1 protease cleaves host IgA1 antibodies and has, for example, been observed to cleave PorA specific IgA1 antibodies bound to the meningococcal surface (Vidarsson *et al.*, 2005). IgA1 protease also cleaves many other proteins (Vitovski & Sayers, 2007). By cleaving Lysosomal-associated membrane protein 1 (LAMP1) in the phagosome IgA1 protease has been found to contribute to intracellular survival in epithelial cells (Lin *et al.*, 1997). After invasion of the epithelium, meningococci have been shown to reside within subepithelial tissue (Read *et al.*, 1995) and have also been observed deep in tonsillar tissue (Sim *et al.*, 2000). Plasminogen, a component of the fibrinolytic system, can after activation cleave components of the extracellular matrix. Binding of plasminogen to the bacterial surface has been shown to increase bacterial invasiveness (Lähteenmäki *et al.*, 2005). Three meningococcal proteins, DnaK, enolase and peroxidase, have been suggested to act as binding partners of plasminogen on the bacterial surface (Knaust *et al.*, 2007).

In the blood stream a pre-requisite for meningococcal survival is the presence of a polysaccharide capsule that protects bacteria from the host immune system (Vogel *et al.*, 1997; Estabrook *et al.*, 1998; Kahler *et al.*, 1998; Geoffroy *et al.*, 2003). However, in a few cases unencapsulated strains have caused meningococcal disease in previously healthy individuals (Hoang *et al.*, 2005; Findlow *et al.*, 2007). Find-

low *et al.* (2007) also demonstrated that an unencapsulated strain recovered from an infected individual showed serum resistance comparable to that of a capsulated reference strain. Increased virulence during bacterial growth in the blood stream is also associated with expression of meningococcal LOS, as strains with disruptions in genes involved in synthesis of LOS, especially of the inner core, are less virulent in animal infection models and are sensitive to serum killing (Kahler *et al.*, 1998; Sun *et al.*, 2000; Geoffroy *et al.*, 2003; Plant *et al.*, 2006). Additionally, acquisition of iron or biosynthesis of amino acids has been associated with meningococcal survival in the blood stream (Sun *et al.*, 2000).

From the blood stream (or directly from the nasopharynx) meningococci can invade the meninges to cause meningitis, by crossing the BBB through the transcellular route or via the choroid plexuses (reviewed in Nassif & So (1995)). In tissue from a fatal case of meningococcal meningitis, meningococci were attached both to endothelial cells in the BBB and the choroid plexus (Pron *et al.*, 1997). The bacteria probably traverse the BBB by stimulating endocytosis on the blood side by the cells composing the membrane and release into the CSF on the other side (Nassif, 1999). *In vitro*, the polysaccharide capsule has been shown to be important for intracellular survival in human brain microvascular endothelial cells (Nikulin *et al.*, 2006; Spinosa *et al.*, 2007).

1.2.5.1 Innate and adaptive host responses to the meningococcus

Host responses to the presence of meningococci involve the activation of the complement system, activation of inflammatory responses (chemokines and cytokines) and activation of coagulation and fibrinolysis. In this section, humoral immune responses to *N. meningitidis* will be discussed. Cellular immune responses, probably playing a smaller role in protection to *N. meningitidis*, will be discussed in Chapter 6. The complement system is required for protection against *N. meningitidis*, and defence against invasive meningococcal disease is primarily mediated through bactericidal antibodies and complement. As a consequence, individuals with deficiencies in

certain components of the complement system, such as properdin and C6–C9, as well as individuals with immunoglobulin deficiencies, are at increased risk of developing meningococcal disease (Emonts *et al.*, 2003). Complement is activated on the surface of the meningococcus by one or more of the three initial complement-activating pathways; the classical, alternative or lectin pathway. Proteins on the meningococcal surface interact with components of the complement cascade, altering their functionality. An overview of the three complement pathways with some of the interaction points is shown in Figure 1.1.

Most of the complement mediated killing of meningococci is initiated by the antibody-dependent classical pathway (Goldschneider *et al.*, 1969), activated by binding of bactericidal antibodies to structures on the bacterial surface. Serogroup B capsular polysaccharide is poorly immunogenic and Welsch & Granoff (2007) have shown that exogenous serogroup B polysaccharide, in most cases, does not inhibit whole blood bactericidal activity, suggesting that bactericidal antibodies in blood are mainly directed against other meningococcal surface structures. However, studies have also indicated that deposition of complement on the meningococcal surface is independent of IgG antibodies to MenB (Drogari-Apiranthitou *et al.*, 2002).

Activation of the alternative and lectin pathways is also evident in response to stimulation with meningococci. During *in vitro* incubation of MenB in human serum considerable amounts of activated factor B (Bb), a serum factor of the alternative pathway, is produced (Drogari-Apiranthitou *et al.*, 2002). Individuals with deficiencies in properdin or factor D, activators of the alternative pathway, are more susceptible to meningococcal disease as well as presenting with more severe symptoms, which suggest that a functional alternative pathway is an important component in protection (Sprong *et al.*, 2006). The initial step in the lectin pathway is mediated by the human serum protein mannose binding lectin (MBL), which has been shown to bind Opa and PorB on the meningococcal surface (Estabrook *et al.*, 2004). Binding of MBL to the meningococcal surface accelerates complement activation and thereby serum killing of *N. meningitidis*, as well as inducing interactions with phagocytotic

cells (Jack *et al.*, 2001a,b). MBL deficiency and specific sequence polymorphisms in the gene encoding MBL have been associated with meningococcal disease (Bax *et al.*, 1999; Hibberd *et al.*, 1999), findings which suggest that the lectin pathway is also important for control of meningococcal disease.

Activation of all three complement pathways results in the cleavage of C3 deposited on the bacterial surface. The same levels of C3 surface-deposition, as well as degradation of C3 to iC3b fragments and C3b molecules on the bacterial surface, have been observed for serum-sensitive and serum-resistant bacterial strains (Jarvis, 1994). The deposition of C3b (probably bound to Opa (Lewis *et al.*, 2008)) and iC3b on the bacterial surface has surprisingly not been shown to be inhibited by capsule, LOS or porins (Drogari-Apiranthitou *et al.*, 2002). Both C3b and iC3b can also function as an opsonin, mediating uptake of the bacteria by phagocytotic cells (opsonophagocytosis). The last step in the complement cascade is the formation of the C5b–9 complement complex (membrane attack complex (MAC)), which following insertion in the outer membrane results in lysis of the bacteria. The polysaccharide capsule may protect the meningococcus from correct insertion of MAC, which is required for efficient lysis of the bacteria. The rate of MAC formation on the MenB surface has been found to be higher in bactericidal than non-bactericidal sera, but correct insertion rather than amounts of MAC seem to be most important for effective bacterial killing (Drogari-Apiranthitou *et al.*, 2002). Individuals with deficiencies in components of the terminal complement complex are more susceptible to meningococcal disease, but may suffer from a less fulminant form (Bartholomew & Shanahan, 1990), emphasising the contribution that activation of host defence mechanisms makes to symptomatic illness. In these individuals, killing of meningococci is possibly mediated through phagocytosis (Ross *et al.*, 1987). Meningococci modulate steps in the complement cascade by binding negative regulators to surface structures. C4bp, an inhibitor of both the classical and lectin pathways, binds to the surface of meningococci in a PorA dependent manner (Jarva *et al.*, 2005). Factor H (fH), a negative regulator of the alternative pathway, has been shown to interact with the meningococcal protein NMB1870 on

the meningococcal surface (Madico *et al.*, 2006). Binding of fH to the meningococcal surface is associated with increased resistance to complement mediated killing and increased survival in blood (Haralambous *et al.*, 2006; Schneider *et al.*, 2006). Additionally, polymorphisms in the fH gene that lead to decreased levels of factor H in human blood plasma have been correlated with increased susceptibility to meningococcal disease (Haralambous *et al.*, 2006). NMB1870 is one of the five components of an experimental MenB vaccine formulation developed by Novartis (Giuliani *et al.*, 2006) (Section 1.4.1).

Activation of proinflammatory (and antiinflammatory) responses in the host is controlled by Toll like receptors (TLR). *N. meningitidis* primarily interacts with TLR2 and TLR4. The meningococcal surface protein PorB has been shown to interact directly with host cell TLR2 (Massari *et al.*, 2006). LOS has been shown to be the main ligand for TLR4 (Zughaier *et al.*, 2004). However, a LOS-deficient meningococcal strain has also been shown to activate TLR4-dependent proinflammatory responses, indicating that additional surface structures interact with TLR4 (Plant *et al.*, 2007). TLRs are important in innate host defences, but uncontrolled proinflammatory activation is detrimental for the host and is one of the hallmarks of fulminant sepsis. For example, expression levels of tumour necrosis factor (TNF)-α, induced by endotoxin (LOS), is correlated with death of patients during sepsis (Waage *et al.*, 1987). Interleukin (IL)-6 is up-regulated in all meningococcal disease patients, but levels have been found to be significantly higher in non-survivors than survivors (Waage *et al.*, 1989). High levels of IL-10, a suppressor of proinflammatory cytokine production, have also been associated with fatality in meningococcal sepsis (Lehmann *et al.*, 1995).

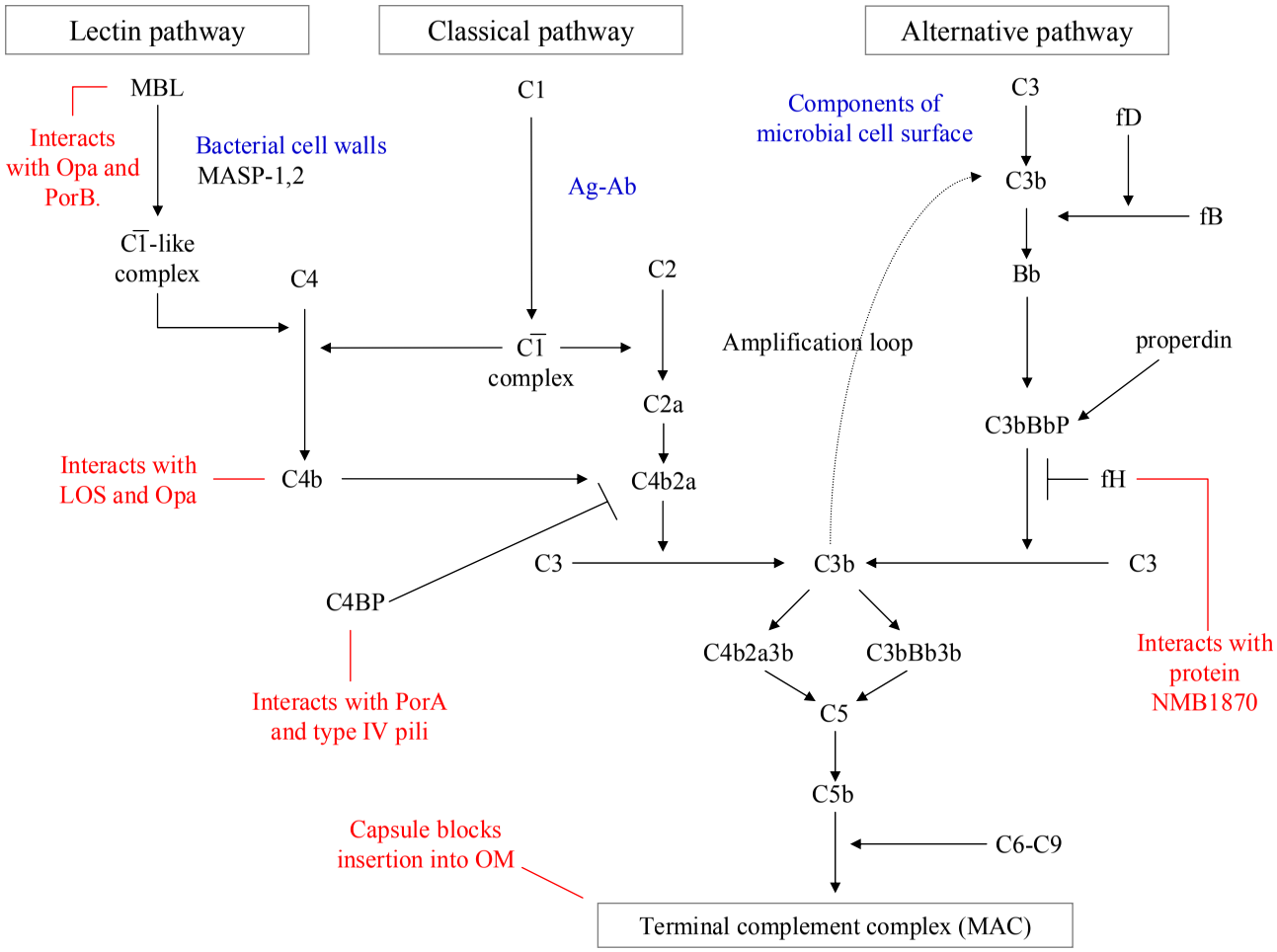


Figure 1.1: Interactions between the human complement system (lectin, classical and alternative pathways) and meningococcal surface structures. Points of interaction between meningococcal surface structures and components of the complement pathways are indicated in red. Indicated in blue are components for which certain polymorphisms or a lack of the component have been shown to increase susceptibility to meningococcal disease. C1 complex is composed of C1q, C1r, and C1s. The C1-like complex in the lectin pathways is composed of MBL and MASP-1,2.

1.2.6 Models of meningococcal disease

Animal models Animal models are useful for exploration of some of the aspects of meningococcal pathogenesis, but none of the available animal models can capture its full complexity, with disease progression from mucosal carriage to septicaemia and/or meningitis. Models of meningococcal disease include: the mouse intra-peritoneal (i.p.) challenge model, the infant rat i.p. infection model, intranasal infection models and infection models using humanized mice. These have mainly been used to model invasive disease in the blood stream. Recent development of transgenic mouse models, expressing human surface receptor CD46 (Johansson *et al.*, 2005) or human transferrin (Zarantonelli *et al.*, 2007), may improve the modelling of meningococcal bacteraemia.

***In vitro* and *ex vivo* models** *In vitro* and *ex vivo* models have also been used to explore specific aspects of meningococcal disease. Human primary or immortalised cell lines have been used to model the interactions between the bacteria and cell types encountered by *N. meningitidis* during different stages of infection. Models to replicate steps in nasopharyngeal colonisation or invasion include cultured epithelial cells or *ex vivo* tissue explants (Stephens *et al.*, 1983; Virji *et al.*, 1992; Read *et al.*, 1995). Transcriptional changes in response to interactions between meningococci and human cells have been studied in both the host (Bonnah *et al.*, 2004; Plant *et al.*, 2004) and the bacteria (Grifantini *et al.*, 2002b; Dietrich *et al.*, 2003) (Section 1.3). Aspects of invasive meningococcal disease can be modelled using animal models (discussed above), as well as cultured immune cells, and in an *ex vivo* whole blood assay (WBA) (Heist *et al.*, 1922; Ison *et al.*, 1995) as well as in serum or plasma. Heist *et al.* (1922) developed the WBA as a simple means for studying bactericidal activity against meningococci. They incubated bacteria in fresh anti-coagulated blood and monitored bacterial survival by counting colony forming units (CFU) at intervals. A minority of individuals' blood allowed bacterial proliferation. Using this assay they defined the terms 'non-killer' and 'killer' as individuals whose blood respectively supported or failed to support the growth of meningococci. Setting aside this labour intensive assay, Goldschneider *et al.* (1969) developed what has become the gold standard in

evaluation of host/meningococcal interaction, the serum bactericidal assay (SBA). In this assay, heat-inactivated[1] serum is incubated with appropriate target strains and complement from various sources[2], and the serum bactericidal titre for each unknown serum is expressed as the reciprocal of the lowest serum dilution yielding a 50% killing as compared to time zero. SBA has become the established way of determining the efficiency of meningococcal vaccines, with a titre of 1:4 considered protective. Ison *et al.* (1995), concerned at the loss of information inherent in tests of serum rather than of whole blood, revived Heist's WBA method, and demonstrated individual differences in susceptibility to meningococcal infection not reflected in SBA. Individuals with a negative SBA to MenB, i.e. non-bactericidal, can still have a positive WBA, i.e. limited meningococcal survival in whole blood (Ison *et al.*, 1999; Welsch & Granoff, 2007), pointing to the importance of MenB killing by phagocytosis (antibody-dependent and/or independent). Peripheral blood mononuclear cells have been used to study, for example, phagocytosis of the bacteria. Using such cells Kalmusova *et al.* (2000) showed that invasive meningococcal strains were able to survive within vacuoles in monocytes/macrophages whereas non-invasive strains were quickly killed. Using mutant strains of bacteria, surface structures important for stimulation of monocytes or protection from phagocytosis have been identified. For example, sialyation of LOS (Unkmeir *et al.*, 2002) and the surface protein NhhA have been suggested to protect the bacteria from phagocytosis (Sjölinder *et al.*, 2008).

Entry of *N. meningitidis* into the CSF requires adhesion to endothelial cells and crossing of the BBB, and these processes have been mimicked *in vitro* using endothelial and more recently meningeal cells (Christodoulides *et al.*, 2002). Adhesion to the BBB has been shown to require the expression of PilC, the adhesive tip of the type IV pilus (Pron *et al.*, 1997). Using such models meningococci have been shown to be encapsulated during passage of the cells and intracellular growth (Nikulin *et al.*, 2006).

[1] To remove endogenous complement
[2] Now conventionally human or baby rabbit

1.3 Transcriptomic studies of infectious disease

Exploring the complex interactions between pathogen and host on a genome-wide scale can increase our understanding of the genes and pathways that are linked to infection and invasion of the host, and may be used as a tool to discover new virulence genes, vaccine candidates and drug targets. Microarray technology, developed in the mid-1990s at Stanford University, can be used to determine the genome-wide transcriptional profile of an organism (Schena *et al.*, 1995). Microarrays are based on the hybridisation of fluorescently labelled targets (one- or two-colour) to gene specific probes on a surface, with the objective to determine the absolute or relative abundance of gene transcripts in the respective samples. Other applications of DNA microarrays include the assessment of gene copy number (array-CGH) (Pinkel *et al.*, 1998), genotyping (Behr *et al.*, 1999), single nucleotide polymorphisms (SNP) analysis (Hacia *et al.*, 1999) and for detection of DNA-protein interactions (ChIP-chip) (Ren *et al.*, 2000).

Microarray technology has been extensively used in the prokaryote field to probe differences due to gene mutations (Thompson *et al.*, 2002; Beliaev *et al.*, 2002; Sebert *et al.*, 2002). Microarrays have also been used to explore, for example, bacterial responses to different perturbations, interactions with host cells, invasive disease or responses to environments mimicking some specific condition encountered by the bacteria during infection (Ye *et al.*, 2000; Smoot *et al.*, 2001; Fisher *et al.*, 2002; Grifantini *et al.*, 2002b; Phadtare *et al.*, 2002; Bina *et al.*, 2003). The first two micro (macro) array papers published in the bacterial field explored the response of *Escherichia coli* to rich or minimal media (Tao *et al.*, 1999) and the drug-induced transcriptome of *Mycobacterium tuberculosis* (Wilson *et al.*, 1999). The sequencing of the first complete meningococcal genome in the year 2000 opened up the possibility of performing functional genomics studies for *N. meningitidis*, such as transcriptomic studies using microarrays (Tettelin *et al.*, 2000). Several groups have used microarrays to study the changes in transcriptional profile when *N. meningitidis* has been subjected to, for example, iron depletion (Grifantini *et al.*, 2003), heat-shock (Guckenberger *et al.*,

2002), and co-culture with human cells (Grifantini *et al.*, 2002b; Dietrich *et al.*, 2003). Grifantini *et al.* (2002b) studied the changes in gene expression at four time-points (30, 60, 120 and 180 min) for three different MenB strains (MC58, 2996, and BZ83) when they adhered to epithelial cells. They found differentially regulated genes (N = 347) mainly to be associated with adhesion, host-pathogen cross talk, amino acid biosynthesis, DNA metabolism. Dietrich *et al.* (2003) carried out similar experiments, comparing differential gene expression of an unencapsulated mutant of MC58 when adherent to epithelial or endothelial cells, using oligo-based microarrays. They found significant changes in gene expression for 72 and 48 genes, respectively. More than 200 meningococcal genes were found to be iron-regulated, including known iron-regulated genes such as *tbpA* and *tbpB*, but also novel genes such as NMB1436–38 (Grifantini *et al.*, 2003). Microarrays have also been used to characterise genes with hypothetical function (Grifantini *et al.*, 2004).

Host cell responses induced by stimulation with *N. meningitidis* have also been studied using microarray technology. The major gene expression changes in response to stimulation with meningococci involve the up-regulation of inflammatory factors. Up-regulation of genes involved in inflammatory processes, as well as cell proliferation, have been observed when epithelial cells are stimulated by piliated meningococci (Linhartova *et al.*, 2006). Stimulation of human primary monocytes with a similar load of heat-killed wild-type *N. meningitidis* or a LOS mutant resulted in differential regulation of 4689 and 72 genes, respectively (Øvstebø *et al.*, 2008), indicating that LOS is the main activator of gene expression changes in response to exposure to dead bacteria. At 100-fold higher load the *N. meningitidis* LOS deficient strain induced differential regulation of the same magnitude as the wild-type strain, indicating a dose response in the stimulatory effect by other surface exposed bacterial factors. However, Øvstebø *et al.* found several genes, including IFN-β, which were specifically up-regulated upon stimulation of monocytes with wild-type meningococci. Co-incubation of *N. meningitidis* with whole human blood resulted in up-regulation of 174 genes, including IL-6 and TNF-α, an activator of inflammatory responses (Pathan

et al., 2004). Meningeal cells, stimulated with secreted meningococcal proteins or co-incubated with live bacteria, expressed genes encoding the inflammatory factors IL-6, a major pyrogen, and IL-8 at higher levels (Wells *et al.*, 2001; Robinson *et al.*, 2004). In addition to changes in immune responses, Bonnah *et al.* (2004) showed that adhesion of *N. meningitidis* to human epithelial cells induced changes in expression of host genes involved in iron homeostasis, indicative of an iron starved state. This is consistent with another study, in which Larson *et al.* (2004) showed that when *N. meningitidis* is incubated with human epithelial cells, degradation of host cell ferritin is increased, providing an iron source for the bacteria.

1.4 Meningococcal vaccines

The severity of meningococcal disease as well as the economic cost of health care (intensive care and long-term care for survivors) has prompted huge efforts to develop preventive measures. Vaccines based on purified capsule polysaccharides have been available since the 1960s for the pathogenic serogroups A, C, Y, and W135. Their major drawback is minimal efficacy in infants and children under 2 years old, at greatest risk of disease, with the exception of serogroup A polysaccharide that offers protection from about three months of age. Although inducing a good immune response in older individuals, protection is of limited longevity. Vaccine boosters have been tested as a way of offering longer lasting protection, but these are not feasible for all serogroups. For example, serogroup C capsular polysaccharide preparations cause immuno hyporesponsiveness upon repeated administration (Granoff *et al.*, 1998). To overcome the limitations with the earlier vaccines, protein-conjugated polysaccharide vaccines have been developed where the capsular polysaccharides are chemically conjugated to a protein. The serogroup C glycoconjugated polysaccharide vaccine is effective in infants and can induce a strong response after only a single dose (Richmond *et al.*, 1999a; Cartwright *et al.*, 2001). The protein conjugated polysaccharide induces a T-cell dependent response, and therefore gives rise to memory T-cells, leading to a long-term protective effect (Richmond *et al.*, 1999a,b) with efficient boosting. Glycoconjugated

vaccines have also been developed for improved prevention of meningococcal disease caused by serogroups A, Y and W135 (Pace & Pollard, 2007). As explained in the next section, however, there is no comparable glycoconjugate vaccine to prevent serogroup B infections, and a radically different approach is needed for this major challenge.

1.4.1 Serogroup B vaccines

The capsular polysaccharide of MenB is virtually non-immunogenic in humans, due to it chemical identity to a human neuronal surface carbohydrate, α-(2→8)-linked poly N-acetyl neuraminic acid (Finne *et al.*, 1983), and therefore the vaccine strategies used for the other pathogenic serogroups are not applicable to MenB. In attempts to increase immunogenicity, the group B polysaccharide has been complexed with OMPs or conjugated to a protein carrier, with limited improvements (Zollinger *et al.*, 1979; Jennings & Lugowski, 1981). There are however concerns that inducing an immune response through such strategies could trigger autoimmunity (Fusco *et al.*, 1998). Trials have been conducted with N-propionylated capsular polysaccharide (Jennings *et al.*, 1986, 1987; Häyrinen *et al.*, 1995; Pon *et al.*, 1997), which might circumvent the possibility of inducing self-reactive antibodies. N-propionylated modified capsular structures were evaluated in a phase I clinical trials (Bruge *et al.*, 2004), but failed to induce protective antibodies to meningococcal strains.

Instead of basing vaccines on the polysaccharide capsule, other strategies have been employed for protection against serogroup B strains. These include the use of various immunogenic surface exposed proteins, as well as formulations of outer membrane vesicles (OMVs), which naturally contain a variety of OMPs, LOS and periplasmic proteins (Danzig, 2004; Vaughan *et al.*, 2006).

1.4.1.1 Lipooligosaccharide

N. meningitidis LOS, also referred to as endotoxin, is a potent activator of the immune system. Several approaches have been taken in attempts to create an effective LOS vaccine. Due to the toxicity of native LOS, elicited by the lipid A moiety of the molecule, modified detoxified structures have been constructed, which have been

tested for their potential to induce a protective immune response. Conjugation of either oligosaccharide structures (Verheul *et al.*, 1993) or truncated LOS inner core structures (Cox *et al.*, 2005) to proteins have been tested as a way of increasing the immunogenicity. In the study by Verheul *et al.* (1993) antibodies to the protein but not to LPS were induced in mice, whereas Cox *et al.* (2005) previously showed that bactericidal antibodies could be induced to the inner core structure of LOS coupled via the lipid A region. A monoclonal antibody raised to an inner core epitope of LOS, conserved in 26 of 34 MenB strains tested (except immunotypes L2, L4, L5, L6), bound to the meningococcal surface (Plested *et al.*, 1999), and was able to confer protection against experimental meningococcaemia in a rat model, but only to strains expressing truncated LOS glycoforms (Plested *et al.*, 2003). Peptide mimetics of LOS structures have also been investigated as a way of inducing protection (Tiwana *et al.*, 2005), but have failed to induce complement-mediated serum bactericidal activity to MenB.

1.4.1.2 Surface exposed proteins

Surface exposed proteins or combinations of several OMPs are currently the most promising candidates for a broad range, if not universal, MenB vaccine. Proteins accessible on the meningococcal surface during colonisation and invasive disease have the potential to induce a host immune response (Zollinger *et al.*, 1974). A large proportion of the bactericidal antibodies in meningococcal patients are directed towards the abundant OMP PorA. The protein has variable expression among strains (van der Ende *et al.*, 1995) and highly variable surface exposed loops (Feavers *et al.*, 1996), which have hampered the development of universally protective vaccine preparations based on PorA. Antigenic variability (or variable surface expression) is a general problem besetting many meningococcal surface exposed proteins.

OMPs which have been explored as vaccine antigens include TbpA (Ferron *et al.*, 1992), NspA (Martin *et al.*, 1997), H.8 (Bhattacharjee *et al.*, 1990) and factor H binding protein (NMB1870) (Masignani *et al.*, 2003). Surface exposed proteins which are induced in response to conditions mimicking environments in the human host are

likely to be good candidates for inclusion in protein based vaccines. Such proteins include those induced under conditions of low iron-availability (Banerjee-Bhatnagar & Frasch, 1990), commonly encountered by *N. meningitidis* in the host. Studies have investigated the potential of several surface exposed iron uptake proteins to induce bactericidal antibodies that provides protection to meningococcal disease. Isolated transferrin binding proteins, TbpA and TbpB, have both been shown to induce an immune response (Ferron *et al.*, 1992; Ala′Aldeen *et al.*, 1994). Antisera raised against one TbpB type have been demonstrated to induce cross-reactive antibodies that bind to the surface and kill a diverse set of strains (Rokbi *et al.*, 1997). Both recombinant TbpA and TbpB have been shown to induce protection to meningococci in a mouse model (West *et al.*, 2001). In mice TbpB were found to induce a stronger bactericidal activity than TbpA, but on the other hand, bactericidal antibodies to TbpA were found to be more cross-reactive. Formulations of TbpB have been tested in phase I clinical trials, but immunogenicity of the protein was limited in humans (Danve *et al.*, 1998). Other proteins that have reached clinical trials include NspA, a conserved meningococcal outer membrane protein, which provided protection against experimental infection in mice (Martin *et al.*, 1997). However, a vaccine comprising recombinant (unfolded) NspA, although inducing bactericidal antibodies in mice, failed to induce bactericidal antibodies in adult volunteers in a clinical trial, possibly due to loss of antigenic epitopes in the linearised protein (Halperin *et al.*, 2007).

The completion of the whole genome sequence of the MenB strain MC58 in 2000 heralded the move into the post-genomic era of vaccine development (Tettelin *et al.*, 2000). Pizza *et al.* (2000) used genome information to identify a large number of putative surface exposed proteins, which were expressed and tested in mice for their ability to induce an immune response. Their actual cellular location was tested by flow cytometry on whole cell meningococci. This approach was termed ‘reverse vaccinology’ by Rappuoli (2000). From the study by Pizza *et al.* (2000) a set of proteins was identified for investigation of their potential as vaccines. Others have used the genomic information combined with new ‘omics’ technologies to identify putative immuno-

genic surface antigens. Proteins that have been investigated include the conserved surface accessible protein NMB0035 (Arenas *et al.*, 2008); the lipoproteins NMB1126 (Niebla *et al.*, 2006) and NMB0928 (Delgado *et al.*, 2007); and the surface protein NMB1468 identified by a proteomic approach (Hsu *et al.*, 2008). Others include a meningococcal OMP named LP2086 (identical to factor H binding protein), which has been explored for its vaccine potential (Fletcher *et al.*, 2004), and shown to offer protection in experimental models of disease (Pillai *et al.*, 2005).

In general, efforts to design vaccines that induce immunity against bacterial surface proteins have met with some drawbacks, including importantly (a) that they do not seem to induce as good immunity in children under 2 years of age (the group most affected by disease) as in adults, and (b) that many surface exposed proteins show a high degree of variability between different strains, through mutation or phase variation, and therefore a vaccine based on these will not give universal protection. Additionally, for many OMPs, abundance and accessibility on the surface of the bacteria have been found to be variable among strains. During all stages of colonisation and infection *N. meningitidis* adapts to the changing environment within the host and evades the immune system by for example phase-variation (due to for example slipped strand mispairing) leading to antigenic variation of proteins, and variable expression of surface antigens. Many putative phase-variable genes have been identified in the genome of MenB (Saunders *et al.*, 2000). Examples of such genes include the pilin gene *pilE* the expression of which has been found to be regulated by phase-variation (Pinner *et al.*, 1991), and that undergoes phase variation in invasive disease (Rytkönen *et al.*, 2004). Introduction of repeats in the coding region of genes due to phase variation can also result in frame shifts, which lead to non-functional truncated gene products (Jonsson *et al.*, 1991) or peptides with varied immunoepitopes. In addition, transcription levels of genes can differ between Neisseria species or meningococcal strains due to other processes. Variable gene expression is often a result of genomic variants in up-stream regions of the gene, such as insertion elements resulting in novel activator binding sites or different strength promoters. An example of the latter is the observed two

fold higher expression of the *lst* gene, encoding a LOS sialyl transferase, in gonococci in comparison to levels in meningococci (Packiam *et al.*, 2006). Uria *et al.* (2008) showed that insertion of an IS1301 element between the capsular biosynthesis locus and the capsular export locus in a MenC strain led to increased expression of adjacent genes and resulted in resistance to killing by bactericidal antibodies induced to the MenC conjugate vaccine as well as other surface structures.

Problems with presenting the antigens in their native form have also hampered attempts to develop vaccines from OMPs. This could be solved by expressing the antigens on the surface of OMVs.

One investigational OMP vaccine developed by Novartis Vaccines, which has passed through phase II clinical trials, shows promising results both in adults and toddlers (Miller *et al.*, 2008; Snape, 2008). The vaccine is based on recombinant surface exposed proteins NadA (NMB1994) and two fusion proteins, NMB2132–NMB1030 and NMB2091–NMB1870 (Giuliani *et al.*, 2006).

1.4.1.3 Outer membrane vesicles (OMVs)

N. meningitidis sheds OMVs (blebs) containing OMPs, LOS and periplasmic proteins, which have similar immunostimulatory effects to live bacteria. OMVs isolated from bacterial cultures and appropriately detoxified can be used as vaccines (Fredriksen *et al.*, 1991). The vaccines developed so far have been more appropriate for use in strain-specific situations such as clonal outbreaks, rather than in a routine immunisation programme aimed at preventing disease caused by all MenB strains. They have been well tolerated and have been used in response to outbreaks of MenB disease in Norway (Bjune *et al.*, 1991) and Cuba (Sierra *et al.*, 1991). Convincing efficacy data derive from recent experience in New Zealand, where a recent epidemic has been controlled using an OMV vaccine based on the epidemic strain NZ98/254. The vaccine was used in mass-vaccination of the population under the age of twenty (Oster *et al.*, 2005). However, immunodominance of a few hypervariable surface proteins, in particular PorA, limits the capacity of OMVs to confer a broad immunity against a wide range of strains. OMVs with modified expression of surface proteins have been

investigated as a way to increase both immunogenicity and strain coverage. OMVs isolated from two strains constructed to express three alleles each of different PorAs (Claassen *et al.*, 1996), have been found to induce bactericidal antibodies in mice. The hexavalent OMV preparation has been demonstrated to induce bactericidal antibodies to the meningococcal strains covered by the PorA types in infants (94%) after four doses (Cartwright *et al.*, 1999) and in children (16-100%) after three doses (de Kleijn *et al.*, 2000). Over-expression of the factor H binding protein NMB1870 on OMVs induced broader protection against meningococci than both the protein alone or wild type OMVs (Hou *et al.*, 2005). O′Dwyer *et al.* (2004) investigated the use of OMVs from commensal Neisseria as vectors for presenting meningococcal antigens in their native form on the surface of OMVs and showed that such OMVs, expressing the vaccine antigen NspA, induced bactericidal antibodies in mice.

1.4.1.4 Live-attenuated vaccines

Live-attenuated vaccines have been used to combat disease caused by for example *Salmonella enterica* (Tacket *et al.*, 2000), and have been suggested as a potential strategy for a MenB vaccine (Tang *et al.*, 1999). Sun *et al.* (2000) identified genes essential for meningococcal growth in the blood stream of infant rats. The ability of two of the attenuated mutant strains ($\Delta siaD\Delta metH$ and $\Delta siaD\Delta rfaF$) to induce an immune response were tested in mice, and were shown to be well tolerated, induce bactericidal antibodies and confer protection to subsequent challenge with wild type meningococci, both homologues and heterologous strains (Li *et al.*, 2004). The meningococcus is strictly a human pathogen, so results in mice are not necessarily highly predictive of the response in humans, and should be interpreted with caution. Vaccines developed using this strategy will naturally require substantial tests of safety before being approved by regulatory authorities.

1.4.1.5 Commensal Neisseria

Since naturally occurring nasopharyngeal colonisation with *N. lactamica* induces a cross-protective immune response to meningococci, intra-nasal vaccination based on

live *N. lactamica* has been suggested (Oliver *et al.*, 2002; Li *et al.*, 2006). OMVs isolated from non-pathogenic *N. lactamica* have also been tested as a vaccine (Gorringe *et al.*, 2005; Finney *et al.*, 2008), but failed to induce bactericidal antibodies to MenB strains in mice.

1.5 Hypothesis and aims

1.5.1 Hypothesis

Vaccine strategies that have successfully reduced the incidence of group C meningococcal disease have had their primary effect through eliciting bactericidal plasma antibody, but have also had a secondary effect through reduction of nasopharyngeal colonisation and carriage (Maiden *et al.*, 2008). However, anti-colonisation strategies to prevent invasive bacterial infection are inherently risky. While a vaccine that targets structures expressed by bacteria on the nasopharyngeal epithelial surface may effectively reduce the incidence of meningococcal disease by reducing colonisation, there remains a risk of occasional invasive infection which can only be neutralised by antibodies in the bloodstream. Significant perturbation of meningococcal ecology with an anti-colonisation vaccine may indeed, in the long run, turn out to be detrimental, as the vaccine-induced immune response may promote the survival of resistant strains expressing alternative antigens or down-regulating antigen expression. For example, an invasive strain of *N. meningitidis* has been identified that lacks the immunodominant surface antigen PorA due to a mutation (Newcombe *et al.*, 1998). While some meningococcal genes expressed during bacteraemia will be as highly expressed by organisms in the nasopharynx, others will be up- or down-regulated in response to the major changes in bacterial environment that follows invasion. Gene products that are expressed more strongly in the blood stream than in the nasopharynx are particularly attractive candidates for inclusion in a vaccine, as they should be less subject to immune selection. A major problem besetting most proposed meningococcal vaccination strategies based on OMPs has been the propensity of the organism to undergo antigenic variation, importantly driven by immune selection pressure (Martin *et al.*,

2000). However septicaemia is, from the organism's perspective, a non-productive end point of meningococcal infection, an evolutionary dead-end from which bacteria do not survive to contribute to the genetic diversity of the species in the nasopharynx of present or future hosts. Therefore, selection pressures operating principally in the bloodstream are less likely to lead to the evolution of vaccine-resistant organisms if their targets are the products of genes expressed largely or solely in the bloodstream.

I hypothesize that any surface exposed gene products that are expressed at a higher level in the bloodstream than on the mucosal surface, where immune selection pressure drives antigenic diversity, should be less variable, and so, attractive as cross-protective vaccine candidates. In the absence of a satisfactory animal model of meningococcal septicaemia, I have chosen to explore the repertoire of meningococcal gene expression in the course of prolonged incubation in human plasma and blood. In this thesis the transcriptome of MenB in *ex vivo* models of septicaemia will be compared to the transcriptional pattern during nasopharyngeal carriage, with the objective of identifying genes expressed largely or solely in the bloodstream. Any such genes that encode a predicted or known surface exposed protein will be explored for their vaccine candidacy.

1.5.2 Aims

- to characterise the expression profile of *N. meningitidis* in models of human septicaemia
- to identify candidate genes which are relatively up-regulated during septicaemia compared to conditions mimicking the carriage state
- to explore cross-protective vaccine candidacy of any surface exposed proteins encoded by such genes

Chapter 2

Materials and methods

2.1 Bacterial strains and growth conditions

2.1.1 Bacterial growth conditions

Neisseria strains were routinely propagated on GC (gonococcal) agar (Difco) supplemented with 1% Vitox (sGC) at 37°C in 5% CO_2. Neisseria strains were grown in Mueller-Hinton (MH) broth supplemented with 1% Vitox (sMH) at 37°C in an orbital incubator at 180 rpm. All experiments with *N. meningitidis* strains were carried out in a biosafety level 3 containment laboratory.

2.1.2 Bacterial strains

Bacterial strains utilised in this work are listed in Table 2.1.

Table 2.1: *Neisseria meningitidis* strains used in this project

Strain	Sequence type	Clonal complex	Designation	Disease	Country
MC58	74	ST-32 complex/ ET-5 complex	B:15:P1.7,16-2	–	UK
BZ 169	32	ST-32 complex/ ET-5 complex	B:P1.5-2,16	invasive †	Netherlands
H44/76	32	ST-32 complex/ ET-5 complex	B:P1.7,16	invasive †	Norway
BZ 83	34	ST-32 complex/ ET-5 complex	B:P1.5-2,10	invasive †	Netherlands
SWZ 107	35	ST-35 complex	B:P1.ND,ND	invasive †	Switzerland
NG H38	36	–	B:P1.ND,3	carrier	Norway

Continued on next page ...

Table 2.1 – continued from previous page

Strain	Sequence type	Clonal complex	Designation	Disease	Country
BZ 232	38	ST-37 complex	B:P1.ND,2	invasive †	Netherlands
NG 6/88	13	ST-269 complex	B:P1.ND,1	invasive †	Norway
NG F26	14	ST-269 complex	B:P1.ND,16	carrier	Norway
BZ 198	41	ST-41/44 complex/ Lineage 3	B:P1.7-2,4	invasive †	Netherlands
NG H15	43	ST-41/44 complex/ Lineage 3	B:P1.19,15-2	carrier	Norway
NG H36	47	ST-41/44 complex/ Lineage 3	B:P1.5-1,2-2	carrier	Norway
BZ 147	48	ST-41/44 complex/ Lineage 3	B:P1.ND,ND	invasive †	Netherlands
297-0	49	ST-254 complex	B:P1.ND,15	carrier	Chile
1000	20	ST-18 complex	B:P1.ND,ND	invasive †	Russia
528	4802	ST-18 complex	B:P1.5-1,10	invasive ‡	Poland
NG P165	–	ST-11/ ET-37 complex	N.D.	–	–
MO1-240185	11	ST-11 complex/ ET-37 complex	B:2a:P1.5,10-8	–	UK
90/18311	11	ST-11 complex/ ET-37 complex	C:P1.5,2-1	–	UK
BZ 133	1	ST-1 complex/subgroup I/II	B:P1.7,16	invasive †	Netherlands
F6124	5	ST-5 complex/ subgroup III	A:P1.20,9	invasive †	Chad
NG E28	26	–	B:4:-	carrier	Norway
2996	–	–	B:2b:P1.2	–	–

†: Unspecified/other; ‡: Meningitis and septicaemia

2.2 Reagents and Kits

Reagents and kits used in this work are described in Table 2.2. Chemicals not listed in the table below were obtained from Sigma or BDH. Solutions were sterilised by autoclaving at 121°C for 15 min. Heat labile reagents were manually filter-sterilised or filtered using a vacuum manifold and 0.22 μm nitrocellulose filters (Millipore).

Table 2.2: Description of reagents and kits

Reagent	**Supplier**
1 kb DNA ladder	Invitrogen
5×First strand buffer	Invitrogen
10× REact 2 buffer	Invitrogen
BigDye v3.1	Applied Biosystems
Cy3 dCTPs Fluorlink	Amersham Biosciences
Cy5 dCTPs Fluorlink	Amersham Biosciences
DNA polymerase I Large (Klenow) fragment	Invitrogen
dNTPs	Invitrogen
DTT	Invitrogen
Fast RNApro Blue kit	QBiogene
Fetal Bovine Serum	Gibco
GC agar	Difco
HotStar *Taq* DNA polymerase	Qiagen
MH broth	Oxoid
Min Elute PCR purification kit	Qiagen
Molecular-grade DNAse I (Units)	Invitrogen
QIAquick gel extraction kit	Qiagen
N. meningitidis antiserum group B	Difco Laboratories
Random hexamer primers	MWG Biotech
RNA 6000 Nano chip and reagents	Agilent
RNAprotect bacterial reagent	Qiagen
RNAse OUT	Invitrogen
RNAse-Free DNase Set	Qiagen
RNeasy Min Elute kit	Qiagen
RNeasy kit	Qiagen
SuperScript III reverse transcriptase (200U/μl)	Invitrogen
TaqMan Universal PCR MasterMix	Applied Biosystems
TaqMan Primers	Applied Biosystems

2.3 Polymerase chain reaction (PCR) and DNA sequencing

2.3.1 PCR

PCR was carried out in a final volume of 30 μl containing 1 μl DNA, 0.75 U HotStar *Taq* DNA polymerase (Qiagen), 0.2 mM dNTPs, 1×PCR buffer with 1.5 mM $MgCl_2$ and 15 pmol of each primer. To increase the stringency of primer binding 10% DMSO was added to reactions. Reactions were performed in a DNA Engine thermal cycler (BioRad). PCR amplification of DNA was carried out as follows. The first cycle consisted of 15 min activation of Taq polymerase at 95°C, 1 min of denaturation at 94°C, annealing at 52°C for 1 min and extension at 72°C for 2 min. The subsequent 29 cycles contained denaturation for 1 min at 94°C, annealing for 1 min at 52°C and extension at 72°C for 2 min. The final cycle consisted of extension at 72°C for 10 min. After amplification by PCR the gene specific fragments were checked on a 1.5% agarose gel (Section 2.4) using TAE (0.04 M Tris acetate, 0.001 M EDTA, pH 8.0) as a buffer and their size compared to the corresponding product from reference strain MC58. Table 2.3 lists all primers used in the project, which were synthesized by MWG-Biotech. Primers used in PCR reactions for amplification of genomic regions for sequencing were designed to bind in flanking regions ∼100–200 base pairs (bp) outside the open reading frame (orf). The primer pairs used in these amplification reactions were designed to match both the genomic sequence of MC58 as well as homologues regions in FAM18 and Z2491 genomes where possible.

2.3.2 Precipitation of amplified products

PCR products were cleaned by polyethylene glycol (PEG) precipitation. PCR products were mixed with 20% PEG/2.5 M NaCl solution to achieve a final concentration of 10.9% PEG and 1.37 M NaCl. The mixture was incubated for 30 min at room temperature (RT) followed by centrifugation at 2750 g for 1 hr (4°C). Pelleted products were further cleaned with 70% Ethanol. Remaining ethanol was removed by an inverse spin for 1 min at 500 g. PCR products were re-dissolved in molecular-grade

water.

Table 2.3: Primers used in PCR reactions

Primer ID	Sequence 5' to 3'	Target gene
NMB0267F	CGGAGGTCAAGAGAAGGTGT	NMB0267
NMB0267R	GCCGTGATTCTCTTTGAAGC	NMB0267
NMB0345F	GAGTATTTCCATACGGCGGACTG	NMB0345
NMB0345R	GCGGTTTTTTTCTGTTTCATGG	NMB0345
NMB0389F	ACAAGGGTGATACCAGGGAG	NMB0389
NMB0390FInt	TCGGTGTGCGTTTCAATGTG	NMB0390
NMB0390RInt	GTTGTTGTAGTTGTCCAAGTCCAGG	NMB0390
NMB0391R	TGTCGTTTTTACGGCGGGTC	NMB0390
NMB0562F	TCAGGAAGATGCCGAAGGTAAG	NMB0563
NMB0563F567	TTCAGCGGGACGATAATCTGCG	NMB0563
NMB0563R1520	GCCCTCCGATTATGAACCAGTC	NMB0563
NMB0564R	CCCTCCGATTATGAACCAGTCC	NMB0563
NMB1040F2	ATCCGGGTGGACAAATAAAC	NMB1040
NMB1040R2	CTGCCCGTACCATCTTTGTC	NMB1040
NMB1882F	AAGCTAAAAGCAGCCTGCAC	NMB1882
NMB1882R3	TCCTTAAATGCGTGTACCTGTG	NMB1882
NMB1961F	TCCAAAAAGGTTCTAAAGGGATGAG	NMB1961
NMB1961R	ACTGTACGAAATCAAAGACTGGCTG	NMB1961

2.3.3 Sequencing

All primer used in sequencing reactions, if different from primers used during standard PCR reaction (Table 2.3), are listed in Table 2.4. Sequencing reactions were performed in 96-well format plates using BigDye v3.1 reaction kit (Applied Biosystems). The sequencing reaction mixture contained 1 μl DNA (PCR product, precipitated as in Section 2.3.2), 1.5 pmol gene-specific primer (forward or reverse direction) and 2 μl BigDye enzyme. The sequencing reaction was carried out in a DNAEngine thermal cycler (BioRad) as follows. The first cycle consisted of 1 sec at 96°C, 10 sec at 96°C, 5 sec at 50°C and 2 min at 60°C. The subsequent 29 cycles contained 10 sec at 96°C, 5 sec at 50°C and 2 (4) min at 60°C. Products were cleaned by precipitation as follows. A mixture of 31 μl H_2O and 64 μl 95% ethanol was added to each well. The plate was incubated in the dark for 15 min at RT followed by centrifugation at 2750 g for 1 hr at 4°C to precipitate products. The pellets were washed with 150 μl 70%

Ethanol. Remaining ethanol was removed by an inverse spin for 1 min at 500 g. The plate was kept at 4°C until sequencing. The sequencing was run on an ABI3730XL sequencer (Sequencing unit, Department of Zoology, University of Oxford). The sequencing trace files generated from each run were checked manually and necessary corrections were made to the sequence-call-errors. Each gene was sequenced on both strands, and in some cases using primers internal to the open reading frame. DNA and protein sequence alignments were carried out using the web based ClustalW programme (Larkin *et al.*, 2007). Sequences were further analysed using the programme Molecular Evolutionary Genetics Analysis (MEGA) (Tamura *et al.*, 2007).

Table 2.4: Primers used in sequencing, if different from primers in Table 2.3

Primer ID	**Sequence 5' to 3'**	**Target gene**
NMB0345F2	GAAGTCTGCCCGAACATTTC	NMB0345
NMB0345RInt	TGGACTTCCTGCGTACCTTTG	NMB0345
NMB0390F1074	ATTGGGACACCGAAGCCTAC	NMB0390
NMB0390F1589	AAAATGGGCGGACATCAG	NMB0390
NMB0390F160	TATTCCGCCGACAGCCACTTAG	NMB0390
NMB0390SeqF	ACAAGGGTGATACCAGGGAG	NMB0390
NMB1882F941	ACCGCTTCAACCAAGACTG	NMB1882
NMB1882FwdInt	GCATCGCCAACTACCAAATC	NMB1882
NMB1882R2Seq	GCGGATTGAAGCGGTAAC	NMB1882
NMB1882R538	TTCAAACAATGGCTTGCG	NMB1882
NMB1961RInt	GTCCAGACTGTCGGTCAAATC	NMB1961

2.4 Gel electrophoresis

Nucleic acids were size-fractionated by agarose gel electrophoresis containing 0.5 μg/ml ethidium bromide and 1×TAE (0.04 M Tris acetate, 0.001 M EDTA, pH 8.0) buffer. Samples were mixed with 0.17 volumes of 6×loading dye, loaded into sample wells and typically electrophoresed at 80–120 volts. The nucleic acids was visualised by placing the gel on a UV transilluminator using instant Polaroid black and white film to record the image.

2.4.1 RNA analysis by capillary gel electrophoresis

1 μl RNA samples were analysed on a RNA 6000 Nano Chip (Agilent) using an Agilent 2100 Bioanalyzer as per manufacturer's instructions (Agilent, Palo Alto, CA) to check quality of isolated RNA. The intensity of 23s and 16s rRNA peaks in relation to a standard was used for RNA quantification, and the ratio between the peak-intensities (23s/16s) used as a control of RNA quality. For RNA without degradation a 23s/16s ratio within the range of 1.4–2 is expected dependent on RNA extraction method and organism. Any contaminating eukaryote RNA in the sample could be detected by peaks in regions corresponding to 28s or 18s rRNA.

2.4.2 Purification of DNA from agarose gels

DNA fragments were visualised by fluorescence of added ethidium bromide, under UV transillumination (302 nm), and excised from gels using a clean scalpel blade. DNA was purified from the agarose using a QIAquick gel extraction kit according to the manufacturer's instructions (Qiagen, UK).

2.5 Extraction of nucleic acids

2.5.1 Isolation of chromosomal DNA

Neisseria strains were grown over night on sGC agar plates. The bacteria were harvested from a single plate and chromosomal DNA isolated using a standard phenol/chloroform method (Sambrook *et al.*, 1989).

2.5.2 Extraction of total RNA

For isolation of total bacterial RNA a bacterial pellet was re-suspended in 1 ml RNApro solution (QBiogen), the solution added to a tube containing Lysis matrix B, and cells lysed by the use of a FastPrep Instrument (QBiogen). Total RNA was isolated using protocol from Fast RNApro Blue kit supplier (QBiogen). RNA samples were further cleaned on a RNA MinElute column or an RNeasy column using on column DNase I treatment following the manufacturer's instructions (Qiagen).

2.6 Nucleic acid manipulations

2.6.1 Quantification of nucleic acid concentration

Quantification of RNA and DNA samples were performed by checking the absorbance at 260 nm using a NanoDrop 1000 instrument (NanoDrop Technologies, Wilmington, DE). The purity of the RNA or DNA was determined by A_{260}/A_{280} and A_{260}/A_{230}. For pure DNA and RNA samples the A_{260}/A_{280} should be 1.8 and 2.0, respectively.

2.7 Microarray procedures

2.7.1 Microarray slides and pre-processing procedures

A *pan*-Neisseria microarray acquired from the Bacterial microarray group at the St George's hospital was used. The microarray has been designed to cover the genome sequence of three strains of *N. meningitidis* and one strain of *N. gonorrhoeae* (Stabler *et al.*, 2005). *N. meningitidis* serogroup B strain MC58 was used as the base strain for the design of the microarray, and therefore all genes from this strain are spotted on the microarray. The MC58 genes were used as a reference to identify any unique or divergent genes in strains FAM18 (MenC), Z2491 (MenA), FA1090 (*N. gonorrhoeae*). Each gene in the gene pool was amplified by PCR, with each product designed to give minimal cross hybridisation to other genes in the pool. There are 2907 genes from MC58, 343 from Z2491, 125 from FAM18 and 404 from FA1090 on the microarray. Quality controlled PCR products are spotted in duplicate on coated glass slides. Before hybridisation microarray slides were pre-hybridised in 3.5×SSC, 0·1% SDS, and 10 mg/ml BSA at 65°C for 20 min before washing in distilled water and a subsequent 1 min wash in isopropanol. The slides were dried by centrifugation at 1500 rpm for 5 min, and stored in a dust-free box for less than 1 hr until hybridisation.

2.7.2 Labelling of total RNA

Complementary DNA (cDNA) was synthesised from 5–10 μg of total RNA using SuperScript III reverse transcriptase (Invitrogen) and random hexamer primers (MWG

Biotech) and labelled directly with either Cy3- or Cy5-dCTP (Amersham Biosciences) as below. In a 0.5 ml microfuge tube 5–10 μg of total RNA was mixed with 3 μg of random hexamers (3 μg/μl, Invitrogen) and made up to a total volume of 11 μl with RNase free water (Qiagen). The sample was incubated in a heat block at 75°C for 5 min and then snap cooled on ice, followed by a brief centrifugation in a microfuge. The reverse transcription reaction mix was prepared by combining 5 μl 5×First Strand Buffer, 2.5 μl of 100 mM DTT, 2.3 μl dNTPs (5 mM dA/G/TTP, 2 mM dCTP), 1.7 μl Cy3- or Cy5-dCTP, and 2.5 μl SuperScript III (200 U/μl). This reaction mix is sufficient for one labelling reaction. The reaction was incubated in dark for 10 min at 25°C followed by incubation at 42°C for 90 min. Cy3- and Cy5- labelled samples were mixed and purified using a MinElute kit following the manufacturer's instructions (Qiagen). The mixture was eluted in 14.9 μl H_2O.

2.7.3 Labelling of genomic DNA

Genomic DNA isolated from stationary phase organisms was fragmented by sonication to obtain sizes between 500-3000 bp. The fragmentation was confirmed by separation on a agarose gel as per Section 2.4. For genomotyping experiments 2 μg was labelled with either Cy5-dCTP or Cy3-dCTP using Klenow fragment (Invitrogen) and random hexamer primers (MWG Biotech). For gene expression experiments, in which genomic DNA was used as a common reference, 0.5 μg genomic DNA was labelled with Cy3-dCTP using Klenow fragment (Invitrogen) and random hexamer primers (MWG Biotech). In a 0.5 ml microfuge tube 0.5-2 μg DNA was mixed with 3 μg of random hexamers (3 μg/μl, Invitrogen) and made up to a total volume of 41.5 μl with molecular biology grade water. The sample was incubated in a heat block at 95 °C for 5 min and then snap cooled on ice, followed by a brief centrifugation in a microfuge. The labelling reaction was prepared by combining 5 μl 10×REact 2 buffer, 1 μl dNTPs (5 mM dA/G/TTP, 2 mM dCTP), 1.5 μl Cy3- or Cy5-dCTP and 1 μl Klenow fragment (3–9 U/μl). This reaction mix is sufficient for one labelling reaction. The reaction was incubated in dark for 120 min at 37°C. Cy3- and Cy5- labelled samples were mixed and purified using a MinElute kit following the manufacturer's

instructions (Qiagen). The mixture was eluted in 14.9 μl H_2O.

2.7.4 Hybridisation and post-hybridisation washes

The mixture of test and reference samples in 14.9 μl H_2O was combined with filtered solutions of 4.6 μl 20×SSC and 2.0 μl 3·6% SDS to achieve a final 23 μl hybridization solution of 4×SSC, 0·3% SDS. Mixed test and reference samples in hybridisation solution (4×SSC, 0·3% SDS) were denatured at 95°C for 2 min followed by a brief centrifugation. The solution was hybridised to a microarray slide under a 22×22mm Lifterslip (Eyrie Scientific). Arrays were incubated in a humidified hybridisation chamber (Corning) placed in a water bath at 65°C for 16–20 hours. Following hybridisation the slides were washed once in 1×SSC, 0·06% SDS at 65°C with agitation for 2 min followed by two washes in 0.06×SSC with agitation for 2 min. Slides were dried by centrifugation in a 50 ml Falcon tube at 1500 rpm for 5 min. Slides were kept in the dark in a dust-free box until scanning.

2.7.5 Scanning and image processing

Microarrays were scanned using a GenePix 4000B laser scanner (Axon Instruments), and fluorescence signals analysed using GenePix Pro 6.1 software (Axon Instruments). Technically flawed spots were removed either automatically by the GenePix software or through manual examination of the array images, and these spots were flagged as 'poor' in the GenePix results files.

2.7.6 Pre-processing and statistical analysis of gene expression data

Data points with a low intensity compared to background (signal-to-noise ratio of $\leq$3) in both channels (and on all arrays) were flagged as poor. Spots on the array that did not pass the initial quality control and filtering procedure on all arrays were down-weighted (0.1) during normalisation and in the following analysis steps. The intensity of each spot was background corrected, each microarray normalised, and the correlation between technical replicates (duplicate spots on each array) calculated using methods implemented in 'Linear models for microarray data' (Limma) (Smyth,

2004). For background correction the method 'normexp' was used. This method gives the lowest false discovery rate and additionally has the advantage of not giving negative values after adjustment (Ritchie *et al.*, 2007). For experiments using genomic DNA as a common reference each slide was normalised to median $\log_2$ ratios. Microarray data from cDNA: cDNA experiments were normalised using locally weighted scatterplot smoothing (Lowess/Loess). Linear models as implemented in the Limma package (Smyth, 2004) for the statistical software R (R Development Core Team, 2008), were used to identify differentially expressed genes. Control spots on the array were removed before fitting the model. P-values were adjusted for multiple testing to control false discovery rate (FDR) using the Benjamini and Hochberg step down correction (Benjamini & Hochberg, 1995). Genes with an adjusted P-value of $\leq$0.01 or $\leq$0.05 (dependent on the experiment) and with a fold change of at least 1.5 are reported as significantly different between conditions or time-points.

2.7.7 Pre-processing and statistical analysis of genomotyping data

Data flagged as absent in GenePix were filtered out, local background (for each spot) intensity was subtracted from the spot intensity and each microarray slide was normalised to median $\log_2$ ratios. The designation of genes in each strain as present, divergent or absent/highly divergent was achieved using Genomotyping Analysis by Charlie Kim (GACK) (Kim *et al.*, 2002). The GACK software uses the normal distribution to calculate a value of estimated probability of presence (EPP) for each gene on the array. A gene is designated as present (1) if the calculated EPP is 100%, as divergent (0) if the calculated EPP is between 0% and 100% and as absent (-1) if the calculated EPP is 0%. Genes in strain MO1-240185 which were identical (or similar) to genes found in MenA strain Z2491 and/or MenC strain FAM18 could also be determined since *pan*-Neisseria arrays (Section 2.7.1) were used in all experiments.

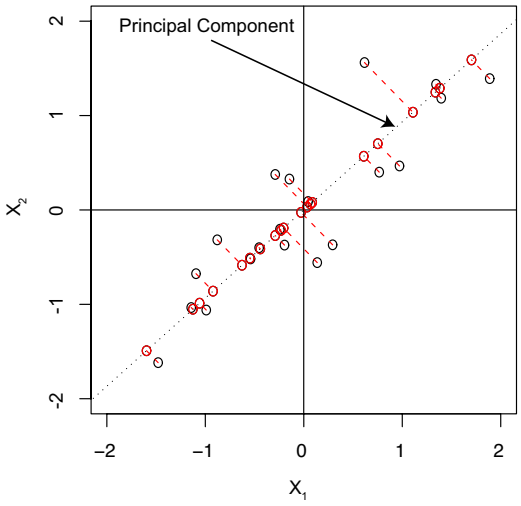


Figure 2.1: Illustration of how data points (black) in a two-dimensional space are projected down orthogonally onto the first principal component. The red points indicate the projection on the component: the position of the projected points on the principal component is equivalent to the score of the observation for component one. Figure adaptated with permission from Rantalainen (2008)

2.7.8 Visualisation (dimensionality reduction) and gene set enrichment analysis

Hierarchical clustering of data on gene or sample level was performed in R using the function `hclust()`. Principal component analysis (PCA) (Figure 2.1) of microarray data was performed in R using the function `prcomp()`. Heat maps were constructed using functions in the R-package gplots, available as part of the gregmisc add-on package bundle (Warnes, 2003). Gene set enrichment analysis to detect differential regulation of groups of genes was performed using the 'Gene set enrichment analysis (GSEA)' software (Mootha *et al.*, 2003) or the function `geneSetTest()` available in the R-package Limma (Smyth, 2004).

2.8 Reverse transcription (RT) PCR for quantification of transcripts

2.8.1 Semi-quantified RT-PCR

OneStep RT-PCR kit (Qiagen) was used according to manufacturer's instructions to confirm expression of a selected set of genes which were differentially expressed in microarray analysis. RNA samples from each time-point were reverse transcribed using random hexamer primers, and the resulting cDNA amplified with gene-specific primers. The resulting products were separated in 2% agarose gels stained with ethidium bromide. The intensity of bands on the gel image was quantified using

ImageQuant software (GE Health Care/Molecular Dynamics). Ratios were calculated for each comparison of interest by dividing the median intensities in the bands on the gel. Primers used are listed in Table 2.5.

Table 2.5: Primers used in semi-quantified RT-PCR

Primer ID	Sequence 5' to 3'	Target gene
NMB0995F	TCAGGGTTGCGAGTTTGGTG	NMB0995
NMB0995R	GAACAAGGAAAGAAAATGGCACG	NMB0995
NMB1988F	CGAACAAACCCTGCTGAAATAC	NMB1988
NMB1988R	ACCTTGAAGCGGTCGTAACG	NMB1988

2.8.2 Comparative real-time RT-PCR (TaqMan analysis)

Before analysis, the RNA was digested 2×15 min with DNase I on an RNeasy column (Qiagen) to remove contaminating genomic DNA. cDNA was synthesised from total bacterial RNA using SuperScript III reverse transcriptase and random hexamer primers (Invitrogen). The RNA was primed with 50 ng random primer per 1 μg of total RNA. For each gene-specific primer and each sample, the TaqMan reaction was performed using 5 ng of input RNA, both for cDNA (reverse transcribed from total RNA) and with total RNA, examined to detect any signal attributable to contaminating DNA. Three biological replicates were assayed for each time-point in blood, and four technical replicates of the TaqMan reaction were performed for each sample. The primers were designed and manufactured using Assay-by-Design (Applied Biosystems) and primers and reporters are listed in Table 2.6 and Table 2.7, respectively. Reporter dye and quencher were FAM and NFQ, respectively. All data were normalised to the expression of 16s rRNA or two genes found to be relatively stable in the microarray experiment (NMB0108 and NMB0184). The data were analysed using the comparative C_T (threshold cycle) method, in which the amount of target normalised to 16s rRNA (in this case) and relative to a 'calibrator' is given by $2^{-\triangle\triangle C_T}$ (Applied Biosystems, 1997). Standard deviations were calculated as described by Bookout & Mangelsdorf (2003).

Table 2.6: Primers used in TaqMan analysis

Forward Primer	Forward Primer Seq.	Reverse Primer	Reverse Primer Seq.	Target gene
NMrrnaA16S-B2F	GCTAATACCGCATACGTCTTGAGA	NMrrnaA16S-B2R	TCGGCCGCTCGAATAGC	NMrrnaA16S
NMB0044-B1F	CCACTTTGAAAACTGCGGACAAC	NMB0044-B1R	CCCAAAATTTAATCAGCGTCGGTTT	NMB0044
NMB0108-M1F	CTCTTTGCAACACTTGGAACACAA	NMB0108-M1R	CATCCCTTTGCCGTGGTTTT	NMB0108
NMB0184-M1F	GGCAAAACCATTTATCTGGCAAACA	NMB0184-M1R	GCACGGGCGGTTTCCATA	NMB0184
NMB0267-M1F	CGCCGCCCACAAAACC	NMB0267-M1R	CGATGACGCTTTTGCCGTTT	NMB0267
NMB0318-B1F	GTTCAGGTTACGCCGCAAAAG	NMB0318-B1R	CGTATCGTCGTGCAAAACCTT	NMB0318
NMB0345-MF	GACAAGAAACCGTCCTTCAAAACC	NMB0345-MR	GGCGATATGCAATGCGTATGC	NMB0345
NMB0390-M1F	GCGTTTCAGACAGTCTCTCCTA	NMB0390-M1R	TCCACGCCGAAGGGATTG	NMB0390
NMB0563-M1F	AGCCGGTTCAACCAACACA	NMB0563-M1R	GCAGTAACGTGTGCGAAGTC	NMB0563
NMB0586-B3F	CCGTCAAACAAAGCAAAGTATCCT	NMB0586-B3R	GCCTTCTTCTTCGGCTTTGAG	NMB0586
NMB1040-B2F	GCAAGCTACCTCACGCTCAAT	NMB1040-B2R	CGGATTGTCGGGATGGTGTT	NMB1040
NMB1164-B2F	CGGTGTTCGTACCCCGATTT	NMB1164-B2R	GGAAGCTGGAGCGGTTGT	NMB1164
NMB1807PON-B2F	AAACGCTTTTACCGGCATTGG	NMB1807PON-B2R	GCCGGACACGACATTGC	NMB1807
NMB1882-B1F	TGCCGACCATCACCGTTAC	NMB1882-B1R	CGTGCCGGAAACAGTGTAG	NMB1882
NMB1961-E1F	GCTCGATTTGACCGACAGTCT	NMB1961-E1R	GCGCGTGTAGCTGTATTTGTC	NMB1961

Table 2.7: Reporter sequences for TaqMan analysis

Reporter 1 Name	Reporter 1 Sequence	Target gene
NMrrnaA16S-B2M2	AAGGTCCCCTGCTTTCT	NMRrnaA16S
NMB0044-B1M1	CCCGCCAGTGTTTACT	NMB0044
NMB0108-M1M2	CAGGGCGAGTATTTG	NMB0108
NMB0184-M1M2	AACGCGCCGGAAAC	NMB0184
NMB0267-M1M1	ATGTGCGCGTAACCAA	NMB0267
NMB0318-B1M2	CCGCACCGTACCGCC	NMB0318
NMB0345-MM2	CCGTTCAAGCCATATTT	NMB0345
NMB0390-M1M1	CATTGCCGCCCAAACC	NMB0390
NMB0563-M1M1	CCCTCCGCATTTCAAG	NMB0563
NMB0586-B3M1	AAGCGACCAAAGGCA	NMB0586
NMB1040-B2M2	CCGTGTCCGTCTTCG	NMB1040
NMB1164-B2M1	CCGTCGGAACATTCG	NMB1164
NMB1807PON-B2M2	AACACCCCAAACATCC	NMB1807
NMB1882-B1M2	CCGTCGTTGGAACTC	NMB1882
NMB1961-E1M2	ATGGCGGCTTCGTCC	NMB1961

2.9 *Ex vivo* infection models

2.9.1 Plasma assay

Fresh frozen plasmas (FFP) that were used in microarray studies were kindly provided by HPA Porton Down and the Department of Haematology at St Mary's Hospital. All other blood plasmas used during this project were collected from adult volunteers (informed consent). For microarray experiments *N. meningitidis* strain MO1-240185 was propagated for 4 hr on sGC agar plates. The cells were harvested into cell culture medium RPMI 1640 (Sigma) to an OD_{600} of approximately 5. An initial load of 1–5×10^7 cfu/ml was added to 20 ml plasma for the 60 min time-point and to 10–20 ml plasma for the 240 min time-point. The same inocula were used for growth in RPMI 1640 (Sigma) supplemented with 10% heat inactivated (hI) FBS. At time-points 60 and 240 min after inoculation an aliquot of the cultures was added to 4 times ice cold medium kept on ice. Bacteria were harvested by centrifugation and RNA was isolated as described in Section 2.5.2. RNA was isolated from at least four independent experiments. To assess the number of viable bacteria aliquots of plasma

were removed at various time-points, 100 μl of appropriate dilutions were plated in triplicate on sGC agar. Results were expressed compared to time zero.

2.9.2 Human blood infection model

Fresh blood for the study was collected from adult volunteers (informed consent) into heparinised tubes. Blood was maintained at 37°C with 5% CO_2, for less than 20 min, until the time course was initiated. The whole blood assay method was adopted from Ison *et al.* (1995). Briefly, about ten colonies of *N. meningitidis* from an overnight sGC agar plate were sub-cultured onto a fresh sGC agar plate and incubated for 4 hours at 37°C with 5% CO_2. The cells were harvested into cell culture medium RPMI 1640 (Sigma) to an OD_{600} of approximately 1–5. Heparinised blood was inoculated with 1–5$\times 10^7$ cfu/ml of meningococci and incubated on a rocking platform at 37°C with 5% CO_2. To assess the number of viable bacteria, aliquots of blood removed at various time-points, and 100 μl of appropriate dilutions in triplicate were plated on sGC agar. Results were expressed compared to time zero.

2.9.2.1 Recovery of bacterial RNA from blood

At time-points 0, 20, 40, 60, 90 and 240 min after inoculation, aliquots of infected blood were added to 4 volumes 5% Saponin (Sigma) in ice cold RPMI 1640 and incubated for 10 min to lyse human cells. The lysate was mixed with RNAprotect bacterial reagent (Qiagen) and cells were harvested by centrifugation at 3500 g for 10 min. The RNA was isolated from the corresponding pellet as described in Section 2.5.2.

2.9.2.2 Biochemical analysis of blood

At the start of a WBA experiment (Section 2.9.2) and at time-points up to 240 min post-infection with meningococci, aliquots of blood were removed for analysis of a number of different constituents. As a control, uninfected blood incubated and treated identically was also included in the analysis. Analysis was performed by the Clinical Biochemistry unit at St Mary's Hospital, London. For analysis of levels of

glucose and lactate 200-400 μl of blood was added to microtainer tube containing sodium fluoride and potassium oxalate and the cells were collected by centrifugation. For analysis of complement (C3, C4 and total haemolytic complement) and iron, 400-500 μl blood was added to a serum-separating microtainer tube and the cells collected by centrifugation. All the samples were kept on ice until analysis. Samples that were going to be analysed for total haemolytic complement were stored at -80°C until analysis. For analysis of blood gases, 600 μl blood was collected in a 1 ml capped syringe and analysed at once in a blood-gas machine (Rapidlab 865).

2.10 pH effect on *N. meningitidis*

About ten colonies of *N. meningitidis* strain MC58 from an overnight sGC agar plate were sub-cultured onto a fresh sGC agar plate and incubated for 4 hours at 37°C with 5% CO_2. Meningococci from solid agar were harvested into 3 ml of Muller-Hinton broth supplemented with IsoVitalex (sMH) to an OD_{600} of 1. The cells were harvested by centrifugation and the bacterial pellet was re-suspended in sMH buffered with 50mM HEPES and adjusted to a pH of 7.4. The culture was further split into two batches, and pH adjusted to either 7.4 or 7.8 using KOH. The pH of the medium was adjusted with KOH solution rather than NaOH to avoid extra sodium ions, which stress cells at high pH (Gerchman *et al.*, 1993). Bacteria were cultured for 20 min at 37°C with 5% CO_2. 5 ml of each liquid culture were added to 2 volumes of RNAprotect bacterial reagent (Qiagen) and the bacteria harvested by centrifugation. RNA was isolated and processed as described in Section 2.5.2. RNA isolated from 6 independent experiments (biological replicates) were analysed using microarrays, as described in Section 2.7.

2.11 Bioinformatic analysis

Public databases containing previously published protein and DNA sequences were searched using the BLAST program (Altschul *et al.*, 1990). Multiple alignments of nucleotide and amino acid sequences were performed using the web based ClustalW

with default settings (Larkin *et al.*, 2007). The amino acid sequence was deduced *in silico* from the nucleotide sequence using the Expasy translate tool (`http://www.expasy.ch/tools/dna.html`) or using tools available in MEGA software (Tamura *et al.*, 2007). TEXshade was used to visualise alignments and representations of protein characteristics and structure (Beitz, 2000). The MC58 (serogroup B), Z2491 (serogroup A) and FAM18 (serogroup C) genomes were interrogated using genome information in the NCBI database (`http://www.ncbi.nlm.nih.gov/genomes`).

Table 2.8: TIGR main role categories

Amino acid biosynthesis	Biosynthesis of cofactors, prosthetic groups and carriers
Cell envelope	Cellular processes
Central intermediary metabolism	DNA metabolism
Energy metabolism	Fatty acid and phospholipid metabolism
Conserved hypothetical proteins	Mobile and extrachromosomal element functions
Protein fate	Protein synthesis
Purines, pyrimidines, nucleosides and nucleotides biosynthesis	Regulatory functions
Transcription	Transport and binding proteins
Unassigned	Unknown function

Proteins were divided into functional groups according to their annotation in the Comprehensive Microbial Resource at 'The Institute for Genomic Research' (TIGR) (Peterson *et al.*, 2001). All TIGR main role categories are listed in Table 2.8. For predictions of cellular location of proteins PSORTb v2.0 (Gardy *et al.*, 2005) and PLSpred (Bhasin *et al.*, 2005) were used. For predictions of signal peptides the SignalP 3.0 server was used (Nielsen *et al.*, 1997; Bendtsen *et al.*, 2004), and for determining the presence and location of twin-arginine signal peptide cleavage sites TatP was used (Bendtsen *et al.*, 2005b). Lipoproteins were predicted using LipoP (Juncker *et al.*, 2003), and non-classically secreted proteins with SecretomeP (Bendtsen *et al.*, 2005a). Secondary structures were predicted using Jnet secondary structure prediction algorithm (Cuff & Barton, 2000). For prediction of transmembrane α-helices either THMMM (Krogh *et al.*, 2001) or TMpred (`http://www.ch.embnet.org/software/TMPRED_form.html`) were used. For prediction of transmembrane β-barrels either BOMP (Berven *et al.*, 2004) or PRED-TMBB (Bagos *et al.*, 2004a,b)

were used.

2.12 Genetic nomenclature

MC58 genes are denominated by the letters NMB followed by a four digit number. In this thesis genes will be annotated by their common name and NMB ID in the first occurrence in each chapter, and thereafter only referred to by their common name.

Chapter 3

MenB growth and corresponding changes in human blood

To model the scenario in naïve hosts, blood from adults with absent bactericidal activity to the meningococcal strains under investigation was used in all microarray studies presented in this thesis. In this chapter is described how whole blood samples from adults were tested, to find individuals whose blood could sustain meningococcal growth (i.e. 'non-killers'). Any changes in composition of 'non-killer' and 'killer' blood samples during incubation with or without the addition of bacteria were also determined. These results were used to explain some of the transcriptional responses to *in vitro* growth in blood (Chapter 6).

3.1 Introduction

During septicaemia, immune defences, both innate and adaptive, are activated along with inflammatory factors. Host immune responses during meningococcal disease have been discussed in Section 1.2.5.1. The hallmark of meningococcal disease is the uncontrolled production of inflammatory factors, such as the pro-inflammatory cytokine TNF-α, which contribute to septic shock through activation of immune cells and the pro-coagulant system, leading to the high mortality. Increases in plasma lactate are commonly observed in patients with severe sepsis and this increase has been correlated with poor outcome (Bakker *et al.*, 1991). The observed increase in plasma lactate was initially suggested to be an effect of tissue hypoxia, but has in

more recent studies been linked to effects of cytokines and local inflammation (Gore *et al.*, 1996; Gutierrez & Wulf, 1996).

3.1.1 *In vitro* (*ex vivo*) models of meningococcal sepsis

Experimental systems exploiting serum bactericidal activity (Goldschneider *et al.*, 1969) or whole blood killing (Heist *et al.*, 1922; Ison *et al.*, 1995) have been used to model meningococcal survival or killing in the bloodstream (Section 1.2.6). Heist *et al.* (1922) coined the terms 'non-killer' and 'killer' to described individuals whose blood respectively supported or failed to support the growth of meningococci in whole blood.

In vitro models that have been used to mimic events during septicaemia include the incubation of whole bacteria or bacterial components, such as LOS, in whole blood or co-incubated with isolated blood cells. Several groups have studied the effects of incubating meningococci, or adding bacterial factors, in whole blood or plasma, mainly focusing on immune and inflammatory responses (Ison *et al.*, 1995; Gagnon *et al.*, 1998; Padrón *et al.*, 1999; Hellerud *et al.*, 2008). Cytokine release during incubation of meningococci in whole blood has been shown to be similar to responses in patients with meningococcal sepsis (Nolte *et al.*, 2002), indicating the relevance of this assay for modelling meningococcal sepsis.

In the developed world meningococcal disease is most common in children under the age of five (Gray *et al.*, 2006; Caro *et al.*, 2007), who generally do not have protective antibodies against *N. meningitidis*. In this chapter whole blood collected from adult donors was tested in the whole blood assay to find individuals whose blood could sustain meningococcal growth. In addition, changes to a number of components of blood (from 'killers' or 'non-killers') were determined during incubation *in vitro* either with or without meningococci.

3.2 Results

3.2.1 Growth of meningococci in blood from adult donors

5–10% of adults in the UK are 'non-killers' of one or several *N. meningitidis* strains: that is, the bacteria proliferate in their anti-coagulated whole blood (Ison *et al.* unpublished). To identify such individuals the survival of MenB strains was initially tested in plasma and/or blood samples donated by twenty adult volunteers. Survival was determined by serial dilution of plasma or blood followed by plating out (100 μl) in triplicate for quantitative determination of CFU (Section 2.9.2). Time-points between 0 to 240 min were explored to determine the behaviour of MenB when incubated over time in blood or plasma. Two individuals (A and B) were found to have essentially absent or low bactericidal activity against the test strain of *N. meningitidis* in both whole blood and plasma. Survival of MenB strain MC58 in non-bactericidal whole blood (A and B) in contrast to survival in bactericidal whole blood (donors C and D) is shown in Figure 3.1.

In fulminant meningococcal sepsis, numbers of bacteria in blood have been determined to be 10^4–10^5 cfu per ml using quantitative direct plating procedures (Zwahlen & Waldvogel, 1984). In a later study, the copy number of meningococcal DNA in blood was found to be 10^5–5×10^8 with a median of 2×10^7 during fulminant meningococcal septicaemia (Øvstebø *et al.*, 2004), indicating potentially higher bacterial loads than determined by plating methods. In the whole blood assay an addition of 10^9 cfu per ml bacteria at the start of the experiment was found to overwhelm the killing capacity of the blood (donor F) (Figure 3.2). A substantially increased survival was observed using 10^9 cfu per ml bacteria in comparison to an initial load of 10^7 cfu per ml bacteria (survival at 90 min 535.48% versus 1.26%). For all studies, the initial inoculum of bacteria was therefore kept to the minimum sufficient to allow the isolation of enough RNA for a microarray profiling experiments (Chapter 5 and 6).

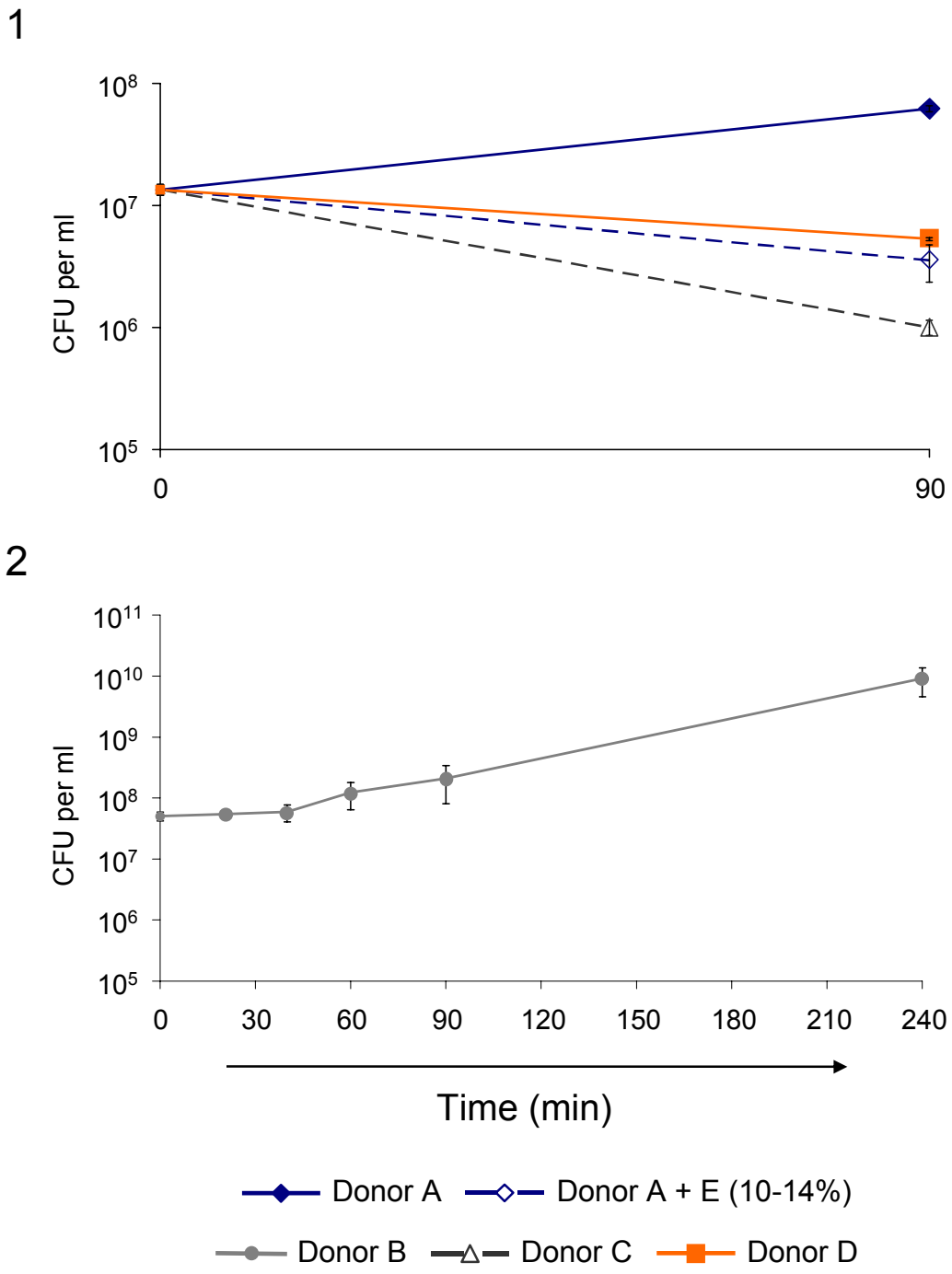


Figure 3.1: Growth of MenB strain MC58 in blood from donor A ('non-killer'), A + bactericidal plasma (E), B ('non-killer'), C ('killer') and D ('killer'). Graph **1** shows the survival or killing of MenB in blood from donor A, A + bactericidal plasma (E), C and D (assay day 2). Graph **2** shows the survival of MenB in blood from donor B (assay day 3). Note that the time scale are different in graph **A** and **B**. An initial load of 10^7 cfu per ml meningococci were added to heparinised blood followed by incubation. CFUs were determined by plating out appropriate dilutions on solid sGC agar. Error bars represent standard deviations of technical replicates (measuring error).

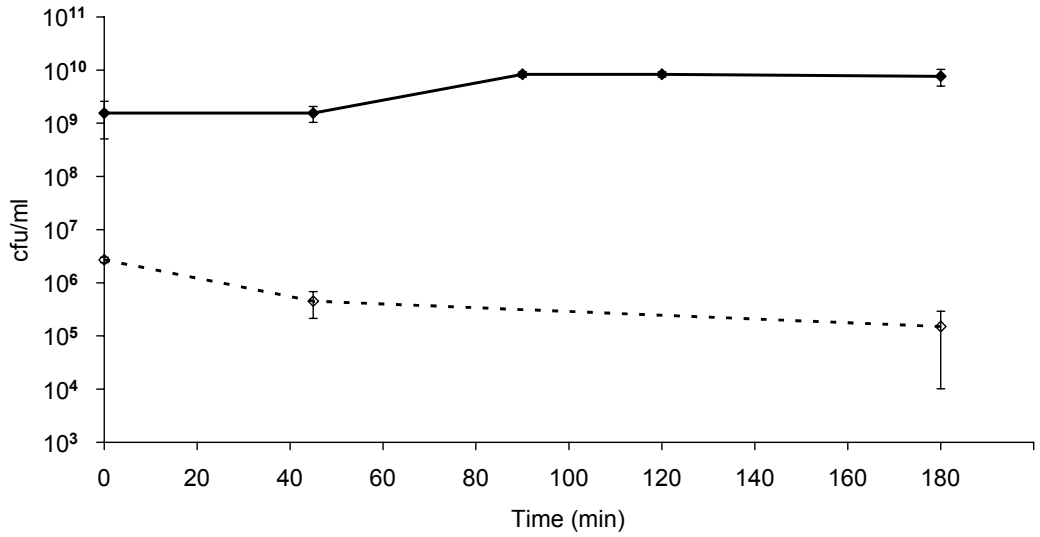


Figure 3.2: The initial load of meningococci affects the bacterial survival over time in blood. At an initial load of 1.6×10^9 cfu per ml (solid line) the bacteria survived up to 180 min in blood from this individual (donor F), whereas at a lower initial load (2.7×10^6 cfu per ml, dashed line) bacteria were killed. Error bars represent standard deviations of technical replicates within assays.

3.2.2 Changes to blood upon infection with meningococci

Since blood composition (carbohydrates, electrolytes) may affect bacterial growth, different constituents of blood were measured using a blood-gas machine or biochemical tests (Section 2.9.2.2). Analyses of blood samples from four individuals, two non-bactericidal (A and B) and two bactericidal (C and D) to MenB strain MC58, were performed before the start of the experiment, and during incubation *in vitro* at time-points until 240 min. These measurements were performed on blood infected with meningococci, as well as on uninfected blood, otherwise treated identically. Growth of meningococci in blood samples from the four individuals are shown in Figure 3.1. To evaluate if there were any differences dependent on whether MC58 was growing or being killed, blood samples collected from donor A was mixed with ∼10% bactericidal plasma (E), which rendered the blood sample bactericidal (Figure 3.1 (1)).

At the start of the experiments all results were within the normal clinical range for the parameters measured, and most of the measured entities, including iron and several electrolytes, stayed within the physiological range during the time course (Tables 3.2 – 3.3). Comparison of physiological changes in bactericidal and non-bactericidal blood samples during the course of the assay did not indicate any relationship between

meningococcal growth and changes in measured entities. For example, no significant change was detected in total levels of complement C3 in blood samples upon incubation with meningococci over a 90 or 240 min time period, independent of whether the blood was bactericidal or non-bactericidal to *N. meningitidis*. This shows, as expected, that C3 is abundantly available in blood and not exhausted during a 240 min WBA, even while meningococci are killed. Levels of soluble total haemolytic complement (THC) were measured at 0, 90 and 240 min in blood samples from three individuals (Donor A, B and C). None of the measured values were outside the normal reference range (23–46 U/ml) for the assay. Levels of extracellular iron in blood did not noticeably change during the course of the experiment. In addition, no significant change was observed in levels of potassium, a marker of haemolysis (Jay & Provasek, 1993), suggesting that this was negligible in the course of a 240 min experiment. Over a 240 min incubation of blood samples, there were significant changes in the concentration of blood glucose and lactate (Figure 3.3). Over the time course blood lactate increased, while plasma glucose decreased. The initial changes, up to 90 min, are most likely independent of bacterial growth since the same change was observed in both infected and uninfected blood samples (Table 3.1). In blood samples containing overwhelming levels of bacteria (fast-growing meningococci) (B, assay 3) (Figure 3.1 (2)), a decrease in the level of lactate was observed between 90 and 240 min.

Table 3.1: Changes in levels of glucose and lactate over time during incubation *in vitro* of infected or uninfected blood samples from all individuals. Levels are given as mean mmol/l with errors given as standard deviations (in parenthesis).

	Time (min)	**Infected blood**	**Uninfected blood**
Glucose	0	5.08 (0.35)	5.40 (0.63)
	90	3.72 (0.27)	3.98 (0.28)
	240	0.98 (0.71)	1.95 (0.07)
Lactate	0	1.88 (0.68)	2.13 (0.86)
	90	4.06 (0.89)	4.70 (0.93)
	240	4.90 (4.22)	8.75 (0.07)

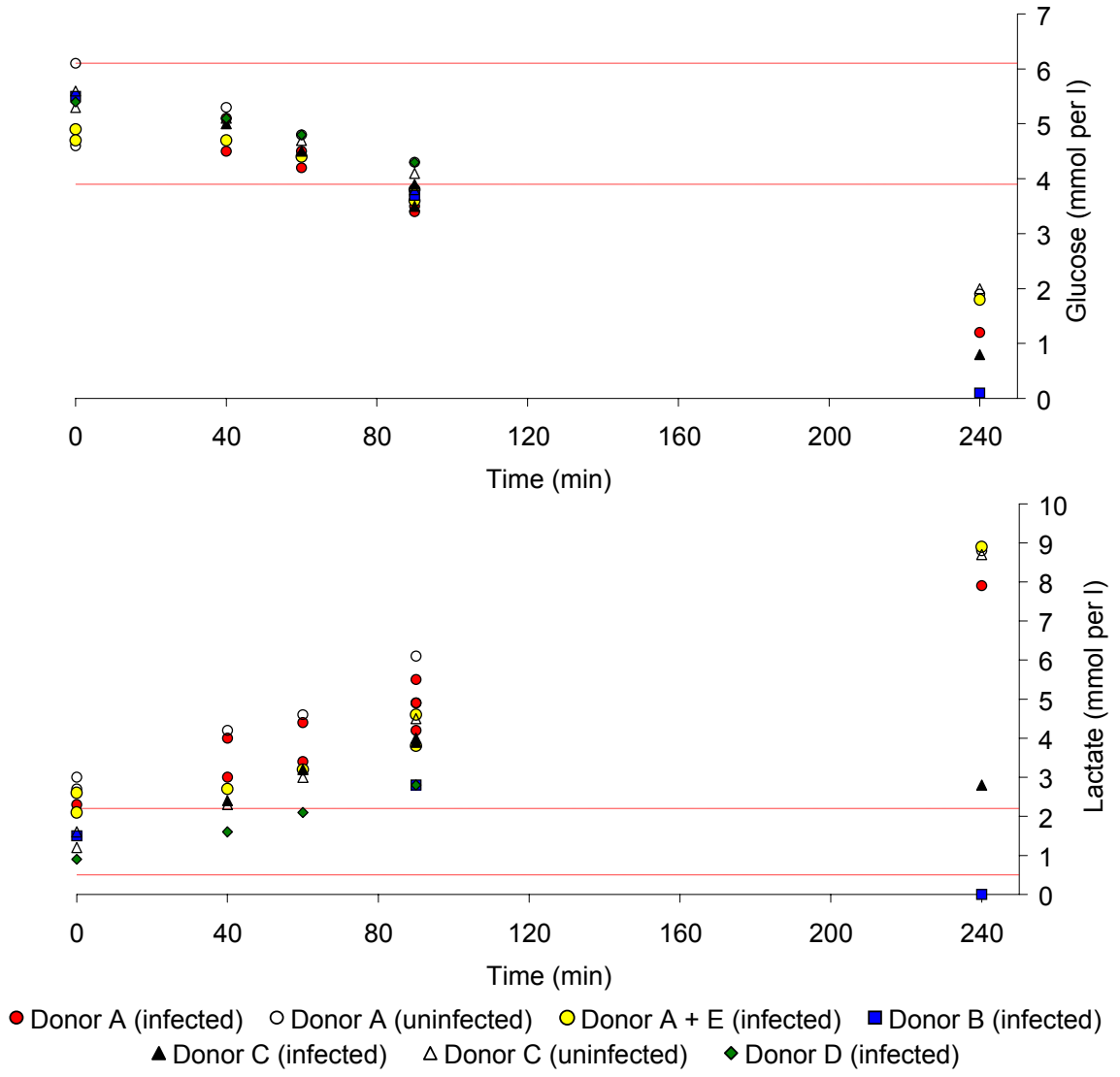


Figure 3.3: Changes in levels of glucose and lactate in blood samples over time. Data for glucose are shown at the top and data for lactate at the bottom in the figure. All solid symbols show data from experiment for blood samples infected with bacteria, and open symbols show data from experiments for blood samples without bacteria (uninfected). Blood glucose levels decreased over time, whereas blood lactate levels increased, with the exception of data at 240 min in one assay (donor B (solid blue square)). Red lines show the normal reference range in blood for this particular analytical method

Table 3.2: Changes in nutrients and complement in blood upon *in vitro* incubation for 90 or 240 min with MenB bacteria (strain MC58) added (infected) or without added bacteria (uninfected). Heparinised whole blood was collected from killers or non-killers of meningococci: individual A (non-killer), individual B (non-killer), individual C (killer) and individual D (mild killer). In some assays using blood from individual A bactericidal plasma from a killer was added to the blood (A + E). Blood was inoculated with 10^7 cfu per ml of MC58 and incubated up to 240 min. All data for time-point 0 min is taken before the addition of bacteria. Groups of rows shaded in the same colour (grey or white) represent assays performed using blood collected from the individual on one occasion.

ID	Assay day	Time (min)	Iron (mmol/l)	Glucose (mmol/l)	Lactate (mmol/l)	C3	CH50
A	1	0	N.D.	6.1	3.0	0.86	
A	1	40	N.D.	5.3	4.2	0.91	
A	1	60	N.D.	4.8	4.6	0.89	
A	1	90	N.D.	4.3	6.1	0.90	
A + MC58	1	40	N.D.	5.1	4.0	0.91	
A + MC58	1	60	N.D.	4.5	4.4	0.91	
A + MC58	1	90	N.D.	3.8	5.5	0.90	
A + MC58	2	0	20	4.9	2.3	0.86	
A + MC58	2	40	19.8	4.5	3	0.84	
A + MC58	2	60	21.2	4.2	3.4	0.84	
A + MC58	2	90	22	3.5	4.2	0.84	
A + E (10%) + MC58	2	0	18.2	4.9	2.1	0.86	
A + E (10%) + MC58	2	40	17.7	4.7	2.7	0.88	
A + E (10%) + MC58	2	60	18.8	4.4	3.2	0.86	
A + E (10%) + MC58	2	90	19.5	3.8	3.8	0.88	
A	4	0	20.8	4.6	2.7	1.00	$\leq 11*$
A	4	90	22.4	3.7	4.9	0.99	34
A	4	240	25	1.9	8.8	1.05	33
A + MC58	4	90	21.8	3.4	4.9	1.00	30
A + MC58	4	240	24.5	1.2	7.9	1.06	26
A + E (10%) + MC58	4	0	20	4.7	2.6	1.04	38
A + E (10%) + MC58	4	90	20.1	3.6	4.6	1.01	32
A + E (10%) + MC58	4	240	23.1	1.8	8.9	1.07	30
B + MC58	3	0	15.1	5.5	1.5	1.00	26
B + MC58	3	90	15.6	3.7	2.8	0.98	34
B + MC58	3	240	17.2	0.1	0	1.02	29
C	2	0	16.6	5.6	1.2	1.04	
C	2	40	17	5.1	2.3	1.02	
C	2	60	17.5	4.7	3	1.03	
C	2	90	18.7	4.1	3.9	1.00	
C + MC58	2	40	17.4	5	2.4	1.01	
C + MC58	2	60	19.5	4.5	3.2	1.04	
C + MC58	2	90	20.1	3.9	4	1.01	
C	3	0	20.5	5.3	1.6	1.08	41

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Table 3.2 – continued from previous page

ID	Assay day	Time (min)	Iron (mmol/l)	Glucose (mmol/l)	Lactate (mmol/l)	C3	CH50
C	3	90	21.7	3.8	4.5	1.08	
C	3	240	24.8	2	8.7	1.07	
C + MC58	3†	90	20.7	3.5	3.9	1.08	42
C + MC58	3†	240	23.3	0.8	2.8	1.11	36
D + MC58	2	0	7.9	5.4	0.9	0.83	
D + MC58	2	40	8.4	5.1	1.6	0.82	
D + MC58	2	60	9.1	4.8	2.1	0.80	
D + MC58	2	90	9.2	4.3	2.8	0.81	

Reference ranges: Glucose: 3.9-6.1 mmol/l, Lactate: 0.5-2.2 mmol/l, C3: 0.75-1.35, CH50: 23-46 U/ml. ∗ Failed assay. † In this assay MC58 survived in donor C's blood.

Table 3.3: Changes in nutrients and electrolytes in blood upon *in vitro* incubation for 90 or 240 min with MenB bacteria (strain MC58) added (infected) or without added bacteria (uninfected) as measured by blood-gas machine. Heparinised blood was collected from killers or non-killers of meningococci: individual A (non-killer), individual B (non-killer), individual C (killer) and individual D (mild killer). In some assays using blood from individual A bactericidal plasma from a killer was added to the blood (A + E). Blood was inoculated with 10^7 cfu per ml of MC58 and incubated up to 240 min. All data for time-point 0 min is taken before the addition of bacteria. Groups of rows shaded in the same colour (grey or white) represent assays performed using blood collected from the individual on one occasion.

ID	Assay day	Time (min)	pH	Glucose (mmol/l)	Lactate (mmol/l)	K^+ (mmol/l)	Na^+ (mmol/l)	Ca^{2+} (mmol/l)	Cl^- (mmol/l)
A + MC58	2	0	7.404	4.8	2.24	4.2	141.9	1.04	103
A + MC58	2	90	7.704	3.2	4.34	4.32	137.5	0.96	105
A + E (10%) + MC58	2	0	7.419	5	2.17	4.11	140.9	1.02	104
A + E (10%) + MC58	2	90	7.695	3.6	3.97	4.26	137.9	0.94	105
A	4	0	7.4	4.5	2.68	3.78	140.4	1.15	102
A	4	90	7.437	3.7	5.45	3.68	140.7	1.11	104
A	4	240	7.352	1.6	8.98	3.78	140	1.15	104
A + MC58	4	90	7.443	3.3	4.9	3.74	138.8	1.11	104
A + MC58	4	240	7.319	0.9	7.63	3.91	141.5	1.16	105
A + E (10%) + MC58	4	90	7.453	3.5	4.95	3.78	139.6	1.09	105
A + E (10%) + MC58	4	240	7.336	1.6	8.57	3.99	142	1.13	106
B	3	0	7.502	5.5	2.28	3.82	138.9	1.19	104
B	3	240	7.486	OR	14.36	4.03	140.8	1.17	104
B + MC58	3	90	7.497	3.6	2.5	3.63	140	1.22	104
B + MC58	3	240	7.259	OR	OR	3.41	144.5	1.36	102
C	2	0	7.379	5.5	1.48	3.94	144.7	1.13	102
C	2	90	7.686	2.8	4.42	4.43	139.8	1.03	105
C + MC58	2	90	7.634	3.5	4.57	4.61	140.2	1.04	105
C + MC58	3†	90	7.499	3.4	3.97	4.06	140.4	1.18	106
C + MC58	3†	240	7.27	OR	2.47	4.31	143.8	1.33	104

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Table 3.3 – continued from previous page

ID	Assay day	Time (min)	pH	Glucose (mmol/l)	Lactate (mmol/l)	K^+ (mmol/l)	Na^+ (mmol/l)	Ca^{2+} (mmol/l)	Cl^- (mmol/l)
C	4	0	7.414	4.8	1.76	4.38	143.1	1.29	104
C	4	240	7.335	1.8	8.43	4.47	144	1.29	104
D + MC58	2	0	7.426	5.5	1.06	4.29	139.1	1.11	103
D + MC58	2	90	7.559	4.2	3.05	4.36	138	1.05	104

Reference ranges: pH 7.35-7.45, Glucose 3.7-5.2, Lactate 0.5-2.0, Na^+ 135-148, K^+ 3.5-5.3, Ca^{2+} 1.13-1.32, Cl^- 98-106. **OR** in the table stands for out of range. † In this assay MC58 survived in donor C's blood

3.2.3 The effect of blood pH on the meningococcal transcriptome

The median pH of freshly drawn heparinised blood samples was 7.4 and increased to 7.54 at 90 min upon incubation at 37°C with 5% CO_2, as determined by measurement in a blood-gas machine (Section 3.2.2). In some cases the pH change was more dramatic with an increase at 90 min to 7.7. This shift towards more alkaline pH was observed both in infected and uninfected blood samples indicating that the addition of bacteria did not contribute to the increase (Figure 3.4). At 240 min there was a decrease in pH in blood, and a significant difference was observed between infected and uninfected blood samples (Figure 3.4). During growth on glucose meningococci produce acetate that decreases the pH of the growth medium (Leighton *et al.*, 2001), which may explain the observed difference.

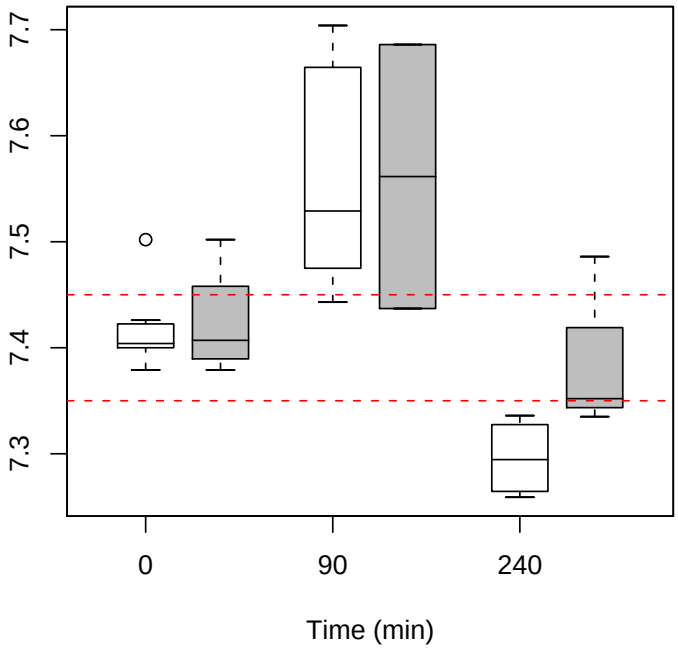


Figure 3.4: pH of blood samples from four different individuals, at 0, 90 and 240 min post collection. The pH of blood was measured using a blood-gas machine. White boxes depict pH in blood samples infected with meningococci, and grey boxes depict pH in uninfected blood samples. Red lines show the normal reference range in blood, for this particular analytical method

Since the pH of blood was found to increase (in a few cases) upon *in vitro* incubation, transcriptional changes induced by subtle differences in pH were assessed to rule out any major effects on the meningococcal transcriptome. Attempts to consistently

buffer pH in blood samples during *in vitro* incubation with meningococci were not successful, so the meningococcal transcriptional changes in response to differences in pH were instead determined in buffered standard broth culture. MenB strain MC58 was sub-cultured from liquid culture (pH 7.4) into broth with a pH of 7.4 or 7.8 and further incubated for 20 min before isolation of RNA as per Section 2.10. Microarray technology was used to analyse the gene expression of meningococci at pH 7.8 versus 7.4 (as described in Section 2.7). A direct comparison (cDNA vs. cDNA hybridisation) was performed. The microarray data were analysed as described in Section 2.7.6. The shift in pH from 7.4 to 7.8 did not induce any major changes in the meningococcal gene expression. Six genes, all down-regulated, were found to be significantly differentially expressed with a fold change of $\geq$1.5 and a P-value of $\leq$0.01 after multiple testing correction (Table 3.4). All of the differentially expressed genes, except *rplD*, have previously been shown to be involved in responses to nitric oxide or anaerobic growth.

Table 3.4: Differentially expressed meningococcal genes in response to growth at pH 7.8 versus 7.4 in sMH broth

ID	Name	Annotation	Fold change	P-value*
NMB0143	*rplD*	50S ribosomal protein L4	-3.03	1.61E-03
NMB0577		NosR-related protein	-1.61	9.17E-09
NMB0578	*nosD*	copper ABC transporter, periplasmic copper-binding protein	-1.52	6.24E-06
NMB1622	*norB*	nitric oxide reductase	-1.95	3.69E-05
NMB1623	*aniA*	major anaerobically induced outer membrane protein	-1.75	6.24E-06
NMB1624	*nirV*	hypothetical protein	-1.82	7.04E-07

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

3.3 Discussion

To be able to model the transcriptional response of *N. meningitidis* in blood or plasma in a naïve host, individuals whose blood sustained meningococcal survival had to be identified. By screening blood samples from twenty adult volunteers, two individuals

were found to have virtually absent bactericidal activity against strain MC58, using an initial load of bacteria of 10^7 cfu/ml.

An initial addition of 10^9 cfu/ml meningococci into blood at the start of the experiment overwhelmed the killing capacity of the blood system. To model septicaemia realistically it was essential to add an initial load of meningococci as close as possible to a clinically relevant load of bacteria in sepsis. Using quantitative direct plating procedures the number of bacteria in fulminant meningococcal sepsis has been suggested to be 10^4–10^5 cfu per ml (Zwahlen & Waldvogel, 1984), while using more recently developed nucleotide based methods, copies of meningococcal DNA have been found to be even higher, between 10^5 and 5×10^8 with a median of 2×10^7 copies per ml during fulminant meningococcal septicaemia (Øvstebø *et al.*, 2004). It is necessary to design experiments so that enough RNA can be extracted for expression analysis, while keeping the bacterial load on an as realistic level as possible. In our experimental setup an initial load of 1–5×10^7 cfu per ml blood (in 5–10 ml of blood) was enough to isolate sufficient amounts of RNA.

To ascertain that no major changes occurred in blood constituents during *in vitro* incubation of infected or uninfected bactericidal or non-bactericidal blood samples, analytes were followed over 240 min time courses, representing the planned time course for the gene expression experiments later carried out (Chapter 6). Levels of complement C3 and soluble THC (CH50) were measured to determine if any difference could be observed between bactericidal and non-bactericidal blood. Neither C3 nor soluble THC changed during the time course, irrespective of whether the blood sample was bactericidal or non-bactericidal. C3 is present in substantial concentrations in the blood, which may explain why no difference could be observed. Furthermore, Jarvis (1994) previously showed that C3 binds to the surface of bacteria in equal amounts, regardless of whether the strain is serum-sensitive or serum-resistant. Difference in bacteriolysis of meningococci has been suggested to be independent of the amount of deposited complement on the surface, but instead to be associated with correct insertion of the MAC complex in the surface of the bacteria (Drogari-Apiranthitou

et al., 2002). During the time course in whole blood, a change was observed in blood glucose (decreased) and in blood lactate (increased). In assays with the fastest bacterial growth, levels of lactate were found to first increase, but then decrease to the 240 min time-point, indicating that bacteria utilised the available lactate as an energy source when a limited amount of glucose was available in the blood. Metabolism of glucose to lactate (1:2 ratio) has previously been observed during *in vitro* incubation of erythrocytes (Gomperts, 1967; Sullivan & Stern, 1983, 1984). Haji-Michael *et al.* (1999) observed increased lactate levels when whole blood was stimulated with LPS, and suggested that the production of lactate may be a by-product of inflammation.

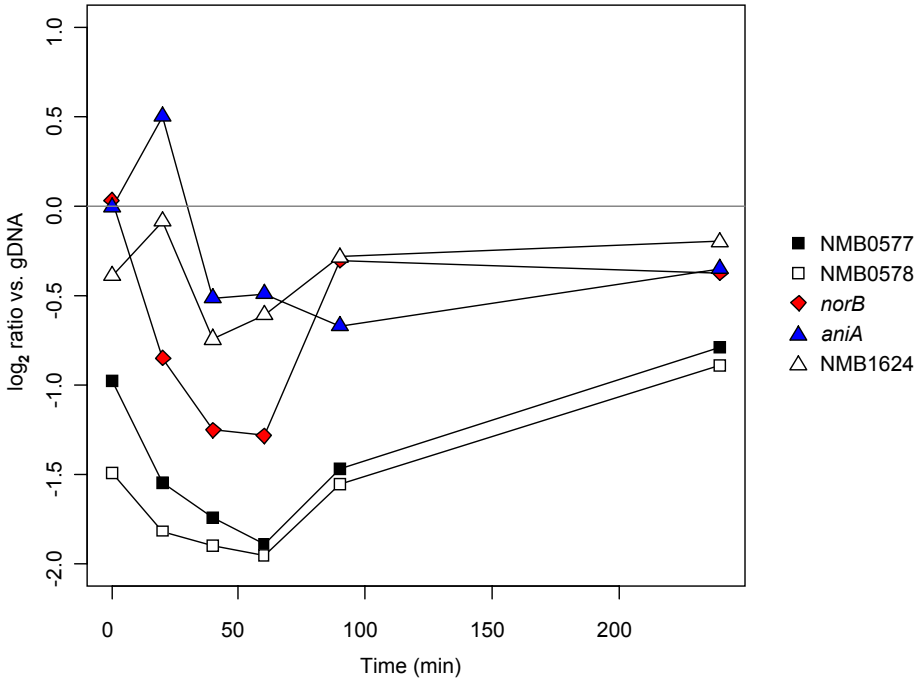


Figure 3.5: Gene expression of pH responsive genes in blood over the time course

In some of the whole blood assays in this study, the pH of blood samples increased during the time course. *N. gonorrhoeae* autolysis has been observed at pH over 8 (Elmros *et al.*, 1976), but, pH in blood never exceeded 7.8 in the experiments presented here. To ascertain that the increased pH of blood did not induce a major shift in gene expression, the meningococcal transcriptional profile in response to incubation at pH 7.8 was determined in comparison to bacteria grown at the physiological pH 7.4. MenB subjected to mildly alkaline pH was shown to respond by down-regulation of a very limited set of genes (N = 6). The expression of five of these genes during

meningococcal growth in blood is shown in Figure 3.5. NMB0577, *aniA* and NMB1623 were down-regulated also in blood at one or more time-points (Chapter 6). On the other hand, expression of *aniA* (NMB1623) and NMB1623 increased to 20 min (the time-point explored in the pH experiment) during growth in blood.

Chapter 4

Genomotyping of MenB strains used in genome-wide transcriptional studies

In this chapter MenB strains that were used in microarray studies were genomotyped. The microarrays that were used in genome-wide transcriptional studies (Chapter 5 and Chapter 6) are based on MenB strain MC58, and since MenB strain MO1-240185 was used in the studies in Chapter 5, the difference in gene content between the two strains was determined. This study was also performed to assess how well MenB strain MO1-240185 was represented on the microarray.

4.1 Introduction

4.1.1 Genomotyping

Genomotyping methods, based on hybridisation to microarrays, can be used to compare the genetic material of bacterial strains. Genomotyping can be used to identify absent or highly divergent genes (a distinction cannot be made) in the so-called 'test' strain compared to the 'reference' strain. Detection is limited to genes represented on the microarray, and the method can not be used for discovery of novel genetic material. Genomotyping has been used to compare Neisserial strains to any of the meningococcal genome sequence strains (Bille *et al.*, 2005; Stabler *et al.*, 2005; Hotopp *et al.*, 2006; Snyder & Saunders, 2006). These, and other studies, have shown that many of the genes encoding meningococcal so-called virulence factors can be

found not only in both disease and carriage-associated isolates of *N. meningitidis* (Schoen *et al.*, 2008), but also in the non-pathogenic *N. lactamica* (Snyder & Saunders, 2006). A few genomic elements have however only been identified in invasive strains of meningococci, including the phage Nf1 associated with hypervirulent lineages (Bille *et al.*, 2005). The phage is thought to contribute to virulence by changing transmission rates in certain age groups.

Data are presented on the difference in gene content, as determined by genomotyping, between *N. meningitidis* serogroup B strains MC58 and MO1-240185. MenB strain MO1-240185 was used in the plasma infection model discussed in Chapter 5 and MenB strain MC58 was used in the blood infection model discussed in Chapter 6.

4.2 Results

4.2.1 Genomic composition of MenB strain MO1-240185

Differences in gene content between MenB strains MC58 (B:15:P1.7,16-2) and MO1-240185 (B:2A:P1.5-10-8), used in the plasma transcriptome study (Chapter 5), were determined by hybridisation of differentially labelled genomic DNA to a *pan*-Neisseria microarray (described in Section 2.7.1). This array is based on strain MC58 genes but also contains meningococcal genes from serogroup C strain FAM18 and serogroup A strain Z2491. The strain MO1-240185 belongs to the ST-11 clonal complex (a hypervirulent lineage associated with invasive disease) and may therefore be expected to have similarity to other strains belonging to this clonal complex, which include the MenC sequence strain FAM18.

Dye-swap experiments were performed to control for possible differences in incorporation of fluorescently labelled Cy5-dCTP and Cy3-dCTP. Normalised $\log_2$ ratios from microarrays were analysed using the software GACK, which calls genes as either absent ('-1'), divergent ('0') or present ('1') (Kim *et al.*, 2002) (Section 2.7.7). Using this approach three IHTs present in the MC58 genome, but absent in FAM18

and Z2491 genomes, were also called as absent in MenB strain MO1-240185. The list of genes called as 'absent' by GACK (N = 165) was compared to a list of stipulated MC58 specific genes from Bentley *et al.* (2007). Eighty-nine out of the ninety-five genes listed were represented in the microarray data after quality control, and of these, all except five were found to be absent from strain M01-240185. This suggests that genes absent in MenB strain MO1-240185 are found specifically in the MenB sequence strain MC58. Data obtained for MenB strain MO1-240185 were compared to publically available genomotyping data for fifty-three Neisseria strains in comparison to MC58 DNA obtained by Hotopp *et al.* (2006). Hierarchical clustering analysis was performed using average linkage and a distance metric based on correlation (1–Pearson correlation), using microarray data collected for strain MO1-240185 and data from Hotopp *et al.* (2006) (Figure A.1). This analysis indicated that strain MO1-240185 is similar to strains of the 'mCGH-3' type defined by Hotopp *et al.* (2006), which also includes MenC sequence strain FAM18. The meningococcal strains in this cluster all contain the so called IHT-E region, as does MO1-240185 (Figure 4.1).

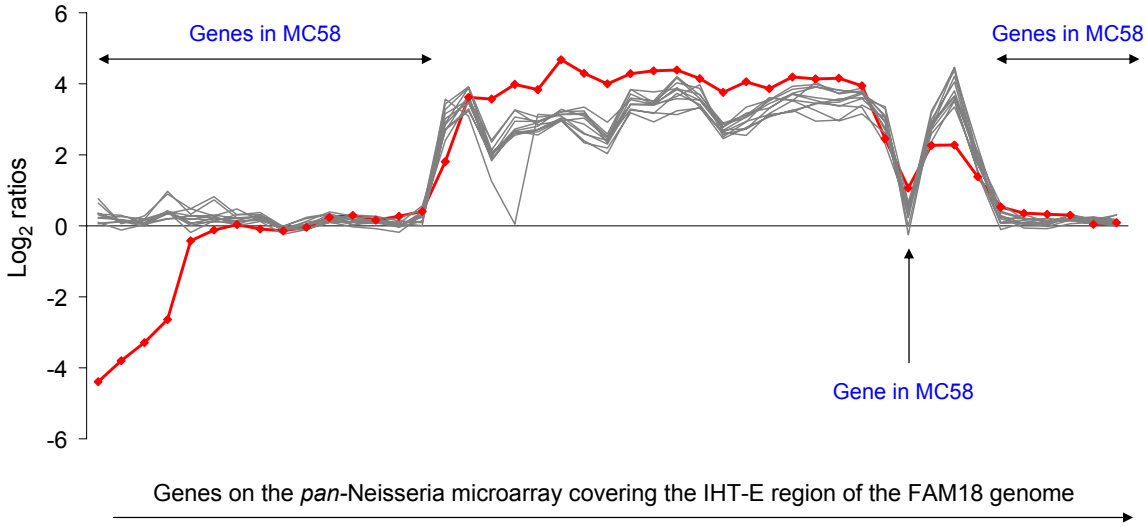


Figure 4.1: Log_2 ratios for genes on the microarray covering the so called Neisserial IHT-E region found in MenC reference strain FAM18. Genomic DNA from all strains was compared to the serogroup B reference strain MC58. Grey lines represent data from Neisserial strains in cluster 'mCGH-3' (data from Hotopp *et al.* (2006)). Data for strain MO1-240185, collected in this study, is depicted in red. Each point in the graph represents a gene on the *pan*-Neisseria microarray. Genes also present in the genome of strain MC58 (MenB) are indicated in the graph.

4.3 Discussion

Comparison of the gene content of MO1-240185 and MC58 revealed that 91% of the genes are shared, i.e. not called as absent by the GACK software. The majority of the genes that were included among the absent genes in MO1-240185 compared to MC58 were genes with hypothetical function or genes in IHT regions. Strain MO1-240185 belongs to the same clonal complex, ST-11/E-37, as the serogroup C strain FAM18, which is represented on the microarray used. As expected, MenB strain MO1-240185 contained several genes found in the FAM18 genome sequence, and hybridised to 68 out of the 125 MenC specific spots on the microarray.

In conclusion, the strain MO1-240185 is comparable to strain MC58 and well represented on the *pan*-Neisseria microarrays used in the genome-wide transcriptional studies.

Chapter 5

Genome-wide transcriptional profiling of serogroup B meningococci in plasma

In this chapter the transcriptional profiling of MenB, upon incubation in whole human non-bactericidal plasma, is presented. The changes in the meningococcal transcriptome were determined by using microarrays. Genes of particular interest were those encoding surface exposed proteins which were up-regulated in this experimental system.

5.1 Introduction

A vaccine based on surface exposed antigenic proteins represents one potential strategy for achieving an effective cross-protective MenB vaccine (discussed in Section 1.4.1.2). Meningococcal genes encoding surface exposed proteins that are up-regulated during infection, or in conditions mimicking *in vivo* bacterial growth, are attractive candidates for inclusion in such a vaccine. The objective of the study presented in this chapter was to identify MenB genes encoding known or putative surface exposed proteins, which were relatively or exclusively up-regulated during incubation in plasma, a simple model of bacteraemia, compared to models of colonisation.

5.1.1 Bacterial growth and survival in serum or plasma

In vitro growth of bacteria in serum or plasma can be used to model bacterial growth in the blood stream of the host. *N. meningitidis* killing by the human host is primarily

mediated through the action of complement to which the bacteria are exposed during *in vitro* incubation in either serum or plasma. *N. meningitidis* surface structures that are associated with increased survival in human serum include the polysaccharide capsule, LOS and surface proteins that interact with human immune defence proteins; for example factor H binding protein encoded by NMB1870 (Vogel *et al.*, 1997; Kahler *et al.*, 1998; Madico *et al.*, 2006). Studies utilising mutagenesis with tagged transposons have also revealed the importance of *de novo* biosynthesis of purine nucleotides and amino acids as well as up-take of iron for bacterial survival both during *in vitro* growth in serum and in animal models of systemic infection (Wang *et al.*, 1996; Fuller *et al.*, 1999; Sun *et al.*, 2000; Samant *et al.*, 2008). Specific genes that are important for meningococcal survival during infection are discussed in greater detail in Chapter 6, as many of them are foremost relevant for bacterial growth in blood.

5.1.2 Microarray studies

Genome-wide transcriptional profiling has been extensively used to study the responses of bacteria to a broad range of biological conditions, both *in vivo* and *in vitro* (Ye *et al.*, 2000; Smoot *et al.*, 2001; Fisher *et al.*, 2002; Grifantini *et al.*, 2002b; Phadtare *et al.*, 2002; Bina *et al.*, 2003). Transcriptional changes in response to plasma or serum exposure have been determined for several human pathogens including *Yersinia pestis* (Chauvaux *et al.*, 2007) and *Klebsiella pneumoniae* (Hsieh *et al.*, 2008). Up-regulated genes included those involved in iron acquisition, thiamine biosynthesis and purine/pyrimidine metabolism. The meningococcal transcriptome in normal human serum (NHS) has previously been explored by Dove *et al.* (2003) and Kurz *et al.* (2003). Dove *et al.* (2003) compared the transcriptional changes in strain Z5463 (serogroup A) upon incubation in 25% NHS for 6 hr to those in meningococci incubated in heat inactivated (hI) serum for 6 hr. The most highly differentially expressed gene was NMA1686, a serogroup A orthologue to MenB gene NMB1475, encoding a hypothetical protein that may be involved in universal stress responses. Kurz *et al.* (2003) investigated the transcriptional changes induced in meningococci upon growth in 100% NHS for 30 min compared to growth in phosphate buffered

saline (PBS) and found 279 genes to be differentially expressed including those encoding proteins involved in categories such as metabolism, transport and translation. Twenty-three of the up-regulated genes encoded known or predicted OMPs, including NMB0586 and NMB1966, encoding components of a Mn^{2+}/Zn^{2+} and a L-glutamate transporter respectively (Monaco *et al.*, 2006; Lim *et al.*, 2008).

In the study presented in this chapter meningococcal gene expression profiles were determined at 60 and 240 min, during incubation in non-bactericidal plasma. The same time-series was studied for meningococci grown in liquid laboratory medium to attempt to identify changes specific to exposure to components of the plasma. The reason for doing a time-series (although sparse) was to explore temporal changes in gene expression upon meningococcal growth in plasma. Two different experimental designs were evaluated; either gene expression in plasma in relation to growth in laboratory medium or in relation to an initial starting point. In these experiments plasma from two individuals were used due to limited availability from each donor. The reason for using plasma rather than blood in the study in this chapter was that plasma studies are easier to standardise than those in blood, as plasma can be stored and used in a set of experiments over time, and that work in plasma is experimentally simpler. In addition, isolation of RNA from bacteria in blood initially proved difficult, and could only be accomplished after extensive testing of different methods. Results from transcriptional profiling during growth in plasma are presented below, with a particular emphasis on genes up-regulated in this growth condition that may encode surface exposed proteins.

5.2 Results

Most adults in the UK have naturally acquired bactericidal antibodies to a range of MenB strains, and accordingly are classified as 'killers' in the serum bactericidal and/or the whole blood assays (further discussed in Chapter 1 and 3). So as to be able to determine genome-wide transcriptional changes during growth in non-immune individuals, plasma samples from adult volunteers and fresh frozen plasma, acquired

through external collaborators (Section 2.9.1), were screened to identify those that could sustain meningococcal growth. The MenB strain MO1-240185 (B:2a:P1.5-10-8) and MenB reference strain MC58 (B:15:P1.7,16-2) were incubated in fresh frozen plasma (FFP) from two different adult individuals, using an initial inoculum of 10^7 cfu/ml. The growth characteristics of each of the strains in fresh frozen plasma from individual 1 (FFP 1) and individual 2 (FFP 2) are shown in Figure 5.1. Each plasma sample supported the sustained growth of strain MO1-240185, but they differed in their ability to support growth of strain MC58. In FFP 1 neither substantial growth nor killing of strain MC58 was observed over 3 hr. On the other hand, FFP 2 was bactericidal to strain MC58. This confirms that bactericidal antibodies to surface structures other than the capsular polysaccharide are of importance for controlling growth of MenB in the circulation. The strain MO1-240185, used in all gene expression studies presented in this chapter, belongs to the ST-11 clonal complex, a hypervirulent lineage responsible for several outbreaks of meningococcal disease. These outbreaks have mainly been associated with serogroup C meningococci. A serogroup C strain of the 2a phenotype belonging to this clonal complex was associated with the increase in meningococcal disease in UK in the 1990s (discussed on page 27), and there is concerns that serogroup B strains of the same phenotype could also emerge as a threat. Therefore studies into the pathogenicity of MenB strains of this phenotype, such as MO1-240185, are important. Plasma from these two individuals (FFP 1 and FFP 2) was used in the genome-wide transcriptional profiling studies presented in the following sections.

In all studies RNA was isolated from bacteria at 60 and 240 min post-inoculation (as described in Sections 2.5.2 and 2.9.1) and the transcriptional profile at each time-point analysed using *pan*-Neisseria microarrays (Section 2.7). The quantity of RNA obtained from meningococci was determined by measuring the absorbance at 260 nm (A_{260}) using a Nanodrop spectrophotometer (Section 2.6.1). The purity of the RNA was determined by the A_{260}/A_{280} ratio. In order to further assess the quality of the RNA, samples were separated on a non-denaturing 1% agarose gel (Section

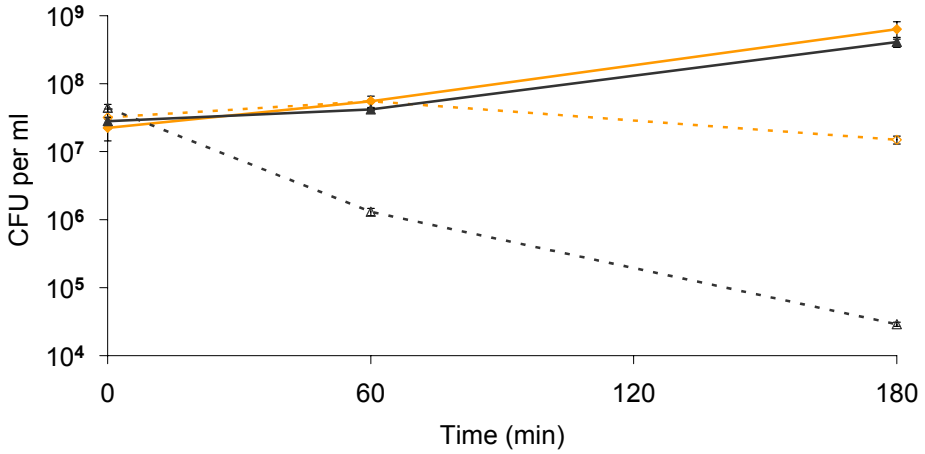


Figure 5.1: Growth over time of MenB strains MO1-240185 and MC58 in fresh frozen plasma from two donors. Data of growth in FFP 1 are shown in orange and data of growth in FFP 2 are shown in dark grey. Data for strain MO1-240185 are represented by a solid line and data for strain MC58 by a dashed line. Number of bacteria at time-point 0 min, 60 min and 180 min were determined by serial dilution and spreading out in triplicate (technical replicates) on solid support for CFU count. In both FFP 1 and FFP 2 growth of strain MO1-240815 and strain MC58 differed. Error bars represent standard deviations of technical replicates.

2.4). On a non-denaturing gel two bands corresponding to 23S rRNA and 16S rRNA are visible (Figure 5.2). The 23S rRNA band should be more intense than the 16S rRNA band in intact good quality RNA (at a ratio of 1.5–2:1). Isolated RNA samples were reverse transcribed to cDNA, labelled with Cy5-dCTP and co-hybridised with Cy3-dCTP labelled MC58 genomic DNA (gDNA) to *pan*-Neisseria microarray slides featuring probes representing the whole meningococcal gene complement of strains MC58, FAM18 and Z2491 and *N. gonorrhoeae* strain FA1090 (as described in Section 2.7).

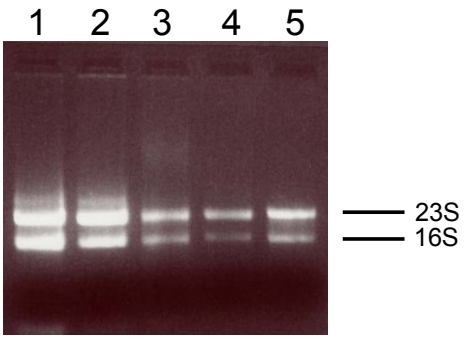


Figure 5.2: Agarose gel image of total RNA isolated from MO1-240185 incubated in fresh frozen plasma (FFP 1) or medium. RNA samples were further treated with DNase I and cleaned up on an RNeasy column before microarray analysis. Lane **1**: FFP 1 60 min; lane **2**: Medium 60 min; lane **3**: FFP 1 240 min; lane **4**: Medium 240 min; lane **5**: 1μg rRNA *E. coli*. Arrows indicate the 16S and 23S rRNA subunits.

In the microarray experiments described in this chapter, a common reference design was used which facilitated comparison between all time-points and conditions.

In all experiments MC58 genomic DNA (gDNA) was used as the reference sample, hybridising to all MenB specific spots on the microarray. The use of gDNA as a common reference has been described and discussed by Kim *et al.* (2002) and Gadgil *et al.* (2005). A representative microarray image of a cDNA versus gDNA hybridisation is shown in Figure 5.3. Microarray data were analysed as outlined in Section 2.7.6. Briefly, data collected from microarrays were pre-processed to remove spots with low fluorescent intensity across all microarray slides. Ratio data were normalised to median $\log_2$ ratios per slide. Normalised data were analysed by means of moderated t-statistics and an empirical Bayes variance adjustment as implemented in the R-package Limma, to find differentially expressed genes (Smyth, 2004; R Development Core Team, 2008). A gene was considered to be significantly differentially expressed between classes when the change was $\geq \pm 1.5$-fold at a FDR of 5% (meaning that less than 5% of the selected genes are expected to be false positives).

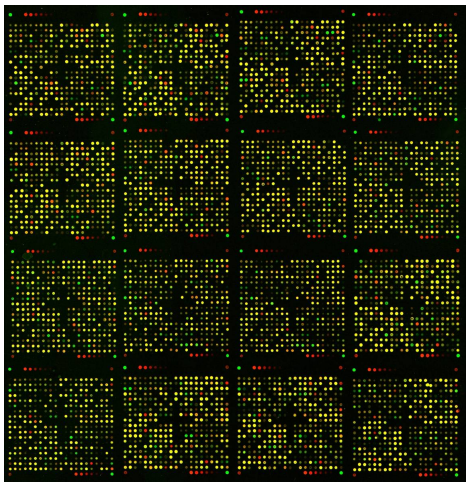


Figure 5.3: A representative microarray image of a cDNA versus gDNA hybridisation. Fluorescently labelled samples were hybridised overnight to a *pan*-Neisseria microarray.

In the following sections, experiments involving FFP 1 and FFP 2 will be described separately. In experiment one, using FFP 1, the gene expression profile of bacteria incubated in plasma was compared to that of bacteria incubated over the same period of time in cell culture medium (Section 5.2.1). In experiment two, using FFP 2, the transcriptional profile of bacteria incubated for different periods in plasma was compared to bacterial gene expression at time-point 0 min (Section 5.2.2).

5.2.1 Transcriptional profile in FFP 1 in relation to gene expression in medium

The gene expression profile of MenB strain MO1-240185 in FFP 1 was compared to bacteria incubated in growth medium RPMI 1640 supplemented with 10% hI fetal bovine serum (medium) in a parallel experiment. The growth of MO1-240185 in plasma was comparable to growth in medium over a 240 min experiment (Figure 5.4). 5–20 μg of RNA could be extracted from meningococci cultured in 6–10 ml

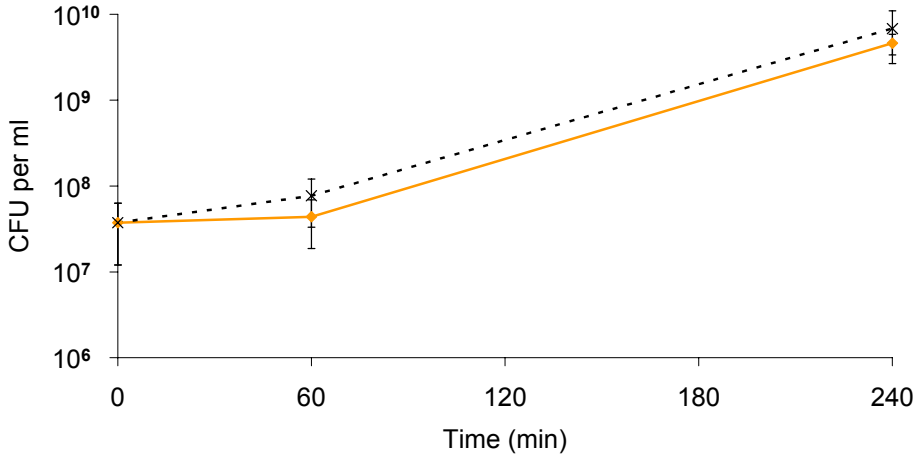


Figure 5.4: Growth over time of MenB strain MO1-240185 in plasma and medium. The solid orange line shows growth in FFP 1 and the black dashed line shows growth in medium after 0, 60 and 240 min incubation. The graph depicts the mean of three assays for time-point 60 min and six assays for time-point 240 min. Error bars represent standard deviations of biological replicates. The growth rate was comparable for meningococcal strain MO1-240185 in plasma and medium.

of plasma or medium. In a representative gel image of RNA samples (Figure 5.2), the two visible bands correspond to 23S rRNA and 16S rRNA, respectively. RNA of sufficient quality and quantity for microarray analysis could be isolated from MenB incubated in both FFP 1 and medium (twenty samples in total). These samples were hybridised onto microarrays (as outlined in Section 2.7). Taking into account the duplicates on the microarray slides, 10 data points were obtained per condition for each gene.

After pre-processing of microarray data, PCA (described in Section 2.7.8), a data dimension reduction method, was performed using gene expression data (mean-centred) from all genes of good quality to provide a multivariate overview of similarities between samples (from plasma and medium) and for a low dimensional visual-

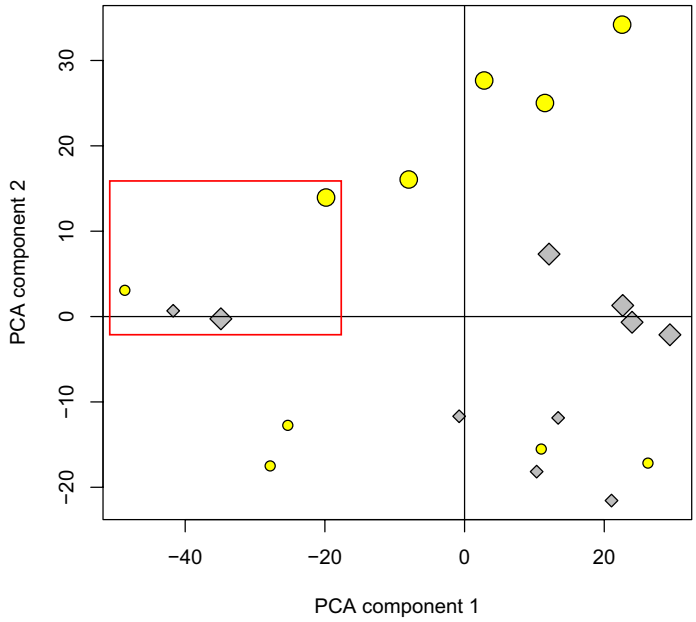


Figure 5.5: PCA of data collected for meningococci incubated in FFP 1 or medium. PCA, with mean-centering, was performed using gene expression data for MC58 genes on microarrays hybridised with RNA from FFP 1 or medium. Data from plasma (FFP 1) are depicted by yellow filled circles, and data from medium are depicted by grey filled diamonds. The smaller symbols represent data from time-point 60 min and the larger symbols represent data from time-point 240 min. The data points in the red rectangle represent samples from assay-day one.

isation of the expression profiles. The score plot of the first two PCA components, showing each assay as one point, is shown in Figure 5.5. Separation in the second PCA component reflects both time (as most samples isolated at 60 and 240 min separate along this component) and growth condition (as data at 240 min for meningococci in either plasma or medium separate along the second component as well). At 240 min, growth condition (plasma or medium) had a greater influence on the transcriptional profile than at 60 min. PCA analysis of the data indicated that microarray data collected for RNA samples isolated on day one (symbols enclosed in rectangle, see Figure 5.5) were different to microarray data for isolation day two to six. However, exclusion of data from day one in the statistical analysis aimed at finding differentially expressed genes, did not significantly alter the results. Therefore, samples from all RNA isolation days were included in the further analysis presented in this chapter. The genes that contributed most to this separation along the first component (indicating large contribution to the pattern seen in Figure 5.5) were NMB0398, NMB0434, NMB0577–79, NMB0810 and *norM* (NMB0812). The gene *norM* encodes an efflux

protein that previously has been shown to protect Neisseria from the toxic effect of cationic compounds (Rouquette-Loughlin *et al.*, 2003). NMB0810, encoding a putative transcriptional regulator, most likely regulates the expression of *norM*. NMB0577 has previously been shown to be regulated by FNR (fumarate and nitrate reduction regulator) upon oxygen-restriction (Bartolini *et al.*, 2006).

Statistical analysis was performed using the Limma package (Section 5.2) to find differences in gene expression between meningococci in plasma and medium. A total of 208 genes was found to be differentially expressed (fold-change $\geq$1.5, FDR of $\leq$0.05) in plasma at 60 min compared to medium. Of these, 74 genes were up-regulated (Supplementary table B.1) and 134 genes were down-regulated (Supplementary table B.2). After 240 min incubation, 260 genes were found to be differentially expressed in plasma compared to medium, using the same inclusion criteria as for the 60 min time-point. There was an even distribution between up-regulated (N = 126) and down-regulated genes (N = 134) at this time-point (Supplementary tables B.3 – B.4). Comparing the differentially expressed genes at 60 and 240 min, it was immediately

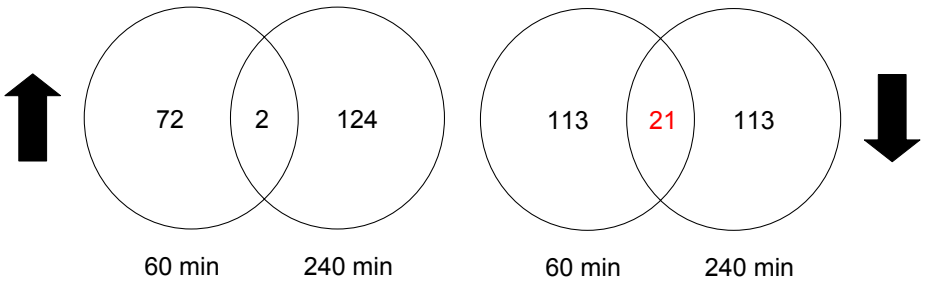


Figure 5.6: Differentially expressed genes at 60 and 240 min in FFP 1 compared to medium. In the figure up-regulated genes are shown to the left and down-regulated genes are shown to the right. The 21 down-regulated genes shared between the two time-points (labelled in red) are listed in Supplementary table B.5.

clear that the transcriptional profiles were different (Figure 5.6). Only two of the up-regulated genes were found to be shared between the two time-points: *cysD-1* (NMB1154) involved in sulphur metabolism and *mtrF* (NMB1719) involved in protection against hydrophobic agents (Veal & Shafer, 2003). Twenty-one genes were consistently down-regulated in plasma (Supplementary table B.5), including genes belonging to the FrpC operon. Another notable category of differentially expressed

genes shared between the two time-points, was those encoding proteins involved in chaperone activities, such as *dnaK* (NMB0554) and *grpE* (NMB0661).

5.2.1.1 The nature of genes differentially expressed in plasma compared to medium

Exploration of sets of genes sharing a common functionality may give clues about biological processes that are important under a certain growth state or condition. Therefore, genes differentially expressed upon growth in FFP 1 were divided into functional categories according to designations established at TIGR (Peterson *et al.*, 2001). In addition, gene set enrichment analysis using the GSEA software (Mootha *et al.*, 2003; Subramanian *et al.*, 2005) was used to determine whether any of the TIGR functional role categories (TFRC) were significantly enriched among the plasma-responding genes (i.e. among up-regulated genes). In GSEA analysis, genes are ranked according to the expression difference between two classes. A non-parametric test is then used to assess whether the null hypothesis can be rejected, the null hypothesis being that the rank ordering of genes is random with regard to the pre-defined gene sets (e.g. functional categories). Any gene sets with high rank order, overrepresented at the top (or bottom) of the list, are correlated with the phenotype. To assess the statistical significance, a permutation test was used to calculate a global P-value, which was then corrected for FDR to give a q-value (a FDR q-value $\leq$0.25 is considered significant by Mootha *et al.* (2003)). Results from GSEA can give an indication of categories of genes that may be important. However, permutations over phenotypes are more effective for larger groups (N $\geq$7 suggested by (Mootha *et al.*, 2003)), so result from GSEA analysis here should be interpreted with some caution. A large proportion of the genes found to be up-regulated at 60 min are annotated as *hypothetical* or *unassigned proteins* (Figure 5.7 (1)). The second largest group of genes contained members involved in *central intermediary metabolism*. In the GSEA analysis, genes encoding functions categorised as *central intermediary metabolism* and *biosynthesis of cofactors, prosthetic groups and carriers* (FDR q-value $\leq$0.10), were significantly enriched and associated with increased expression during meningo-

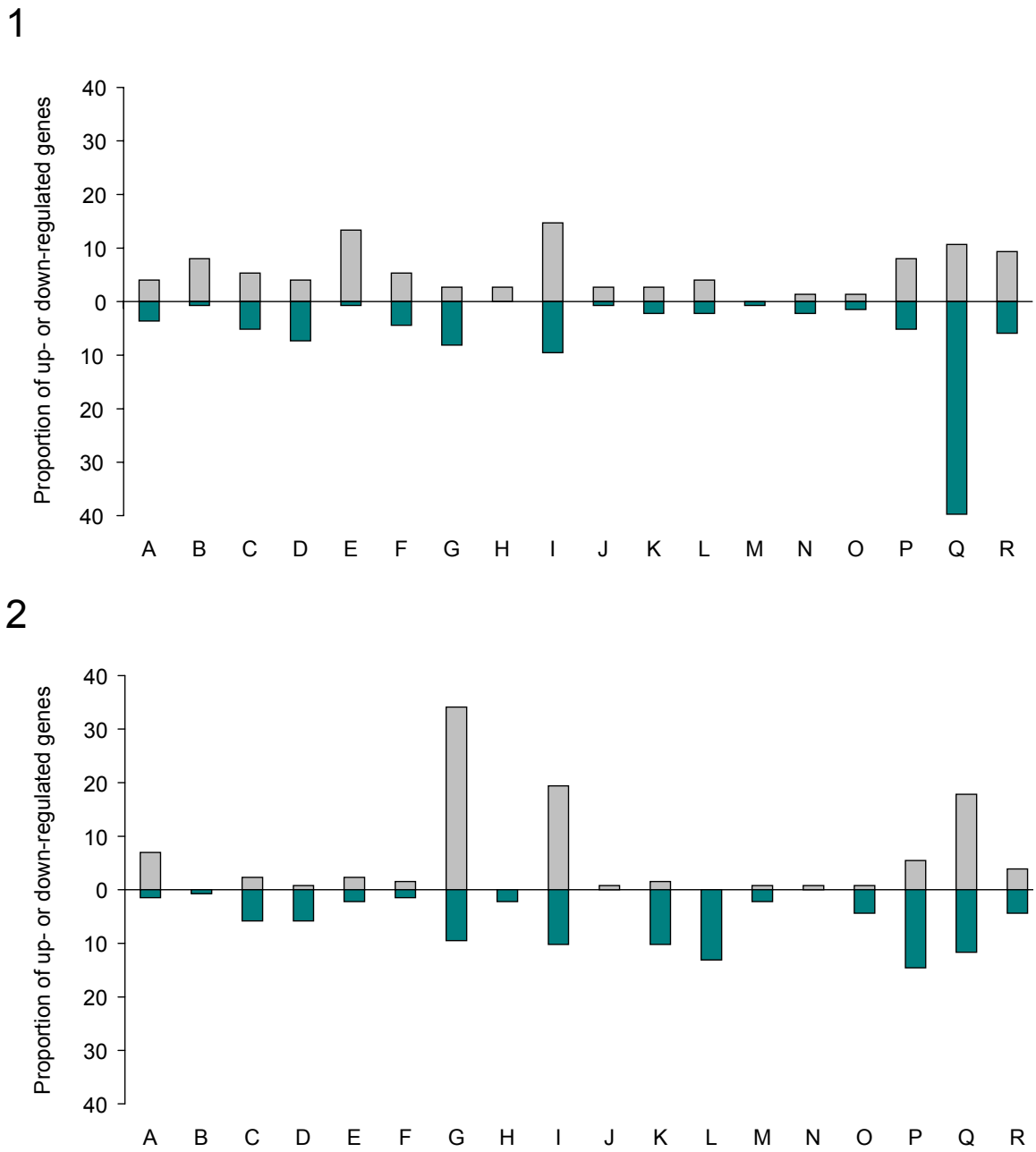


Figure 5.7: Differentially expressed genes at 60 (**1**) and 240 min (**2**) in FFP 1 compared to medium divided into functional categories, according to their TIGR designations. Up-regulated genes are depicted by light-grey bars and down-regulated genes by green bars. Numbers shown on the Y-axis represent the percentage each category constitutes of the total number of either up- or down-regulated genes. Columns: **A**. Amino acid biosynthesis, **B**. Biosynthesis of cofactors, prosthetic groups, and carriers, **C**. Cell envelope, **D**. Cellular processes, **E**. Central intermediary metabolism, **F**. DNA metabolism, **G**. Energy metabolism, **H**. Fatty acid and phospholipid metabolism, **I**. Hypothetical proteins, **J**. Mobile and extrachromosomal element functions, **K**. Protein fate, **L**. Protein synthesis, **M**. Purines, pyrimidines, nucleosides, and nucleotides biosynthesis, **N**. Regulatory functions, **O**. Transcription, **P**. Transport and binding proteins, **Q**. Unassigned, **R**. Unknown function.

coccal growth in plasma compared to medium at 60 min. At 240 min many of the genes found to be up-regulated encoded proteins involved in *energy metabolism* (Figure 5.7 (2)) and this category was also significantly enriched (FDR q-value = 0.05) in GSEA analysis. Genes annotated in the three TFRCs *protein synthesis*, *transcription* and *protein fate*, were common among the down-regulated genes following 240 min incubation in plasma. This may indicate a slower growth of meningococci in plasma compared to medium, although this was not reflected in CFUs at 240 min (Figure 5.4). In GSEA analysis the same three categories were found to be associated with up-regulation in the medium-grown meningococci (FDR q-value $\leq$0.11).

5.2.1.2 Known or putative surface exposed proteins

Genes found to be up-regulated in plasma were divided into categories according to predicted (or known) cellular locations, based on experimental data or *in silico* predictions. Algorithms: PSORTb, PSLpred and the bioinformatics tools available at CBS (`http://www.cbs.dtu.dk/services`) were used as laid out in Section 2.11 for *in silico* predictions. All MC58 genes that encoded a protein with a signal peptide or which were attributed as encoding non-cytoplasmic proteins are listed in Table 5.1. Genes encoding proteins that have been identified in preparations of OM or OMVs, or experimentally confirmed to be on the surface of the bacteria are also included in Table 5.1. Fifty such genes were up-regulated in these experiments.

The gene NMB1971, encoding a predicted OMP, was up-regulated at 60 min in plasma. NMB1971 has previously been found to be up-regulated in an *in vitro* model of colonisation (Grifantini *et al.*, 2002b), and a NMB1971 knock-out strain has been shown to be attenuated in an infant rat model of infection (Sun *et al.*, 2000). Taken together these results suggest an important function for the gene product in meningococcal survival *in vivo* or in virulence. NMB0787 and NMB0995, the products of which have previously been shown to be surface exposed on intact meningococci by flow cytometry (following 2 hr adherence to epithelial cells) (Grifantini *et al.*, 2002b), were up-regulated at 60 min during growth in plasma. The gene *cysK* (NMB0763), up-regulated at 60 min, encodes a protein that has been identified in both OM and

OMVs preparations (Ferrari *et al.*, 2006; Williams *et al.*, 2007), suggesting a possible surface exposed location despite that the protein is predicted to be located in the cytoplasm using prediction algorithms.

Of the genes up-regulated at 240 min during growth in plasma, *mapA* (NMB0390), *nspA* (NMB0663), *nadA* (NMB1994)[1], *mspA* (NMB1998), NMB1144, NMB1624 and NMB2050, all encode proteins with a known or predicted outer membrane location. The products of *nadA* and *mspA* are both annotated as auto-transporter adhesin/invasins and have been shown to be involved in adherence to epithelial cells (Capecchi *et al.*, 2005; Turner *et al.*, 2006). NadA has also been shown to mediate adherence to monocytes and macrophages (Franzoso *et al.*, 2008). NadA is one of five components of a experimental meningococcal vaccine (Giuliani *et al.*, 2006). NMB1220, up-regulated at 240 min, encodes a protein that is not predicted to be located in the meningococcal OM, but, which in previous studies has been shown to be exposed on the meningococcal surface (Pizza *et al.*, 2000). This protein was found to function as a ligand for the class A macrophage scavenger receptor (Peiser *et al.*, 2006).

In proteomic studies, meningococcal proteins not predicted to be located in the outer membrane have been identified in OM preparations (Williams *et al.*, 2007). Seventeen of the genes up-regulated at 240 min encode proteins identified in the OM fraction by Williams *et al.* (2007). These genes included *gdhA* (NMB1710) and NMB1035. *gdhA* encodes a NADP-specific glutamate dehydrogenase (described on page 107). NMB1035, encoding a putative lipoprotein with unknown function, has previously been shown to be up-regulated in response to MenB incubation in normal human serum for 30 min when compared to bacteria incubated in PBS (Kurz *et al.*, 2003).

[1]The common name of NMB1994, *nadA*, is shared with NMB0394 (*nadA*) encoding a quinolinate synthetase, but the denomination *nadA* in this thesis will hereafter refer to NMB1994.

Table 5.1: Up-regulated genes at 60 or 240 min during growth in FFP 1 (indicated by Up in the table) that encode proteins with a known or putative location in the periplasm or the outer membrane of the bacteria. Genes that have been found in experimental studies to be located on the surface of the bacteria or detected in protein preparations of OMVs or OM are also included in the table.

		Time-point (min)			PSORTb	PSLpred	CBS	
ID	**Name**	**60**	**240**	**Experimental data**	**Location**	**Location**	**Prediction**	**SignalP**
NMB0041	*tbpA*		Up	OM (Williams *et al.*, 2007)	Periplasmic	Periplasmic	SignalP	√
NMB0085	*gltS*		Up	OMVs (Williams *et al.*, 2007)	Inner membrane	Inner membrane	TMHMM	
NMB0311			Up	OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB0318	*farA*		Up		Unknown	Outer membrane	SignalP	√
NMB0390	*mapA*		Up	OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Cytoplasmic	Cytoplasm	
NMB0392	*nadB*	Up			Periplasmic	Periplasmic	Cytoplasm	
NMB0401	*putA*		Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0500			Up		Unknown	Extracellular	Cytoplasm	
NMB0530		Up			Cytoplasmic	Periplasmic	Cytoplasm	
NMB0631			Up	OMVs (Ferrari *et al.*, 2006; Vipond *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB0663	*nspA*		Up	OM and OMVs (Williams *et al.*, 2007; Ferrari *et al.*, 2006)	Outer membrane	Outer membrane	SignalP	√
NMB0763	*cysK*	Up		OM and OMVs (Williams *et al.*, 2007; Vipond *et al.*, 2006; Vaughan *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasm	
NMB0776			Up		Unknown	Periplasmic	SignalP	√
NMB0787		Up		Surface located (Grifantini *et al.*, 2002b); OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Periplasmic	Periplasmic	LipoP	√
NMB0920	*icd*		Up	OMVs (Williams *et al.*, 2007)	Unknown	Periplasmic	Cytoplasm	
NMB0923			Up		Periplasmic	Periplasmic	LipoP	√
NMB0950	*sdhA*		Up	OM and OMVs (Williams *et al.*, 2007)	Multiple locations	Periplasmic	Cytoplasm	
NMB0954	*gltA*		Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0995		Up		Surface located (Grifantini *et al.*, 2002b)	Unknown	Cytoplasmic	Cytoplasm	
NMB1035			Up	OM (Williams *et al.*, 2007)	Unknown	Periplasmic	LipoP	√
NMB1144			Up		Unknown	Outer membrane	SignalP	√
NMB1150	*ilvD-1*		Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1195		Up			Unknown	Outer membrane	Cytoplasm	
NMB1207	*bfrA*		Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	

Continued on next page ...

Table 5.1 – continued from previous page

ID	Name	Time-point (min) 60	240	Experimental data	PSORTb Location	PSLpred Location	CBS Prediction	SignalP
NMB1220			Up	Surface located (Pizza *et al.*, 2000), Macrophage receptor (Peiser *et al.*, 2006), OMVs (Williams *et al.*, 2007; Vipond *et al.*, 2006)	Inner membrane	Cytoplasmic	Cytoplasm	
NMB1371	*argD*		Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1388	*pgi-1*		Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1393	*edd*		Up	OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB1437			Up	OMVs (Vipond *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasm	
NMB1572	*acnB*		Up	OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB1590			Up	OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB1620			Up	OMVs (Williams *et al.*, 2007)	Unknown	Periplasmic	TatP	√
NMB1623	*aniA*		Up		Periplasmic	Periplasmic	LipoP	√
NMB1624			Up		Unknown	Outer membrane	SignalP	√
NMB1672		Up			Unknown	Periplasmic	Cytoplasm	
NMB1674		Up			Periplasmic	Periplasmic	TatP	√
NMB1677			Up		Unknown	Periplasmic	SecretomeP	
NMB1690			Up	OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB1710	*gdhA*		Up	OM and OMVs (Williams *et al.*, 2007; Ferrari *et al.*, 2006; Vaughan *et al.*, 2006)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1723	*fixP*		Up		Unknown	Periplasmic	TMHMM	√
NMB1805	*cyc*		Up	OM (Williams *et al.*, 2007)	Periplasmic	Periplasmic	SignalP	√
NMB1849	*carA*		Up	OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB1971		Up			Outer membrane	Outer membrane	SignalP	√
NMB1994	*nadA*		Up		Outer membrane	Outer membrane	SignalP	√
NMB1998	*mspA*		Up	OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	√
NMB2050			Up	OMVs (Williams *et al.*, 2007)	Outer membrane	Cytoplasmic	Cytoplasm	
NMB2051	*petC*		Up		Unknown	Periplasmic	TMHMM	√
NMB2053	*petA*		Up	OM (Williams *et al.*, 2007)	Inner membrane	Periplasmic	SecretomeP	
NMB2096	*mqo*		Up	OMVs (Williams *et al.*, 2007; Ferrari *et al.*, 2006)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB2099		Up			Cytoplasmic	Outer membrane	Cytoplasm	

OM or OMVs in the table indicate that the protein has been detected in outer membrane or outer membrane vesicle preparations, respectively. Predictions from algorithms PSORTb and PSLpred are shown together with predictions from CBS. Denominations from CBS: SignalP, the protein encodes a signal peptide; TMHMM, the protein encodes transmembrane helices; LipoP, the protein is a lipoprotein; SecretomeP, the protein is secreted through a non-classical i.e. not signal peptide triggered protein secretion and TatP, the protein has a twin-arginine signal peptide cleavage site.

5.2.1.3 Virulence factors

Tettelin *et al.* (2000) identified 104 established or putative determinants of meningococcal virulence. The major *N. meningitidis* virulence factors are capsule, LOS and the type IV pilus. None of the genes associated with these surface structures were up-regulated in plasma-grown organisms. Genes associated with biosynthesis and transport of the polysaccharide capsule were found to be down-regulated following 60 min incubation in plasma, but the genes were still expressed at reasonable high levels (signal intensity in comparison to gDNA). A gene (*pglB* (NMB1820)) encoding a protein involved in pilin glycosylation was found to be up-regulated at 60 min. A mutant in *pglB* has previously been shown to be more sensitive to killing in serum. The increased sensitivity was not found to be linked to decreased pilin glycosylation, but was instead attributed to a decrease in expression of the polysaccharide capsule (Kahler *et al.*, 2001). Three of Tettelin's genes (NMB0995, *bfrA* (NMB1206) and *nadA*), were up-regulated in plasma-grown organisms. NMB0995, up-regulated at 60 min, encodes a macrophage infectivity potentiator-related protein. The other two genes (*bfrA*, encoding bacterioferritin A, and *nadA*) were up-regulated at 240 min.

5.2.1.4 Meningococcal adaptation to the plasma environment

Genes involved in bacterial metabolic functions were affected by growth in plasma. Such genes included those involved in amino acid biosynthesis, energy metabolism and associated with responses to oxygen availability.

Amino acid biosynthesis and acquisition Amino acids are essential for many cellular processes - e.g. protein synthesis, peptidoglycan cross-linking (alanine) and nitrogen transfer (glutamate, glutamine) (Hebeler & Young, 1976; Boehlein *et al.*, 1994). Biosynthesis of amino acids has been shown to be important for bacterial survival at sites where their availability is limited (Wang *et al.*, 1996; Fuller *et al.*, 1999; Sun *et al.*, 2000). In some pathogens, limitation of amino acids has been shown to induce the expression of virulence associated genes (Wagner & Mulks, 2006). For example, *N. meningitidis* has been reported to require L-glutamic acid, L-arginine,

glycine, L-serine and (for some strains) L-cysteine under laboratory growth conditions (Catlin, 1973). Using genomic information Baart *et al.* (2007) predicted that *N. meningitidis* is most likely able to synthesise all amino acids, with the possible exception of serine. Ten genes encoding products functioning in biosynthesis of either the aromatic, aspartate family, glutamate family or pyruvate family of amino acids have previously been identified as essential to allow sustained bacteraemia in an infant rat model (Sun *et al.*, 2000). Four of the genes (*aroG* (NMB0307), *aroE* (NMB0358), *ilvD-2* (NMB1188) and *gdhA*) found in the study by Sun *et al.* (2000) were up-regulated during incubation in plasma. The gene *aroE*, encoding a shikimate 5-dehydrogenase, was up-regulated at 60 min. The three other genes were up-regulated at the 240 min time-point: *ilvD-2*, encoding a dihydroxy-acid dehydratase; *aroG*, encoding a phospho-2-dehydro-3-deoxyheptonate aldolase; and *gdhA*, encoding a NADP-specific glutamate dehydrogenase. An additional eight genes associated with synthesis of amino acids (*cysK*, *dapE* (NMB1530), *ilvE* (NMB0337), *ilvD-1* (NMB1150), *leuC* (NMB1036), *proB* (NMB1069), *argD* (NMB1371), *argF* (NMB1573)) were up-regulated in plasma-grown meningococci at either 60 or 240 min. Genes up-regulated at 240 min were primarily associated with degradation or synthesis of glutamate (*argD*, *argF*, *proB*, *gdhA*) and branched chained amino acids (*ilvE*, *ilvD-1*, *leuC*). In addition, several genes encoding transporters of amino acids were up-regulated during growth in plasma (presented in the following paragraphs).

The gene *gdhA*, up-regulated at 240 min in bacteria grown in plasma is involved in glutamate metabolism and synthesis, and its gene product, an L-glutamate dehydrogenase, converts 2-oxoglutarate and NH_4^+ to L-glutamate (or the reverse reaction). At the same time-point *amtB* (NMB0615), encoding an ammonium transporter, was up-regulated. Ammonia can be used as a nitrogen source by bacteria and can be utilised in the synthesis of, for example, L-glutamate. The transcription of *gdhA* has been shown to be initiated from two separate promoters (Pagliarulo *et al.*, 2004). The gene *gdhR* (NMB1711), encoding the transcriptional regulator that activates transcription of *gdhA* from one of its two promoters, was also up-regulated in plasma at 240 min.

Pagliarulo *et al.* (2004) showed that the transcriptional activation of *gdhA* by GdhR can be repressed by the binding of 2-oxoglutarate to the promoter, which results in blockage of activator binding. GdhR has also been shown to regulate the transcription of the operon NMB1966–1962, encoding components of a L-glutamate transporter, which has been suggested to function at low sodium levels (Monaco *et al.*, 2006). The operon was not significantly differentially expressed in plasma, but instead *gltS* (NMB0085), involved in L-glutamate uptake at higher sodium concentrations, was up-regulated in plasma-grown organisms. Genes (NMB0787–89) encoding a putative ATP-binding cassette (ABC) transporter of polar amino acids were up-regulated at 60 min, suggesting that there may be free amino acids available for uptake, at least initially. Alternatively, up-regulation of these genes may suggest that there is a lack of amino acids in plasma which the bacteria respond to by increasing expression of amino acid transport systems. NMB0787–89 have been shown to be up-regulated by growth in L-glutamine (Friedrich *et al.*, 2007), which may indicate that L-glutamine, the most abundant amino acid in plasma (Tsai & Huang, 1999), is transported by this ABC transporter. Research in gonococci suggests that the substrate-binding component, encoded by NMB0787 in MC58, is essential for bacterial viability (Du & Arvidson, 2006).

At the later time-point during incubation in plasma, *putP* (NMB0402) (encoding a sodium/proline symporter) was found to be up-regulated. L-proline can be degraded by the gene product of *putA* (NMB0401) (also up-regulated at 240 min) to 1-pyrroline-5-carboxylate and further to L-glutamate. These transcriptional changes point to a requirement of L-glutamate by the later time-point in plasma. However, it may be that differential regulation of genes associated with L-glutamine and L-glutamate results from differences in glutamine and glutamic acid concentrations in plasma and culture medium RPMI 1640.

In a study by Grifantini *et al.* (2002a) genes involved in sulphur metabolism and acquisition were found to be induced when *N. meningitidis*, but not *N. lactamica*, adhered to epithelial cells. Up-regulation of these genes results in increased synthesis

of the sulphur-containing amino acids cysteine and methionine, the universal start of proteins. Genes involved in sulphur metabolism, including *cysD-1* and *cysN-1* (NMB1153), were here up-regulated at 60 min.

At the 240 min time-point many of the induced genes belong to the functional category *energy metabolism* (significant by GSEA analysis) (Figure 5.7 (2)). The enrichment of this TFRC was apparently related to an increase in oxidative phosphorylation, since up-regulation was observed for genes encoding the NADH dehydrogenase I (*nuo* genes), ubiquinol-cytochrome c reductase (*pet* genes), succinate dehydrogenase (*sdh* genes) and cytochrome cbb_3 oxidase (*fix* genes). This suggests an increase in energy requirement at later time-points in plasma. Up-regulation could also indicate either increased levels of the ferric uptake repressor (Fur) (Fur can also function as an activator) and/or available iron as both Fur and iron-replete conditions previously have been shown to induce expression of these genes (Delany *et al.*, 2006). Genes encoding the ATP synthase F1 and an electron transfer flavoprotein were, however, down-regulated.

Neisseria primarily degrades glucose through the pentose phosphate or the Entner-Doudoroff pathway (Morse & Hebeler, 1978). The tricarboxylic acid (TCA) cycle is generally not active until glucose is exhausted in the medium or the cells reach stationary phase of growth. Nine genes encoding proteins involved in the TCA cycle were up-regulated at 240 min, suggesting that the bacteria utilised the TCA cycle for energy production.

Iron uptake Iron uptake is important for meningococcal survival in the human host (Rohde & Dyer, 2003). During growth *in vivo*, meningococci can sequester iron from the human iron binding proteins transferrin (Schryvers & Morris, 1988b), pre-dominant in serum, and lactoferrin, found mainly on mucosal surfaces and at sites of infection (Schryvers & Morris, 1988a). The two genes, *tbpA* (NMB0461) and *tbpB* (NMB0460), encoding the transferrin binding protein, were not up-regulated at either time-point upon growth in plasma in comparison to growth in medium. The gene NMB0034, encoding a putative high-affinity iron transporter, was, however, up-

regulated in plasma-grown organisms at 60 min. This gene has previously been shown to be up-regulated under iron-depleted conditions (Grifantini *et al.*, 2003). After 240 min incubation in plasma, genes encoding proteins involved in iron uptake were found to be down-regulated. These included genes encoding the transferrin binding protein and the lactoferrin binding protein (*lbpA* (NMB1540) and *lbpB* (NMB1541)). Delany *et al.* (2006) used microarrays to identify genes induced by both iron and Fur, or by the transcriptional regulator alone. 20 of the genes up-regulated in plasma at 240 min were found by Delany *et al.* to be induced both by iron and Fur. An additional seven genes (up-regulated during growth in plasma) are induced only by Fur. This suggests that levels of available iron and of the regulator Fur were higher in plasma than in medium at this time-point.

Responses to changes in oxygen availability After 240 min incubation, up-regulated genes included *mapA* and *aniA* (NMB1623). Both genes have previously been shown to be positively regulated by FNR under oxygen-limiting conditions (Bartolini *et al.*, 2006). The gene *mapA* encodes a surface exposed 85 kDa maltose phosphorylase involved in catalysing the cleavage of maltose to glucose and β-glucose-1-phosphate, the latter subsequently converted into β-D-glucose-6-phosphate by a β-phosphoglucomutase, encoded by *pgmB* (NMB0391). β-D-glucose-6-phosphate can be used for energy utilisation in the pentose phosphate or the Entner-Doudoroff pathway. A *mapA* knock-out strain was found to have a 3-log reduction in blood survival in a mouse model of infection compared to wild-type (Bartolini *et al.*, 2006). The gene *aniA* encodes a nitrite reductase that degrades nitrite to nitric oxide (NO). Besides positive regulation by FNR, *aniA* is negatively regulated by the NO-sensitive repressor NsrR (encoded by NMB0437) (Rock *et al.*, 2007; Heurlier *et al.*, 2008). This gene was found to be up-regulated at 60 min during growth in plasma. Two other genes, *dnrN* (NMB1365) and NMB1624, which were up-regulated at 240 min in plasma, have also been reported to be negatively controlled by NsrR (Rock *et al.*, 2007; Heurlier *et al.*, 2008). The gene NMB1624, transcribed down-stream of *aniA*, has similarity to *nirV* genes and may have a nitrite reductase function and *dnrN*

is similar to proteins functioning in protection against NO damage (Heurlier *et al.*, 2008).

5.2.1.5 Stress induced responses and cell detoxification

Genes encoding proteins involved in responses to oxidative stress or other general stresses have previously been associated with survival in animal models of infection and *in vitro*/*ex vivo* infection models (Herbert *et al.*, 2002; Bron *et al.*, 2004). Some of the genes up-regulated during growth in plasma encode chaperones involved in protein folding or degradation. *clpA* (NMB0836), up-regulated at 60 min, encodes the ATP binding subunit of a two-component ATP-dependent Clp protease which contributes to the elimination of damaged or aggregated proteins (Singh *et al.*, 2000). The product of *dsbB* (NMB1649), up-regulated at 240 min, plays a critical role in returning DsbA, involved in disulphide bond formation of periplasmic proteins, to its active state (Whitley & von Heijne, 1993).

Other meningococcal transcriptional changes at 240 min that might be a response to stresses encountered by the bacteria during growth in plasma included up-regulation of the operon NMB1436–38, as well as a putative transcriptional regulator (encoded by NMB1891), known to be responsive to various stresses. Products of NMB1436–38 have been shown to be involved in meningococcal responses to oxidative stress (Grifantini *et al.*, 2004). Deletion of the operon NMB1436–38 resulted in a strain with increased sensitivity to killing by polymorphonuclear leukocytes and impairment in growth in the blood stream of adult mice (Grifantini *et al.*, 2004). NMB1891 has previously been shown to be up-regulated in heat-shock (Guckenberger *et al.*, 2002). The MenB gene has sequence similarity to the xenobiotic response element (XRE) family of transcriptional regulators, which has been reported to be involved in responses to a wide variety of stresses including acidic pH (Tran *et al.*, 2005), salt (Pflüger *et al.*, 2007), nitrogen availability (Veit *et al.*, 2006) and radiation (Liu *et al.*, 2003).

Efflux proteins protect bacteria from the effects of toxic compounds, such as antibacterial factors secreted by host cells. Genes significantly induced during growth

in plasma included *farA* (NMB0318) and *mtrF*, both encoding efflux proteins. In addition, a putative drug/metabolite transporter (encoded by NMB1459) was up-regulated at 60 min.

5.2.1.6 Proteins with putative or hypothetical function

Forty-three percent of the genes in strain MC58 are annotated as hypothetical proteins or proteins with unknown function. The products of more than one quarter (53 out of 200) of MenB-specific genes up-regulated during growth in plasma are annotated as hypothetical proteins, emphasising that many genes associated with virulence are still poorly characterised.

Two such genes up-regulated at 60 min have previously been observed to have elevated expression upon exposure to serum (NMB0260) (Kurz *et al.*, 2003) or in contact with epithelial cells (NMB0305) (Grifantini *et al.*, 2002b). Three of the genes up-regulated at 240 min (NMB0228, NMB0252 and NMB1590), have previously been reported to be differentially expressed upon incubation in NHS versus PBS (Kurz *et al.*, 2003). During meningococcal growth in FFP 1, NMB0277 and NMB0776 were up-regulated at 60 min or 240 min respectively. A sequence repeat found in the upstream regions of both these genes has been suggested to be involved in transcriptional induction on host cell contact (Deghmane *et al.*, 2003; Morelle *et al.*, 2003). However, Morelle *et al.* (2003) showed that neither of the genes is necessary for attachment to host cells. NMB0277 is annotated as the virulence factor MviN, due to sequence similarity to the mouse virulence family (MVF) of proteins, and its gene product may function in drug efflux. NMB0776 is similar to the *gly1* gene identified in gonococci, encoding a protein of unknown function which, when mutated, results in strains with decreased cytotoxicity to host cells (Arvidson *et al.*, 1999). The genes up-regulated at 60 min also included NMB1532, encoding a protein with a putative haemerythrin, oxygen-binding, domain. Interestingly, a *Staphylococcus aureus* protein with a haemerythrin domain has been shown to be involved in bacterial adaptation to low oxygen environments (Throup *et al.*, 2001).

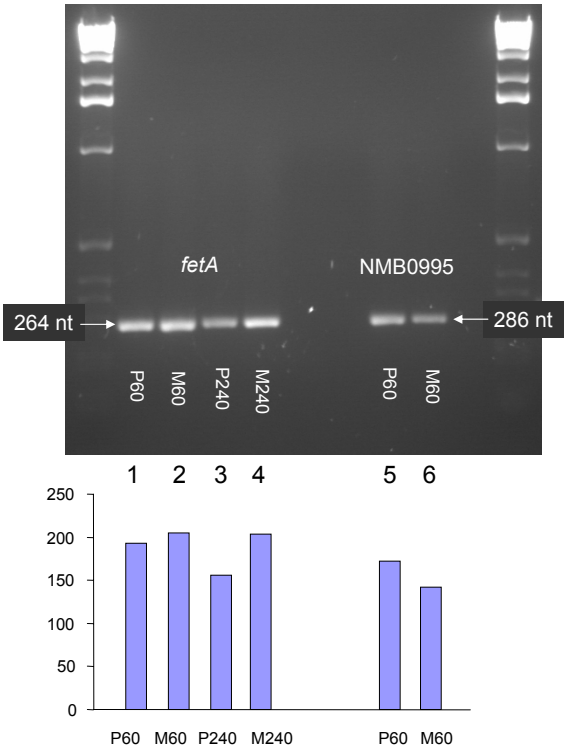


Figure 5.8: Agarose gel image of products from semi-quantitative RT-PCR using RNA isolated from meningococci incubated in FFP 1 (P) or medium (M) for 60 and 240 min (Section 5.2.1). Samples were separated on a 2% agarose gel, with a 1 kb ladder. Samples in lane 1–4 were amplified using primers specific for *fetA*. Samples in lane 5–6 were amplified using primers specific for NMB0995. Height of bars in graph corresponds to the median intensity of each band as determined by quantification with ImageQuant software.

5.2.1.7 Validation of microarray data

Expression of two of the genes found to be differentially expressed upon growth in FFP 1 from the microarray study was validated by semi-quantitative RT-PCR (Section 2.8.1). These were NMB0995 and *fetA* (NMB1988) (encoding an iron-regulated outer membrane protein). *fetA* was down-regulated in plasma at 240 min (compared to all other conditions and time-points) and NMB0995 was up-regulated in plasma at 60 min (compared to all other conditions and time-points). For gene NMB0995, the ratio in median intensity was 1.21 between the product amplified from an aliquot of RNA isolated from plasma-grown bacteria at 60 min and the product amplified from an aliquot of RNA isolated from medium grown bacteria at 60 min. For gene *fetA* the median intensity for the product from RNA isolated from plasma at 240 min was 0.76 of the median intensity for RNA isolated from medium grown organisms at 240 min, and ~ 0.8 compared to median intensity for products amplified from RNA recovered

from bacteria in either plasma or medium at 60 min time-point.

5.2.2 Transcriptional profile in FFP 2 in comparison to time-point 0 min

The transcriptional profile after incubation for 60 and 240 min in FFP 2 was compared to that at time-point zero. RNA was isolated at 0, 60 and 240 min from four independent experiments. The quantity of RNA that could be extracted from meningococci in FPP 2 was 10–20 μg. The quality of the isolated RNA was determined by separation on an agarose gel (Section 2.4). Each of the isolated RNA samples were analysed on microarrays.

Processing of microarray data and statistical analysis to find differentially expressed genes was performed as described in Section 5.2. Meningococcal gene expression in plasma at 60 and 240 min post-inoculation was compared to transcription at time-point zero. Following 60 min incubation in FFP 2, 378 genes were differentially expressed with a fold-change of $\geq$1.5 and a FDR of $\leq$0.05. Applying the same cut off, 199 genes were found to be differentially expressed at 240 min (Supplementary tables B.6–B.9). Of the differentially expressed genes, 108 were shared between the two time-points in plasma (Figure 5.9, Supplementary table B.10). Genes that were found to be up-regulated at both time-points included NMB0787–89, encoding a putative amino acid transporter; *leuD* (NMB1034) and *leuC*, involved in biosynthesis of branched chain amino acids; and NMB1040, an outer membrane protein with an unknown function. These genes will be presented in greater detail in subsequent sections.

To explore the functional roles of genes associated with meningococcal growth in plasma, genes differentially expressed in response to bacterial growth in FFP 2 were divided into categories according to their TIGR designations (Figure 5.10), and the microarray data were analysed using the GSEA software (described on page 100) to test for significantly overrepresented TFRCs. Meningococcal growth in plasma at 60 min was associated with an enrichment of genes (with higher expression than at t = 0 min) that are involved in the following TFRCs: *purines, pyrimidines, nucleosides*



Figure 5.9: Venn diagram of genes differentially expressed in FFP 2 at 60 and 240 min compared to the 0 min time-point. Up-regulated genes are shown to the left and down-regulated genes are shown to the right in the figure. Genes that were up- or down-regulated at both time-points in FFP 2 are listed in Supplementary table B.10.

and nucleotides biosynthesis; *protein synthesis*; *protein fate*; *transcription*; *biosynthesis of cofactors, prosthetic groups and carriers*; *hypothetical proteins*; and *DNA metabolism* (FDR q-value $\leq$0.25). Genes involved in *energy metabolism* and *cellular processes* were associated with up-regulation at t = 0 min (FDR q-value $\leq$0.194) compared to 60 min during growth in FFP 2, indicating a down-regulation of genes in those TFRCs at 60 min. None of the TFRCs were found to be over-represented among genes with increased expression at 240 min. However, genes annotated in the category *transcription* were significantly enriched (FDR q-value 0.058) among genes up-regulated at time-point 0 min (in relation to the 240 min time-point), which suggest that transcription was reduced at the later time-point in FFP 2.

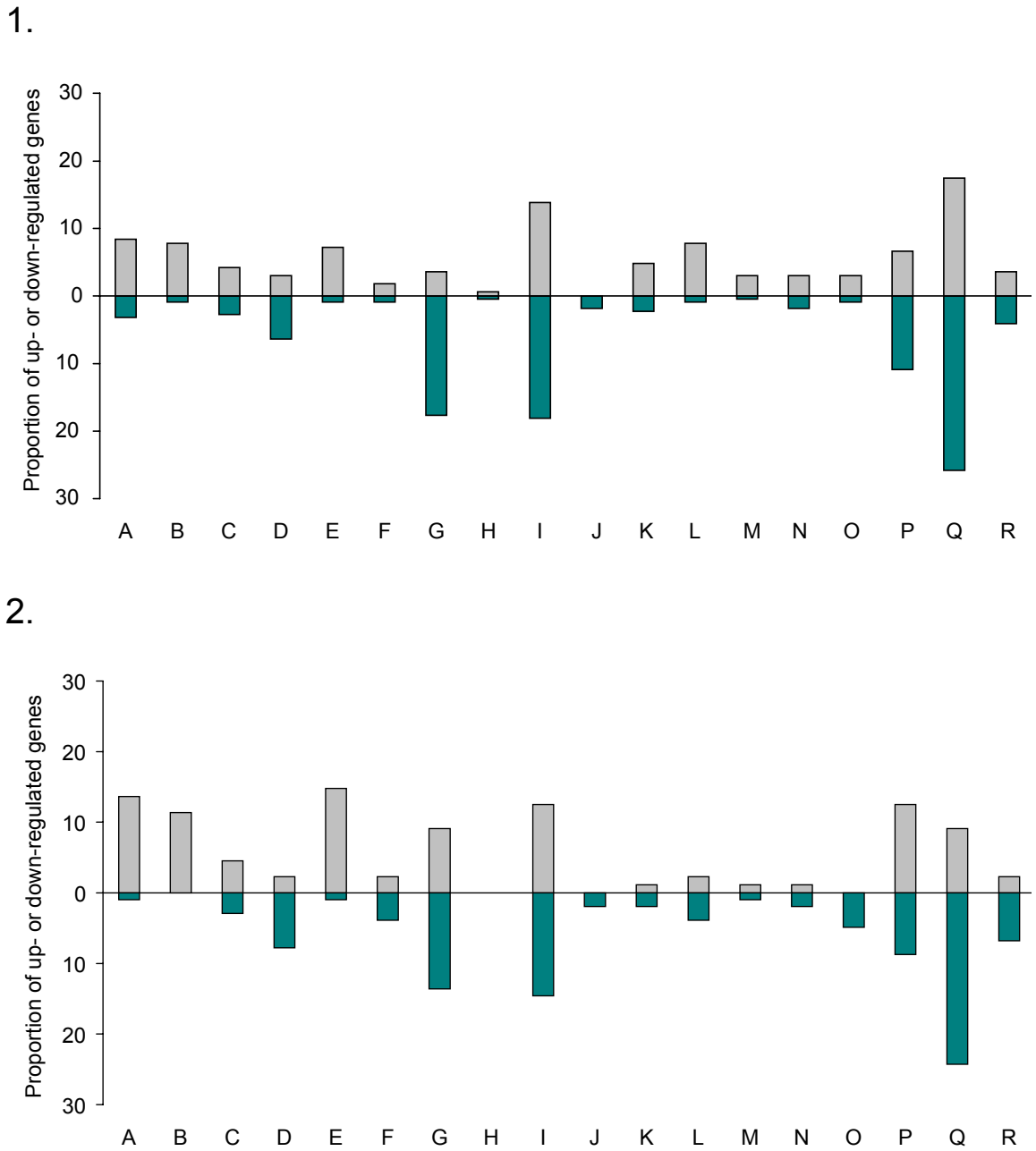


Figure 5.10: Differentially expressed genes at 60 (1) and 240 min (2) in FFP 2 compared to time zero divided into functional categories, according to TIGR designations. Up-regulated genes are depicted by light-grey bars and down-regulated genes by green bars. Numbers shown on the Y-axis represent the percentage each category constitutes of the total number of either up- or down-regulated genes. Columns: **A**. Amino acid biosynthesis, **B**. Biosynthesis of cofactors, prosthetic groups, and carriers, **C**. Cell envelope, **D**. Cellular processes, **E**. Central intermediary metabolism, **F**. DNA metabolism, **G**. Energy metabolism, **H**. Fatty acid and phospholipid metabolism, **I**. Hypothetical proteins, **J**. Mobile and extrachromosomal element functions, **K**. Protein fate, **L**. Protein synthesis, **M**. Purines, pyrimidines, nucleosides, and nucleotides biosynthesis, **N**. Regulatory functions, **O**. Transcription, **P**. Transport and binding proteins, **Q**. Unassigned, **R**. Unknown function.

5.2.2.1 Known or putative surface exposed proteins

Genes found to be up-regulated in plasma were divided into categories according to predicted (or known) cellular locations, based on experimental confirmations or *in silico* predictions. Several different prediction algorithms were used to form a consensus about cellular location (Section 2.11). All MC58 genes found to encode a protein with a signal peptide or which were attributed as encoding proteins located in the periplasm or the outer membrane, are listed in Table 5.2. In addition, genes encoding proteins that have been identified in preparations of OM or OMVs, or experimentally confirmed to be on the surface of the bacteria are also included in Table 5.2. Sixty-six such genes were up-regulated in these experiments.

Seven genes (NMB0866, NMB0872, NMB1533, NMB1961, NMB1962, NMB1966 and NMB1977) encoding proteins predicted to have an outer membrane location, were up-regulated at 60 min in plasma. The protein encoded by NMB0872 contains tetratricopeptide repeat (TPR) domains, commonly involved in protein–protein interactions (Blatch & Lässle, 1999). A surface exposed autotransported serine protease A (AspA) (encoded by NMB1969), was up-regulated at the 240 min time-point. Reactive antibodies to AspA have been detected in acute sera from patients (Turner *et al.*, 2002). Three more genes up-regulated at the 240 min time-point encode proteins with a known or predicted surface location. They were NMB0293, encoding a TonB-dependent receptor; *mapA*, encoding an outer membrane located maltose phosphorylase; and *mlp* (NMB1898), encoding a lipoprotein. Interestingly, the gene *mapA* was also up-regulated at 240 min during incubation in FFP 1 in comparison to medium. Three genes (NMB0821, NMB1040 and NMB1073), encoding proteins with a predicted outer membrane location, were up-regulated at both time-points in plasma. Additionally, NMB0787 and NMB0995, which were both consistently up-regulated during incubation in FFP 2, have previously been established by Grifantini *et al.* (2002b), using flow cytometry, to encode surface located products.

Table 5.2: Up-regulated genes at 60 or 240 min during growth in FFP 2 (indicated by Up in table) that encode proteins with a known or putative location in the periplasm or the outer membrane of the bacteria. Genes that have been found in experimental studies to be located on the surface of the bacteria or detected in protein preparations of OMVs or OM are also included in the table.

		Time-point (min)			PSORTb	PSLpred	CBS	
ID	**Name**	**60**	**240**	**Experimental data**	**Location**	**Location**	**Prediction**	**SignalP**
NMB0010	*pgk*	Up	Up	OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0085	*gltS*		Up	OMVs (Williams *et al.*, 2007)	Inner membrane	Inner membrane	TMHMM	
NMB0086			Up	OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Multiple locations	Cytoplasmic	Cytoplasm	
NMB0142	*rplC*	Up		OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Periplasmic	SecretomeP	
NMB0174	*valS*	Up		OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0214	*prlC*	Up		Surface located (Grifantini *et al.*, 2002b). OM and OMVs (Williams *et al.*, 2007; Ferrari *et al.*, 2006; Vaughan *et al.*, 2006)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0267		Up			Unknown	Periplasmic	SignalP	√
NMB0284	*purB*		Up	OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB0292		Up		OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB0293			Up		Outer membrane	Outer membrane	SignalP	√
NMB0317		Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0337	*ilvE*		Up	OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB0389	*galM*		Up		Periplasmic	Periplasmic	Cytoplasm	
NMB0390	*mapA*		Up	OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Cytoplasmic	Cytoplasm	
NMB0392	*nadB*	Up			Periplasmic	Periplasmic	Cytoplasm	
NMB0468	*speA*	Up	Up		Multiple locations	Periplasmic	Cytoplasm	
NMB0540	*aspC*		Up	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0554	*dnaK*	Up		Plasminogen binding (Knaust *et al.*, 2007). OM and OMVs (Williams *et al.*, 2007; Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006)	Unknown	Periplasmic	Cytoplasm	
NMB0561	*grpE*	Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Periplasmic	Cytoplasm	
NMB0563	*apbE*	Up			Unknown	Extracellular	LipoP	√
NMB0720	*thrS*	Up		OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	

Continued on next page ...

Table 5.2 – continued from previous page

ID	Name	Time-point (min) 60	240	Experimental data	PSORTb Location	PSLpred Location	CBS Prediction	SignalP
NMB0749			Up		Periplasmic	Periplasmic	SignalP	√
NMB0763	*cysK*		Up	OM and OMVs (Williams *et al.*, 2007; Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasm	
NMB0787		Up	Up	Surface located (Grifantini *et al.*, 2002b). OM and OMVs (Williams *et al.*, 2007; Ferrari *et al.*, 2006)	Periplasmic	Periplasmic	LipoP	√
NMB0802	*metB*	Up	Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0815	*purA*	Up		OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0819		Up			Unknown	Periplasmic	SecretomeP	
NMB0820		Up			Unknown	Periplasmic	SecretomeP	
NMB0821		Up	Up		Unknown	Outer membrane	TMHMM	
NMB0840		Up			Unknown	Periplasmic	Cytoplasm	
NMB0866		Up			Outer membrane	Extracellular	SignalP	√
NMB0872		Up		OMVs (Ferrari *et al.*, 2006)	Unknown	Outer membrane	SignalP	√
NMB0876			Up	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0922	*lst*	Up		Surface located (Shell *et al.*, 2002). OMVs (Vipond *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasm	
NMB0943	*metF*	Up		OMVs (Vipond *et al.*, 2006)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB0995		Up	Up	Surface located (Grifantini *et al.*, 2002b)	Unknown	Cytoplasmic	Cytoplasm	
NMB1017	*sbp*		Up		Periplasmic	Periplasmic	LipoP	√
NMB1020		Up			Cytoplasmic	Extracellular	Cytoplasm	
NMB1031	*leuB*	Up		OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1035		Up		OM (Williams *et al.*, 2007)	Unknown	Periplasmic	LipoP	√
NMB1037	*gshA*	Up	Up	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1040		Up	Up	OMVs (Ferrari *et al.*, 2006)	Unknown	Outer membrane	SignalP	√
NMB1044	*fpr-1*	Up		OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB1070	*leuA*	Up		OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1073		Up	Up	OM and OMVs (Williams *et al.*, 2007)	Unknown	Outer membrane	Cytoplasm	
NMB1150	*ilvD-1*	Up		OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1252	*purM*	Up		OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1297	*mltD*	Up	Up		Unknown	Periplasmic	SignalP	√

Continued on next page ...

Table 5.2 – continued from previous page

ID	Name	Time-point (min) 60	240	Experimental data	PSORTb Location	PSLpred Location	CBS Prediction	SignalP
NMB1347	*suhB*	Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1371	*argD*		Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1389			Up	OMVs (Vaughan *et al.*, 2006; Vipond *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasm	
NMB1437			Up	OMVs (Vipond *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasm	
NMB1533		Up		OM and OMVs (Williams *et al.*, 2007; Ferrari *et al.*, 2006)	Periplasmic	Periplasmic	LipoP	√
NMB1538	*rpoD*	Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1565		Up			Unknown	Periplasmic	SignalP	√
NMB1643	*infB*	Up		OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	SecretomeP	
NMB1680	*aroC*	Up		OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB1800		Up	Up		Unknown	Extracellular	SecretomeP	
NMB1898	*mlp*		Up	OMVs (Ferrari *et al.*, 2006)	Unknown	Periplasmic	LipoP	√
NMB1961		Up		OM (Williams *et al.*, 2007); OMVs (Ferrari *et al.*, 2006)	Outer membrane	Outer membrane	SignalP	√
NMB1962		Up		OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasm	
NMB1969	*aspA/nalP*		Up	OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006)	Outer membrane	Extracellular	LipoP	√
NMB1977		Up		OM (Williams *et al.*, 2007)	Unknown	Outer membrane	LipoP	√
NMB2000	*hslO*	Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasm	
NMB2053	*petA*	Up	Up	OM (Williams *et al.*, 2007)	Inner membrane	Periplasmic	SecretomeP	
NMB2129	*argG*		Up	OM and OMVs (Williams *et al.*, 2007; Ferrari *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasm	

OM or OMVs in the table indicate that the protein has been detected in outer membrane or outer membrane vesicle preparations, respectively. Predictions from algorithms PSORTb and PSLpred are shown together with predictions from CBS. Denominations from CBS: SignalP, the protein encodes a signal peptide; TMHMM, the protein encodes transmembrane helices; LipoP, the protein is a lipoprotein and SecretomeP, the protein is secreted through a non-classical i.e. not signal peptide triggered protein secretion.

5.2.2.2 Virulence factors

The expression of meningococcal virulence factors identified by Tettelin *et al.* (2000) (described in Section 5.2.1.3) during growth in plasma were explored. Genes involved in biosynthesis or transport of the polysaccharide capsule were found to be down-regulated upon incubation in FFP 2, but were still expressed at reasonably high levels (compared to common reference). Eight of the Tettelin genes were found to be up-regulated compared to t = 0 at either or both time-points in plasma. These included *lst* (NMB0922), encoding an alpha-2,3-sialyltransferase and NMB0995, encoding a putative macrophage infectivity potentiator-related protein, as well as three genes (*lgtF* (NMB1704), *rfaK* (NMB1705) and *lgtA* (NMB1929)), which encode proteins involved in synthesis of LOS (up-regulated at 60 min). Genes up-regulated at 60 min also included *pglF* (NMB0596), encoding a putative O-antigen ligase involved in glycosylation of pilin (Power *et al.*, 2006). A *pglF* mutant has increased sensitivity to cationic antimicrobial peptides (CAMPs) (Tzeng *et al.*, 2005a).

5.2.2.3 Meningococcal adaptations to incubation in FFP 2

Initial responses at 60 min included up-regulation of genes involved in *protein synthesis* and *protein fate*, indicating an initial adaptation to the environment. Genes involved in *protein synthesis* were over-represented among plasma-responding genes (using GSEA) at 60 min. The genes that contributed most to the enrichment of this category were *infB* (NMB1643) (encoding a translation initiator) and two genes (*prfC* (NMB0626) and *prfA* (NMB1686)) encoding peptide release factors 1 and 3, respectively.

Amino acids are essential in many processes in the cell and biosynthesis of amino acids are important for bacterial pathogenesis (described in greater detail in Section 5.2.1.4). During incubation in FFP 2, several of the genes up-regulated at 60 and 240 min were associated with biosynthesis of branched-chain amino acids (valine, leucine and isoleucine), including the genes *leuD* and *leuC*. These genes encode the small and large subunit of an isopropylmalate isomerase that forms part

of the leucine-biosynthesis pathway. At 240 min, genes such as *aspC* (NMB0540) and *tyrB* (NMB1678), involved in biosynthesis of aromatic amino acids, were also up-regulated. Both these genes encode aminotransferases functioning mainly in the breakdown of amino acids. Besides genes associated with amino acid biosynthesis, genes encoding amino acid transporters were also found to be up-regulated. The operon NMB0787–89, encoding an amino acid ABC transporter that may specifically bind polar amino acids, was up-regulated at both time-points in plasma. Similarly, at both time-points, genes encoding proteins involved in L-glutamate transport were up-regulated. NMB1966, encoding a component of a L-glutamate ABC transporter, was up-regulated at 60 min and *gltS*, encoding a sodium/glutamate symporter, was found to be up-regulated at 240 min (also up-regulated at 240 min in FFP 1).

As with meningococcal responses to growth in FFP 1 (described on page 108), genes up-regulated at 60 and 240 min during growth in FFP 2 included some involved in sulphur metabolism (involved in the synthesis of S-adenosylmethionine (SAM), methionine and N-formylmethionyl-tRNA) and *sbp* (NMB1017) involved in acquisition of sulphate (up-regulated at 240 min).

Samant *et al.* (2008) have previously identified *de novo* biosynthesis of purine and pyrimidine to be essential for bacterial growth in serum. After 60 min growth in plasma three genes (*purA* (NMB0815), *purK* (NMB1019) and *purM* (NMB1252)) involved in purine biosynthesis were found to be up-regulated. Analysis of the microarray data with the function `geneSetTest()` in the Limma package (Smyth, 2004), which implements an alternative approach to gene set enrichment analysis, using moderated t-statistics for each gene from the statistical analysis as input values, indicated that genes involved in purine biosynthesis were significantly more likely to be up-regulated in plasma at 60 min than at time-point 0 min (P-value = 0.00186).

Energy metabolism and exploitation of nutrient sources Gene expression changes at the later time-point during incubation in plasma indicate that nutrient sources may have become limited, or alternatively that increased energy was required due to increased bacterial growth. Up-regulation was, for example, observed for *relA*

(NMB1735), encoding a protein that mediates accumulation of (p)ppGpp in response to nutrient-limitation. *N. gonorrhoeae* RelA-dependent accumulation of (p)ppGpp occurs under amino acid, energy source and phosphate starvation (Fisher *et al.*, 2005). Additionally, comparison of the expression of genes involved in energy metabolism at 60 and 240 min (Section 5.2.3) revealed significant up-regulation of genes encoding proteins involved in the TCA cycle. Genes (NMB0388 and *gluP* (NMB0535)) encoding putative transporters of carbon sources (maltose and glucose/galactose respectively) were induced over the time course in plasma. Genes involved in exploitation of alternative carbohydrate sources such as maltose were also found to be up-regulated over time during growth in plasma. At 240 min NMB0470, encoding a C4-dicarboxylate transporter, was also found to be up-regulated. This protein is thought to transport 2-oxoglutarate and/or malate, which can be used for energy utilisation in the TCA cycle. The gene has previously been found to be up-regulated during exposure to serum (Kurz *et al.*, 2003). A C4-dicarboxylate permease has previously been shown to be up-regulated when *Pasteurella multocida* is grown in medium supplemented with haemoglobin (Paustian *et al.*, 2002).

At the 240 min time-point, several of the up-regulated genes encoded proteins involved in biosynthesis of the cofactor thiamine (*thiC* (NMB2040), *thiO* (NMB2068), *thiE* (NMB2069) and *thiG* (NMB2071)), a possible response to amino acid availability. Thiamine acts as a co-enzyme in steps in the TCA cycle and the pentose phosphate pathway, as well as in metabolism of branched-chain amino acids. Differential regulation of *thiC*, involved in thiamine biosynthesis, has previously been observed in *Yersinia pestis*, when incubated in plasma as compared to broth (Chauvaux *et al.*, 2007).

Iron acquisition and metabolism Genes encoding proteins involved in iron uptake, including *lbpAB* and *hmbR* (NMB1668), were down-regulated at 60 min in comparison to expression at 0 min. However, seven of the genes (NMB0316–17, NMB0821, NMB0861–62, NMB0865–66) that were up-regulated at 60 min during growth in plasma have previously been found to have increased expression under

iron limiting conditions and to be under transcriptional regulation by Fur (Grifantini *et al.*, 2003). Between 60 and 240 min, genes involved in iron uptake were found to be induced (Section 5.2.3).

Stress-induced functions Following 60 min incubation in plasma, genes encoding chaperones were up-regulated. These included *prlC* (NMB0214), encoding oligopeptidase A; *lon* (NMB1231), encoding an ATP-dependent protease; and *clpX* (NMB1372), encoding the ATP-binding subunit of an ATP-dependent protease.

Polyamines have been shown to be involved in many processes in the cell, for example protection to oxidative damage (Shah & Swiatlo, 2008). Shah *et al.* (2008) previously found that *potD*, encoding the binding-domain of a polyamine transporter, is highly up-regulated in a murine model of pneumococcal bacteraemia. In this study, the genes *potB* (NMB0611) and *potC* (NMB0612), encoding components of a polyamine transporter, were found to be up-regulated at 60 min.

Efflux proteins play a role in the protection of bacteria from toxic compounds. Following 60 min incubation in plasma, NMB0393, encoding a multidrug resistance protein thought to confer protection to quaternary ammonium compounds, was found to be up-regulated.

Bacteria respond to hyperosmolarity by increasing influx of potassium, a process followed by an increase in glutamate synthesis (genes required for synthesis were up-regulated between 60 and 240 min time-points) to counteract the high level of cations (Dinnbier *et al.*, 1988). After the initial phases K^+ is transported out of the cell by KefB or other non-specific efflux pumps (Kempf & Bremer, 1998). The gene *trkA* (NMB1614), encoding a potassium transport system, was found to be up-regulated at 60 min during incubation in plasma in this study. Chen *et al.* (2004) previously showed that the *Vibrio vulnificus* TrkA protein is involved in serum resistance, which was attributed to its ability to counterbalance the change in osmotic pressure occurring as a result of insertion of MAC. Up-regulation of *trkA* during growth in FFP 2 could reflect a general bacterial response to changes in osmolarity, but alternatively could be a response to counteract insertion of MAC.

The gene *dnrN* (described in Section 5.2.1.4), shown to be under NsrR transcriptional repression by Heurlier *et al.* (2008), was up-regulated in plasma-grown organisms at 240 min. This may indicate that meningococci were subjected to oxygen limitation or nitric oxide at the later time-point in plasma. The products of the operon NMB1436–38 involved in meningococcal protection to oxidative stress were, however, up-regulated at 240 min, although this may be related protection to iron toxicity rather than oxidative stress (Grifantini *et al.*, 2004). NsrR also represses the transcription of *norB* (NMB1622), when nitric oxide levels are low. In gonococci, *norB* has been shown to be repressed by an additional regulator, with features of a member of the ArsR family of transcriptional regulators (Isabella *et al.*, 2008). Isabella *et al.* (2008) showed that the binding of the ArsR regulator, to its binding site in the *norB* up-stream region, can be abolished by the binding of the regulator Fur to the same site. An orthologue of the gonococcal *arsR* gene, NMB0398, was found to be up-regulated following 240 min incubation in plasma.

5.2.2.4 Regulatory proteins

Only a few transcriptional regulators have been characterised in *N. meningitidis* and relatively few genes, encoding putative regulatory proteins, were found to be differentially expressed during growth in plasma. At 60 min three of the up-regulated genes (*phoQ/misS* (NMB0594), NMB1561, NMB2055) and three of the down-regulated genes (NMB1049, NMB1303, NMB1843) encode putative or established transcriptional regulators. *phoQ/misS* encodes a sensor histidine kinase, which together with the product from NMB0595 (the DNA-binding response regulator) makes up a two-component regulatory system, one of only four that have been identified in the *N. meningitidis* strain MC58 genome. This has been suggested to respond to levels of Mg^{2+} (Johnson *et al.*, 2001), although this has subsequently been called into question (Tzeng *et al.*, 2006). A knock-out mutation of NMB0595 resulted in attenuated growth in mice (Newcombe *et al.*, 2004), which may relate to changes in the LOS inner core structure observed for the mutant (Tzeng *et al.*, 2006). The transcriptional profile of an NMB0595 mutant under laboratory conditions has been reported

both by Newcombe *et al.* (2004) and Tzeng *et al.* (2008). Fourteen of the genes that these authors found to be down-regulated, were up-regulated at 60 min in FFP 2, perhaps induced in response to activation of the two-component system. NMB1843, here found to be down-regulated during plasma growth, has previously been shown to be up-regulated in contact with endothelial cells (Dietrich *et al.*, 2003), and confirmed to repress transcription of *nadA* (Schielke *et al.*, 2008). Differentially expressed genes at 240 min included three genes encoding transcriptional regulators, one up-regulated (*relA*) and two down-regulated (NMB0398, *lrp* (NMB1650)). *lrp* encodes a protein with similarity to members of the family of leucine-responsive regulatory proteins. In *E. coli* Lrp is a global regulator that regulates the expression of genes involved, for example, in metabolism of amino acids and peptide transport (Calvo & Matthews, 1994), but the functionality of Lrp may not be the same in other bacterial species (Lintner *et al.*, 2008). In *E.coli* Lrp has been shown to repress the expression of *metK*, the gene product of which is important for the synthesis of SAM (Newman *et al.*, 1998). During incubation in plasma, *metK* (NMB1799) was up-regulated after 240 min, which may suggest a similar regulation in meningococci. However, genes under regulation of Lrp in *N. meningitidis* have not yet been determined.

5.2.2.5 Hypothetical genes and proteins with putative functions

At both time-points in plasma a large proportion of the differentially expressed genes is categorised into the groups *hypothetical proteins* or *unassigned* (70 in total). A few of the genes with a putative function based on sequence similarity, or genes that have previously been observed to be differentially regulated in meningococcal growth under other environmental conditions, are described below.

Two genes up-regulated at 60 min in plasma, NMB0292 and NMB0438, may be involved in responses to changes in oxygen availability, as their products incorporate domains involved in such processes. NMB0292 encodes a protein with a putative nitroreductase domain and the NMB0438 gene product has a haemerythrin domain, which is commonly associated with oxygen binding.

Some of the genes that were up-regulated at 60 min have previously been observed

to be differentially expressed in other *in vitro* infection models. *creA* (NMB1335), *suhB* (NMB1347) and NMB1912 have previously been found to be up-regulated on contact with epithelial cells (Grifantini *et al.*, 2002b; Deghmane *et al.*, 2003; Morelle *et al.*, 2003). *creA* and *suhB* both have regulatory elements in their upstream region, which have been suggested to be associated with gene regulation in response to contact with host cells (Deghmane *et al.*, 2003; Morelle *et al.*, 2003). NMB1336, up-regulated at 60 min during growth in plasma, has previously been found to be up-regulated upon exposure to serum (Kurz *et al.*, 2003).

Some of the up-regulated genes annotated as encoding hypothetical proteins have sequence similarity to transporters. NMB0531, encoding a putative transporter of the Na+/H+ NhaC family type and NMB1426, encoding a protein with similarities to Mn^{2+}/Fe^{2+} transporters, were up-regulated at 60 min. At 240 min NMB0213, encoding a putative ion channel, was up-regulated. This type of ion channel has been shown to provide protection to hypoosmotic shock (Pivetti *et al.*, 2003).

5.2.3 Progressively regulated genes during growth in FFP or medium

To determine meningococcal responses to longer incubation in human plasma or medium, microarray data collected for the 240 min time-point were compared to data collected for the 60 min time-point. Separate analysis was performed for each of the three growth conditions (FFP 1, FFP 2 or medium). The overlap between the differentially expressed genes from each comparison is shown in the Venn diagram in Figure 5.11. Fifty-eight of the up-regulated genes were only found to be up-regulated during incubation in plasma (FFP 1 and FFP 2). All genes that were found to be up-regulated in FFP 1 and/or FFP 2 at 240 min versus 60 min are listed in Supplementary table B.11. Many of the up-regulated genes from 60 to 240 min, during incubation in plasma, may reflect changes in nutrient availability. Growth in plasma was associated with up-regulation of genes involved in the TCA cycle (Figure 5.12). Under growth in broth or medium *in vitro*, Neisseria primarily degrades glucose via the Entner-Doudoroff (80%) or the pentose phosphate pathway (20%) (Morse &

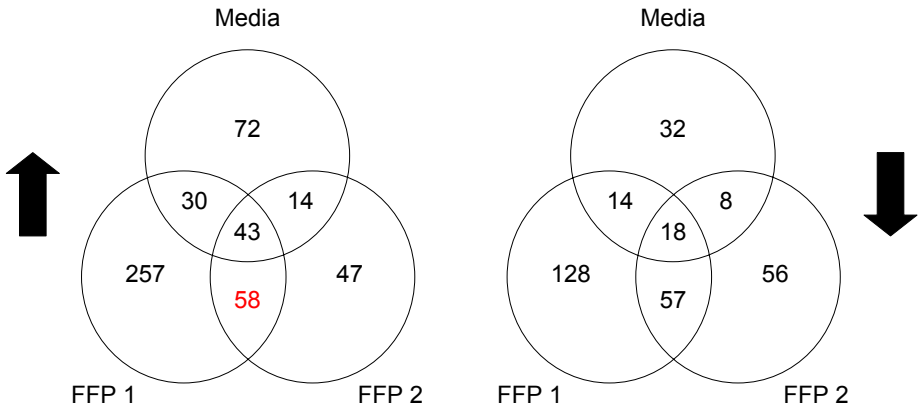


Figure 5.11: Venn diagram of differentially expressed genes at 240 min versus 60 min in plasma (FFP 1 or FFP 2) or medium. Up-regulated genes are shown to the left and down-regulated genes to the right in the figure. Fifty-eight meningococcal genes (number coloured in red) were up-regulated in both FFP 1 and FFP 2.

Hebeler, 1978). The TCA cycle is generally not active until glucose is exhausted in the medium or the cells reach the stationary phase of growth. Eight of the up-regulated genes, that were specifically up-regulated in plasma-grown organisms, encode proteins in the TCA cycle, including four genes (NMB0948–51), encoding subunits of succinate dehydrogenase, a membrane-bound enzyme that converts succinate to fumarate under aerobic growth. *sdhA* (NMB0950) and *sdhC* (NMB0948) have been shown by Mellin *et al.* (2007) to be under transcriptional regulation by *nrrF*, a Fur-repressed small RNA (sRNA), and to be expressed under iron-depleted conditions. Genes that were specifically up-regulated in plasma from 60 to 240 min also included three encoding transporters: *gluP* a putative glucose/galactose transporter; *lctP* (NMB0543) a lactate permease; and *amtB* a putative ammonium transporter. Increased expression of *lctP* may suggest that the meningococcus utilises lactate as an energy source at the later time-point during growth in plasma. In the cell, lactate can be degraded to pyruvate, which can be used in amino acid biosynthesis or in the TCA cycle. Pyruvate can be converted to phosphoenol pyruvate, which can be used in sialic acid synthesis together with N-acetyl-mannosamine (ManNAc). Expression of the lactate permease has previously been shown to be important for nasopharyngeal colonisation (Exley *et al.*, 2005a), as well as survival in the bloodstream (Exley *et al.*, 2005b). The gene *gluP*, found to be up-regulated in plasma, has previously been found to be progressively up-regulated when meningococci interact with epithelial cells (Grifantini *et al.*,

2002b). The observed up-regulation of a putative ammonium transporter suggests that the meningococcus has an increased need for nitrogen at later time-points in plasma. Up-regulation was also observed for *gdhA*, encoding a protein engaged in synthesis of L-glutamate from ammonia and 2-oxoglutarate (Figure 5.12).

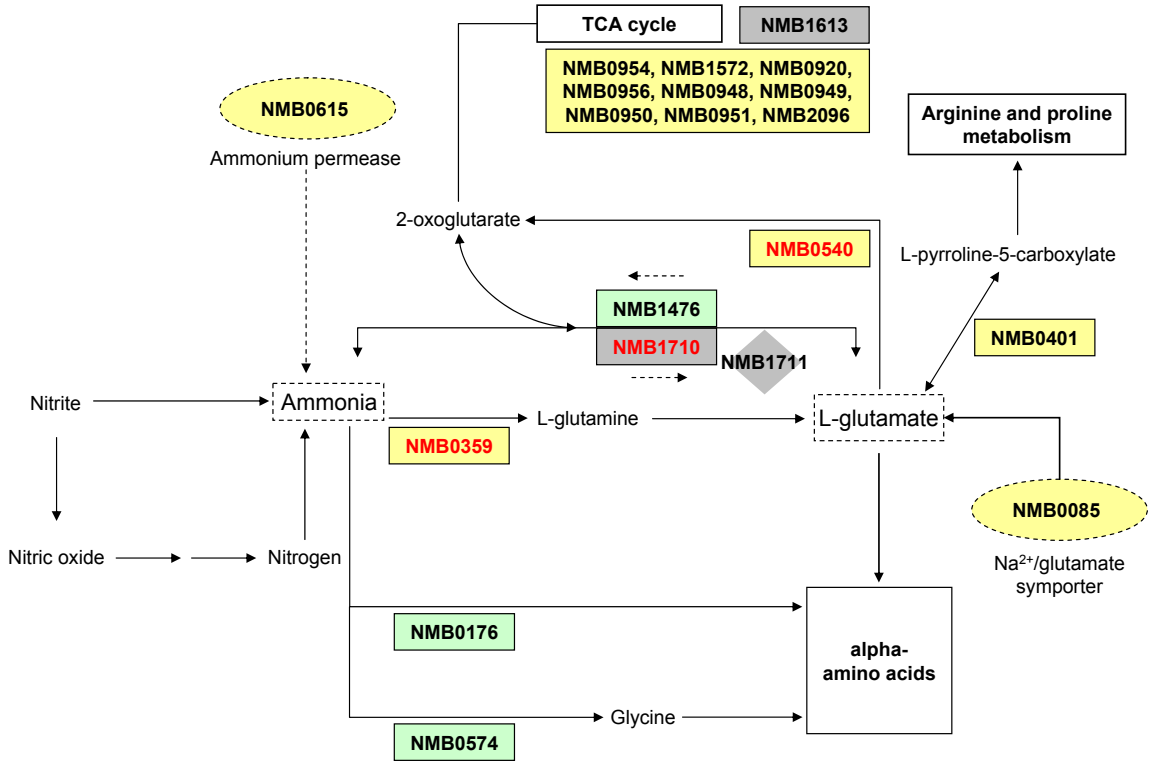


Figure 5.12: Up-regulated genes in plasma or medium at 240 min versus 60 min mapped to pathways for metabolism of nitrogen and L-glutamate, and related to the TCA cycle. Yellow boxes indicate genes that were up-regulated in both FFP 1 and FFP 2. Grey boxes indicate genes that were up-regulated in FFP 1. Green boxes indicate genes that were up-regulated in FFP 2. Genes coloured in red were also found to be progressively up-regulated during growth in medium. The diamond shape indicates a positive regulator and the oval shape indicates a transporter.

Meningococcal genes involved in iron-utilisation were also found to be up-regulated over time during growth in both plasma and medium. However, a few of these genes were only found to be differentially expressed in plasma-grown organisms, including *hemO* (NMB1669) that encodes a haem oxygenase involved in the extraction of iron from haem. Haem can be transported across the *N. meningitidis* membrane by the haemoglobin receptor (HmbR) or HpuA/B (not present in the MC58 genome) in a TonB-dependent manner (Stojiljkovic *et al.*, 1995). Uptake of haem can also occur independently of TonB, using an unknown mechanism (Desai *et al.*, 2000). The gene

encoding the haemoglobin receptor was found to be progressively up-regulated during growth in FFP 2.

5.3 Discussion

In this chapter the transcriptional profile of a MenB strain MO1-240185 was determined in a model of infection in a non-immune ('non-killer') individual. This model was realised by incubating MenB organisms at 37°C in plasma samples (FFP 1 and FFP 2) and determining bacterial transcriptional changes.

In the first set of experiments, the gene expression of MenB strain MO1-240185 in FFP 1 was compared to the gene expression of MenB in cell culture medium with an addition of fetal bovine serum, since meningococci showed similar growth under these two conditions. The gene expression profiles of meningococci incubated in plasma or medium were similar at the 60 min time-point. By 240 min the difference in gene expression between meningococci in plasma or medium was greater, despite similar growth characteristics of strain MO1-240185 in plasma and medium. In the second study, the gene expression in FFP 2 was compared to the gene expression of bacteria recovered from solid agar at time-point 0 min. The gene expression changes in this study were most dramatic at 60 min. However, it should be noted that the two FFPs differ in composition, as indicated by their different killing of strain MC58 (Figure 5.1). Since the same common reference sample (gDNA) was used in each of the microarray hybridisations it is possible in principle to make a number of comparisons between time-points and conditions. Unfortunately, direct comparison of data from the two studies could not be accomplished due to relatively high inter-experimental variation, a problem frequently encountered in microarray experiments. Instead lists of differentially expressed genes under each condition were compared to find genes that consistently respond to exposure to plasma.

The study presented in this chapter was partly designed to be a proof of principle for later work in whole human blood (presented in Chapter 6), to find optimal conditions for growth and to explore the isolation of meningococcal RNA from human

biological fluids. The advantages of using plasma rather than blood are that studies in plasma are easier to standardise. For example, plasma can be stored, unlike blood that has to be used directly after collection. It is therefore possible to use plasma collected on one occasion in a set of experiments. A difficulty in gene expression studies is finding a suitable reference condition. In this study the gene expression of meningococci incubated in plasma was compared to either of two reference conditions: medium supplemented with hI fetal bovine serum or to a time-point 0 min (initial inoculum). In other similar studies rich broth (Hsieh *et al.*, 2008; Chauvaux *et al.*, 2007), heat-inactivated serum (Dove *et al.*, 2003) or culture medium (Kurz *et al.*, 2003) have been used as reference conditions. In Chapter 6, in which the genome-wide transcriptional changes in whole human blood are presented, the second experimental design was chosen, i.e. meningococcal gene expression in blood was compared to expression at a time-point 0 min as well as between sampling points in the time course.

In this chapter MenB strain MO1-240185 was used in all microarray experiments. The primarily reason for using this strain rather then MenB sequence strain MC58 (represented on the microarray) was that MO1-240185 (but not MC58) survived in plasma available to us at the time of the experiments. In microarray experiments in blood (Chapter 6) MenB strain MC58 was used as this strain survived in blood samples from the two individuals used in that study (donors A and B).

5.3.1 Global impact of growth in plasma on meningococci

First, genes encoding surface exposed proteins identified to be up-regulated in this genome-wide transcriptional study are discussed, as identification of such genes was the primary objective of the study presented in this chapter. Second, the general themes in changes to the meningococcal transcriptome upon incubation in plasma are discussed.

Surface exposed proteins The primary aim of the work presented in this chapter was to identify genes expressed during infection that might be exposed on the

surface of the bacteria. Genes encoding surface exposed proteins that are specifically up-regulated during infection identify candidates for inclusion in a MenB vaccine (described in detail in Section 1.4.1.2). In the study presented here, several genes encoding known or putative surface exposed proteins were found to be up-regulated during meningococcal growth in plasma in relation to different control conditions (Table 5.1 and Table 5.2). Meningococcal growth in either FFP 1 or FFP 2 resulted in up-regulation of NMB0787 and NMB0995, encoding surface exposed proteins (Grifantini *et al.*, 2002b), by 60 min. Both these genes were also up-regulated at the later time-point (240 min) in FFP 2. Both these genes were previously observed to be up-regulated in contact with epithelial cells (Grifantini *et al.*, 2002b). Five of the genes encoding putative surface exposed proteins (*gltS*, *mapA*, *argD*, NMB1437 and *petA* (NMB2053)) were found to be up-regulated by the later time-point (240 min) in both plasma samples. NMB1035 encodes a putative lipoprotein, which has been identified in preparations of outer membrane proteins (Williams *et al.*, 2007) and was up-regulated at 60 min in FFP 2 and at 240 min in FFP 1. The gene *nspA*, encoding an outer membrane protein that previously has been identified as a vaccine candidate (Martin *et al.*, 1997), was up-regulated at 240 min after incubation in FFP 1.

An aim of this study was to explore the differences in meningococcal gene expression in a model of bacteraemia compared to the gene expression in a model of colonisation, to identify genes encoding surface exposed proteins that respond in particular to conditions to which meningococci are exposed in the blood stream. Despite several attempts to isolate RNA from meningococci adherent to epithelial cells, bacterial RNA could not be recovered of sufficient quality and quantity for microarray analysis. Data were therefore compared to microarray data from two studies in which the meningococcal transcriptional profile in contact with cultured epithelial cells has been characterised (Grifantini *et al.*, 2002b; Dietrich *et al.*, 2003). Genes that encode proteins that may have a cellular location in the periplasm or outer membrane, and that are down-regulated at some time-point in contact with cells, are listed in Table 5.3. Genes that were found to be up-regulated during meningococcal growth in

plasma, but which were down-regulated when meningococci adhered to epithelial cells included *mapA*, *dnaK*, *purA*, *rplY* (NMB0876), *bfrA*, NMB1220, NMB1533, *mspA* and *hslO* (NMB2000).

Some of the genes, encoding (known or putative) surface-exposed proteins, which were up-regulated during incubation in plasma were not reported to be differentially expressed upon attachment to epithelial cells in the studies by Grifantini *et al.* (2002b) and Dietrich *et al.* (2003). These genes included NMB0821, NMB0866, NMB1040, NMB1073, *mlp*, NMB1961, *aspA* and *nadA*. NadA is one of the five components of a experimental MenB protein based vaccine (Giuliani *et al.*, 2006). The sequence variability of *mapA*, NMB1040 and NMB1961 is explored using direct sequencing from a collection of meningococcal strains in Chapter 7.

Surface structures Genes encoding proteins involved in capsular polysaccharide synthesis and export to the bacterial surface were found to be down-regulated in FFP 1 at 60 min in comparison to medium; however the genes were still expressed at high levels. A few genes encoding other meningococcal surface structures were, however, up-regulated during growth in plasma, for example, three genes involved in LOS biosynthesis (up-regulated at 60 min in FFP 2). Glycosylation of pilin has previously been shown to protect meningococci from CAMPs and serum killing (Kahler *et al.*, 2001; Tzeng *et al.*, 2005a). In response to incubation in both FFP 1 and FFP 2 genes involved in pilin glycosylation were found to be up-regulated at 60 min.

Table 5.3: Comparison of meningococcal gene expression in FFP 1 and FFP 2 to gene expression of bacteria in contact with epithelial cells. Only genes that encode proteins that may be surface exposed are listed in the table. Abbreviations: OM, outer membrane; IM, inner membrane; C, cytoplasmic; P, periplasmic; L, Lipoprotein; U, no clear location; TM, Trans-membrane helices.

		FFP1 vs medium		**FFP2 vs. 0 min**		**16HBE14 cells**[A]				**HEp-2 cells**[B]	**Cellular location**				
ID	**Name**	**60 min**	**240 min**	**60 min**	**240 min**	**30 min**	**60 min**	**120 min**	**180 min**	**360 min**	**PSORTb**	**PLSpred**	**CBS**	**SignalP**	**Experimental**
NMB0390	*mapA*		Up		Up				Down		OM	C	C	N	In OMV preparations[A,D]
NMB0554	*dnaK*			Up		Down					U	P	C	N	Plasminogen-binding[E]. In preparations of OM[C] and OMVs[C,D,F,G]
NMB0763	*cysK*	Up			Up	Down		Up	Up		U	C	C	N	In preparations of OM[C] and OMVs[C,D,G]
NMB0787		Up		Up	Up	Down		Up	Up	Up	P	P	L	Y	Surface located by flow cytometry[A]. In preparations of OM[C] and OMVs[C,F]
NMB0815	*purA*			Up		Down	Down				C	C	C	N	In OM and OMV preparations[C]
NMB0876	*rplY*				Up	Down					C	C	C	N	In OMV preparations[C,F]
NMB1207	*bfrA*		Up			Down		Down	Down		C	C	C	N	In OM and OMV preparations[C]
NMB1220			Up			Down					IM	C	C	N	Surface located by flow cytometry[H]. In preparations of OM[C] and OMVs[C,G]
NMB1533				Up		Down	Down				P	P	L	Y	In preparations of OM[C] and OMVs[C,F]
NMB1723	*fixP*	Down	Up				Down		Down		U	P	TM	Y	
NMB1998	*mspA*		Up							Down	OM	OM	–	Y	In OMV preparations[C,D]
NMB2000	*hslO*			Up						Down	C	C	C	N	In OM preparations[C]
NMB2051	*petC*		Up			Down	Down		Down		U	P	TM	Y	

A (Grifantini *et al.*, 2002a), B (Dietrich *et al.*, 2003), C (Williams *et al.*, 2007), D (Vaughan *et al.*, 2006), E (Knaust *et al.*, 2007), F (Ferrari *et al.*, 2006), G (Vipond *et al.*, 2006), H (Pizza *et al.*, 2000)

Meningococcal adaptation to growth in plasma For a general overview of meningococcal responses to growth in plasma, data were analysed using the GSEA software (Mootha *et al.*, 2003). Following incubation of MenB in both plasma samples (FFP 1 and FFP 2) for 60 min, genes in the TFRCs *biosynthesis of cofactors, prosthetic groups and carriers* were overrepresented among plasma-responding genes. The subset of genes in the category that contributed mostly to the enrichment was involved in porphyrin metabolism, including the genes *cysG-1* (NMB1156) and *cysG-2* (NMB1194), encoding siroheme synthases; *hemC* (NMB0539), encoding a porphobilinogen deaminase; and *hemH* (NMB0718), encoding a ferrochelatase. The TFRC *central intermediary metabolism* was specifically overrepresented in microarray data from FFP 1 at 60 min. Upon incubation in FFP 2 six additional TFRCs were found to be enriched at 60 min: *purines, pyrimidines, nucleosides and nucleotides*; *protein synthesis*; *protein fate*; *transcription*; *hypothetical proteins*; and *DNA metabolism*. Performing the same analysis for data from the 240 min time-points one TFRC *energy metabolism* was found to be enriched among the up-regulated genes only during incubation in FFP 1. In each respective reference condition (meningococci grown in medium and the time-point 0 min) genes involved in *transcription* were found to be significantly enriched among up-regulated genes, indicating down-regulation of this TFRC at 240 min in both plasma (FFP 1 and FFP 2). This suggests a slowing of growth in plasma, although this was not yet reflected in the CFU counts at 240 min. In meningococci incubated in medium (at 240 min) two more TFRCs - *protein fate* and *protein synthesis* - were found to be significantly enriched among up-regulated genes in relation to bacteria in FFP 1, which may also relate to changes in growth phase in plasma. GSEA analysis has provided some interesting results as presented and discussed here. However, it should be noted that the sample numbers in this study are somewhat limited, and results should be treated with caution. To confirm the GSEA results a larger study would be necessary, but this was not feasible for the project presented here. Nonetheless the results should be indicative of the major trends in the data.

During incubation in FFP 1 NMB0034, encoding a putative high-affinity iron transporter, was up-regulated at 60 min compared to organisms grown in medium. In general, however, incubation of meningococci in plasma did not result in a significant up-regulation of iron-responsive genes, such as those encoding iron-uptake systems. Instead, genes including *fetA* and *tbpAB* were down-regulated during growth in FFP 1 at 240 min, as well as at 60 min in FFP 2. These genes have previously been found to be down-regulated in iron-replete versus iron-depleted conditions in microarray experiments performed by Grifantini *et al.* (2003). In the FFP 1 study, the down-regulation of these genes might be explained by the release of iron into the plasma through bacterial lysis, or through release of bound iron from proteins such as transferrin, or alternatively by the consumption of iron in the medium after 240 min incubation. For example *fetA* was also found to be down-regulated after 240 min incubation in plasma compared to the 60 min time-point in FFP 1, which suggests that the effects seen were the result of increased availability of iron in plasma, rather than its consumption by the media-grown organisms to which the transcriptome was compared. In the FFP 2 study, the down-regulation of these genes at 60 min may perhaps be explained by the iron-replete status of the bacteria at the start of the experiment.

During growth in plasma, genes associated with responses to lower oxygen tension were up-regulated both in comparison to medium and to t = 0. After 60 min incubation in plasma, two of the genes (NMB0438 and NMB1532) found to be up-regulated encode proteins with a haemerythrin domain, commonly associated with oxygen binding. Other proteins with this domain have been shown to be involved in bacterial adaptations to exposure to low oxygen environments (Throup *et al.*, 2001). During incubation in FFP 1, two genes (*mapA* and *aniA*), previously shown by Bartolini *et al.* (2006) to be regulated by FNR under micro-aerobic conditions, were up-regulated in comparison to incubation in medium. Bacteria in FFP 1 and medium were cultured under identical conditions, and showed similar growth rates, so the oxygen availability should have been comparable. Britigan *et al.* (1985) have

previously shown that growth of *N. gonorrhoeae* in the presence of serum considerably increases bacterial oxygen consumption. Therefore the induction of these genes might be explained by the fact that meningococci in plasma consume oxygen more rapidly than in medium, possibly due to higher levels of oxidative phosphorylation, as indicated by up-regulation of energy metabolism genes at 240 min in FFP 1.

Amino acid synthesis is necessary for bacterial survival in the host (Wang *et al.*, 1996; Fuller *et al.*, 1999; Sun *et al.*, 2000). In this study, meningococcal growth in plasma was associated with differential regulation of genes involved in amino acid biosynthesis, underlining the importance of these genes in infection. The major transcriptional changes observed for this group of genes occurred at 240 min during incubation in FFP 1 and at both time-points during incubation in FFP 2.

Other processes that previously have been found to be important during bacterial growth in infection models include sulphur metabolism (Grifantini *et al.*, 2002a) and *de novo* nucleotide biosynthesis (Samant *et al.*, 2008). In the present studies using FFP 1 and FFP 2, genes involved in sulphur metabolism and acquisition were found to be up-regulated, at 60 min during incubation in FFP 1 and at 60 and 240 min during incubation in FFP 2. Up-regulation of these genes results in increased synthesis of sulphur containing amino acids, such as methionine.

In a recent study by Samant *et al.* (2008) all mutants attenuated in growth in serum were shown to map to genes involved in *de novo* biosynthesis of purine and pyrimidine. Consistent with this finding, meningococcal genes involved in purine biosynthesis were found to be up-regulated at 60 min during incubation in FFP 2 compared to the start position at t = 0 (bacteria grown on solid nutrient agar).

In this study time-responsive genes were also established by comparing the gene expression at the late to the early time-point upon meningococcal incubation in plasma. Comparison of the gene expression profiles at 60 and 240 min in either plasma (FFP 1 and FFP 2) or medium revealed up-regulation of fifty-eight genes specific for plasma-grown organisms. Many of these may be up-regulated as a response to changes in nutrient availability. Genes encoding components of the TCA cycle were found to

be progressively up-regulated, including *acnB* (NMB1572) encoding a bifunctional aconitate hydratase 2. This gene is known to be sensitive to iron-depletion in *E. coli* (Varghese *et al.*, 2003). Genes encoding proteins for uptake of haem iron (*hmbR* and *hemO*) were also found to be progressively up-regulated during meningococcal growth in plasma (FFP 2). Up-regulation of genes involved in up-take of lactate (*lctP*) and alternative sugar sources, as well as the TCA cycle, indicates that glucose was not the main source of energy at the later time-point in plasma.

5.3.2 Conclusions

The transcriptional response of MenB strain MO1-240185 to incubation in non-bactericidal human plasma from two individuals has been characterised. This model has provided an initial platform for studying meningococcal transcription as bacteria proliferate in the bloodstream, a basis for understanding aspects of the pathogenesis of meningococcal sepsis.

In the studies presented in this chapter genes encoding surface exposed proteins, or putatively surface exposed proteins, were identified amongst genes up-regulated in plasma. These proteins represent potential vaccine targets. The sequence variability of three of these proteins (*mapA*, NMB1040 and NMB1961) are presented in Chapter 7.

In the next phase of my project, this work was refined so as to be able to study meningococcal gene expression in non-bactericidal fresh human blood. In experiments mimicking bacterial infections, reference conditions are invariably difficult. Based on experiments in plasma, I decided to include additional time-points in the blood study (Chapter 6) to improve the monitoring of meningococcal transcriptional responses, thereby improving characterisation of temporal expression patterns.

Chapter 6

Genome-wide transcriptional profiling of *Neisseria meningitidis* serogroup B in blood

In this chapter the gene expression of meningococci in the whole blood *ex vivo* model of sepsis is presented. Genes of particular interest were those, encoding surface exposed proteins, which were up-regulated in this experimental system. In Chapter 5 meningococcal transcriptional changes upon incubation in plasma were discussed. Incubating bacteria in plasma to model septicaemia has the obvious drawback of ignoring potentially important interactions of meningococci with cellular constituents of blood. In young children, with no or low bactericidal activity to meningococci, cellular immune responses may be of great importance, and this can be modelled with the use of fresh anti-coagulated non-bactericidal whole human blood. The model is more complex than plasma due to the presence of cells as well as a richer microenvironment, with metabolic changes over time induced by both the human and bacterial cells. The complexity of the system makes the experimental procedures and standardisation of the experiments harder to achieve, compared to the plasma model.

6.1 Introduction

Killing of *N. meningitidis* in its human host is mainly due to complement-mediated processes, discussed in Section 1.2.5.1. However, in young children serum bactericidal

activity is generally low, even after recent meningococcal infection (Pollard *et al.*, 1999). Cellular immunity is correspondingly important in limiting disease in young children. Additionally, individuals with poor serum bactericidal activity against *N. meningitidis* can still show phagocytic activity, either opsonic or non-opsonic (Welsch & Granoff, 2007). Since cellular immunity through phagocytic activity may be important for control of bacterial growth, especially in individuals without bactericidal antibodies, it is of interest to study the transcriptional changes that occur in bacteria propagated in blood compared to transcriptional changes in plasma (Chapter 5).

6.1.1 Components of blood and cellular and humoral immune responses

While *N. meningitidis* is predominantly an extracellular bacterium, it can adhere to and invade human host cells, and survive within cells. On the mucosal surface, and when crossing the blood-brain barrier, the meningococcus interacts with epithelial and endothelial cells and encounters phagocytotic cells such as macrophages. In the bloodstream meningococci come in contact with erythrocytes and leukocytes. *N. meningitidis* in blood is primarily killed by complement-mediated bactericidal effects, but also by antibody dependent and independent phagocytosis (Estabrook *et al.*, 1998). Both host and bacterial factors may modulate the susceptibility of the bacterium to phagocytosis. For example host receptors for lipo-oligosaccharide (LOS) may be important for effective phagocytosis of meningococci, since a LOS-deficient mutant strain is less susceptible to phagocytosis (Uronen-Hansson *et al.*, 2004), but this could potentially also be an effect of changes in cell surface-exposed lipoproteins or membrane composition in the mutant (Steeghs *et al.*, 2001). Heyderman *et al.* (1999) previously showed that incubation of meningococci *in vitro* in whole blood stimulates activation of neutrophils, leading to increased phagocytosis of the bacteria. Stimulation of whole blood with *N. meningitidis* or isolated OMVs increases the production of reactive oxygen species (ROS) (Mirlashari *et al.*, 2002). ROS stimulate the recruitment of phagocytic cells, but can also kill bacteria directly after phagocytosis.

Meningococcal septicaemia is characterised by high levels of cytokines such as tumour necrosis factor (TNF)-α, a pro-inflammatory factor, and interleukin (IL)-6, IL-8 and IL-10 (Waage *et al.*, 1987, 1989; Lehmann *et al.*, 1995). A similar pattern of cytokine release is observed *in vitro*, on incubation of meningococci in whole human blood (Ison *et al.*, 1995). Expression of chemokines, regulators of leukocyte traffic, during meningococcaemia has been shown to relate to the amount of detectable LOS in the blood stream (Schall & Bacon, 1994; Møller *et al.*, 2005). *N. meningitidis* sheds OMVs in large quantities during growth in blood, which contribute to the overwhelming immune response during meningococcal sepsis (Devoe & Gilchrist, 1973). OMVs contain LOS, OMPs, periplasmic proteins and probably some entrapped cytoplasmic proteins (Williams *et al.*, 2007). During growth in blood, bacteria are also exposed to factors secreted by cells, such as antibacterial proteins and peptides. Neutrophils secrete the antimicrobial factors lactoferrin, bactericidal/permeability-increasing protein, serprocidins, cathelicidins and defensins; whereas platelets secrete antimicrobial effectors (platelet microbicidal proteins), which target the bacterial cytoplasmic membrane (Levy, 2000).

6.1.2 Bacterial genes associated with survival in *in vivo* and *ex vivo* models of sepsis

Encapsulation protects bacteria, such as meningococci, from both bactericidal and phagocytotic killing during growth in the bloodstream (Read *et al.*, 1996; Vogel *et al.*, 1996). Genes associated with several functional categories, such as amino acid biosynthesis, iron uptake and regulatory function, have also been demonstrated to be important for *N. meningitidis* survival in the bloodstream. Studies in other bacteria have also demonstrated the importance of genes associated with metabolism in adaptation to new environments during growth *in vivo*. A few of these categories are discussed below.

Lipooligosaccharide expression LOS, also referred to as endotoxin, is a potent stimulant of the immune system (Brandtzaeg *et al.*, 1989). Mutant meningococci with disruption of genes involved in LOS biosynthesis, or in genes involved in its

sialyation, are attenuated in the bloodstream or during growth in serum (Vogel *et al.*, 1996; Sun *et al.*, 2000; Li *et al.*, 2004). Attenuation associated with disruption of genes functioning in LPS biosynthesis has also been reported for other organisms, including *E. coli* K1 (Gonzalez *et al.*, 2001) and *P. multocida* (Harper *et al.*, 2003). Knock-out of the *E. coli* gene *rfaL*, encoding an O antigen ligase, resulted in attenuation in systemic infection in an infant rat model (Gonzalez *et al.*, 2001). Mutants in either the *P. multocida* gene *dcaA*, encoding a phosphoethanolamine transferase, or pm1294, encoding a heptosyl transferase, were demonstrated to result in attenuation in a mouse septicaemia model (Harper *et al.*, 2003).

Iron uptake and transport systems Iron is an essential co-factor in the cell, but can be toxic at high levels. Iron is therefore sequestered to iron binding proteins. Most of the available iron in the human host is bound to proteins such as transferrin or lactoferrin, which results in low levels of free iron for bacteria to utilise. Low levels of free iron in the host can be seen as a bacteriostatic factor since it limits bacterial growth (Weinberg, 1984). However, most bacterial species have developed ways of scavenging iron bound to human proteins and genes involved in iron or haem uptake and utilisation are essential for bacterial survival *in vivo* (Li *et al.*, 2005). The meningococcus encodes several systems for uptake of various forms of bound iron, including transferrin binding proteins (TbpA and TbpB) (Schryvers & Morris, 1988a), lactoferrin binding proteins (LbpA and LbpB) (Schryvers & Morris, 1988b) and a haemoglobin receptor (HmbR) (Stojiljkovic *et al.*, 1995). *N. meningitidis*, an obligate human pathogen, only binds iron from human forms of transferrin and lactoferrin (Schryvers & Gonzalez, 1989). In order to establish meningococcal sepsis in animal models, an additional iron source is required (human transferrin or iron dextran) (Oftung *et al.*, 1999). Haemoglobin is normally only found within cells, but may be released during lysis of erythrocytes. A *N. meningitidis hmbR* (NMB1668) mutant has previously been shown to be attenuated in the infant rat model, indicating that uptake of haemoglobin may be of importance during bacterial growth in the bloodstream (Stojiljkovic *et al.*, 1995). *N. meningitidis* strains with disruptions in any

of the genes encoding the TonB system (*tonB* (NMB1730), *exbB* (NMB1729), *exbD* (NMB1728)) have also been found to be attenuated in their ability to cause bacteraemia in an infant rat model (Sun *et al.*, 2000). The TonB system, located in the inner membrane, is required for utilisation of transferrin, lactoferrin and haemoglobin (Stojiljkovic & Srinivasan, 1997), and functions by transducing the energy that induces conformational changes in TonB-dependent outer membrane transporters.

In addition to iron, bacteria require other nutrients (e.g. amino acids, oligopeptides) that they must acquire from their environment. Sun *et al.* (2000) isolated eight meningococcal mutants attenuated in an infant rat bacteraemia model that mapped to genes encoding transporters including: NMB0792, a sodium: dicarboxylate/sulphate transporter; NMB1240, an ATP-binding component of an ABC transporter; NMB1362, a putative oxalate/formate antiporter; NMB1612, a substrate-binding protein of a polar amino acid transport system; and NMB1515, a putative transporter. Additionally, meningococci with disabling mutations in the NMB1966 operon, encoding a L-glutamate transporter, have previously been found to be attenuated in growth in the bloodstream (Chow, 2006; Monaco *et al.*, 2006).

Amino acid biosynthesis Synthesis of amino acids is important for bacterial survival during growth in body sites where exogenous sources of amino acids are limited, such as in the bloodstream. On the other hand, blood is fairly rich in peptides that may be absorbed and degraded by the bacterium. Biosynthesis of branched amino acids (isoleucine, leucine and valine) has been demonstrated to be important for several pathogens during *in vivo* growth, including *Actinobacillus pleuropneumoniae* (Fuller *et al.*, 1999), *Pseudomonas aeruginosa* (Wang *et al.*, 1996) and *M. tuberculosis* (Hondalus *et al.*, 2000). *S. enterica* requires synthesis of aromatic amino acids for survival *in vivo* (Günel-Ozcan *et al.*, 1997; Sebkova *et al.*, 2008). Amino acid biosynthesis has also been shown to be important during meningococcal bacteraemia. Eleven out of seventy-three attenuated genes identified by screening insertional mutants in an infant rat bacteraemia model were associated with amino acid biosynthesis, including genes involved in biosynthesis of branched amino acids (*ilvI* (NMB1577),

ilvD (NMB1188)), aromatic amino acids (*aroB* (NMB1814), *aroC* (NMB1680), *aroD* (NMB1446), *aroE* (NMB0358), *aroG* (NMB0307)) and glutamate (*gdhA* (NMB1710)) (Sun *et al.*, 2000).

Nucleotide biosynthesis *De novo* nucleotide biosynthesis is important for bacterial survival in animal models of disease, as well as during *in vitro* growth in serum. *N. meningitidis* with a transposon insertion in *purL*, involved in purine biosynthesis, was attenuated in growth compared to wild-type in an infant rat model of disease (Sun *et al.*, 2000). Mutations in *E. coli*, *S. typhimurium* and *Bacillus anthracis* resulting in an attenuated phenotype in heat-inactivated human serum were all found to map to genes involved in either purine or pyrimidine ribonucleotide biosynthesis (Samant *et al.*, 2008). Mutations of genes involved in *de novo* purine or pyrimidine biosynthesis in *P. multocida* (Harper *et al.*, 2003) and *E. coli* (Li *et al.*, 2005) have also been reported to result in attenuation in septicaemia models.

Stress induced functions and cell detoxification During growth in blood microbes are subjected to environmental stresses such as the action of reactive oxygen species released by phagocytic cells. Deletions in bacterial genes encoding chaperones or other proteins involved in protection against oxidative damage or environmental stresses have previously been found to result in virulence attenuation in animal models of sepsis. Polissi *et al.* (1998) found that disruptions of genes encoding transporters of polyamines, which act as antioxidants in the bacterial cell, led to attenuation in growth of *Streptococcus pneumoniae* in the blood stream. Studies in *E. coli* (Gonzalez *et al.*, 2001) and *Haemophilus influenzae* (Rosadini *et al.*, 2008) have indicated that *dsbA*, which is involved in disulphide bond formation (Whitley & von Heijne, 1993), is important for survival and infection *in vivo*. The *H. influenzae* gene *dsbB*, encoding the second component of the DsbA–B system, has also been demonstrated to be required for bacteraemia in an animal model (Herbert *et al.*, 2002). The meningococcal operon NMB1436–38, encoding proteins involved in protection against oxidative stress, may be important for growth in the bloodstream since a mutant is attenuated in a mouse model of infection (Grifantini *et al.*, 2004). Genes important for *H.*

influenzae bacteraemia in the infant rat model of human invasive disease included a putative zinc protease involved in degradation of proteins and peptides (Herbert *et al.*, 2002). In addition, genes encoding oxidoreductases have been shown to be required for septicaemia caused by *E. coli* (Li *et al.*, 2005).

Miscellaneous genes important for meningococcal survival *in vivo* Other genes that have been found to be important for meningococcal survival in the bloodstream include NMB0345, encoding a putative a cell-binding factor; *ntrY* (NMB0114), encoding a nitrogen regulation protein (Sun *et al.*, 2000); *phoP/misS* (NMB0595), encoding the DNA binding part of a two-component system (Newcombe *et al.*, 2004); and *luxS* (NMB1981), encoding a S-ribosylhomocysteinase (Winzer *et al.*, 2002). Sun *et al.* (2000) identified seventy-three such *N. meningitidis* genes by screening insertional mutants *in vivo* in an infant rat model, of which sixteen encoded hypothetical proteins, including NMB0840 and NMB1564 (both discussed later in this chapter).

6.1.3 Bacterial transcriptional changes in *ex vivo* or animal models of sepsis

One early study of meningococcal gene expression used the strategy of differential fluorescence induction (DFI) developed by Valdivia & Falkow (1996) (using modified organisms with inserted promoter–GFP constructs) to evaluate genes up-regulated in a whole blood model (at 5 hr) (Webb, 2001). Genes found to respond the most to growth in blood (as determined by changes in fluorescence) were *pilMNOPQ* (NMB1808–12), *pilC1* (NMB1847), NMB1449, *frpC* (NMB1415), *groEL* (NMB1972) and *groES* (NMB1973).

Functional genomics (transcriptomics, proteomics etc.) technologies, like microarrays, can be used to study the global responses of bacteria in models of infection. Genome-wide transcriptional changes in the course of septicaemia (*ex vivo* or *in vivo*) have been studied in a few pathogens, including group A *Streptococcus* (GAS) (Graham *et al.*, 2005), *S. pneumoniae* (Orihuela *et al.*, 2004) and *P. multocida* (Boyce *et al.*, 2002). Such studies have revealed adaptive transcriptional changes during bacteraemia that confer apparent survival advantages.

Graham *et al.* (2005) determined the repertoire of gene expression of GAS in human blood at 30, 60 and 90 min in comparison to 0 min. They found genes involved in purine biosynthesis to be highly up-regulated during growth in blood, with the three most strongly up-regulated genes involved in purine biosynthesis. Gene expression peaked at 30 min indicating an involvement in the early adaptation to the environment. Several genes encoding membrane transporters were found to be up-regulated during growth in blood, including systems for transport of manganese, zinc and iron. Over time the most significant group of changing genes were those encoding cofactors. Down-regulated genes over the time course included those involved in glycolysis, which they attribute to the high levels of nutrients in blood, and in cell envelope biogenesis (including peptidoglycan biosynthesis), which they suggest relates to decreased growth rate in blood.

Orihuela *et al.* (2004) recovered *S. pneumoniae* RNA from the blood of mice with severe experimentally-induced sepsis. In comparison to bacteria grown in rich broth, transcriptional changes included up-regulation of genes involved in biosynthesis of purine and folic acid and those encoding manganese and zinc transporters. These authors' microarray data further underline the importance of biosynthesis of purines during growth in blood. No genes involved in transport of iron were found to be up-regulated in this system.

Boyce *et al.* (2002) found sixteen *P. multocida* genes to be consistently up-regulated during sepsis in poultry, in comparison to growth in rich medium. Up-regulated genes included *asnA*, encoding an ammonia-dependent asparagine synthetase; *purH*, involved in purine biosynthesis; and *gdhA*, encoding a glutamate dehydrogenase. Boyce *et al.* (2002) observed limited overlap between *P. multocida* genes found to be differentially expressed in septicaemia and genes that had previously been demonstrated to respond to growth under iron limitation.

So far no studies have been published in which genome-wide transcriptional changes in *N. meningitidis* have been determined in the course of septicaemia, either in *in vivo* or in *ex vivo* models. In this chapter I present the genome-wide transcriptional

profile of a MenB strain during culture in whole human blood, an *ex vivo* model of bacteraemia. To model the scenario in naïve hosts, representing young children, meningococci were incubated in non-bactericidal whole human blood. Genes of particular interest were those, encoding surface exposed proteins, which were up-regulated in this experimental system.

6.2 Results

Blood samples from a total of 20 adult volunteers were screened for the ability to sustain growth of MenB strain MC58. Two of the adult blood donors tested in this whole blood assay (WBA) (Section 2.9.2) were found to have almost no bactericidal activity to strain MC58. The growth characteristics of strain MC58 in non-bactericidal blood from individual A (female) and B (male) are shown in Figure 6.1 (these are the same donor A and B as in Chapter 3). The survival of MC58 in blood from these two individuals varied slightly between assays, but in most assays a 10-fold to 100-fold increase in bacterial number was observed during a 240 min time course. In contrast, during incubation of meningococci in blood from a 'killer', no bacteria were detectable by 60 min. Thirty ml of fresh non-bactericidal heparinised pre-prandial blood samples from adult donors were infected with meningococcal strain MC58 (B:15:P1.7,16-2) in late exponential phase of growth, at an initial inoculum of 10^7 cfu per ml, and incubated for up to 240 min. At the starting point, and up to 240 min post-infection, changes in the blood biochemistry were measured (data presented in Section 3.2.2). At 20, 40, 60, 90 and 240 min post-infection, bacterial RNA was isolated from the blood of donor A, following differential lysis of human cells, for a genome-wide transcriptional study as described in Section 2.9.2. The time course experiment was replicated on six separate occasions over a period of two months. For analysis of a subset of genes using comparative real-time RT-PCR, RNA isolated from meningococci incubated in blood from both donor A and B were used (Section 6.2.4). RNA was isolated from bacteria in blood using the method described in Section 2.9.2, and the quality of the isolated RNA was determined by analysis on a Bioanalyzer (described in Section

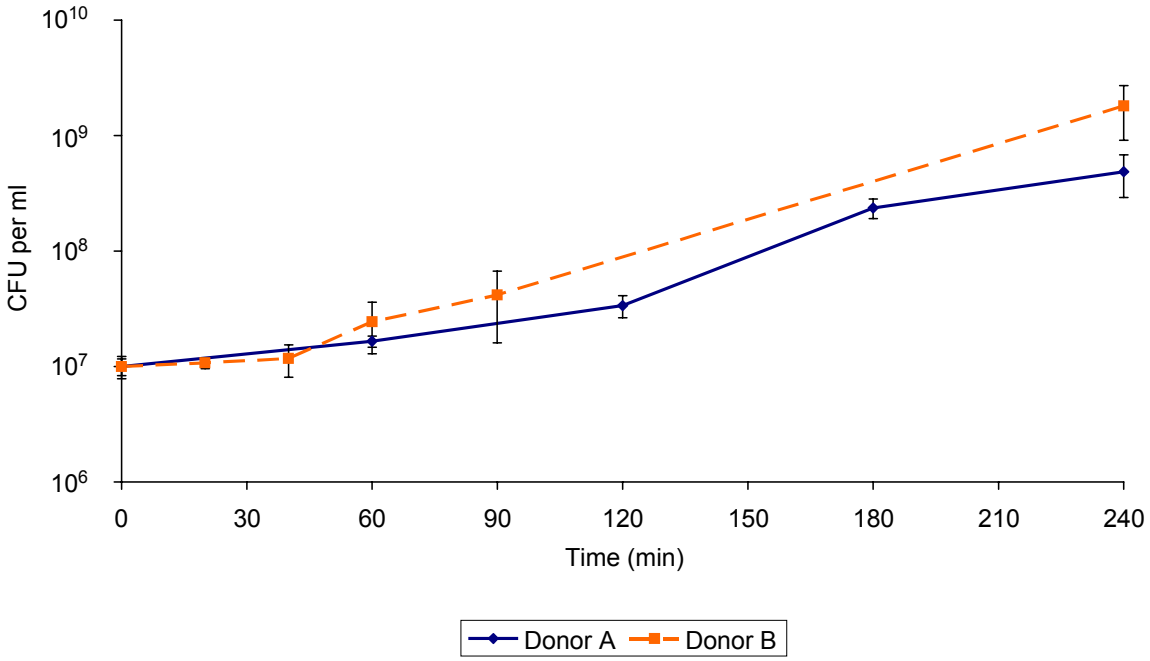


Figure 6.1: Survival of MenB strain MC58 in blood from donor A and donor B up to 240 min. Data presented for each individual are from one representative whole blood assay, with CFUs determined at different time-points. An initial load of 10^7 cfu per ml was added to heparinised blood followed by incubation up to 240 min. CFUs were determined by plating out appropriate dilutions in triplicate on solid sGC agar. Error bars represent standard deviations of technical replication.

2.4.1) (Figure 6.2). In the genome-wide transcriptional study, blood from only donor A was used for two main reasons: firstly, due to the cost of performing microarray studies and secondly, that insufficient amounts of RNA for microarray analysis could be isolated from bacteria incubated in blood from donor B.

6.2.1 MenB transcriptome in blood from donor A

RNA isolated from meningococci incubated in blood from donor A was reverse transcribed, fluorescently labelled and hybridised to *pan*-Neisseria microarrays as described in Section 2.7. Genomic DNA isolated from strain MC58 was used as a common reference in all hybridisations. Data collected from microarrays were filtered to remove spots with low intensity on all microarray slides and normalised to median $\log_2$ ratios on each slide. After pre-processing and normalisation of the data, statistical analysis was carried out using Bayesian linear models, as implemented in the software package Limma (Smyth, 2004), to identify differentially expressed genes (see Section 2.7.6 for details). A gene was considered to be significantly changed between

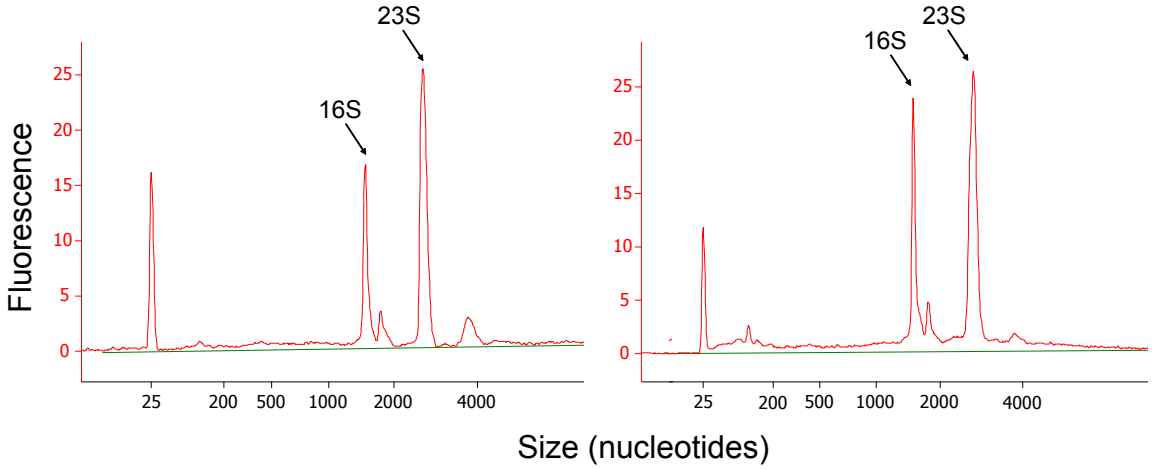


Figure 6.2: Bacterial RNA samples isolated from blood were analysed on an Agilent 2100 Bioanalyzer using RNA 6000 Nano assay chip. Abundance is given as fluorescence (relative to an RNA ladder) and plotted against migration time that has been converted into a nucleotide (nt) estimate of product size relative to an RNA ladder. To the left is an electropherogram of RNA isolated from meningococci in blood from individual A at the 20 min time-point and to the right is an electropherogram of RNA isolated from meningococci in blood from individual B at the 90 min time-point. The peak at 25 nt represents the marker added to all samples. Peaks at 1800 nt may represent contaminating human rRNA (18S) in the samples.

conditions or time-points when the change was $\geq \pm 1.5$-fold at a FDR of ≤ 0.01. The data were further analysed using gene enrichment methods that determine the significant differential regulation of a set of genes, rather than gene-by-gene. Two different methods were used for gene enrichment: the GSEA software developed by Mootha *et al.* (2003) (described on pages 100 and 135) or the function `geneSetTest()` available in the R-package Limma developed by Smyth (2004). PCA, a data dimension reduction method, was performed using gene expression data (mean-centred) from all genes of good quality to provide a multivariate overview of similarities between samples and for a low dimensional visualisation of the expression profiles (Section 2.7.8). The first two PCA components are shown in the score plot in Figure 6.3. The microarray data separated in space along the first PCA component according to time-point in blood (samples from later time-points are further to the left along the first component) and along the second PCA component according to experimental condition (incubation in blood or at t = 0 bacteria re-suspended in RPMI 1640 culture medium). Microarray data collected for time-points 20, 40 and 60 min during growth in blood had similar overall transcription profiles, indicated by the fact that

all samples from these time-points cluster in the bottom right corner in Figure 6.3.

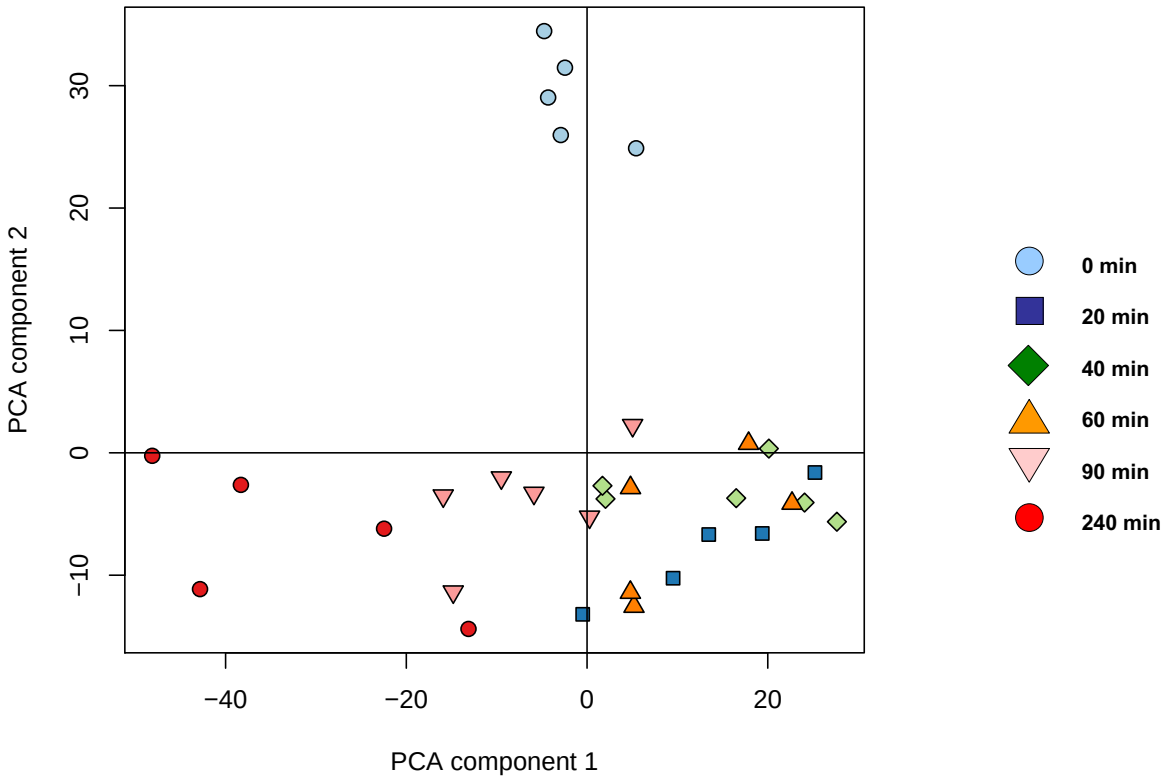


Figure 6.3: Principal component analysis of the gene expression in blood or on solid agar using data from all MC58 genes on all microarray slides. The PCA score plot reveal a separation of the time-points in blood along the first component (from right to left: early to late time-points), which points to differences in their overall transcriptional profile. Each symbol represents one biological replicate (five to six biological replicates per time-point). PCA component 1 explains 33.3% and PCA component 2 explains 16.8% of the variance in the data.

6.2.2 Nature of differentially expressed genes in comparison to time-point 0 min

Data presented in the following sections represent the comparison of the gene expression profile during incubation in blood to the gene expression profile at 0 min. Transcriptional changes between time-points or in relation to an early time-point in blood (t = 20 min) are presented in Section 6.2.3. In all of the comparisons, up-regulated meningococcal genes encoding known or putative surface exposed proteins will first be described, as identification of such proteins were the primary aim of this project.

At all time-points (20, 40, 60, 90 and 240 min) during *ex vivo* culture in blood, an extensive remodelling of the meningococcal transcriptome was observed, compared to

the transcriptional profile of bacteria at the time of inoculation. Using the inclusion criteria, 1135 genes (50% of the genome) were found to be differentially expressed at one or more time-points in response to exposure to human blood relative to time-point 0 min. All differentially expressed genes, grouped by their TIGR designations (Peterson *et al.*, 2001), are listed in Appendix C (Tables C.1–C.17). At each time-point, there was a relatively even distribution between up- and down-regulated genes. The proportion of differentially expressed genes that are associated with each TFRC is listed in Table 6.1. Most of the genes up-regulated in blood were associated with the following TFRCs: *hypothetical proteins*; *protein synthesis*; *energy metabolism*; and *transport and binding proteins*. The same categories, with the exception of *protein synthesis*, were also most common among the down-regulated genes. At 20 min, a large proportion of the up-regulated genes encode proteins involved in *energy metabolism*, at a much higher level than at later time-points. One plausible explanation for this up-regulation may be an initial energy requirement for adaptation to the new environment. At 90 min, there was a relative increase compared to the earlier time-points in transcription of genes encoding proteins involved in *biosynthesis of cofactors, prosthetic groups and carriers*, components of *central intermediary metabolism* and *regulatory functions*. The proportion of the up-regulated genes associated with either *DNA metabolism* (6.62%) or *cell envelope* (5.96%) was increased at the 240 min time-point. Analysis was performed using the GSEA software (Mootha *et al.*, 2003) to find TFRCs that were significantly associated with differential regulation during incubation in blood. Categories with a FDR q-value of ≤ 0.25 (the cut-off suggested by Mootha *et al.* (2003)) are listed in Table 6.2. None of the TFRCs were found to be significantly enriched among genes with higher expression at the 90 and 240 min time-points than at 0 min.

Table 6.1: Differentially expressed genes in blood versus time-point 0 min categorised into functional groups according to their TIGR designations. Numbers listed in the table (%) gives the proportion of each TFRC among up- or down-regulated at each time-point. Underlined numbers indicate categories found at a higher proportion among up- or down-regulated genes at specific time-points during incubation in blood, than the total proportion in the MC58 genome (column two).

		Proportion (%) of up- or down-regulated genes (at each time-point)				
TFRC	**MC58 (%)**	**20 min**	**40 min**	**60 min**	**90 min**	**240 min**
Up-regulated						
Amino acid biosynthesis	3.42	5.56	6.54	7.63	6.63	2.65
Biosynthesis of cofactors, prosthetic groups and carriers	4.08	2.96	2.31	2.97	8.84	5.96
Cell envelope	5.39	2.96	4.62	3.39	4.42	5.96
Cellular processes	5.92	1.85	3.85	1.69	2.76	4.64
Central intermediary metabolism	1.75	1.11	2.31	2.12	3.87	1.99
DNA metabolism	4.43	3.70	3.08	3.39	3.31	6.62
Energy metabolism	7.15	11.85	8.85	7.20	4.42	6.62
Fatty acid and phospholipid metabolism	1.36	1.48	1.54	2.12	1.10	0.99
Conserved hypothetical proteins	14.12	10.37	14.23	11.86	20.44	13.25
Mobile and extrachromosomal element functions	3.20	–	–	–	1.10	4.97
Protein fate	3.24	2.96	6.92	6.36	5.52	2.65
Protein synthesis	5.39	22.59	15.77	17.37	4.97	3.97
Purines, pyrimidines, nucleosides and nucleotides biosynthesis	1.80	2.59	3.85	3.81	2.76	2.98
Regulatory functions	2.37	1.85	1.54	1.69	2.76	2.32
Transcription	1.71	4.81	3.85	4.66	2.21	1.66
Transport and binding proteins	5.52	6.30	6.15	5.93	6.63	5.63
Unassigned	23.24	11.85	10.77	12.71	12.71	21.52
Unknown function	5.92	5.19	3.85	5.08	5.52	5.63
Down-regulated						
Amino acid biosynthesis	3.42	1.74	1.81	1.17	1.03	3.88
Biosynthesis of cofactors, prosthetic groups and carriers	4.08	0.87	0.90	1.17	0.68	1.83
Cell envelope	5.39	1.74	2.26	2.33	3.77	4.11
Cellular processes	5.92	9.57	9.05	9.34	9.25	7.76
Central intermediary metabolism	1.75	1.30	1.36	1.17	1.37	1.37
DNA metabolism	4.43	1.74	2.26	2.33	3.08	3.88
Energy metabolism	7.15	13.04	12.22	10.12	9.93	11.64
Fatty acid and phospholipid metabolism	1.36	0.43	–	0.39	1.37	1.60
Conserved hypothetical proteins	14.12	15.65	11.76	14.79	12.33	10.73

Continued on next page ...

Table 6.1 – continued from previous page

TFRC	MC58 (%)	Proportion (%) of up- or down-regulated genes (at each time-point) 20 min	40 min	60 min	90 min	240 min
Mobile and extrachromosomal element functions	3.20	1.30	1.81	1.56	2.40	1.14
Protein fate	3.24	2.17	1.36	1.17	1.71	2.74
Protein synthesis	5.39	0.43	0.45	0.39	0.34	6.85
Purines, pyrimidines, nucleosides and nucleotides biosynthesis	1.80	0.43	0.90	0.78	1.03	1.60
Regulatory functions	2.37	4.35	3.62	3.50	5.14	3.20
Transcription	1.71	0.87	0.90	0.78	0.68	1.60
Transport and binding proteins	5.52	9.13	9.05	8.17	6.16	5.94
Unassigned	23.24	28.70	32.58	33.07	32.88	25.57
Unknown function	5.92	6.52	7.69	7.78	6.85	4.57

Table 6.2: TFRCs significantly enriched (FDR q-value of ≤ 0.25), using GSEA analysis, among genes that were expressed at higher levels during meningococcal growth in whole blood.

TFRC	20 min	40 min	60 min
Amino acid biosynthesis	√	√	√
Biosynthesis and biosynthesis of cofactors, prosthetic groups and carriers			√
Purines, pyrimidines, nucleosides, and nucleotides biosynthesis	√	√	√
Protein fate		√	√
Protein synthesis	√	√	
Transcription	√		√

Categories that were found to be significant during blood at 20, 40 and/or 60 min in comparison to gene expression at time-point 0 min are indicated by a tick (√)

6.2.2.1 Surface structures and surface exposed proteins

During incubation in blood, transcriptional profiles of genes encoding components of the cell envelope were found to alter substantially, suggesting that the surface of the organism was undergoing extensive re-modelling. There was a complex sequence of changes, for example, in the expression of genes involved in pilus biogenesis –

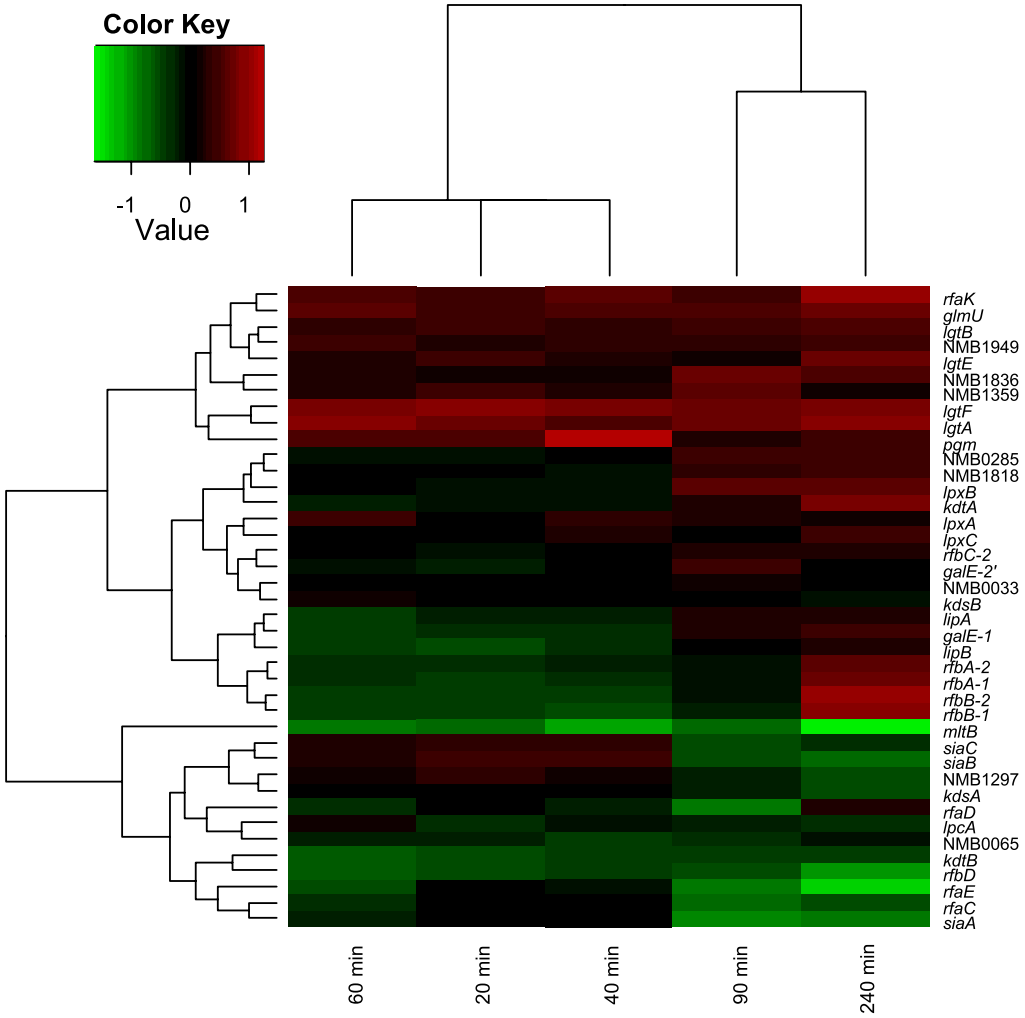


Figure 6.4: Heat map (Section 2.7.8) visualising gene expression of genes associated with LOS and polysaccharide capsule at time-points 20 to 240 min during incubation in blood, relative to expression at the 0 min time-point. Values represent $\log_2$ ratios.

an initial increase in expression, followed in many cases by subsequent waning. An overview of gene expression (in relation to time-point 0 min) of all genes annotated in TIGR subrole category *biosynthesis and degradation of surface polysaccharides and lipopolysaccharides*, is shown in Figure 6.4. Two separate clusters can be seen in the figure, one representing the early time-points (20–60 min) and one representing the later time-points (90 and 240 min). The separation corresponds mainly to increased expression of seven genes (*lipAB*, *galE-1*, *rfbA-1*, *rfbA-2*, *rfbB-1* and *rfbB-2*) and decreased expression of the capsule biosynthesis genes (Figure 6.5) at the later time-points. Surprisingly, none of the genes involved in capsule biosynthesis and surface expression were found to be significantly up-regulated in this experimental system

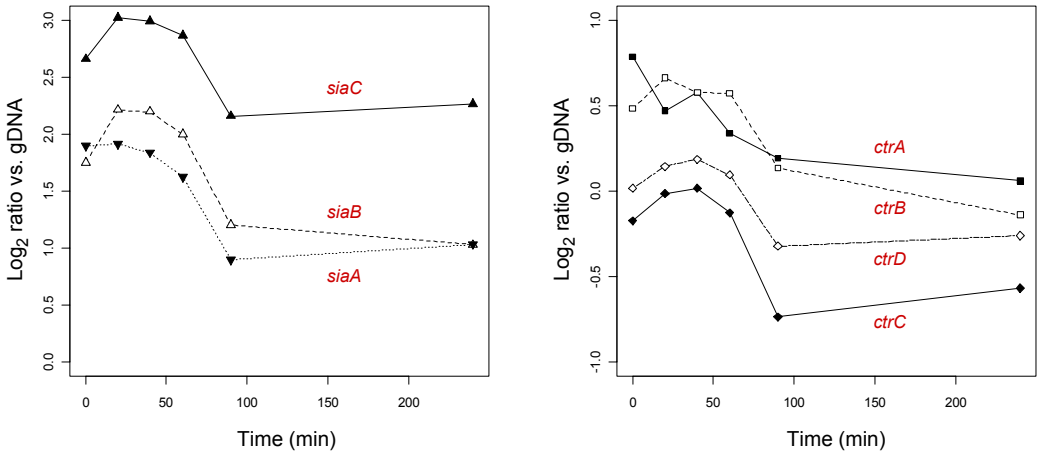


Figure 6.5: Transcriptional profiles over time of genes involved in biosynthesis (left) and export to the surface (right) of the capsular polysaccharide in comparison to the common reference (gDNA).

(Supplementary table C.3), although their expression was slightly elevated at early time-points (20 and 40 min) (Figure 6.5). At all time-points, genes the products of which are required for biosynthesis of the capsular polysaccharide were expressed at high absolute levels, emphasising an important caveat in interpretation: if capsular genes, or any other genes, are already highly expressed at time 0 min, no change may be detected. This does not of course mean that such genes are necessarily expressed at low level in blood.

In bacteria incubated in blood, *lst* (NMB0922), encoding the sialyl-transferase responsible for sialylation of LOS, was found to be up-regulated at 20 to 60 min. Loss of LOS sialyation in meningococci, due to a knock out of *lst*, is associated with a 50% increase in serum sensitivity (Kahler *et al.*, 1998). At this point, it has not been confirmed that up-regulation of *lst* correlates with actual increased sialylation. Several other genes associated with the capsule, LOS or surface structures were found to be significantly up-regulated in response to incubation in blood. These included two genes involved in inner core LOS biosynthesis: *lgtF* (NMB1704), up-regulated at 20 min and *rfaK* (NMB1705), up-regulated at 20, 40 and 90 min. As well as a phosphoglucomutase (encoded by *pgm* (NMB0790)) involved in for example LOS biosynthesis, which was up-regulated at 40 and 60 min. The gene *lipB* (NMB0083) (responsible for proper translocation and surface expression of lipidated capsule poly-

mer) was up-regulated at 60 and 90 min.

Known or putative surface proteins with increased expression in blood–grown organisms Genes found to be up-regulated in blood were sorted by cellular locations, based on experimental confirmations or *in silico* predictions. Predictions of cellular location were performed using a combination of algorithms: PSORTb, PSLpred and bioinformatics tools available at CBS (`http://www.cbs.dtu.dk/services`), as described in Section 2.11. All genes that encode a signal peptide (identifying a protein transported past the inner membrane), or which encode proteins assigned to a periplasmic or outer membrane location, are listed in Supplementary table C.18. Genes encoding proteins that previously have been identified in preparations of OMPs or OMVs, or that have been experimentally confirmed to be located on the surface, are also included in the table. In total 231 such genes were up-regulated during meningococcal growth in blood. Of those, 163 encode proteins that may be located on the surface under some conditions. Genes encoding surface antigens that were found to be up-regulated in blood included: *pilB* (NMB0044), *mapA* (NMB0390), *mntC* (NMB0586), *potD-2* (NMB0623), *purL* (NMB1996), NMB0464, NMB0787, NMB0964, NMB0995, NMB1040, NMB1126/1164, NMB1332, NMB1523, NMB1875, NMB1882, NMB1961 and NMB2132. NMB0464, also known as *pldA* or OMP1A, was found to be up-regulated at 20 to 60 min. NMB0464 encodes a putative outer membrane located phospholipase A that has been assessed for its vaccine potential, however, anti-OMP1A serum has not been found to have protective activity (Bos *et al.*, 2005). NMB0995 encodes a putative macrophage infectivity potentiator (MIP). A gonococcal homologue of the protein has previously been shown to be a surface exposed lipoprotein with peptidyl-prolyl cis-trans isomerase (PPIase) activity that is important for bacterial survival in macrophages (Leuzzi *et al.*, 2005). NMB2132, encoding a transferrin binding protein-related protein, is one of the components of a MenB protein based vaccine currently in clinical trials (Giuliani *et al.*, 2006). NMB1332, up-regulated at 240 min (also up-regulated between 90 and 240 min), encodes a carboxy-terminal peptidase that has been described as a virulence

factor (Tettelin *et al.*, 2000). The gene product of NMB1332 has been identified in protein preparations of both OM (Williams *et al.*, 2007) and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007), which suggests that the protein may be accessible on the surface of the bacteria. Two genes, NMB0497 and NMB1779, both encoding the secreted component (TpsA, hemagglutinin/hemolysin-related protein) of two-partner secretion (TPS) systems in MC58, were found to be up-regulated at 240 min. TpsA is known to be transported to the extracellular space by TpsB (encoded by either NMB1762 or NMB1780). TPS systems have recently been shown to contribute to *N. meningitidis* adherence to epithelial cells (Schmitt *et al.*, 2007) and antibodies reactive to the secreted component have been detected in patient sera (van Ulsen *et al.*, 2008).

6.2.2.2 Meningococcal adaptation to the blood environment

An initial increase in protein synthesis (at 20, 40 and 60 min), as well as major changes in metabolism, was observed upon culture of meningococci in blood. The increase in transcription of genes involved in protein and amino acid synthesis may reflect environmental adaptation. Blood is relatively rich in carbon and energy sources, such as carbohydrates and peptides, but contains very limited free amino acids. The proportion of up-regulated genes that were involved in protein synthesis was reduced at 90 and 240 min in blood (Table 6.1).

During bacterial growth in the bloodstream, glucose is the most readily available energy source, whereas lactate has been suggested to be an important energy source during meningococcal colonisation of the nasopharynx (Exley *et al.*, 2005a). However, similar levels of lactate and glucose can be found in blood and explants of nasopharyngeal tissue (Exley *et al.*, 2005a). The TCA cycle is generally not active during meningococcal growth in medium with glucose (Morse & Hebeler, 1978), but is, however, active when lactate is used for energy. Growth in a combination of lactate and glucose has been shown to render the meningococcus more resistant to serum, most likely due to an increase in LOS sialyation and production of the capsular sialic acid

(Exley *et al.*, 2005b). Besides lactate and glucose, meningococci can utilise pyruvate (Grossowicz, 1945) and maltose (Bartolini *et al.*, 2006) as energy sources. During *in vitro* incubation of human blood, with or without inoculated meningococci, a decrease in levels of glucose and an increase in levels of lactate (L-lactate) were observed between 0 and 240 min (Chapter 3, Figure 3.3), most likely related to host cell metabolism. The *N. meningitidis* gene *lctP* (NMB0543), encoding a lactate permease, was not found to be up-regulated in response to the increase in plasma lactate, but was instead found to be down-regulated at the 20 and 240 min time-points in blood. The gene expression of *lctP* did increase slightly at 40 and 60 min in blood in comparison to 20 min, but the change was not significant ($\leq$1.5-fold, FDR $\geq$0.01). The gene *lldA* (NMB1377), encoding a L-lactate dehydrogenase involved in utilisation of L-lactate, was however found to be up-regulated in blood between 20 and 40 min (Section 6.2.3.1).

During incubation in blood, an initial up-regulation of *mapA*, up to 40 min, was observed. *mapA* encodes a maltose phosphorylase that catalyses the cleavage of maltose to glucose and β-glucose-1-phosphate (further described on page 110). This gene was also up-regulated during meningococcal growth in plasma at 240 min (Sections 5.2.1.4 and 5.2.2.1).

Amino acid biosynthesis and acquisition Amino acid biosynthesis is important for bacterial pathogenesis (described in Section 6.1.2). Five of the eleven genes (*aroC*, *aroG*, *gdhA*, *metF* (NMB0943) and *metH* (NMB0944)) in this category found to be attenuated by Sun *et al.* (2000) in an infant rat model of infection, were up-regulated at least once during the time course in blood.

During growth in blood (at 20 to 60 min) up-regulated genes also included those encoding an L-glutamate transporter, as well as a NADP-linked glutamate dehydrogenase (encoded by *gdhA*) involved in assimilation of nitrogen and glutamate metabolism, which is highly active in conditions of poor glutamate-availability. Glutamate is central for bacterial growth as it is required for assimilation of nitrogen (essential for synthesis of amino acids), involved in defence against osmotic stress

(that may be of importance in the bloodstream) (Dinnbier *et al.*, 1988) and can also serve as a carbon source. Blood is poor in amino acids, but contains ammonia which *N. meningitidis* can also use for assimilation of nitrogen *in vitro* after adaption to a glutamate-free medium (Jyssum, 1959). In meningococci *gdhA* has been shown to be positively regulated by GdhR (described on page 107), an activation that can be blocked by binding of 2-oxoglutarate (a TCA cycle intermediate) to the same site (Pagliarulo *et al.*, 2004). *N. meningitidis* also expresses a NAD-linked glutamate dehydrogenase (encoded by *gluD* (NMB1476)), which functions in catabolism of L-glutamate to 2-oxoglutarate and NH_4^+. Over time *gdhA* and genes encoding the glutamate transporter were down-regulated, indicating that intracellular levels of 2-oxoglutarate increased (which may relate to increasing use of lactate for energy). Increased expression (significantly up-regulated at 240 min vs. 20 min) was instead observed for the gene encoding the NAD-linked glutamate dehydrogenase (active at lower levels of glucose and higher levels of L-glutamate (Pagliarulo *et al.*, 2004)). At 90 and 240 min during growth in blood, a nitrogen assimilation regulatory protein (encoded by *ntrX*) was up-regulated. NtrX is part of the NtrX/NtrY two-component system which regulates nitrogen metabolism in response to nitrogen limitation (Pawlowski *et al.*, 1991). A way for the bacteria to increase intracellular nitrogen, which can then be used in other processes in the cell, may be through catabolism of L-glutamate by NAD-linked glutamate dehydrogenase. It is also possible that L-glutamate may become increasingly available in the extracellular milieu due to conversion from L-glutamine by host cells (a reaction catalyzed by a glutaminase (Kovacevic & McGivan, 1983)).

Degradation of peptides may be one mechanism whereby the organisms acquire amino acids from blood, besides *de novo* biosynthesis. In addition to increased expression of genes associated with amino acid biosynthesis, *prlC* (NMB0214), encoding an oligopeptidase, was up-regulated at 40, 60 and 90 min in blood. At 240 min, a proline iminopeptidase (encoded by *pip* (NMB0927)) was up-regulated, which catalyses removal of the N-terminal proline from peptides.

Genes encoding amino acid transporters were also found to be up-regulated during growth in blood. These included NMB0787–89, an operon encoding a putative ABC transporter of polar amino acids, which was found to be up-regulated in blood at 20 to 90 min. The operon has previously been shown to be up-regulated in response to growth in L-glutamine (Friedrich *et al.*, 2007), the most abundant amino acid in blood (Tsai & Huang, 1999). Additionally, a transporter of L-glutamate, encoded by *gltS* (NMB0085), was up-regulated at 20 min. During incubation in blood the gene *hisJ* (NMB1612), encoding the periplasmic amino acid-binding protein of a putative ABC transporter, was found to be up-regulated after 40 min, but was down-regulated at the 240 min time-point. Upon incubation beyond 90 min in blood, *mtr* (NMB2031), encoding a tryptophan transporter, was found to be up-regulated. A *P. multocida* tryptophan transporter (Mtr) has previously been found to be up-regulated in response to growth in the presence of haemoglobin (Paustian *et al.*, 2002). In relation to time-point 0 min, NMB0177 (at 20 and 60 min) and *putP* (NMB0402) (at 40 min) were found to be down-regulated. They encode alanine and proline symporters, respectively.

Genes involved in sulphur metabolism were found to be up-regulated in response to human plasma (Chapter 5). A similar transcriptional response was also observed for meningococci incubated in human blood. Following 240 min incubation in blood, *sbp* (NMB1017) and *cysW* (NMB0880) were found to be up-regulated. They encode components of a transporter involved in acquisition of sulphate from the extracellular milieu. Incubation in blood also led to consistent up-regulation of *metH* and *metF*, the gene products of which are involved in methionine biosynthesis.

Nucleotide biosynthesis Genes involved in nucleotide biosynthesis have been found to be essential for *in vitro* growth in serum (Samant *et al.*, 2008) and in animal models of disease (Sun *et al.*, 2000). The function `geneSetTest()`, available in the R-package Limma (Smyth, 2004), was used to test if genes involved in *de novo* purine or pyrimidine ribonucleotide biosynthesis were also significantly associated with meningococcal growth in blood. Moderated t-statistics for each gene from the

Limma statistical analysis were used as input data to the function. Genes involved in purine biosynthesis were found to have significantly higher expression at 20, 40, and 60 min in blood compared to expression at time-point 0 min (P-value ≤ 0.01). The enrichment was most significant at early time-points (20 and 40 min) in blood. Likewise, genes involved in pyrimidine biosynthesis were up-regulated in blood compared to growth on solid agar and significantly enriched at time-points 20, 40, 60 and 90 min.

6.2.2.3 Transport systems

In addition to genes encoding amino acid-transporters, incubation in blood induced differential regulation of several other genes encoding transport machinery. Up-regulated genes encoded transporters of cations (*kefB* (NMB0209), NMB1271, *trkA* (NMB1614) and *mntABC* (NMB0588–86)) and anions (NMB0378, *cysW*, *sbp*). *mntC*, encoding the periplasmic substrate binding component of a putative transporter of Mn^{2+} and/or Zn^{2+}, was up-regulated in blood at 40 min. Orthologues of *mntABC* in *N. gonorrhoeae* have previously been shown to encode a transporter that transports Mn^{2+} and Zn^{2+} with equal affinity (Lim *et al.*, 2008). Mutations in *mntC* have been shown to result in meningococcal strains with increased sensitivity to $O_2^{\cdot -}$ killing (Seib *et al.*, 2004). The product of *trkA* up-regulated during growth in blood at 20 to 90 min (also up-regulated in FFP 2 (page 124)), has been suggested to counteract the osmotic changes associated with insertion of MAC into the bacterial OM (Chen *et al.*, 2004).

Genes encoding iron-binding proteins (e.g. *tbpA* (NMB0461), *tbpB* (NMB0460) and *hmbR*) were not up-regulated during incubation in blood in relation to a time-point 0 min. However, some of the genes involved in iron uptake were found to be progressively up-regulated as a response to bacterial growth in blood (Section 6.2.3). Transcriptional profiles in blood of genes encoding systems required for iron uptake (and some putative iron uptake systems) are shown in Figure 6.6. During growth in blood, *fetB2* (NMB1880) and NMB1882, encoding two proteins potentially involved

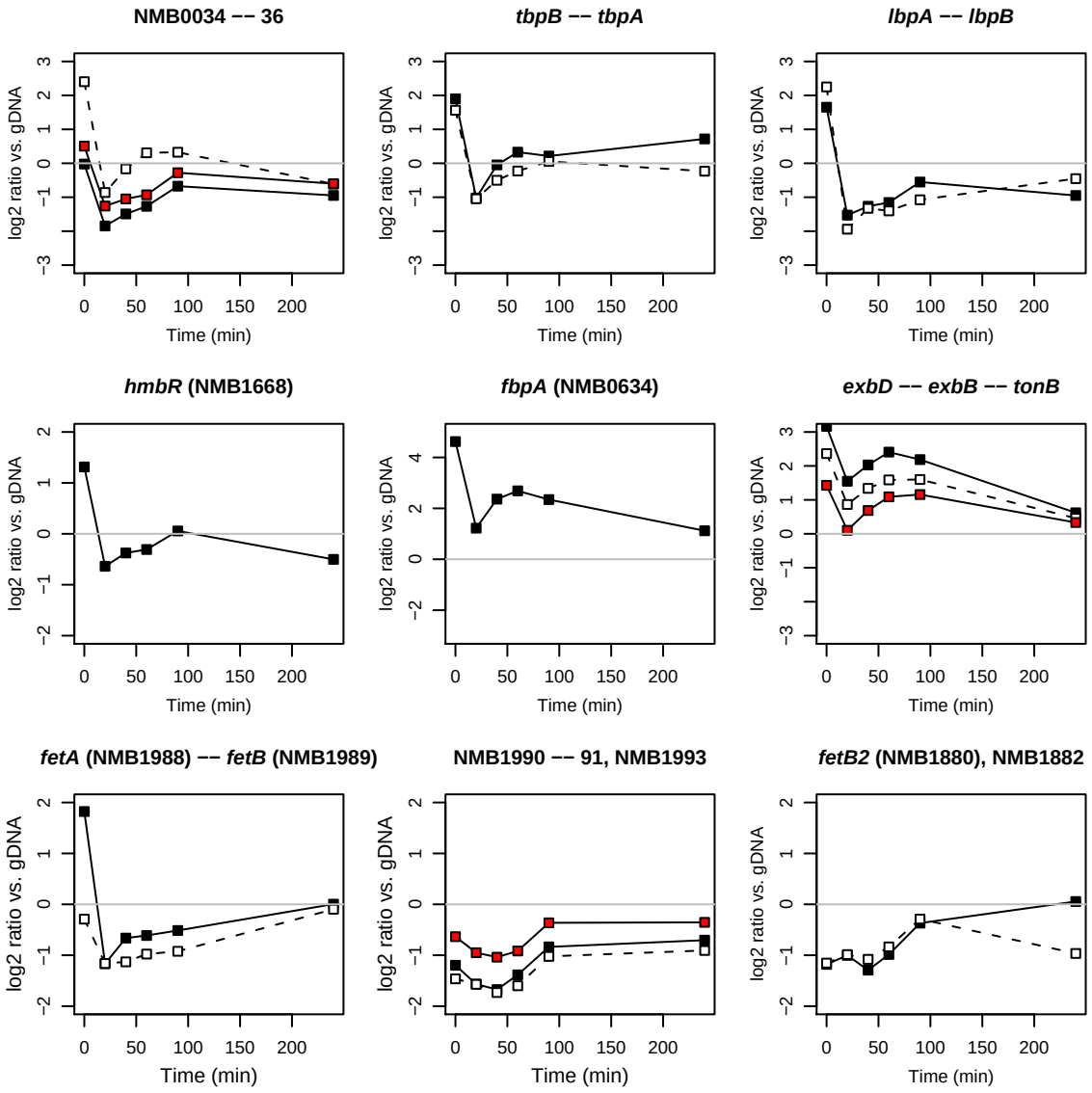


Figure 6.6: Transcriptional profiles of iron uptake genes (and putative iron uptake genes) over time. Y-axis shows $\log_2$ ratios versus gDNA. In each subfigure (in order as listed in title): gene (1) is represented by solid lines and filled squares, gene (2) is represented by dashed lines and empty squares and gene (3) is represented by solid lines and filled red squares.

in iron uptake, were found to be up-regulated. NMB1882, up-regulated at 90 min, encodes a putative ferric siderophore receptor protein and *fetB2*, up-regulated at 240 min, encodes a periplasmic solute-binding protein component of a putative ABC transporter. NMB1882 has been shown to be required for meningococcal intra-cellular survival and transcytosis through a cell layer (Quattroni *et al.*, 2007).

6.2.2.4 Virulence factors

Twenty-five of the genes that were up-regulated at one or several time-points in blood are listed as virulence factors in Tettelin *et al.* (2000) (Table 6.3). Two of these genes, both encoding unrelated opacity proteins (Opa and Opc), will be presented in greater depth. Stephens & McGee (1983) observed that colonies of clinical isolates from blood or CSF generally are transparent, which relates to down-regulation of opacity proteins through phase-variation or other processes. Both *opcA* (NMB1053), encoding a surface exposed opacity protein, and NMB0926 (Opa) were up-regulated at early time-points in blood, but gene expression waned over time. Although NMB0926 is annotated with a frame-shift in the MC58 genome sequence, antibodies to this protein have been detected in sera from patients (Steller *et al.*, 2005), suggesting expression of the protein on the surface of at least some meningococcal strains during invasive infection. Lewis *et al.* (2008) demonstrated that Opa interacts with C3b and C4b on the meningococcal surface, and speculate that it is therefore advantageous for meningococci to down-regulate surface expression of opacity proteins to avoid complement-mediated killing, which could explain down-regulation of NMB0926 at later time-points during growth in blood in this study. OpcA is immunogenic in humans (Rosenqvist *et al.*, 1993), and clinical isolates from the bloodstream have been found to express lower levels of the protein (Achtman *et al.*, 1991).

Table 6.3: Genes that were differentially expressed during growth in blood at 20, 40, 60, 90 and/or 240 min in comparison to time-point 0 min, which are annotated as virulence factors in Tettelin *et al.* (2000). Only genes with an adjusted P-value ≤ 0.01 in the statistical analysis are listed in the table.

ID	Name	Annotation	Microarray data ($\log_2$ ratio)				
			20 min	40 min	60 min	90 min	240 min
NMB0014	*kdtA*	3-deoxy-D-manno-octulosonic-acid transferase					0.851
NMB0018	*pilE*	pilin	0.837	0.714	0.723		
NMB0051	*pilU*	twitching motility protein	0.618	0.603			-0.602
NMB0179	*fabZ*	(3R)-hydroxymyristoyl-(acyl-carrier-protein) dehydratase			0.596		0.614
NMB0199	*lpxB*	lipid-A-disaccharide synthase				0.691	0.657
NMB0216	*katA*	catalase		2.656	2.750	3.301	2.483
NMB0393		multidrug resistance protein				0.816	
NMB0586		putative adhesin		0.649			
NMB0922	*lst*	alpha-2,3-sialyltransferase	1.159	0.809	0.913		0.686
NMB0926		opacity protein (authentic frameshift)		0.673			-0.884
NMB0995		macrophage infectivity potentiator-related protein	1.337	0.958	0.741	0.734	0.729
NMB1053	*opcA*	class 5 outer membrane protein	1.124	1.068			

Continued on next page ...

Table 6.3 – continued from previous page

ID	Name	Annotation	Microarray data (log_2 ratios) 20 min	40 min	60 min	90 min	240 min
NMB1206	*bfrB*	bacterioferritin B	2.482	1.193	0.633		1.001
NMB1207	*bfrA*	bacterioferritin A	2.186	0.969			1.164
NMB1332	*prc*	carboxy-terminal peptidase					1.217
NMB1704	*lgtF*	beta-1,4-glucosyltransferase	0.960	0.844	0.884	0.760	0.806
NMB1705	*rfaK*	alpha-1,2-N-acetylglucosamine transferase		0.654			1.041
NMB1779		hemagglutinin/hemolysin-related protein					1.057
NMB1880	*fetB2*	putative ABC transporter, periplasmic solute-binding protein					1.233
NMB1882		TonB-dependent receptor				0.866	
NMB1926	*lgtE*	lacto-N-neotetraose biosynthesis glycosyl transferase					0.725
NMB1928	*lgtB*	lacto-N-neotetraose biosynthesis glycosyl transferase					0.600
NMB1929	*lgtA*	lacto-N-neotetraose biosynthesis glycosyl transferase	0.726		0.904	0.779	0.974
NMB1961		VacJ-related protein	0.742	0.691	0.721	0.634	
NMB2132		transferrin binding protein-related protein	0.661				

6.2.2.5 Stress responses and cell detoxification

Some of the changes in meningococcal gene expression that were observed over the 240 min time course of incubation in blood may be attributed to MenB stress responses. At the 40 and 60 min time-points, several genes encoding chaperones were found to be up-regulated: *grpE* (NMB0561), *dnaJ* (NMB0059), *dnaK* (NMB0556), *dsbC* (NMB0550), *groEL* (NMB1972) and *groES* (NMB1973). Additionally the expression of *katA* (NMB0216), encoding catalase, increased greater than 8-fold after 40 min incubation in blood, and stayed consistently up-regulated until 240 min. This may reflect the fact that one aspect of host defence to which these organisms are exposed in this whole blood experimental system is the oxygen free radical burst derived from specialised phagocytic cells (polymorphonuclear leukocytes; PMNs) (Klebanoff *et al.*, 1983). Several other genes, the products of which have previously been reported to protect against oxidative stress, were also found to be up-regulated. One of these, *pilB*, encodes a protein with methionine sulfoxide reductase and disulphide reductase activity. The PilB protein is found both in a secreted form (with three domains) associated with the outer membrane and a cytoplasmic form (with two domains). The central and C-terminal domains of the protein have previously been shown to have methionine sulfoxide reductase (Mrs) A and MrsB activities, respectively, which

mediate the repair of proteins damaged by sulfoxidation of methionine residues. The N-terminal domain, specifically expressed in the secreted form, has been shown to display a disulphide reductase activity that can recycle Msr activities (Wu *et al.*, 2005). The operon NMB1436–38, the gene products of which have been suggested to be involved in protection against oxidative stress, was found to be up-regulated at all time-points between 20 and 90 min. Grifantini *et al.* (2004) demonstrated that knocking out the operon resulted in increased sensitivity to killing by PMNs and impaired growth in the bloodstream of mice compared to wild-type (Grifantini *et al.*, 2004).

Polyamines are involved in many processes in the cell, including protection against oxidative damage and responses to nitric oxide-mediated immune functions (Shah & Swiatlo, 2008). The *S. pneumoniae potD* gene, encoding the substrate-binding protein, has previously been found to be highly expressed in a murine model of bacteraemia (Shah *et al.*, 2008). During meningococcal growth in blood, four genes - *potB* (NMB0611), *potC* (NMB0612), *potD-2* and *potD-3* (NMB1594), encoding proteins involved in transport of polyamines - were found to be up-regulated at 20 to 60 min.

Protection against host antibacterial factors is, in part, mediated through efflux proteins. During meningococcal growth in blood, *farA* (NMB0318), encoding a component of an efflux protein (FarAB) mediating resistance to long-chained fatty acids, was found to be up-regulated after 90 and 240 min. The FarAB complex is located in the bacterial inner membrane and requires the outer membrane protein MtrE, also used by the *mtrCDE*-encoded efflux pump (NMB1714–16), for export of fatty acids from the cell (Lee & Shafer, 1999). An additional two genes encoding multidrug resistance proteins were found to be up-regulated during growth in blood: NMB0393, up-regulated at 90 min and *norM* (NMB0812), up-regulated at 240 min. The NorM efflux protein has been shown to be involved in protection against toxic effects of cationic compounds during *in vitro* incubation (Rouquette-Loughlin *et al.*, 2003). The multidrug efflux protein NMB0393 was also found to be up-regulated during growth in FFP 2 (described on page 124).

Another group of genes found to be up-regulated in blood-grown organisms were those belonging to the category of *DNA metabolism*. Up-regulation of these genes was most prominent at the 240 min time-point (6.6%). Genes up-regulated at 240 min included *mutY* (NMB1396), encoding an A/G-specific adenine glycosylase, and *mutS* (NMB2160), encoding a DNA mismatch repair protein. *N. meningitidis mutS* mutants have been shown to undergo an increased rate of phase variation (Alexander *et al.*, 2004). The gene *xseB* (NMB0262), encoding the small subunit of an exodeoxyribonuclease, was found to be significantly up-regulated at 60 and 240 min. Expression of *xseB* was also found to be increased at 20, 40 and 90 min, but, at these time-points the up-regulation was not significant in the statistical test. *xseB* has previously been demonstrated to be up-regulated when meningococci attach to epithelial cells (Grifantini *et al.*, 2002b; Morelle *et al.*, 2003). Morelle *et al.* (2005) have demonstrated in a later study that increased expression of *xseB* (as a result of either host cell contact or IPTG-induced over expression) is associated with increased phase-variation, which may be advantageous during invasive disease to avoid recognition by the host's immune system. At the 240 min time-point, many of the up-regulated genes - for example, NMB0805, NMB1050 and NMB1251 - encode proteins with transposase function. Transposase genes NMB1050 and NMB1251 have previously been found to be up-regulated during *N. meningitidis* attachment to epithelial cells (Grifantini *et al.*, 2002b).

6.2.2.6 Transcriptional regulation

Thirty-two genes annotated as regulators were differentially expressed during incubation in blood (Supplementary table C.14), twelve of which were up-regulated at least once during the time course in blood. Up-regulated genes included NMB0737 (at 20, 60, 240 min), encoding a putative HPr kinase phosphatase, and NMB1816 (at 60, 90 min), annotated as an NrdR family transcriptional regulator. HPr kinase phosphatases have previously been shown to regulate carbohydrate metabolism through the PTS system in Gram positive bacteria (Warner & Lolkema, 2003). NMB1816 is homologous to a group of transcriptional regulators, including the *E. coli* regulator

YbaD that consists of a Zn-ribbon and ATP-cone domain. This group of regulators has been shown to act as repressors of Nrd operons, encoding ribonucleotide reductases (Grinberg *et al.*, 2006; Torrents *et al.*, 2007).

Six genes (NMB0398, NMB0573, NMB1007, NMB1249–50 and NMB1303) encoding putative regulators were down-regulated at four or more of the time-points in blood, in comparison to expression at time-point 0 min. An orthologue of NMB0398, annotated as a ArsR family regulator, has been demonstrated to act as a repressor of *norB* in *N. gonorrhoeae*. This repression can be reversed by binding of the regulator Fur to the same site (Isabella *et al.*, 2008). During incubation in blood, *norB* (NMB1622) was down-regulated at 20, 40 and 60 min, but showed increased gene expression between 60 and 90 min in blood. NMB0398 has previously been demonstrated to be induced by exposure to H_2O_2 (Grifantini *et al.*, 2004). NMB0573 encodes a protein predicted to belong to the AsnC family of regulators (Lrp homologue). NMB0573 has previously been shown to respond to growth under low nutrient conditions, especially under limited amino acid availability. The octameric assembly of the protein, essential for DNA binding, has been suggested to be stabilised by L-methionine and L-leucine, suggesting that it may respond to limitation of these amino acids (Ren *et al.*, 2007). In *E. coli*, transcription of *asnC*, encoding a similar regulator, responds to levels of asparagine and the nitrogen assimilation control protein (Nac) (Poggio *et al.*, 2002). NMB1249–NMB1250, genes that encode the components of one of only four of the complete two-component systems in the MC58 genome, were down-regulated at all time-points (20 to 240 min) during incubation in blood. NMB1249 encodes a putative nitrate/nitrite sensory protein (NarX) and NMB1250 encodes as a LuxR family regulator. The equivalent two-component system in gonococci has previously been shown to regulate the transition from aerobic to anaerobic growth (Lissenden *et al.*, 2000). NMB1303, annotated as a MerR family regulator and 99% identical at the amino acid level to the gonococcal gene *nmlR* (NGO0602), was also found to be down-regulated at all time-points. NmlR has been shown to act as a redox-responsive Zn^{2+}-dependent repressor, regulating the expression of itself

and three other genes: *adhC*, encoding a class III alcohol dehydrogenase; *copA*, encoding a Cu^{2+}-exporting ATPase; and *trxB*, encoding a thioredoxin reductase, which in strain MC58 are the annotations of genes NMB1304, NMB1325, NMB1324 respectively (Kidd *et al.*, 2005). During incubation in blood, NMB1304 was found to be down-regulated at all time-points from 20 to 240 min, but differential regulation of NMB1324 and NMB1325 could not be determined, due to poor quality of data for these two genes recovered from all microarrays.

6.2.2.7 Putative or unassigned function

The largest proportion of genes differentially expressed in response to incubation in blood fall into the categories *conserved hypothetical* or *unassigned proteins*, a reminder that many genes associated with meningococcal biology and virulence are still poorly characterised. Two of these genes, which either have significant sequence similarity to proteins with known function or have previously been shown to be differentially expressed in conditions mimicking meningococcal *in vivo* growth, are briefly discussed here. NMB0840 of unknown function, has previously been shown to be required for bacterial growth in an infant rat model of sepsis (Sun *et al.*, 2000), and was up-regulated at 20 to 60 min during culture in blood. NMB1475 was found to be highly up-regulated in blood at all time-points after 20 min. Its product has sequence similarity (44% identity) to universal stress response proteins in other prokaryotes and also similarity to ABC transporters of Co^{2+} (44% identity). Transcription of the gene has previously been shown to be altered during prolonged incubation in NHS (Dove *et al.*, 2003).

6.2.3 Meningococcal genes progressively up-regulated on incubation in blood

Since a common reference design was used in the microarray study, gene expression differences between time-points in blood could also be determined. Two different comparisons were performed. Firstly, the gene expression changes between consecutive time-points in blood were determined, to find time-responsive genes (Supplementary

tables C.19 – C.35). Secondly, the gene expression at 40, 60, 90 and 240 min post-infection was compared to transcription at 20 min in blood, to account for initial adjustments to the new environment (Supplementary tables C.37 – C.53). Genes that were found to be differentially expressed (between time-points or relative to t = 20 min) will be presented in the following sections. Up-regulated meningococcal genes encoding known or putative surface exposed proteins will first be described, as identification of such proteins were the primary aim of the study presented here.

6.2.3.1 Transcriptional changes between time-points in blood

Comparison of the gene expression at adjacent time-points during incubation in blood showed that major shifts in gene expression occurred between most time-points during the time course, although, very few genes changed significantly between 40 and 60 min. An overview of the proportion of the up-regulated genes annotated in each TFRCs is shown in Figure 6.7.

Surface exposed proteins As in Section 6.2.2.1 all up-regulated genes were divided into cellular locations (Supplementary table C.36). Of the up-regulated genes, 74 encode proteins that are either known or predicted to be located in the outer membrane or for which experimental data suggest a possible surface exposed location. Genes up-regulated between 20 and 40 min, encoding putative surface exposed proteins, included: NMB0035, encoding a lipoprotein (Arenas *et al.*, 2006); NMB0345, previously found to be essential in an animal model of bacteraemia (Sun *et al.*, 2000); NMB0946, encoding a peroxiredoxin 2 family protein/glutaredoxin previously found to contribute to plasminogen binding to the bacterial surface (Knaust *et al.*, 2007); and NMB0964, encoding a putative TonB-dependent receptor (described on page 172). NMB0345 is most likely co-transcribed with NMB0342–47, of which NMB0346 was also found to be significantly up-regulated at 40 min (as well as at 60 min in comparison to 20 min; Section 6.2.3.2). In acute phase sera collected from patients with meningococcal disease, reactive antibodies were found to, for example, NMB0345 and *mntC* (Litt *et al.*, 2004), suggesting that these proteins are expressed on the bacterial

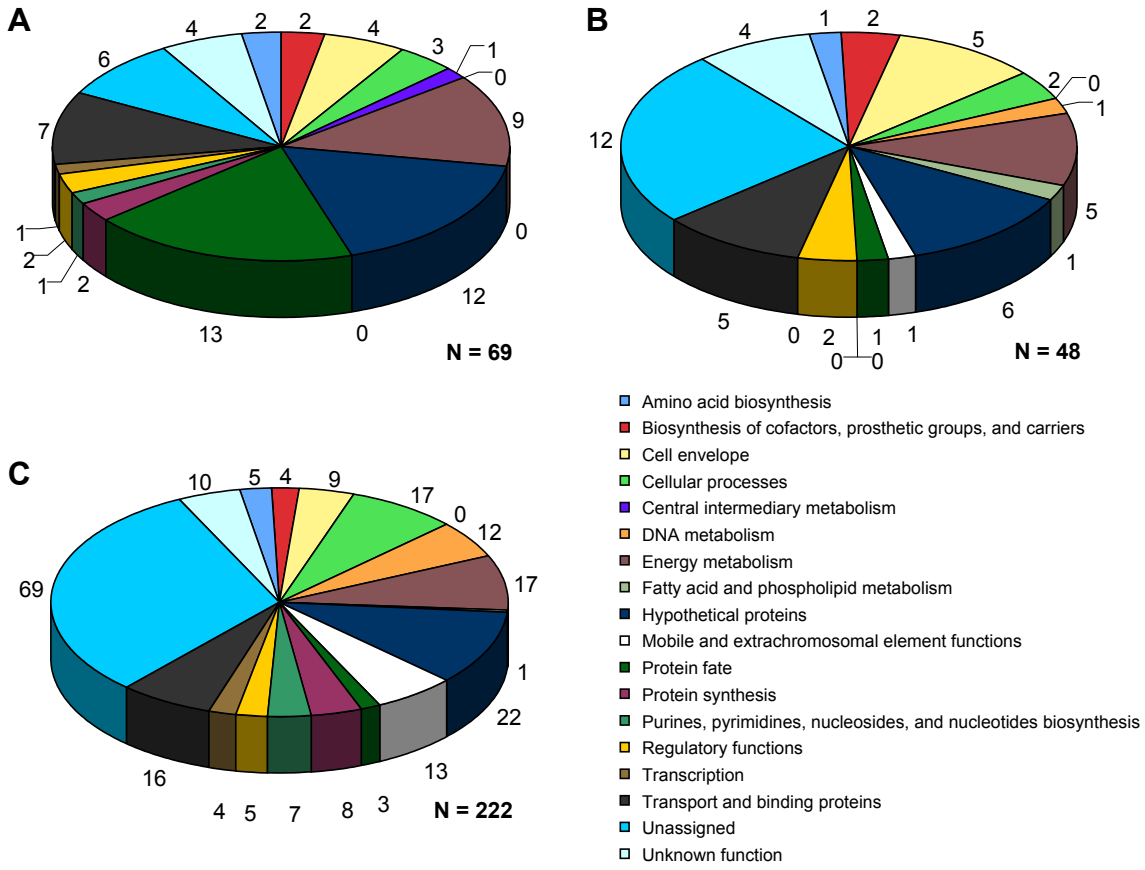


Figure 6.7: TFRCs represented among genes up-regulated between time-points in blood. **A.** 20 → 40 min; **B.** 60 → 90 min; and **C.** 90 → 240 min. Numbers in the figure reflect the number of genes in each category. Data for 60 min in relation to 40 min are not included as no genes were up-regulated in this comparison. Some of the genes are annotated in more than one TFRC.

surface during bacterial growth in the host. Proteins involved in iron-transport such as the surface exposed immunogenic proteins TbpA and TbpB were also found to be up-regulated between 20 and 40 min.

Genes up-regulated at later time-points, encoding putative surface exposed proteins, included *gcp* (NMB1802) and secreted components (TpsA) of the TPS system. A possibly secreted O-sialoglycoprotein endopeptidase, encoded by *gcp*, was found to be up-regulated between 60 and 90 min. A similar protein (50% sequence identity) in *Mannheimia haemolytica* serotype A is secreted and forms the basis for an experimental cattle vaccine against infection with *M. haemolytica* A1 (Pandher & Murphy, 1996; Shewen *et al.*, 2003). The *M. haemolytica* protein can interact with leukocyte surface antigens CD34, CD43, CD44 and CD45 (Abdullah *et al.*, 1992). However, this protein may not be secreted in other Gram-negative bacteria (Watt *et al.*, 1997). Two *tpsA*-

type genes (NMB0497 and NMB1779) were significantly up-regulated between 60 and 90 min. Incubation of bacteria for 240 min resulted in further increase in the level of expression of genes encoding both the transporter (TpsB) and the secreted part (TpsA) of a TPS system. Up-regulated genes included the *tpsA*-type genes NMB0493, NMB497/NMB1779 and NMB1214 and the *tpsB*-type genes NMB0496 (truncated) and NMB1762. The different meningococcal TPS systems have recently been shown to contribute to bacterial adherence to epithelial cells mediated through the secreted TpsA, suggesting that the protein may remain surface associated (Schmitt *et al.*, 2007). van Ulsen *et al.* (2008) suggested that TPS system II (*tpsA* genes NMB0493, NMB1768; *tpsB* gene NMB1762) and III (*tpsA* gene NMB1214) are associated with invasive strains, whereas TPS system I (*tpsA* genes NMB0497/NMB1779; *tpsB* genes NMB0496 and NMB1780) is ubiquitous. They found reactive antibodies to TPS domains in blood from four out of fourteen meningococcal patients (NMB1768 (1), NMB0493 (2) and NMB1214 (1)), indicating secretion of the proteins *in vivo*.

The largest group of genes (N = 13) up-regulated between 20 and 40 min were those annotated in the TFRC *protein fate* (Figure 6.7 (A)). MenB gene expression changes resulting from incubation in blood between 20 and 40 min were analysed using the GSEA software. In this analysis, genes in the following five TFRCs were significantly enriched among genes with increased expression at 40 min: *protein fate*; *amino acid biosynthesis*; *purines, pyrimidines, nucleosides and nucleotides biosynthesis*; *cellular processes*; and *regulatory functions*. Two of the most highly up-regulated (> 3.5-fold) genes between 20 and 40 min were the catalase gene (*katA*) and NMB1475, which may have a role in general stress responses. Incubation in blood between 20 and 40 min also resulted in increased expression of the genes *lldA*, encoding an L-lactate dehydrogenase, and *ppsA* (NMB0618), encoding a phosphoenol pyruvate synthase, both involved in pyruvate metabolism. L-lactate dehydrogenase catalyses conversion of L-lactate to pyruvate, which may be further converted by phosphoenol pyruvate synthase to phosphoenol pyruvate, utilised in the biosynthesis of sialic acid. Increased expression of these genes may reflect the increased levels of lactate observed over

time during *in vitro* incubation in blood (Chapter 3, Figure 3.3). Genes encoding proteins involved in iron uptake, such as *tonB*, *fbpA* (NMB0634) and *tbpB*, were among those up-regulated between 20 and 40 min in blood. *N. meningitidis* growth in blood may be associated with an increased need for zinc, as four of the up-regulated genes between 20 and 40 min are associated with either zinc-deficient conditions or zinc uptake. Expression of *rpmJ2* (NMB0941) and *rpmE2* (NMB0942), encoding alternative ribosomal proteins, was elevated between 20 and 40 min. Transcription of *rpmE2* and *rpmJ2* has previously been shown to increase under conditions of zinc-deficiency in other bacteria (Nanamiya *et al.*, 2004; Owen *et al.*, 2007; Shin *et al.*, 2007). NMB0964, encoding a TonB-dependent receptor, has been shown to be up-regulated under conditions of low zinc-availability (Stork *et al.*, 2008), and was found to be up-regulated here. *mntC*, encoding a putative Zn^{2+}/Mn^{2+} transporter, was also found to be up-regulated.

Only three genes were found to be differentially expressed between 40 and 60 min in blood, all down-regulated. Significant down-regulation was observed for the genes *pgm*, encoding phosphoglucomutase involved in *biosynthesis and degradation of surface polysaccharides and lipopolysaccharides*; *xseA* (NMB1363), encoding the large subunit of exodeoxyribonuclease involved in degradation of DNA; and NMB1564, encoding a protein with a putative redox function. Sequence alignment indicated similarity between NMB1564 and proteins with an osmotically inducible protein C (OsmC) domain. Other proteins with this domain have been shown to respond to a variety of stresses, such as osmotic stress (Gutierrez & Devedjian, 1991) and oxidative stress (Lesniak *et al.*, 2003). In meningococci, NMB1564 has previously been shown to be up-regulated in response to a temperature shift (37°C to 45°C) (Guckenberger *et al.*, 2002), as well as being found to be essential for bacteraemia in an infant rat model (Sun *et al.*, 2000). These studies indicate that the NMB1564 gene product may be involved in stress responses, although its function is not yet known. The limited number of significantly differentially regulated genes between 40 and 60 min suggest that bacteria at these two time-points in the time course were in a stable phase

of growth (after initial adaptations to the blood environment) in which only minor changes occurred in the transcription levels of genes. Although there are few significantly differentially regulated genes between 40 and 60 min the overall transcriptome is unlikely to be identical.

In contrast to the limited gene expression changes occurring between 40 and 60 min, more substantial changes were seen between 60 to 90 min, with up-regulation of 46 genes and down-regulation of 43 genes. The five most common TFRCs among genes up-regulated between 60 and 90 min were: *unassigned*; *hypothetical proteins*; *transport and binding proteins*; *cell envelope*; and *energy metabolism* (Figure 6.7 (B)). Several of the up-regulated genes encode proteins annotated in the category *biosynthesis and degradation of surface polysaccharides and lipo-polysaccharides*, including *lipA*, the gene product of which is required for proper translocation of the capsular biopolymer to the bacterial surface (Tzeng *et al.*, 2005b). This gene was previously found to be up-regulated in response to meningococcal intracellular growth (Spinosa *et al.*, 2007). Genes encoding the proteins involved in biosynthesis of the capsular polysaccharide were found to be down-regulated, although still expressed at high absolute levels (Figure 6.5) (discussed on page 155).

At 90 min, two genes encoding regulatory proteins were up-regulated in comparison to their respective gene expression at 60 min. These were *ntrX* (NMB0115), encoding a nitrogen assimilation regulatory protein, and NMB1792, encoding a putative sensor histidine kinase. In *Azorhizobium caulinodans*, the NtrX/NtrY two-component system regulates nitrogen metabolism in response to nitrogen limitation (Pawlowski *et al.*, 1991), so up-regulation of meningococcal NtrX may indicate an increased need for nitrogen at later time-points in blood. Transcription of *ntrY* was also increased, but not significantly ($\log_2$ ratio = 0.552; adjusted P-value = 0.0183). Sun *et al.* (2000) showed that this transcriptional regulator is essential for sustained bacteraemia in an infant rat model.

Corresponding to the observed induction of zinc-regulated meningococcal genes between 20 and 40 min in blood, the putative ZupT low-affinity transporter (NMB0175),

transporting zinc from the periplasm to the cytoplasm, was up-regulated at 90 versus 60 min. In *E. coli*, a similar protein has been shown to also transport other metal cations, such as Fe^{2+} and Co^{2+} (Grass *et al.*, 2005).

Two genes associated with cell detoxification, *norM* and *farB* (NMB0319), were found to be up-regulated in response to incubation in blood. Up-regulation of these genes may be a response to anti-bacterial factors secreted by the human cells in blood. Time-responsive genes also included some encoding secreted factors as well as secretion systems, such as *hlyB* (NMB1400) and *hlyD* (NMB1738), the gene products of which have been shown by Wooldridge *et al.* (2005) to form a secretion system that translocates cytolysins. Change in transcription of *tolC* (NMB1737), encoding the third component of the system, did not reach significance in the statistical test (adjusted P-value = 0.063) but expression was at a similar level to *hlyB* and *hlyD* (1.8-fold change). The *hlyD* gene was also found to be progressively up-regulated during growth in plasma (FFP 1 and FFP 2).

The five most common TFRCs among genes up-regulated between 90 and 240 min were: *unassigned*; *hypothetical proteins*; *energy metabolism*; *cellular processes*; and *transport and binding proteins* (Figure 6.7 (C)). Up-regulation of genes involved in cellular processes primarily reflects an increased expression of genes encoding the NADH dehydrogenase I (*nuo* genes). Up-regulated genes annotated in the TFRC *cellular processes* also included *sodB* (NMB0884), encoding a [Fe,Mn]-cofactored superoxide dismutase involved in cell detoxification. Significant up-regulation was also observed for *clpP* (NMB1312), encoding a protease involved in protein degradation.

Some of the changes in gene expression between the 90 and 240 min time-points may reflect differences in either the growth phase of the bacteria or the availability of nutrients at the later time-point. For example genes involved in glycolysis/glucogenesis and peptidoglycan synthesis were found to be down-regulated at 240 min (compared to 90 min), which may suggest a slowing in bacterial growth, although this was not reflected in CFUs at 240 min. Down-regulation of genes in these two functional categories was also observed for GAS incubated in blood (Graham *et al.*, 2005).

A significant down-regulation was also observed for genes associated with synthesis of pyruvate family amino acids. A regulatory protein encoded by NMB0573 (described on page 167), that may respond to amino acid availability, was also up-regulated.

6.2.3.2 Comparison of gene expression at later time-points in blood to the 20 min time-point

Meningococcal gene expression at 40, 60, 90 and 240 min was compared to the gene expression at the earliest time-point in blood, at 20 min (Supplementary tables C.37 – C.53). As expected, the greatest changes occurred between 20 min and the last time-points (90 and 240 min) in the time course. Up-regulation in relation to 20 min was investigated in more detail by determining the proportion of genes belonging to each TFRCs (Figure 6.8).

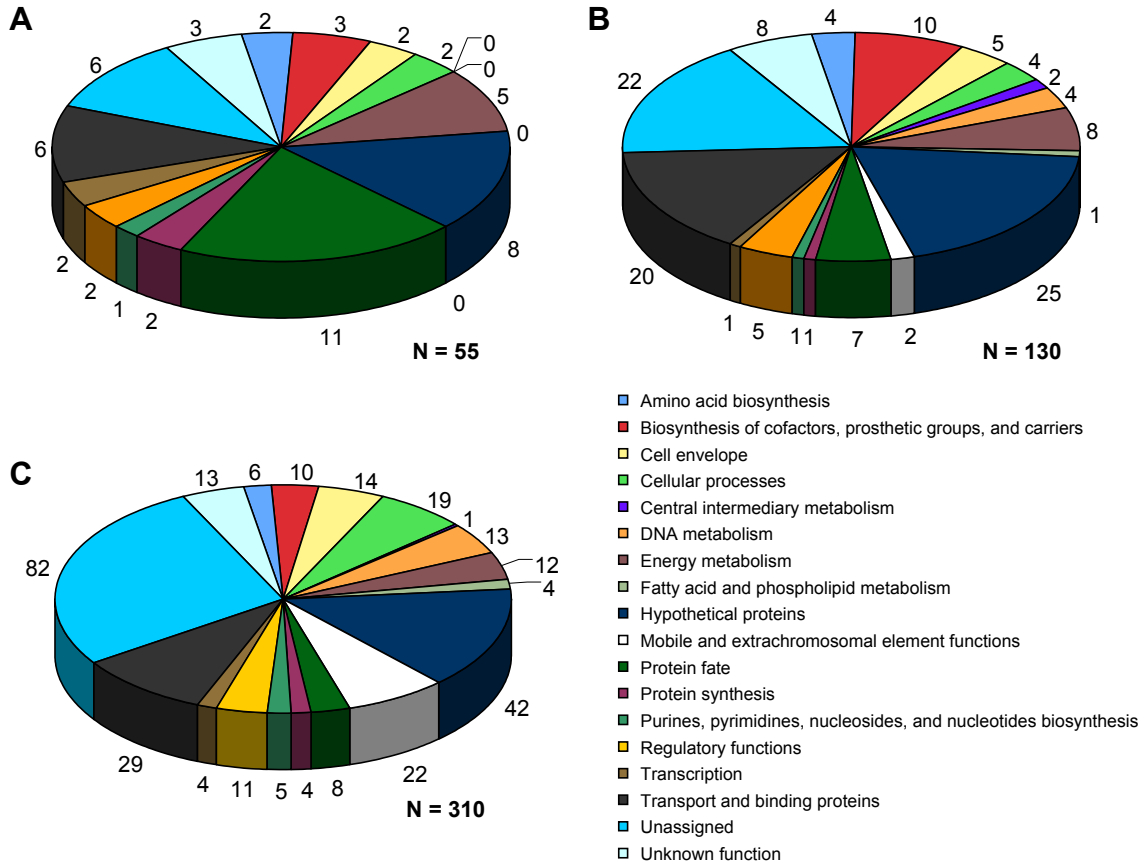


Figure 6.8: TFRCs represented among genes up-regulated at 60, 90 and 240 min relative to 20 min. **A** 60 vs. 20 min, **B** 90 vs. 20 min and **C** 240 vs. 20 min. Numbers in the figure reflect the number of genes in each category. Observe that some genes may be annotated in more than one TFRC.

The majority of the up-regulated genes were annotated in TFRCs *unassigned*

or *hypothetical proteins*. TRFC *transport and binding proteins* were also common among genes up-regulated at all time-points. Changes relative to 20 min, observed at all time-points, included up-regulation of genes involved in iron uptake, such as *tbpA*, *hmbR* and NMB0034. Concordantly, NMB1378, encoding a putative DNA binding protein that has been shown to be regulated by iron depletion as well as other environmental cues such as hydrogen peroxide and contact with cells (Basler *et al.*, 2006), was also found to be up-regulated at 40, 60, 90 and 240 min. A larger proportion of genes with elevated expression was associated with *protein fate* and *amino acid biosynthesis* at 60 min than at 20 min (significantly enriched in GSEA analysis). At the 90 min time-point, a large proportion of the down-regulated genes was associated with *protein synthesis*, indicated by enrichment of this TFRC among genes up-regulated at 20 min when compared to the 90 min time-point.

Surface exposed proteins and structures As in Section 6.2.2.1, all up-regulated genes were divided into cellular locations (Supplementary table C.54). Seventy-five genes (excluding data for 40 vs. 20 min) encoding known or potential surface exposed proteins (identified by *in silico* predictions or by experimental data), were up-regulated during the extended time course in blood. These included NMB0035 (up-regulated at 60–240 min), NMB0345–46 (up-regulated at 60 min) and NMB0964 (up-regulated at 60 and 90 min). These were also up-regulated between 20 and 40 min during growth in blood and have already been described in Section 6.2.3.2.

Other genes up-regulated at later time-points in blood included NMB1882, up-regulated at 90 min (described on page 162) and NMB0655, up-regulated at 240 min. NMB0655, annotated as a hypothetical protein, has been found to be up-regulated in contact with epithelial cells and identified on the bacterial surface by flow cytometry (Grifantini *et al.*, 2002b). Genes encoding the secreted part (TpsA) and the transporter part (TpsB) of a two component system (TPS) were also up-regulated at 90 and 240 min during incubation in blood. The functions of products of these genes have already been discussed in Section 6.2.3.1 (page 171). The secreted part may, in some cases, remain bound to the bacterial surface, where it can act as

a surface receptor (Schmitt *et al.*, 2007). At the two last time-points in the time course, the genes of operon NMB2003–2004 with sequence similarity to the *lgrAB* operon, encoding membrane proteins involved in control of murein hydrolase activity and penicillin tolerance (Groicher *et al.*, 2000), were also up-regulated.

However, many genes encoding various meningococcal surface structures and OMPs were found to be down-regulated at these two time-points. These included genes encoding the type IV pilus (e.g. *pilE* and *pilQ*), as well as genes encoding OMPs such as *opcA* and *rmpM* (NMB0382). Down-regulation of *opcA*, encoding the class 5 outer membrane protein, could reflect a response to human complement (Section 6.2.2.4). Expression of capsule polysaccharide biosynthesis genes was also down-regulated at later time-points during incubation in blood (Figure 6.5). In contrast, genes involved in LOS biosynthesis (e.g. *galE*, *kdtA*), as well as *lipA* and *lipB* (required for translocation of the capsular polysaccharide), were up-regulated at 90 and 240 min.

Energy metabolism Some of the changes in gene expression after 20 min may relate to the increase observed in blood lactate. The L-lactate dehydrogenase gene (*lldA*) was found to be up-regulated at all time-points after 20 min. As at 40 min (Section 6.2.3.1), the phosphoenol pyruvate synthase gene, *ppsA*, was also up-regulated at 60 min. The gene products of *lldA* and *ppsA* are involved in the conversion of L-lactate to phosphoenolpyruvate (L-lactate $\xrightarrow{LldA}$ pyruvate $\xrightarrow{PpsA}$ phosphoenolpyruvate). Unlike the response to growth in plasma (Section 5.2.3), genes encoding proteins involved in the TCA cycle were down-regulated over time (at 90 and 240 min) during incubation in blood, rather than up-regulated.

Cell detoxification Besides the up-regulation of *katA* at all time-points beyond 20 min, *arsC* (NMB0005) an oxidoreductase (annotated as an arsenate reductase from sequence similarity) was found to be up-regulated at 240 min. *sodC* (NMB1398), encoding a periplasmic [Cu,Zn]-cofactored superoxide dismutase involved in extracytoplasmic protection to oxidative stress, was found to be down-regulated at time-points 90 and 240 min. However, the gene encoding the cytoplasmic [Fe,Mn]-cofactored superoxide dismutase SodB, was up-regulated between 90 and 240 min.

Genes encoding various multidrug efflux proteins were induced at the later time-points (90 and 240 min). These included *farAB*, *norM* and the *mtrCDE*-encoded efflux pump. *N. gonorroheae* FarAB, the fatty acid receptor, similar to FarAB in MenB, mediates protection against bactericidal long chain fatty acids (Lee & Shafer, 1999). Responses at the last time-point also included up-regulation of DNA metabolism genes such as *mutT* (NMB0453) and *mutY*.

6.2.4 MC58 gene expression in blood analysed by comparative real-time RT-PCR

Comparative real-time RT-PCR was used to validate a subset of the microarray data obtained using donor A's blood (Section 6.2.1), and was used to assess the expression of a set of meningococcal genes upon incubation in blood from donor B (Section 6.2.4.1). This can provide some information of inter-individual variation. All genes explored in this analysis were selected because they encode proteins located in the periplasm or on the surface of the meningococci and may have vaccine potential (discussed in Section 6.3.2). Comparative real-time RT-PCR experiments were conducted using the TaqMan platform as described in Section 2.8.2. For each gene-specific primer and each sample, the TaqMan reaction was performed both with cDNA (reverse transcribed from total RNA) and with total RNA, used to subtract any signal attributable to contaminating DNA. The data were analysed using the comparative C_T (threshold cycle) method, in which the amount of target normalised to a control (16s rRNA) and relative to a 'calibrator' is given by $2^{-\triangle\triangle C_T}$ (Described in Section 2.8.2) (Applied Biosystems, 1997). Standard deviations were calculated as in Bookout & Mangelsdorf (2003). As for microarray data, the gene expression at time-points 40, 60, 90 and 240 min in blood were compared to gene expression at both 0 min and 20 min. Data from TaqMan analysis and microarray analysis for the subset of genes are listed in Table 6.4 (comparison to 0 min) and Table 6.5 (comparison to 20 min). Log_2 ratio data from microarray experiments are included for all time-points, but as before only data points with an adjusted P-value ≤ 0.01 and a fold change of ≥ 1.5 are considered to be significantly differentially expressed. The gene expression of nine of the genes, in

comparison to 0 min, is shown in Figure 6.9.

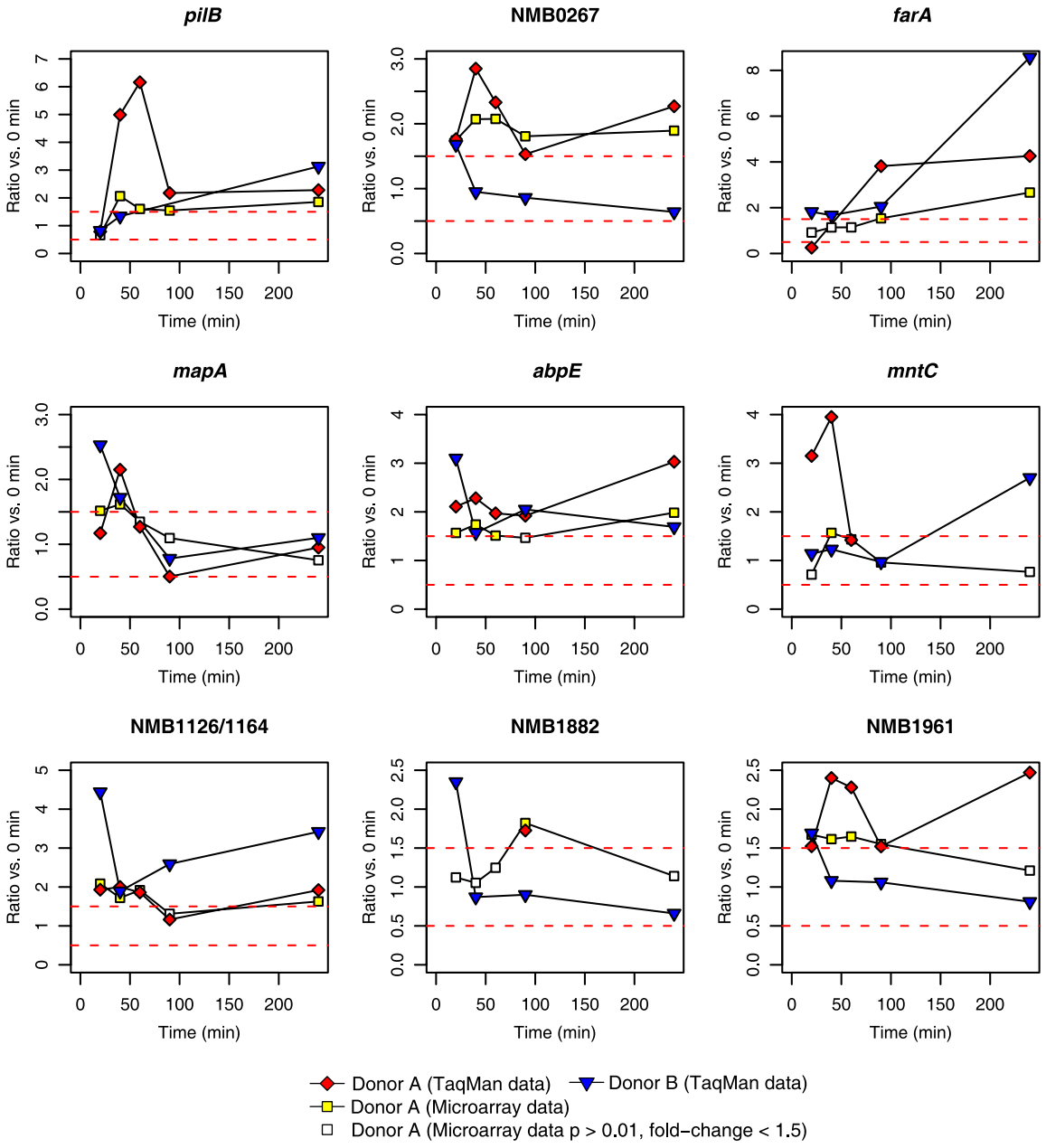


Figure 6.9: Gene expression of nine MenB genes in blood in comparison to 0 min, as measured by TaqMan and microarray technology. For TaqMan analysis each point represents the average of three biological replicates, with each assay performed four times (technical replication). Data from donor B for time-point 90 min are only based on an experiment on one occasion and are only included for illustrative purposes. Data from microarray analysis that were not found to be significant in the statistical analysis, using Limma, are represented by open symbols. Symbols falling above or below the red dotted lines (at 0.5 and 1.5) have a 1.5-fold change in expression.

6.2.4.1 Transcriptional profile in blood from donor B

RNA was isolated from meningococcal strain MC58 after incubation in blood collected from a second donor (B, male). The growth of MC58 in blood from this individual is shown in Figure 6.1. Although MC58 showed a similar pattern of unrestricted growth in blood from donors A and B, less RNA could be recovered from meningococci in blood from donor B. The quality of the meningococcal RNA was analysed on a Bioanalyzer (Figure 6.2). Expression of eleven genes (*pilB*, NMB0267, *farA*, NMB0345, *mapA*, *apbE* (NMB0563), *mntC*, NMB1040, NMB1164, NMB1882 and NMB1961) was determined using comparative real-time RT-PCR. Gene expression data for time-points 0, 20, 40 and 240 min were collected from RNA isolated in three independent experiments (biological replicates), performed on three different days, with the exception of data included for gene expression at 90 min in donor B's blood, which are only based on an experiment on one occasion. As before, the gene expression changes at 40, 90 and 240 min were determined relative to 0 min (Table 6.4) and 20 min (Table 6.5). TaqMan data collected for strain MC58 incubated in blood from donor B were compared to data collected for donor A, analysed using microarrays and comparative real-time RT-PCR.

Expression of most of the genes tested changed at one or more time-points during incubation in blood from donor B, relative to gene expression at 0 or 20 min. The expression profile of *farA* and NMB1164 over time is similar for data from microarray and TaqMan analysis (donor A) and also similar for the two donors (A and B). Comparison of data from donor B to data from donor A revealed differences in the temporal expression of genes. The overall pattern of gene expression in blood from donor A and B was found to be most different at the 40 min time-point in comparison to 0 min (Pearson correlation = – 0.2). Genes that were found to have a different expression pattern (in comparison to 0 min) during MenB incubation in blood from the two donors (A and B) are listed below:

- The gene expression of *pilB* was only elevated at 240 min during incubation in blood from donor B, whereas in blood from donor A the gene was up-regulated at all time-points beyond 20 min.

- *mntC*, encoding a component of a zinc/manganese transporter, was up-regulated after 20 and 40 min (by TaqMan analysis) during growth in blood from donor A. In blood from donor B, expression of the gene increased later (at 240 min).
- During incubation in blood from donor A, the genes NMB0267 and NMB1882 were found to be significantly up-regulated from 20 min and 90 min, respectively, whereas in blood from donor B, expression of the genes transiently increased after 20 min incubation.
- The initial expression at 20 min of *mapA* was higher during incubation in blood from donor B than in blood from donor A.
- NMB1961, up-regulated during incubation in blood from donor A, was only slightly elevated at one time-point (20 min) during incubation in blood from donor B.

Potential explanations for these differences are discussed in Section 6.3.3.

6.2.4.2 Validation of microarray data

RNA isolated from meningococci incubated in blood samples from donor A was used to validate the microarray data. RNA was isolated from independent samples not used in microarray analysis, and validation was performed using the TaqMan platform. The results of the TaqMan analysis of individual genes broadly agreed with the findings based on microarray hybridisations. However, the following discrepancies were found:

- By TaqMan analysis, expression of NMB0345 was increased at 240 min compared to 20 min, while it was not significantly changed as assessed by microarray analysis.
- By TaqMan analysis, expression of *mapA* was not increased at 20 min compared to 0 min, while it was significantly increased as assessed by microarray analysis. In addition, expression of *mapA* was increased at 40 min compared to 20 min, while it was not significantly changed as assessed by microarray analysis.
- By TaqMan analysis, expression of *mntC* was significantly increased at 20 min compared to 0 min, while it was reduced as assessed by microarray analysis. However, at 40 min, the results were concordant.
- By TaqMan analysis, expression of NMB1040 was unchanged at 90 min compared to 0 min, while it was modestly increased as assessed by microarray analysis.
- By TaqMan analysis, expression of NMB1961 was increased at 240 min compared to 0 min, while it was not significantly changed as assessed by microarray analysis.

Table 6.4: TaqMan and microarray analysis to characterise the expression of a subset of genes during growth in blood over time in comparison to time-point 0 min. For TaqMan data $SD_{Fold-change}(SD_{F.-c.})$ indicates how variable expression of the specific gene are among biological replicates. For microarray data significance of up- or down-regulation is indicated by a P-value (adjusted for multiple testing). Data for 90 min from donor B are based on only one biological replicate, so the standard deviation in this case only reflects technical variation in the assay. Microarray data which are underlined indicate those genes that were not significantly differentially expressed at that particular time-point.

			20 vs. 0		**40 vs. 0**		**60 vs. 0**		**90 vs. 0**		**240 vs. 0**	
Technology	**Donor**	**Gene**	**Ratio**	**$SD_{F.-c.}$/ P-value**	**Ratio**	**$SD_{F.-c.}$/ P-value**	**Ratio**	**$SD_{F.-c.}$/ P-value**	**Ratio**	**$SD_{F.-c.}$/ P-value**	**Ratio**	**$SD_{F.-c.}$/ P-value**
Microarray	A	NMB0044	0.661	2.31E-02	2.064	4.08E-05	1.603	1.01E-02	1.546	8.36E-03	1.854	5.20E-04
TaqMan	A		0.787	0.174	4.990	0.80	6.161	1.67	2.174	0.398	2.281	0.47
TaqMan	B		0.821	0.11	1.335	0.28	N.D.	N.D.	N.D.	N.D.	3.129	0.67
Microarray	A	NMB0267	1.744	4.44E-05	2.071	8.89E-08	2.075	3.02E-07	1.807	9.28E-06	1.894	2.63E-06
TaqMan	A		1.762	0.542	2.854	0.46	2.329	0.54	1.531	0.133	2.270	0.36
TaqMan	B		1.682	0.216	0.951	0.13	N.D.	N.D.	0.859	0.192	0.635	0.17
Microarray	A	NMB0318	0.917	6.39E-01	1.139	4.19E-01	1.142	4.41E-01	1.535	3.26E-03	2.664	1.35E-09
TaqMan	A		0.255	0.061	N.D.	N.D.	N.D.	N.D.	3.816	0.532	4.257	0.81
TaqMan	B		1.824	0.41	1.670	0.27	N.D.	N.D.	2.049	0.310	8.566	1.45
Microarray	A	NMB0345	0.470	5.24E-05	0.768	1.23E-01	0.889	5.91E-01	0.500	1.23E-04	0.382	3.79E-07
TaqMan	A		0.340	0.081	0.899	0.15	0.644	0.33	N.D.	N.D.	0.677	0.16
TaqMan	B		0.490	0.094	1.233	0.33	N.D.	N.D.	0.583	0.119	0.659	0.13
Microarray	A	NMB0390	1.517	8.46E-05	1.616	3.58E-06	1.348	4.84E-03	1.096	4.05E-01	0.754	5.70E-03
TaqMan	A		1.171	0.261	2.151	0.33	1.270	0.15	0.503	0.072	0.951	0.25
TaqMan	B		2.532	0.55	1.719	0.21	N.D.	N.D.	0.779	0.110	1.100	0.21
Microarray	A	NMB0563	1.567	2.65E-06	1.740	7.67E-09	1.512	1.47E-05	1.466	2.77E-05	1.983	3.34E-11
TaqMan	A		2.108	0.401	2.280	0.43	1.967	0.40	1.920	0.175	3.029	0.69
TaqMan	B		3.101	0.62	1.566	0.23	N.D.	N.D.	2.049	0.334	1.694	0.32
Microarray	A	NMB0586	0.711	5.66E-03	1.568	1.54E-04	1.435	3.63E-03	0.960	7.74E-01	0.765	2.46E-02
TaqMan	A		3.150	0.751	3.954	0.70	1.421	0.35	N.D.	N.D.	N.D.	N.D.

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Table 6.4 – continued from previous page

Technology	Donor	Gene	20 vs. 0		40 vs. 0		60 vs. 0		90 vs. 0		240 vs. 0	
			Ratio	$SD_{F.-c.}$/ P-value	Ratio	$SD_{F.-c.}$/ P-value	Ratio	$SD_{F.-c.}$/ P-value	Ratio	$SD_{F.-c.}$/ P-value	Ratio	$SD_{F.-c.}$/ P-value
TaqMan	B		1.144	0.274	1.229	0.22	N.D.	N.D.	0.967	0.201	2.702	0.65
Microarray	A	NMB1040	1.323	5.42E-02	1.193	2.23E-01	1.424	2.52E-04	1.464	2.15E-04	1.318	4.55E-02
TaqMan	A		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.881	0.107	N.D.	N.D.
TaqMan	B		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.857	0.119	N.D.	N.D.
Microarray	A	NMB1164	2.086	2.06E-04	1.719	4.38E-03	1.918	1.05E-03	1.311	1.86E-01	1.628	5.96E-03
TaqMan	A		1.930	0.546	2.002	0.31	1.864	0.35	1.163	0.176	1.922	0.34
TaqMan	B		4.435	0.87	1.895	0.26	N.D.	N.D.	2.595	0.358	3.416	0.63
Microarray	A	NMB1807	0.617	1.21E-02	0.571	1.22E-03	0.702	7.27E-02	0.823	2.86E-01	0.684	4.06E-02
TaqMan	A		N.D.	N.D.	0.635	0.13	0.613	0.17	N.D.	N.D.	N.D.	N.D.
TaqMan	B		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Microarray	A	NMB1882	1.123	5.48E-01	1.053	7.84E-01	1.247	2.04E-01	1.822	1.67E-04	1.140	4.31E-01
TaqMan	A		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.726	0.231	N.D.	N.D.
TaqMan	B		2.349	0.52	0.865	0.13	N.D.	N.D.	0.900	0.134	0.657	0.15
Microarray	A	NMB1961	1.672	6.10E-04	1.615	8.05E-04	1.648	9.56E-04	1.551	2.21E-03	1.211	1.95E-01
TaqMan	A		1.523	0.350	2.403	0.44	2.275	0.46	1.521	0.189	2.475	0.46
TaqMan	B		1.687	0.29	1.079	0.09	N.D.	N.D.	1.062	0.164	0.806	0.16

N.D. Not determined.

Table 6.5: TaqMan and microarray analysis to characterise the expression of a subset of genes during growth in blood over time in comparison to time-point 20 min. For TaqMan data $SD_{Fold-change}(SD_{F.-c.})$ indicates how variable expression of the specific gene is among biological replicates. For microarray data significance of up- or down-regulation is indicated by a P-value (adjusted for multiple testing). Data for 90 min from donor B are based on only one experiment, so the standard deviation in this case only reflects technical variation in the assay. Microarray data which are underlined indicate those genes that were not significantly differentially expressed at that particular time-point.

			40 vs. 20		**60 vs. 20**		**90 vs. 20**		**240 vs. 20**	
Technology	**Donor**	**Gene**	**Ratio**	**$SD_{F.-c.}$/ P-value**	**Ratio**	**$SD_{F.-c.}$/ P-value**	**Ratio**	**SD/ P-value**	**Ratio**	**$SD_{F.-c.}$/ P-value**
Microarray	A	NMB0044	3.122	1.45E-08	2.425	1.47E-05	2.339	2.00E-06	2.804	5.54E-08
TaqMan	A		5.844	0.935	7.216	1.960	2.546	0.466	2.671	0.545
TaqMan	B		1.821	0.379	N.D.	N.D.	N.D.	N.D.	4.406	0.95
Microarray	A	NMB0318	1.243	3.38E-01	1.246	3.52E-01	1.674	5.96E-04	2.907	1.21E-10
TaqMan	A		N.D.	N.D.	N.D.	N.D.	2.367	0.330	2.641	0.504
TaqMan	B		0.924	0.180	N.D.	N.D.	1.799	0.272	4.739	0.80
Microarray	A	NMB0345	1.634	9.37E-03	1.893	2.63E-03	1.065	7.89E-01	0.813	2.66E-01
TaqMan	A		2.726	0.450	1.951	0.450	N.D.	N.D.	2.054	0.478
TaqMan	B		2.620	0.383	N.D.	N.D.	1.239	0.253	1.400	0.27
Microarray	A	NMB0390	1.065	7.63E-01	0.888	5.34E-01	0.723	1.79E-03	0.497	8.16E-10
TaqMan	A		2.061	0.313	1.217	0.321	0.482	0.069	0.911	0.242
TaqMan	B		0.687	0.085	N.D.	N.D.	0.311	0.044	0.739	0.21
Microarray	A	NMB0586	2.206	7.65E-09	2.018	5.38E-07	1.350	1.34E-02	1.076	5.75E-01
TaqMan	A		2.394	0.425	0.861	0.209	N.D.	N.D.	N.D.	N.D.
TaqMan	B		1.378	0.250	N.D.	N.D.	1.085	0.225	2.398	0.57
Microarray	A	NMB1882	0.938	8.44E-01	1.111	7.97E-01	1.623	2.82E-03	1.016	9.35E-01
TaqMan	A		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TaqMan	B		0.372	0.058	N.D.	N.D.	0.387	0.058	0.283	0.07
Microarray	A	NMB1961	0.965	9.19E-01	0.986	9.85E-01	0.928	7.04E-01	0.724	4.04E-02
TaqMan	A		1.589	0.292	1.505	0.302	1.006	0.125	1.637	0.304
TaqMan	B		0.641	0.051	N.D.	N.D.	0.631	0.098	0.479	0.09

N.D. Not determined.

6.3 Discussion

In this study, *N. meningitidis* was incubated in anti-coagulated non-bactericidal whole blood collected from adult donors. Growth of meningococci in this *ex vivo* model of septicaemia induced extensive re-modelling of the bacterial transcriptome. In comparison with corresponding time-points for meningococci incubated in plasma (Chapter 5), there was a greater response to growth in blood. An overview of the overlap between genes differentially expressed in blood and plasma (FFP 2) at corresponding time-points is shown in Figure 6.10.

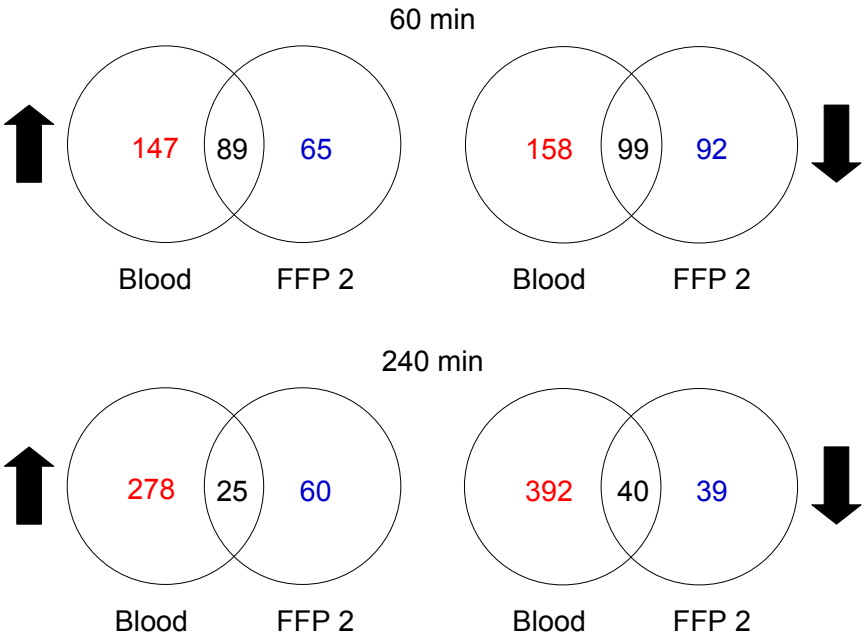


Figure 6.10: Venn diagrams showing the overlap of differentially expressed genes at 60 and 240 min during incubation in blood and FFP 2, in relation to 0 min.

First, transcriptional changes relating to meningococcal adaptations to growth in blood and bacterial responses to host factors will be discussed. In subsequent sections the focus will be on potential surface exposed proteins, as identification of such proteins was the main objective of this study.

6.3.1 Global impact of growth in blood on meningococci

During growth in blood, the majority of genes with altered expression levels, at all time-points/comparisons were annotated in TFRCs *hypothetical proteins* and *unassigned*, reflecting the large proportion of the meningococcal genome that are still of

unknown functionality. Many genes involved in key metabolic functions were also affected by blood exposure, such as those associated with synthesis of amino acids, uptake of trace elements and energy production. The functional group *amino acid biosynthesis* was also significantly enriched in GSEA analysis in comparisons of 20, 40 and 60 min to the 0 min time-point. Up-regulated genes in this TFRC included those involved in synthesis of glutamate, methionine, and branched chain amino acids. One of the up-regulated genes *gdhA*, a NADP-linked glutamate dehydrogenase, has previously been observed to be up-regulated both during meningococcal growth in plasma (in this project) and when *P. multocida* was incubated in blood (Boyce *et al.*, 2002). In GSEA analysis the TFRCs *purines, pyrimidines, nucleosides and nucleotides* and *protein synthesis* were also significantly enriched at the three first time-points in blood.

During incubation in blood many genes associated with *transport and binding* were observed to be up-regulated. In relation to the 20 min time-point, this reflects an up-regulation of genes encoding proteins involved in, for example, iron transport. Other groups of genes progressively up-regulated during the time course in blood (in relation to the 20 min time-point or between adjacent time-points) included those responding to conditions of low zinc availability and those involved in protein fate.

In blood, most iron is available bound to transferrin, which can be assimilated by meningococci through the agency of transferrin binding protein. *N. meningitidis* expresses a haemoglobin receptor and may also acquire iron from this source in the context of haemolysis. At t = 20 min, genes encoding iron uptake proteins were found to be down-regulated, in comparison to time-point 0 min, a finding which may be explained by the bacteria having already adapted to an iron-deplete state at the time of inoculation by expressing these genes at high levels. Results from other studies in which transcriptional profiling has been used to explore the bacterial responses during growth in blood has also indicated limited up-regulation of iron-regulated genes (Boyce *et al.*, 2002; Orihuela *et al.*, 2004). However, previous studies have shown iron-uptake to be essential in meningococcal invasive disease (Section 6.1.2).

Over the time course in blood, iron regulated genes were found to be progressively up-regulated after the 20 min time-point. For example, at 90 min *hmbR*, encoding a haemoglobin receptor, was found to be up-regulated in comparison to time-point 20 min. Up-regulation of *hmbR* may suggest that haemolysis occurred during *in vitro* incubation of blood over this period of time. This was not gross, however, as concentration of potassium, a marker of haemolysis, did not change significantly over the 240 min time course (Section 3.2.2). However, up-regulation of *hmbR* may not be associated with haemolysis, but instead reflect a need by the bacteria to acquire iron, resulting in up-regulation of genes encoding iron-uptake systems. This highlights one of the limitations of genome-wide transcriptional profiling, i.e. that it is in most cases not possible to directly draw conclusions about causal mechanisms from patterns of differential gene expression. However, microarray experiments are invaluable for finding genes associated with a certain perturbation or growth condition, and useful for generating new hypothesis.

Responses to host factors during incubation in blood. In the bloodstream, meningococci are exposed both to complement (modelled in plasma) and host cells and factors secreted by such cells. Some transcriptional changes during meningococcal growth in blood may be responses to anti-bacterial factors secreted by host cells, or other toxic compounds. For example, during prolonged incubation in blood genes encoding efflux proteins were up-regulated. In addition *lipA*, up-regulated at later time-points in blood, has previously been found to up-regulated in response extended growth in broth with sub-lethal levels of the CAMP protegrin PG-1, as well as to intracellular growth (Spinosa *et al.*, 2007).

Binding of plasminogen to the bacterial surface and subsequent activation to plasmin (a serine protease that cleaves host fibrinogen) has been shown to increase invasiveness of bacteria (Lähteenmäki *et al.*, 2005; Cole *et al.*, 2006). Activated plasmin can also degrade complement (Seya *et al.*, 1985) and has been shown to degrade C3b and immunoglobins when bound to the surface of bacteria (Sodeinde *et al.*, 1992; Rooijakkers *et al.*, 2005). Plasminogen binds to the surface of meningococci (Ullberg

et al., 1992), where it is activated by host factors. An increase in formation of plasmin is observed early in meningococcal invasive infection and in infection models (up to 2 hr). This activation is, however, rapidly decreased due to increased expression of the plasminogen activation inhibitor pAI-1 (at approximately 3 hr in infection models) (Brandtzaeg *et al.*, 1990). Three of the genes (*eno* (NMB1285), *dnaK*, NMB0946) that were up-regulated in blood encode proteins that have been demonstrated to interact with human plasminogen *in vitro* (Knaust *et al.*, 2007). However, these proteins also perform important functions in meningococcal metabolism and maintenance, so up-regulation in blood may not necessarily indicate increased expression of these proteins on the surface of the bacteria. Curiously, all three proteins are predicted to be located and to function in processes in the cytoplasm. Other cytoplasmic proteins that have also been demonstrated to be on the bacterial surface under some conditions include GroEL chaperonin (Hsp60) (Yamaguchi *et al.*, 1996), and reactive antibodies to this protein have been identified in convalescent sera from meningococcal patients (Pannekoek *et al.*, 1993). Apparent surface location of these proteins (as well as others predicted to be cytoplasmic) emphasises the difficulty in accurately predicting the cellular location of bacterial proteins using *in silico* methods.

In recent studies, TPS systems have been shown to be involved in interactions with host cells (Schmitt *et al.*, 2007; Talà *et al.*, 2008) and to be associated with invasive clinical isolates (van Ulsen *et al.*, 2008). The secreted component of the system (TpsA; hemagglutinin/hemolysin-related protein) has also been found to be bound to the meningococcal surface, where the protein can act as an adhesin. During growth in blood in this study, genes encoding TPS systems were found to be up-regulated. A similar up-regulation was also observed in an earlier study of meningococcal transcriptional responses to growth in blood (Webb, 2001).

The abundance of some surface exposed meningococcal proteins is governed by phase-variation (described in Section 1.4.1.2), which may regulate transcription or translation of the gene. Down-regulation of genes encoding antigenic surface proteins, or variation of surface epitopes, is used by meningococci as a way of evading the

host immune system. OpcA, down-regulated over time in blood, has been shown to be regulated at the transcriptional level by phase-variation (Sarkari *et al.*, 1994). Down-regulation of *opcA* may therefore represent a bacterial response to host immune responses in blood.

6.3.2 Potential surface exposed proteins

The primary aim of this study was to find genes, encoding surface exposed proteins, which were up-regulated in blood, a model of septicaemia, while being down-regulated or not differentially expressed in contact with epithelial cells, a model of colonisation. Data were therefore compared to a published data set, reporting the pattern of MenB gene expression upon extended contact with epithelial cells (Grifantini *et al.*, 2002b). The set of meningococcal genes found to be up-regulated in response to incubation in human blood was compared to the set of genes differentially expressed in contact with epithelial cells (Supplementary table C.55), particularly focusing on those differentially expressed after 3 hr in the colonisation model. (Imperfect as it may be, this time-point in Grifantini *et al.*'s experimental system, is the one most nearly representing conditions of prolonged meningococcal colonisation of the nasopharynx). *In silico* prediction of protein location was used to identify genes encoding proteins with a putative surface location. Eight genes up-regulated in blood were down-regulated at 3 hr in contact with epithelial cells. These were *rpsO* (NMB0609), *rplT* (NMB0723), *bfrA* (NMB1207), *bfrB* (NMB1206), *pilP* (NMB1811), *atpD* (NMB1934), *atpG* (NMB1935) and *mapA*. The *mapA* gene was selected for further studies based on the fact that it was up-regulated at more then one time-point during growth in blood, as well as that its gene product is predicted to be located in the outer membrane of the bacteria.

Several genes, encoding (known or putative) surface-exposed proteins, which were up-regulated during incubation in blood were not reported to be differentially expressed upon attachment to epithelial cells in the study by Grifantini *et al.* (2002b) (Supplementary table C.56). These genes included *abpE*, *mlp*, NMB0345, NMB0821, NMB0866, NMB1040, NMB1882 and NMB1961.

Based on the putative location of proteins, as well as their expression pattern in blood and in contact with epithelial cells, seven genes (*mapA*, NMB0345, NMB0267, *abpE*, NMB1040, NMB1882 and NMB1961) were selected for further studies (Chapter 7). The expression of these genes was also measured during growth in blood samples from a second individual (donor B).

6.3.3 Confirmation of the transcriptional profile in blood using comparative real-time RT-PCR

Comparative real-time RT-PCR was used to confirm the microarray data in blood from donor A, as well as to determine the expression of a select set of genes in RNA isolated from meningococci incubated in blood from a second individual (donor B), to get a measure of variability in gene expression of these genes during incubation of meningococci in different individuals' non-bactericidal blood. In this study the assumption is made that there is limited variability in meningococcal response to incubation in 'non-killer' blood from healthy adult individuals.

Using comparative real-time RT-PCR, data from microarray analysis (donor A) could be confirmed for most of the genes tested. Comparison to data collected for meningococci incubated in blood from donor B indicated that many of the genes tested were up-regulated, in at least one time-point, during incubation in blood samples from this donor as well. However, the temporal expression of the genes was not always identical. Some potential explanations for the observed differences in transcription of the subset of genes during meningococcal growth samples from these two individuals are discussed below. Firstly, differences in availability of nutrients in blood samples from donor A and B may result in some of the changes seen, such as the difference in expression of NMB1961, which is most likely regulated by GdhR, a glutamate responsive regulator (Monaco *et al.*, 2006). Secondly, even though strain MC58 can survive in blood samples from these two individuals, which is assumed to result from an absence of bactericidal antibodies, the individuals could have antibodies to meningococcal surface structures which although non-bactericidal could through binding to the surface of the bacterium induce a response resulting in changes in the

transcriptional profile of the meningococcus. In addition, other immune responses in blood may differ between the two individuals, which may affect the bacteria. Thirdly, the transcription profile of a subset of genes, based on a pre-selection criteria (potential surface exposure), was investigated in meningococci incubated in blood samples from donor B. In deciding about this group of genes those with more variable expression may have been selected. It is possible that the results do not reflect the general response of meningococci in blood, which may be more similar in the two different blood samples. Ideally the genome-wide transcriptional profile should have been explored in meningococci during growth in blood samples from donor B. However, problems with isolating sufficient amounts of RNA for microarray experiments after growth in blood samples from this individual meant that this could not be achieved. It may be that the difference in amount of RNA that could be isolated (even though similar growth was observed for the bacteria) was a result of alteration in bacterial metabolism, resulting in less RNA expressed per cell.

6.3.4 Conclusions and future work

Criticisms of the model and the study Bacterial growth rate and nutritional status of the bacteria initially inoculated into the blood must be anticipated critically to influence the transcriptional changes that are recorded subsequently. In this study, gene expression of meningococci in blood was compared to time-point 0 min (start point), but also between time-points in blood. The selection of the conditions defining the zero time-point (bacteria being scraped off plates after standardised culture, re-suspended in RPMI 1640 culture medium, added to fresh blood and immediately processed for RNA extraction) has been pragmatic, as the most relevant reference point achievable in practise. The approach is comparable to the experimental design employed by Graham *et al.* (2005) in examining the transcriptome of group A Streptococcus in whole human blood. In addition, meningococci on the nasopharyngeal mucosal surface (the condition I ultimately wish to compare to the bacterium in blood) are almost certainly more similar to meningococci on solid agar than to those grown in liquid culture. In similar studies others have used as a reference standard

the no more physiological condition of organisms incubated in rich liquid medium (Orihuela *et al.*, 2004). Regardless of the conditions chosen for preparing organisms to start the experiment, the use of a common reference design allows comparisons of gene transcription not only of later time-points to time zero, but also any other pair wise comparisons, for example, comparison of gene expression at 90 min in blood to that at 20 min. Since a comparison to time-point zero or any other control condition have their respective disadvantages, data have also been presented of gene expression differences between later time-points and 20 min, as well as the gene expression between time-points, as a way of detecting genes progressively up-regulated in the system. An alternative experimental design would be to propagate bacteria in minimal medium designed to mimic the mucosal environment before inoculation in blood. However there is currently insufficient information on how to develop such a recipe without extensive further investigation.

Conclusions and future work To this end, DNA microarray analysis was utilised to quantify transcriptional changes in among all known genes in the genome of *N. meningitidis* strain MC58 in response to culturing in whole human non-bactericidal blood. At all time-points in blood there was an extensive remodelling of the transcriptome compared to the starting point. Genes up-regulated during meningococcal incubation in blood included those associated with adaptations to the host environment such as those characterised in functional groups *amino acid biosynthesis* and *protein synthesis*. Up-regulation was also observed for genes involved in *cell detoxification*, expression of surface structures, and those encoding transporters.

Blood used in this study was collected from adult donors. Since meningococcal disease mostly affects young children and teenagers, it might be suggested that blood from younger individuals should have been used, but this was not practically possible. However, this was modelled satisfactorily by identifying healthy adult 'non-killers' of the MenB strain under consideration, this phenotype assumed to reflect a lack of bactericidal antibodies and so to model the naïve, infant, state. It would be of interest to characterise the meningococcal transcriptome in blood samples collected

from additional individuals. However, to model the variation in meningococcal gene expression in response to incubation in blood from different individuals, blood from an estimated number of at least 10–20 non-killing volunteers[1] would be required. Identifying 20 adults with non-killing capacity would have required screening of blood from ∼400 individuals assuming ∼5% are 'non-killers', as suggested by Ison *et al.* (unpublished). This was not within the scope of this project.

I propose that the *ex vivo* whole blood model can be used to model some responses of the bacteria during septicaemia, while conceding that that it cannot completely replicate the complex environment encountered by the bacteria during infections. The *ex vivo* whole blood model has been used with the objective to find new antigens - new targets of interest for vaccines. From this work, seven candidate genes were selected for further studies, presented in Chapter 7. Identification of targets (for example metabolic genes) for other therapeutic interventions such as anti-bacterial drugs could also be envisioned from the work presented here.

[1]Further transcriptional profiling studies are needed to calculate the exact number of individuals

Chapter 7

Sequence variability of genes encoding OMPs up-regulated in infection models

Sequence variability among meningococcal strains is an important issue in the assessment of candidate protein vaccine antigens. Our hypothesis is that meningococcal genes encoding surface expressed proteins which are up-regulated during growth in blood to a greater extent than during colonisation, may be less variable than those highly expressed on the epithelial surface, where the bacteria will be subjected to immune selection pressure (Section 1.5.1). In this chapter a set of genes encoding proteins of putative or known surface location, which were found to be up-regulated during incubation of MenB in plasma (Chapter 5) and blood (Chapter 6), are investigated for sequence variability to test this hypothesis.

7.1 Introduction

The sequence variability of meningococcal genes has proved a major challenge to the development of universal protein based MenB vaccines. Intense immune-mediated selection pressure operating at the mucosal surface colonised by meningococci has led to the evolution of mechanisms of evasion including phase-variation and sequence plasticity. Antigenic variability intra- or inter-strains occurs through gene sequence variability, but also through variable expression of gene products, variable abundance of proteins (Moe *et al.*, 1999), or proteins being poorly accessible on the meningococcal surface (Arenas *et al.*, 2008). Only the sequence variability of genes has been

explored in this project. Meningococcal sequence variability can be exemplified by the immunodominant meningococcal OMPs PorA and PorB, used in sero and serosubtyping, which both have been shown to have high sequence variability in surface exposed loops. Sequencing of the three variable loop regions (VR1, VR2 and VR3) in PorA have so far yielded 185, 508 and 13 different variants, respectively. Sequence variants of PorA VR1 and VR2 are stored in a public database that can be assessed and interrogated through a webpage interface (`http://neisseria.org/nm/typing/pora`) (Russell *et al.*, 2004), and a limited set of sequence variants found in VR3 are available at `http://www.smprl.scot.nhs.uk/PorA_VR3.htm` (Clarke *et al.*, 2003). PorA induces protective antibodies in humans (Peeters *et al.*, 1996), but sequence variability markedly limits the usefulness of PorA as a cross-protective vaccine (Feavers *et al.*, 1996).

7.2 Results

7.2.1 Bioinformatic analysis of publically available sequences of the selected genes

A first indication of potential variability of a gene may be obtained quickly from a comparison of MenB sequences to homologues in the sequenced Neisserial genomes of meningococcal strains Z2491 (serogroup A) and FAM18 (serogroup C), as well as gonococcal strain FA1090. Local sequence alignments were performed, using BLAST, at both nucleotide (blastn) and protein (blastp) level (Altschul *et al.*, 1990). All of the MC58 genes listed in Table 7.1 have more than 90% identity (amino acid (aa) level) to the genes from strains Z2491 and FAM18. No gonococcal strain FA1090 protein with sequence similarity to *mapA* (NMB0390) was found in the non-redundant data base at NCBI.

Table 7.1: Sequence alignment of seven protein sequences of MenB strain MC58 with protein sequences of *N. meningitidis* strains Z2491 and FAM18 and *N. gonorrhoeae* strain FA1090 to find orthologous proteins.

ID	Length (aa)	Z2491	FAM18	FA1090
NMB0267	239	Id. = 230/239, Pos. = 232/239	Id. = 228/239, Pos. = 234/239	Id. = 219/239, Pos. = 223/239, Gaps = 11/239
NMB0345	288	Identical	Identical	Id. = 284/288, Pos. = 286/288
NMB0390	752	Id. = 733/752, Pos. = 739/752	Id. = 733/752, Pos. = 741/752	No Hit
NMB0563	351	Id. = 347/351, Pos. = 348/351	Id. = 349/351, Pos. = 350/351	Id. = 339/351, Pos. = 345/351
NMB1040	426	Id. = 423/426, Pos. = 425/426	Id. = 424/426, Pos. = 424/426,	Id. = 396/426, Pos. = 402/426
NMB1882	725	Id. = 719/725, Pos. = 721/725	Id. = 720/725, Pos. = 722/725	Id. = 695/725, Pos. = 703/725
NMB1961	275	Identical	Identical	Id. = 263/277, Pos = 265/277, Gaps = 2/277

Id. in the table stands for Identities and Pos. in the table stands for Positives i.e. amino acids with similar characteristics.

7.2.2 Sequence variability of genes up-regulated in models of infection

The regions containing the genes listed in Table 7.1 were amplified from genomic DNA prepared from 23 meningococcal strains: 21 MenB strains, one MenA strain and one MenC strain (Table 2.1), as described in Section 2.3. The strains used for sequencing were from a diverse collection of well characterised strains, mainly isolated in Europe. Figure 7.1 shows the relationship between STs represented in the strain collection. Any differences in the size of the amplified fragments were observed upon separation in a 2% agarose gel (Section 2.4) before direct sequencing of the amplicons as described in Section 2.3.3. Sequence variability was defined as differences in sequence compared to the gene sequence in MenB reference strain MC58.

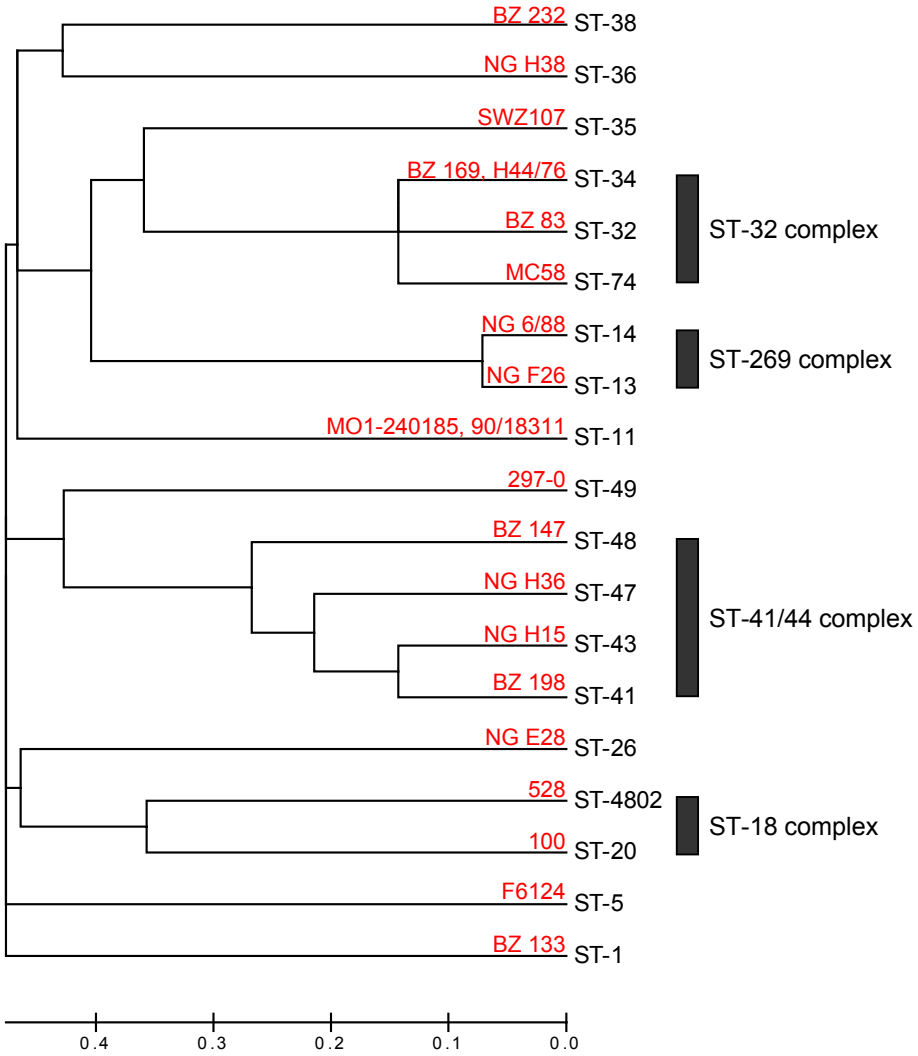


Figure 7.1: Balanced tree of STs constructed of MLST data from the database at `http://pubmlst.org/neisseria` using neighbour joining algorithm and edited in MEGA (Tamura *et al.*, 2007). Names of the meningococcal strains that were used in sequencing are listed in red next to their respective ST designation (details in Table 2.1).

7.2.2.1 Sequence variability of NMB0345

NMB0345 encodes a putative cell binding factor, possibly a member of the peptidyl-prolyl isomerase family (30% sequence similarity). Its product has a predicted outer membrane location and has been found in OMV preparations from meningococci (Ferrari *et al.*, 2006) and from *N. lactamica* (Vaughan *et al.*, 2006). NMB0345 has been shown to be important for survival in the blood stream, at least in an infant rat model (Sun *et al.*, 2000). The gene was found to be progressively up-regulated in whole human blood (Chapter 6), with a peak in gene expression at 40 and 60 min. NMB0345 is 100% identical, at the amino acid level, to homologous genes in

Z2491 and FAM18 genomes and 98% identical to a FA1090 homologue (Table 7.1). Secondary structures (predicted using Jnet (Cuff & Barton, 2000)) mapped to the protein sequence (with amino acids shaded by functional characteristics) are shown in Figure 7.2.

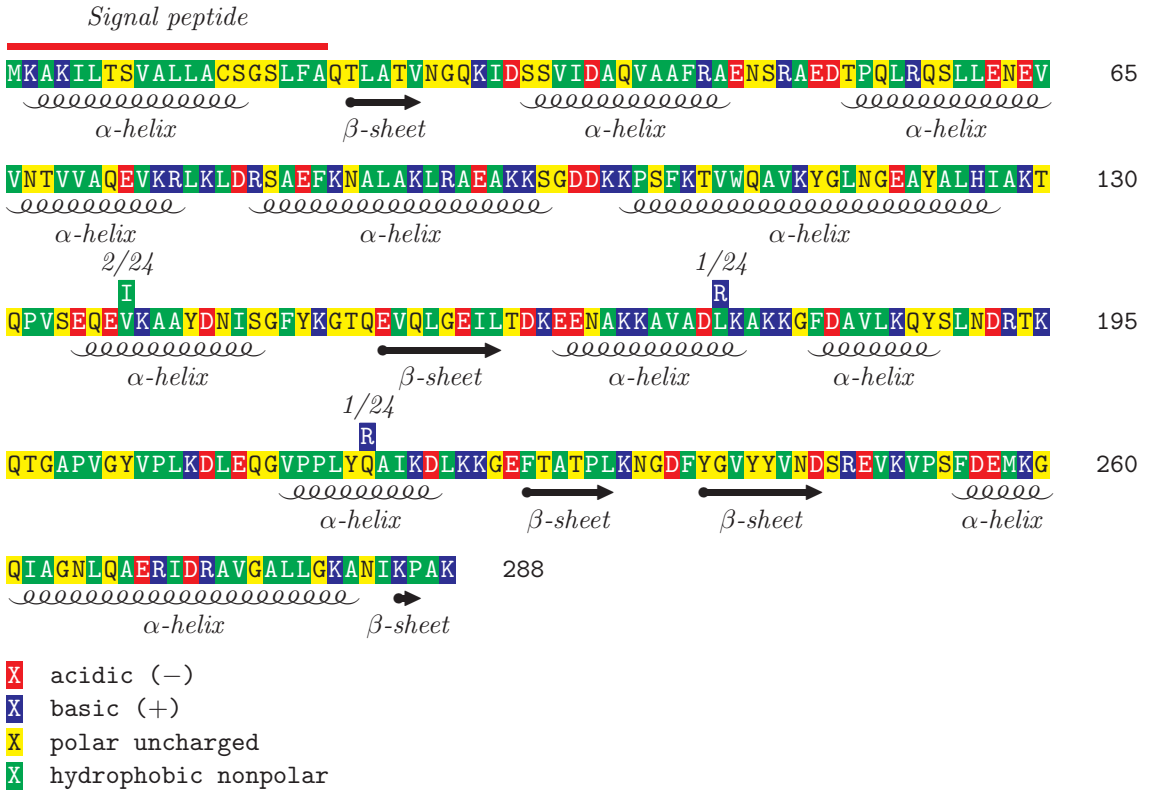


Figure 7.2: Secondary structure predictions for NMB0345 using Jnet algorithm (Cuff & Barton, 2000) mapped to the sequence of the gene (produced using TEXshade package (Beitz, 2000)). Amino acids are coloured by their functional characteristics. Symbols above the primary sequence show positions of three variable residues (and their frequency) among strains (see multiple alignment in Figure D.1 for details).

The genomic region containing NMB0345 could be amplified from DNA isolated from all the strains (Table 2.1), and no difference in the sizes of the amplified fragments could be observed upon separation in an agarose gel. Direct sequencing of the products revealed limited sequence variability. The amino acid sequences were deduced from the nucleotide sequences. Multiple sequence alignment of the amino acid sequences from all strains is shown in Figure 7.3 (detailed alignment in Supplementary figure D.1). Only three non-synonymous variants were found, resulting from single nucleotide variants at positions 412, 527 and 653. All three variants are located

in domains of NMB0345 predicted to form α-helices (Figure 7.2).

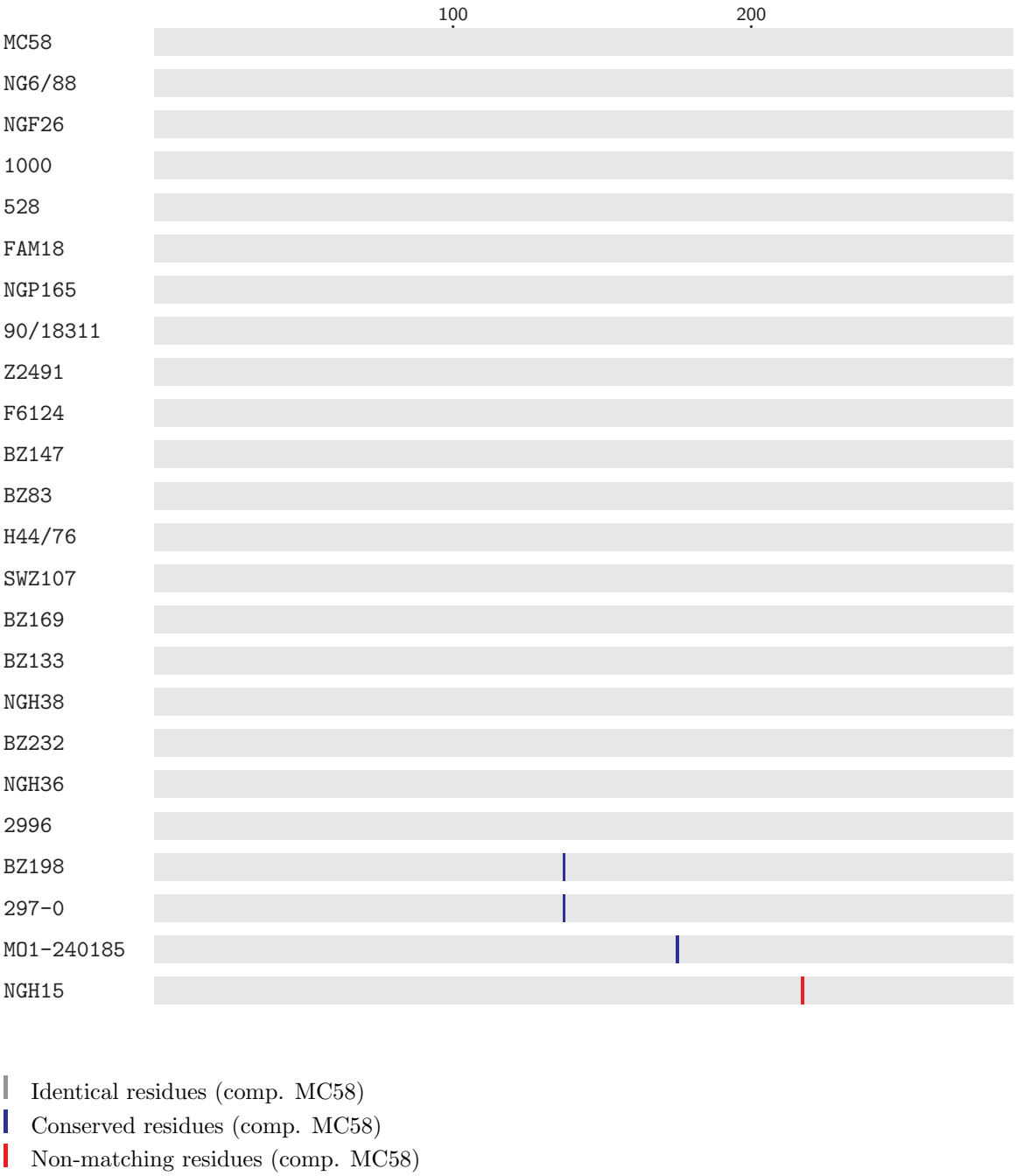


Figure 7.3: Alignment fingerprint of the 24 amino acid sequence of NMB0345 produced using the TEXshade package (Beitz, 2000). Residues that are identical, conserved or non-matching to the MC58 sequence are indicated.

7.2.2.2 Sequence variability of *mapA* (NMB0390)

The gene *mapA* encodes a protein annotated as maltose phosphorylase, with a predicted outer membrane position. It was found to be differentially regulated during

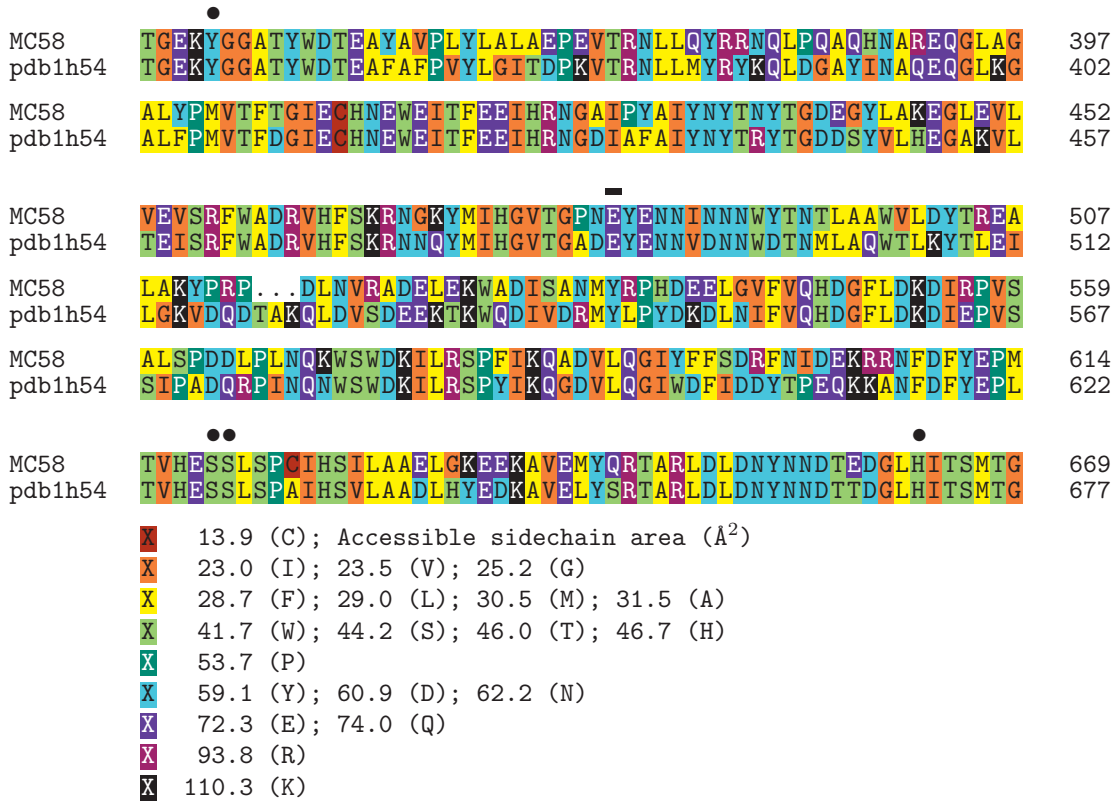


Figure 7.4: Alignment of the central domain of *mapA* with a maltose phosphorylase from *L. brevis*. The catalytic residue (E_{482}) and residues (Y_{347}, S_{619}, S_{620}, H_{663}) in the active site (in the *L. brevis* protein) are indicated by a bar and bullets respectively. The figure was constructed using TEXshade (Beitz, 2000)

incubation in both human blood and plasma (Chapter 5 and Chapter 6). In blood, *mapA* was significantly up-regulated at 20 and 40 min in comparison to expression at time-point 0 min. The expression of *mapA* was found to be highest at the 40 min time-point in blood. Sequence alignment of protein sequences from meningococcal strains MC58 (serogroup B), Z2491 (serogroup A) and FAM18 (Serogroup C) indicate that the protein is fairly conserved in meningococci, but there is no homologue in gonococcal strain FA1090 (Table 7.1). The protein is similar in sequence (59.2% over 743 aa) to a maltose phosphorylase from *Lactobacillus brevis*, the three dimensional structure of which has been determined (PDB identity: 1h54) (Egloff *et al.*, 2001). The catalytic residue (E_{482}) and residues in contact with phosphate in the active site (Y_{347}, S_{619}, S_{620}, H_{663}), which are identical in both the proteins are indicated in protein alignments (Figures 7.4 and 7.8).

Using primers designed to bind the genomic sequence of MenA, MenB and MenC sequence strains up and downstream of *mapA*, the gene could be amplified from genomic DNA isolated from all but four of the strains (NG 6/88, NG F26, NG H38, NG H36) listed in Table 2.1. This indicates that genomic region flanking the gene is the same in most strains. Using primers designed to bind within the *mapA* orf, fragments of the same size (1585 bp) as for strain MC58 could be amplified from genomic DNA isolated from all of these four strains, indicating the presence of *mapA* (Figure 7.5). The fragments from these four strains have not yet been sequenced.

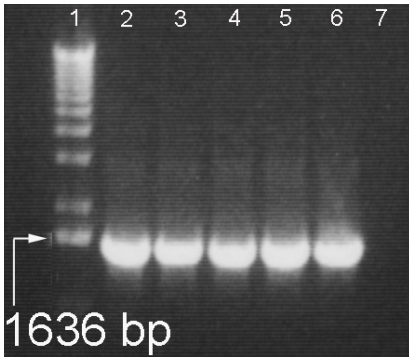


Figure 7.5: Agarose gel image of fragments of gene *mapA* amplified from genomic meningococcal DNA using primers NMB0390FInt and NMB0390RInt (Table 2.3) designed to bind within the gene. Lane 1: 1kb DNA ladder, Lane 2: strain MC58 (positive control), Lane 3: strain NG 6/88, Lane 4: strain NG F26, Lane 5: strain NG H38, Lane 6: strain NG H36, Lane 7: no DNA (negative control).

Multiple sequence alignment of deduced protein sequences from the other 15 meningococcal strains (Figure 7.8) showed that *mapA* is conserved among most of the strains. Identical amino acid sequences were found in the majority of the select meningococcal strains, indicating that in fact it is the MC58 *mapA* sequence that is the most dissimilar. Figure 7.7 shows the characteristics of residues, highlighting potential differences arising from sequence variability among strains. One of the sequence polymorphisms resulting in a amino acid difference in comparison to the MC58 sequence is shown in Figure 7.6.

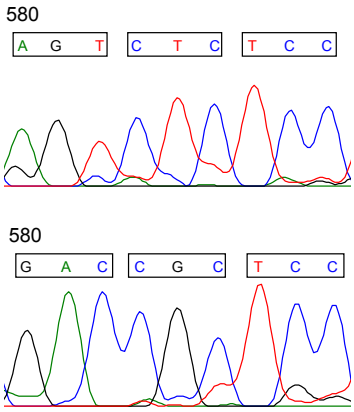


Figure 7.6: Sequence traces (nucleotide position 580 to 588) for strains MC58 (top) and MO1-240185 (bottom) showing a site of variation found in five of the meningococcal strains, which resulted in an amino acid change from SLS to DRS.

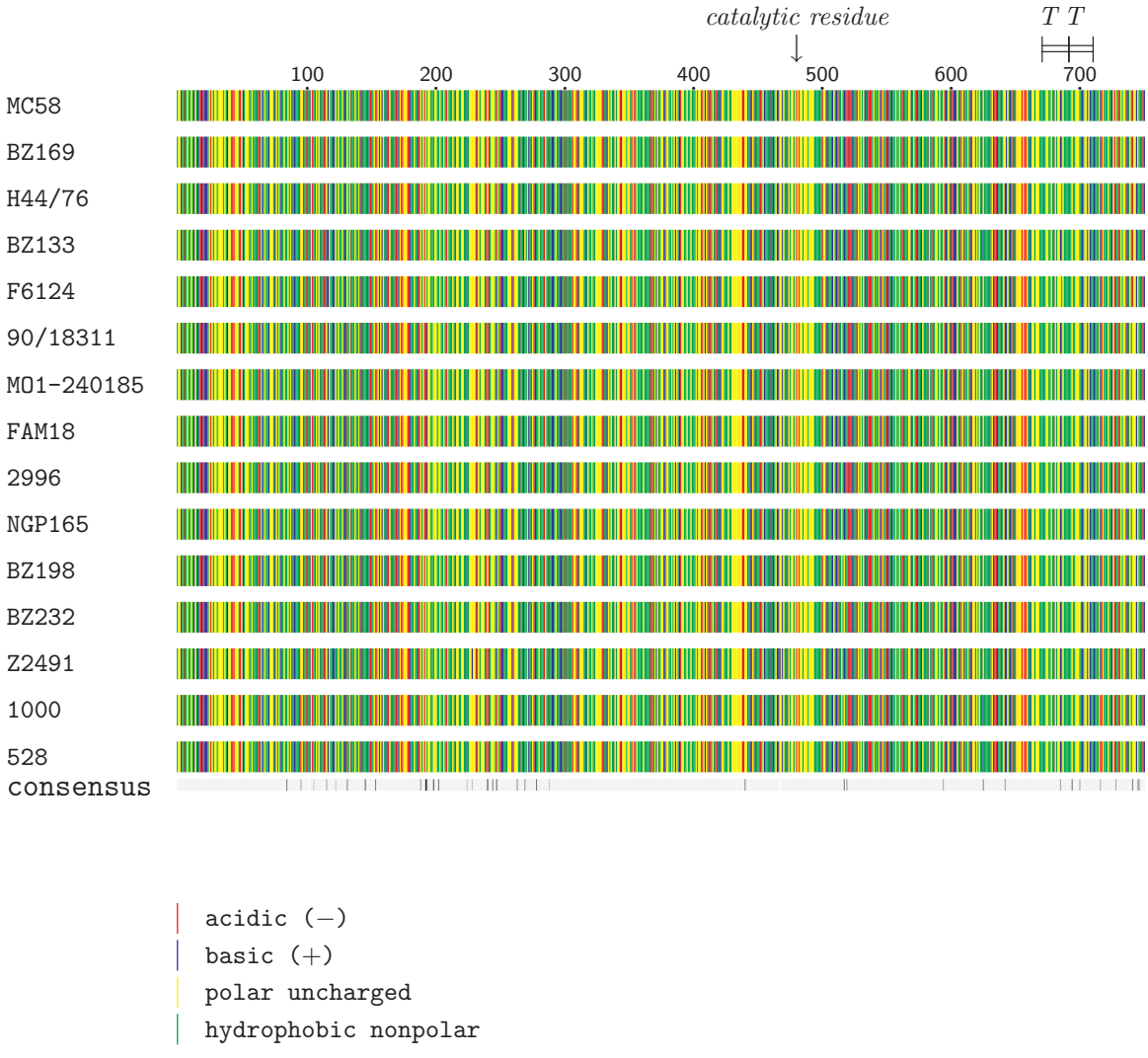


Figure 7.7: Graphical representation of the multiple alignment of protein sequences of *mapA* (constructed using TEXshade (Beitz, 2000)), showing the properties of amino acids and visualising regions of charged, hydrophobic or hydrophilic nature. Transmembrane helices, indicated by T in the figure, were predicted using TMpred (`http://www.ch.embnet.org/software/TMPRED_form.html`). In the consensus line a lighter colour represent similar residues, whereas a darker colour indicates less conserved residues.

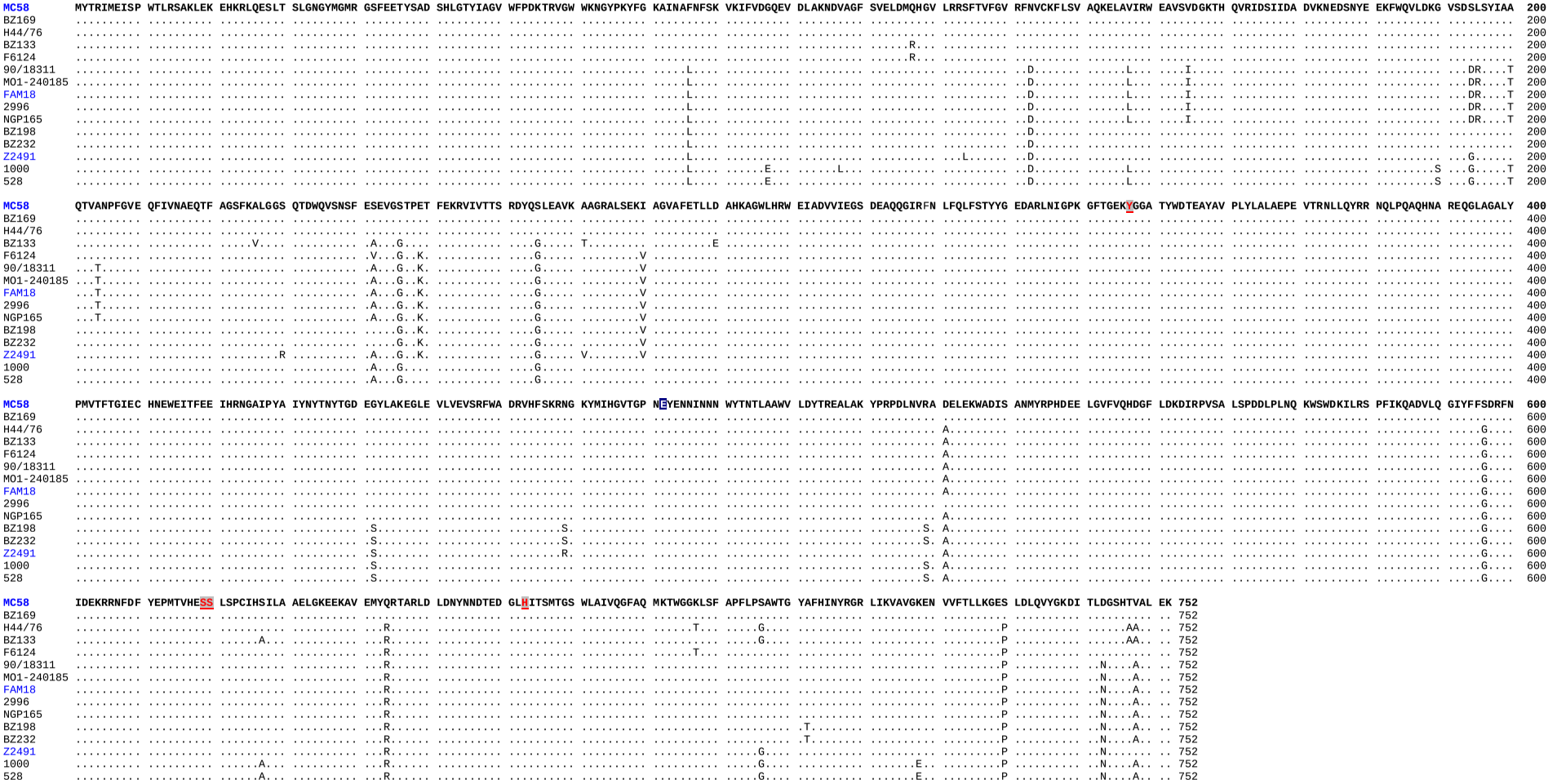


Figure 7.8: Multiple sequence alignment of *mapA* amino acid sequences from 15 meningococcal strains, including MC58 (labelled in blue), using ClustalW with default settings. The amino acid sequences were compared to the sequence from strain MC58. All strains used were MenB strains, except F6124 (MenA), 90/18311 (MenC) and NG P165 (NT). The MenA (strain Z2491) and MenC (strain FAM18) genome sequence strains were also included for comparison (labelled in blue). The location of residues in the active site (shaded in grey) and the catalytic residue (shaded in dark blue) are indicated in the alignment.

7.2.2.3 Sequence variability of NMB1040

NMB1040 encodes a hypothetical protein, with similarity to several proteins of unknown function found in proteobacteria. The first 20 amino acids of NMB1040 encode a probable signal peptide, but the cellular location of this protein is unclear. Pfam analysis (Sonnhammer *et al.*, 1997) suggests that the protein contains a domain similar to the SCP-like extracellular protein domain, which have a putative Ca^{2+} chelating function (Milne *et al.*, 2003). NMB1040 was found to be up-regulated upon incubation in plasma (FFP 2) at 60 and 240 min (Section 5.2.2) and was also found to be moderately up-regulated in blood (Chapter 6). Sequence alignment of NMB1040 to the genomic sequences of Neisseria strains FAM18, Z2491 and FA1090 indicate that the protein is well conserved (Table 7.1).

The genomic region containing NMB1040 could be amplified from all strains (Table 2.1), and no size difference of the amplified fragments was observed upon separation in a 2% agarose gel. Sequencing of the region showed that the gene has limited sequence variability, at least in the collection of strains used in this study. Amino acid sequences of the gene NMB1040 in 25 meningococcal strains were aligned using ClustalW (Larkin *et al.*, 2007). The multiple sequence alignment of the deduced amino acid sequences (Figure 7.9) indicate that the protein is well conserved. Figure 7.10 shows the characteristics of residues over the full length of the protein, highlighting potential differences arising from sequence variability among strains. The most common sequence variant found in 17 strains is a change from serine to arginine in position 76 of the MC58 sequence.

```
MC58        --------MK SLFIRLLLLG SAAGVFYHTQ NQSLPAGELV YPSAPQIRDG GDALHYLNRI RAQIGLHKLA HAPVLENSAR RHASYLTLNP EDGHGEHHPD NPHYTAQKLT  102
NGH36       --------.. .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  102
NGH15       --------.. .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  102
NGH38       --------.. .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  102
BZ133       --------.. .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  102
BZ147       --------.. .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  102
297-0       --------.. .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  102
BZ198       --------.. .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  102
NG6/88      --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
NGF26       --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
SWZ107      --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
FAM18       LPRREDGI.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  110
1000        --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
528         --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
NGP165      --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
2996        --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
90/18311    --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
NGE28       --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
MO1-240185  --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
Z2491       --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
F6124       --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
BZ232       --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
BZ169       --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
BZ83        --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102
H44/76      --------.. .......... .......... .......... .......... .......... .......... .......... ...R...... .......... ..........  102

MC58        ERTRLAGYLY NGVHENISTE EEAAESSDSD IRTQQRQVDG LMSAIYHRLS LLDRHTDEAG AAFVRENGKT VLVFNQGNGR FERHCAQGRN QPEAGRKYYR NACHNGAVVY  212
NGH36       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
BZ133       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
297-0       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
BZ198       .H........ .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
FAM18       .......... .......... .......... .......... .......... .......... .......... ........S. .......... .......... ..........  220
1000        .......... .......... .......... .......... .......... .......... .......... ........S. .......... .......... ..........  212
528         .......... .......... .......... .......... .......... .......... .......... ........S. .......... .......... ..........  212
NGP165      .......... .......... .......... .......... .......... .......... .......... ........S. .......... .......... ..........  212
2996        .......... .......... .......... .......... .......... .......... .......... ........S. .......... .......... ..........  212
90/18311    .......... .......... .......... .......... .......... .......... .......... ........S. .......... .......... ..........  212
NGE28       .......... .......... .......... .......... .......... .......... .......... ........S. .......... .......... ..........  212
MO1-240185  .......... .......... .......... .......... .......... .......... .......... ........S. .......... .......... ..........  212
Z2491       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
F6124       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
BZ232       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  212

MC58        TDEAMPAQEL LYTAYPVGSG ALPYFHGERP DPVPEYEITG NPASIDFSEA AGKITMKSFK LYQGKNEIRP VRVLTAGNDP NGRLTAYQFA LFPLKPLEYG TLYTAVFDYV  322
NGH36       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
BZ133       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
297-0       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
BZ198       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
FAM18       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  330
1000        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
528         .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
NGP165      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
2996        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
90/18311    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
NGE28       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
Z2491       .......... ........N. .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
F6124       .......... ........N. .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
BZ232       .......... ........N. .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
BZ169       .......... ........N. .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
BZ83        .......... ........N. .......... .......... .......... .......... .......... .......... .......... .......... ..........  322
H44/76      .......... ........N. .......... .......... .......... .......... .......... .......... .......... .......... ..........  322

MC58        RNGRRAQAKW QFRTRKPDYP YFEVNGGETL AVRKGEKYFI HWRGRWCLEA CTRYTYRQRP GSRLSIGRHE AGGIVFSVDG MAGSRITLAP EGETERGVTL YLQD 426
NGH36       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
BZ133       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
297-0       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
BZ198       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
FAM18       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 434
1000        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
528         .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
NGP165      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
2996        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
90/18311    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
NGE28       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
Z2491       .......... .......... .......... .......... .......... .......... .......... .......K .......... .......... .... 426
F6124       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
BZ232       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .... 426
```

Figure 7.9: Multiple sequence alignment of NMB1040 amino acid sequences in 25 meningococcal strains, including MC58 (labelled in blue), using ClustalW with default settings. The amino acid sequence in MenB sequence strain (MC58) was used as reference sequence. All strains used were MenB strains, except F6124 (MenA), 90/18311 (MenC) and NG P165 (NT). Sequences of the homologues genes in serogroup A (Z2491) and serogroup C (FAM18) sequence strains were also included in the alignment (blue text).

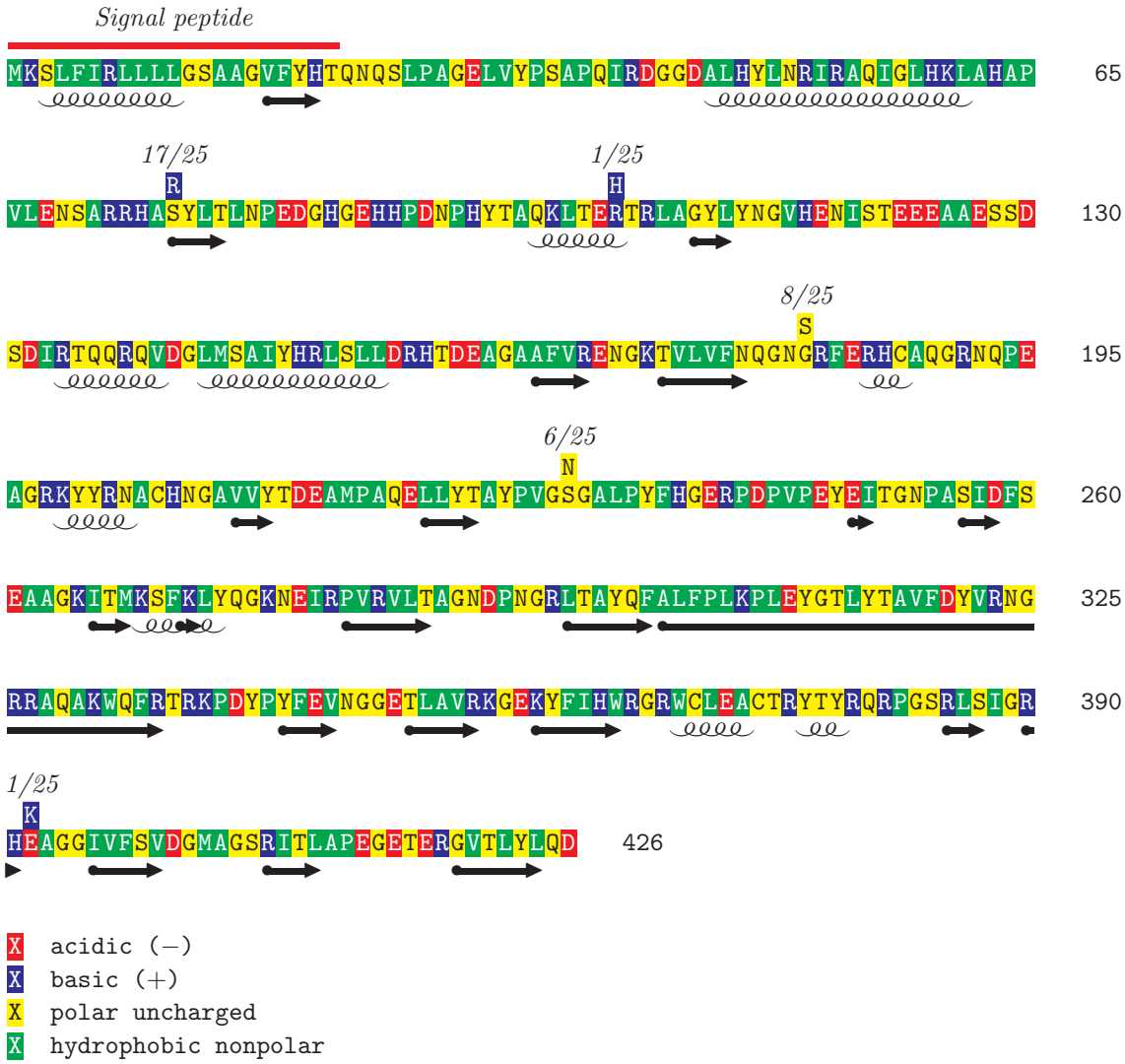


Figure 7.10: Secondary structure predictions for NMB1040 using the Jnet algorithm (Cuff & Barton, 2000) mapped to the sequence of the gene (constructed using TEXshade (Beitz, 2000)). Amino acids are coloured by their functional characteristics. Symbols above the primary sequence show positions of variable residues (and their frequency) among strains (see multiple alignment in Figure 7.9 for details).

7.2.2.4 Sequence variability of NMB1882

NMB1882, annotated as a TonB-dependent receptor, was found to be up-regulated upon growth in blood. The protein is predicted to be located in the outer membrane (by PSORTb v2.0 (Gardy *et al.*, 2005)) and contains membrane-spanning β-barrels (PRED-TMBB (Bagos *et al.*, 2004a,b)). The location within the protein of these β-barrels is shown in Figures 7.11 and 7.12. The corresponding proteins in MenA and MenC sequence strains (Z2491 and FAM18) are 99% identical to the MC58 protein.

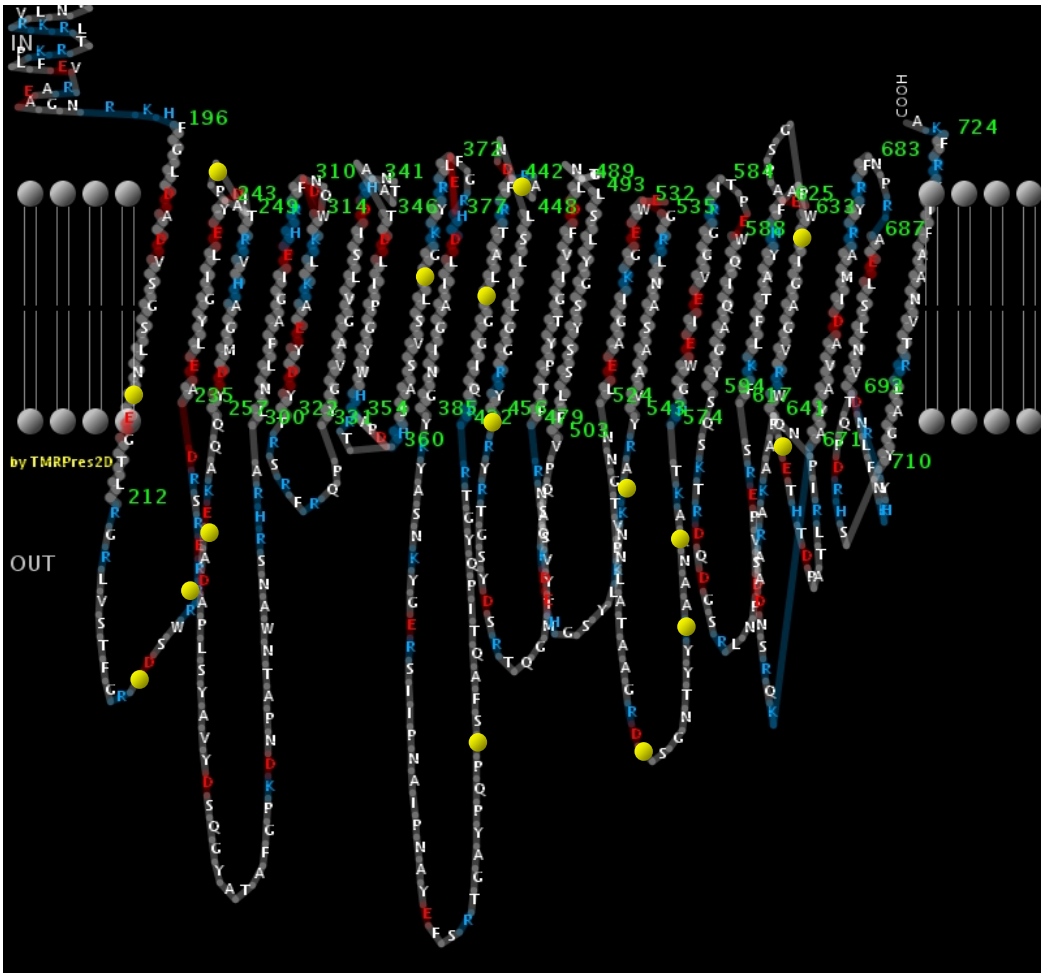


Figure 7.11: Topology model of membrane protein NMB1882. Representation of putative membrane spanning β-barrels in NMB1882 predicted using PRED-TMBB. The image represents the result from the posterior decoding method. Yellow balls indicate residues with sequence variability (details in Figure 7.13). Positively charged amino acids are coloured blue and negatively charged are coloured red.

A genomic region containing the gene was sequenced in the collection of meningococcal strains. The gene could be amplified from all strains. A multiple sequence alignment of the 14 protein sequences [1], deduced from the nucleotide sequence, and the NMB1882 homologues in MenA strain Z2491 and MenC strain FAM18 is shown in Figure 7.13. For some of the strains the complete sequence of the full length protein has not been determined. This was due to undetermined bases in the sequence trace, even though the sequencing of these amplicons was repeated at least twice. However, overall only limited sequence variability was found for NMB1882 in this set of meningococcal strains. Figure 7.12 shows the functional characteristics of amino

[1] inadequate quality sequence obtained from the remaining five amplified DNA segments

acids over the full length of the protein, highlighting potential differences arising from 19 sequence variants found. Ten of these variants is located in any of the predicted surface exposed loops (Figures 7.11 and 7.12), with the eighth loop (residues 544 – 573) being the most variable. This suggests that the eighth loop may be of antigenic significance.

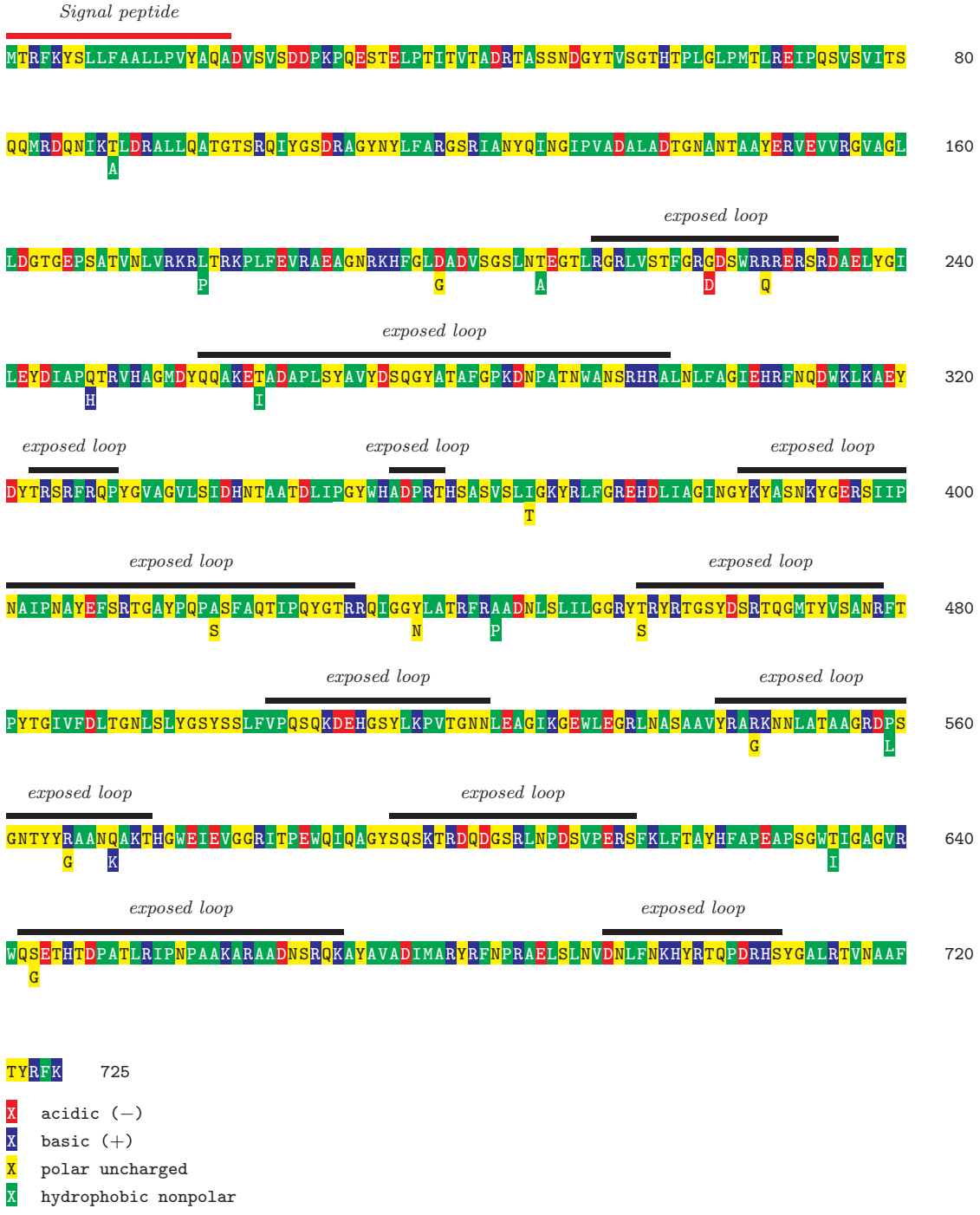


Figure 7.12: Secondary structure predictions for NMB1882 using the Jnet algorithm (Cuff & Barton, 2000) mapped to the sequence of the gene (constructed using TEXshade (Beitz, 2000)). Amino acids are coloured by their functional characteristics. Symbols below the primary sequence show positions of variable residues (and their frequency) among strains (see multiple alignment in Figure 7.13 for details).

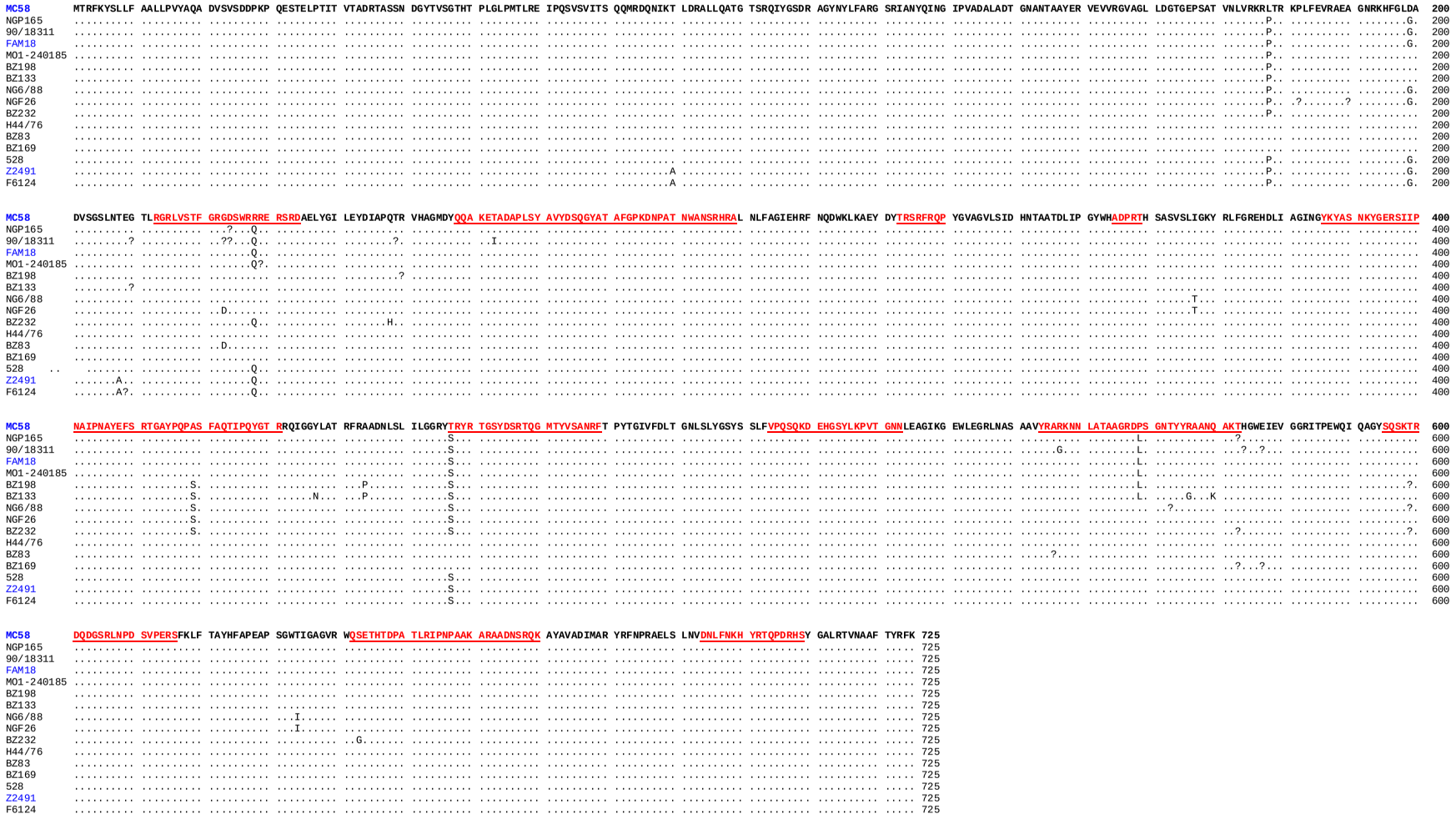


Figure 7.13: Multiple sequence alignment of NMB1882 amino acid sequences in 16 meningococcal strains, including MC58 (labelled in blue), using ClustalW with default settings. The amino acid sequence in MenB sequence strain (MC58) was used as reference sequence. All strains used were serogroup B strains, except F6124 (MenA), 90/18311 (MenC) and NG P165 (NT). The MenA (strain Z2491) and MenC (strain FAM18) genome sequence strains were also included for comparison (labelled in blue). Predicted external loops are indicated in red in the alignment.

7.2.2.5 Sequence variability of NMB1961

NMB1961 was found to be up-regulated in whole human blood at 20, 40, 60 and 90 min post-infection (Chapter 6). NMB1961 was also up-regulated at 60 min during incubation in plasma (Chapter 5). The gene encodes a 275 amino acids protein annotated as a VacJ-like lipoprotein through its sequence similarity to a domain in VacJ, a *Shigella flexneri* outer membrane lipoprotein required for intercellular spreading (Suzuki *et al.*, 1994). NMB1961 is predicted to be located in the outer membrane, and the protein has previously been detected in OMVs isolated from *N. meningitidis* (Ferrari *et al.*, 2006). Alignment of the sequence (both nucleotide and amino acid) from MenB strain MC58 versus the homologues in a MenA strain (Z2491) and a MenC strain (FAM18) indicated that the gene is highly conserved in meningococci (Table 7.1). The nucleotide sequence of the Z2491 homologue is 99% identical over 765 of 768 nucleotides, but is 12 nt longer then the MC58 gene due to a perfect repeat of the sequence GGAACACAACCT at the extreme 3' end of the gene. The protein sequences are identical in MC58 and FAM18 over the whole length of the protein, and in Z2491 up to position 275 and the FA1090 amino acid sequence is 94% identical to NMB1961 from MC58. Secondary structures (predicted using Jnet (Cuff & Barton, 2000)) mapped to the protein sequence (with amino acids shaded by characteristics) is shown in Figure 7.14.

Genomic regions containing the gene were amplified from the whole set of diverse strains (Table 2.1). The nucleotide sequences and the corresponding deduced amino acid sequences were aligned using the programme ClustalW (Larkin *et al.*, 2007) (Supplementary figure D.4), and differences to the MC58 sequence recorded. Ten different nucleotide polymorphisms were found in NMB1961. The most common single nucleotide polymorphisms were changes at positions 216 (9 out of 20 strains), 393 (17 out of 20 strains), 570 (5 out of 20 strains) and 591 (10 out of 20 strains). The nucleotide sequence from the serogroup A strain F6124 was 12 nucleotides longer than the MC58 sequence due to repeat at the extreme 3' end of the gene, also found in serogroup A genome sequence strain Z2491 (Alignment in Supplementary figure D.3).

Polymorphisms in the coding sequence only contributed to one variant in the amino acid sequence. The variant at position 8 (F_8 to A_8) were found in strains 297-0, BZ 232 and F6124. Additionally, the protein is 4 aa longer in strain F6124 (and Z2491) than the MC58 protein, due to the repeat.

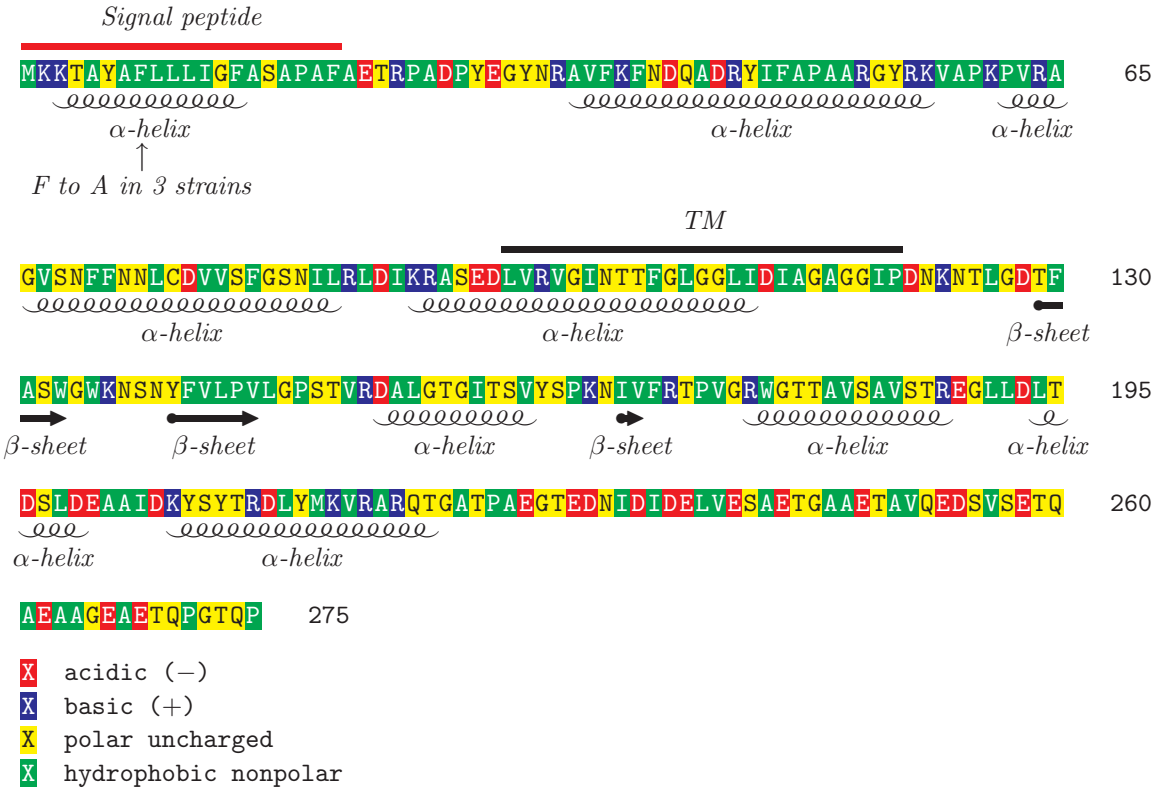


Figure 7.14: Secondary structure predictions for NMB1961 using the Jnet algorithm (Cuff & Barton, 2000) mapped to the sequence of the gene (constructed using TEXshade (Beitz, 2000)). Amino acids are coloured by their functional characteristics.

7.2.2.6 Sequence variability of *apbE* (NMB0563)

The gene *apbE* (NMB0563) is annotated as a membrane-associated lipoprotein involved in thiamine biosynthesis. The protein is most likely membrane-anchored, either in the inner or outer membrane. The gene was found to be consistently up-regulated during incubation in whole human blood (Chapter 6) and during growth in FFP 1 (Chapter 5). NMB0563 is 99% identical, at the amino acid level, to homologues genes in Z2491 and FAM18 genomes and 96% identical to a FA1090 homologue (Table 7.1).

Genomic regions containing the gene could be amplified from all meningococcal strains except NG 6/88, NG F26, SWZ107 and NG H36, using primers designed to

bind MenB strain MC58, MenA strain Z2491 and MenC strain FAM18. Direct sequencing of the gene in 18 meningococcal strains revealed that *apbE* is well conserved (Figure 7.15). The most commonly found variant was a change from N_{269} to S_{269} in 12 of the strains (Figure D.2).

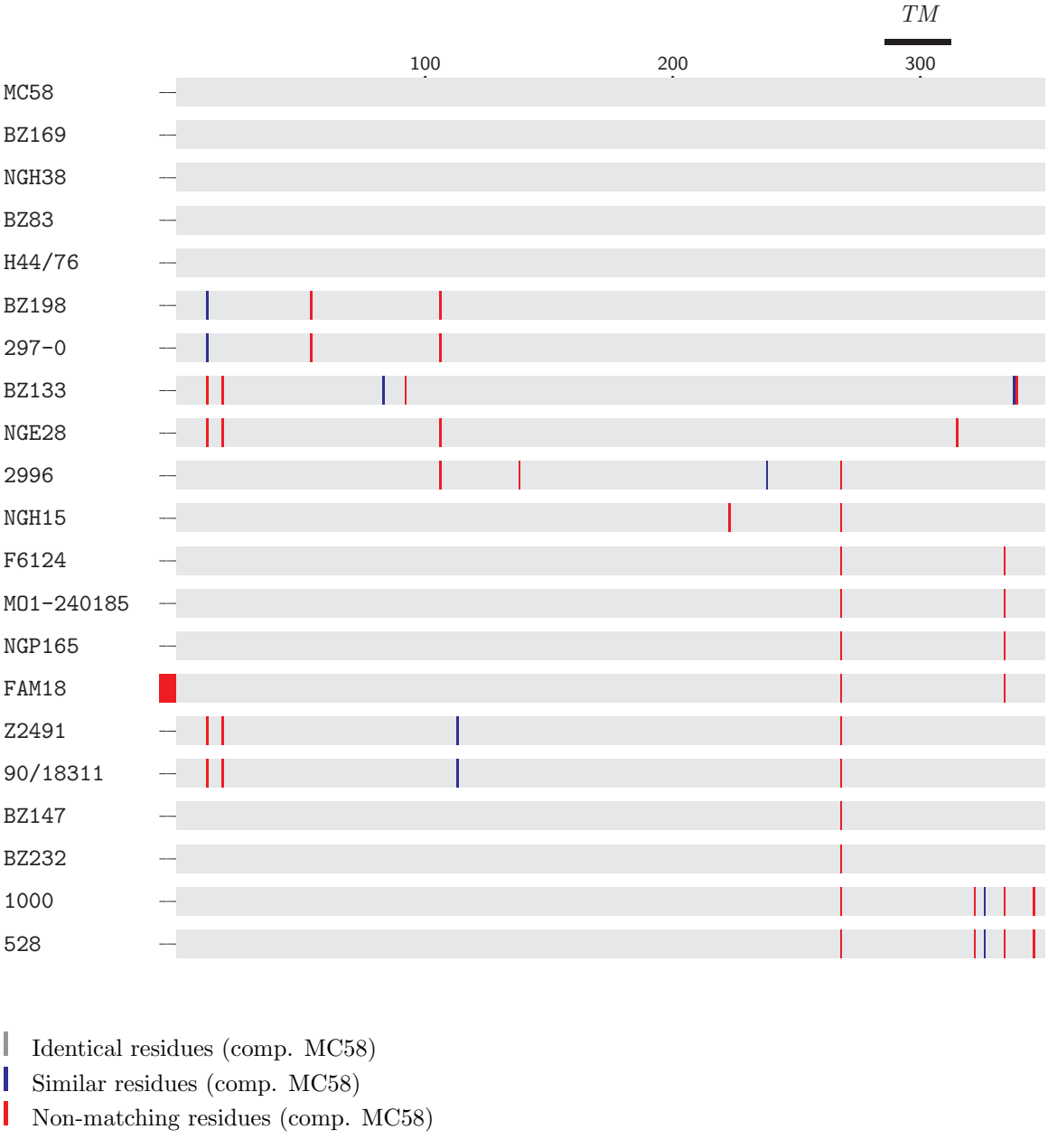


Figure 7.15: Alignment fingerprint of the 21 amino acid sequence of *apbE* produced using the TEXshade package (Beitz, 2000). Residues that are identical, similar or non-matching the MC58 sequence are indicated (based on ClustalW alignment). The position of a transmembrane helix, indicated by TM in the figure, was predicted using TMpred (`http://www.ch.embnet.org/software/TMPRED_form.html`).

7.2.2.7 Sequence variability of NMB0267

NMB0267 forms part of an operon together with NMB0266 and NMB0268. The gene was found to be up-regulated at all time-points in blood and plasma. Its product has sequence similarity to the so-called rare lipoprotein A (RlpA), a member of a family of lipoproteins that are anchored to the inner membrane facing the periplasm and which functions in cell membrane biogenesis. NMB0267 is fairly well conserved in both meningococci and gonococci and is 96% and 95% identical, at the amino acid level, to homologous genes in the Z2491 and FAM18 genomes and 92% identical to a FA1090 homologue (Table 7.1).

Direct sequencing of the gene in the collection of meningococcal strains showed that NMB0267 is well conserved, although four variants were found towards the C-terminal of the protein (Figure 7.16). Figure 7.17 shows the characteristics of residues over the full length of the protein, highlighting a difference in hydrophobicity in the C-terminal of the protein arising as a result of the different sequence variants in fourteen of the strains in comparison to strain MC58.

```
MC58        MTLTRKTLFL LTAAFGTHSL QTASADAVVK AEKLHASANR SYKVAGKRYT PKNQVAEFTQ TGNASWYGGR FHGRKTSGGE RYDMNAFTAA HKTLPIPSYV  100
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ198       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
297-0       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
Z2491       .......... .....I..F ........R .......... .......... .......... .......... .......... .......... ..........  100
F6124       .......... .....I..F ........R .......... .......... .......... .......... .......... .......... ..........  100
1000        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
528         L......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
FAM18       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGP165      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGE28       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGH36       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
90/18311    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
2996        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ232       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100

MC58        RVTNTKNGKS VIVRVNDRGP FHGNRIIDVS KAAAQKLGFV NQGTAHVKIE QIVPGQSAPV AENKDIFIDL KSFGTEHEAQ AYLNQAAQNF AVSSSGTNLS  200
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ198       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
297-0       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
Z2491       .......... .......... .......... .......... .......... .......... .......... .......... .........L .S.A.NP...  200
F6124       .......... .......... .......... .......... .......... .......... .......... .......... .........L .S.A.NP...  200
1000        .......... .......... .......... .......... S......... ........L. .......... .......... .........L .S.A.NP...  200
MO1-240185  .......... .......... .......... .......... S......... ........L. .......... .......... .........L .S.A.NP...  200
528         .......... .......... .......... .......... S......... ........L. .......... .......... .........L .S.A.NP...  200
NGH38       .......... .......... .......... .......... S......... ........L. .......... .......... .........L .S.A.NP...  200
FAM18       .......... .......... .......... .......... S.........  .....K.... .......... .......... ..........  .A.T.SP..A  200
NGP165      .......... .......... .......... .......... S.........  .....K.... .......... .......... ..........  .A.T.SP..A  200
NGE28       .......... .......... .......... .......... S.........  .....K.... .......... .......... ..........  .A.T.SP..A  200
NGH36       .......... .......... .......... .......... S.........  .....K.... .......... .......... ..........  .A.T.SP..A  200
90/18311    .......... .......... .......... .......... S.........  .....K.... .......... .......... ..........  .A.T.SP..A  200
2996        .......... .......... .......... .......... S.........  .....K.... .......... .......... ..........  .A.T.SP..A  200
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... ..........  .A.A.SP..A  200
BZ232       .......... .......... .......... .......... .......... .......... .......... .......... ..........  .A.A.SP..A  200

MC58        VEKRRYEYVV KMGPFTSQER AAEAEAQARG MVRAVLTAG 239
BZ83        .......... .......... .......... ......... 239
H44/76      .......... .......... .......... ......... 239
SWZ107      .......... .......... .......... ......... 239
NGF26       .......... .......... .......... ......... 239
BZ169       .......... .......... .......... ......... 239
NG6/88      .......... .......... .......... ......... 239
BZ198       .......... .....A.... .......... ......... 239
297-0       .......... .....A.... .......... ......... 239
NGH15       .......... .....A.... .......... ......... 239
Z2491       .......... .....A.... .......... ......... 239
F6124       .......... .....A.... .......... ......... 239
1000        ....H..... .....S.... .......... ......... 239
MO1-240185  ....H..... .....S.... .......... ......... 239
528         ....H..... .....S.... .......... ......... 239
NGH38       ....H..... .....S.... .......... ......P.. 239
FAM18       I......... .....A.... .......... ..QT..... 239
NGP165      I......... .....A.... .......... ..QT..... 239
NGE28       I......... .....A.... .......... ..QT..... 239
NGH36       I......... .....A.... .......... ..QT..... 239
90/18311    I......... .....A.... .......... ..QT..... 239
2996        I......... .....A.... ........H. ..QT..... 239
BZ147       I......... .....A.... .......... ......... 239
BZ232       I......... .....A.... .......... ......... 239
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Figure 7.16: Multiple sequence alignment of NMB0267 amino acid sequences in 24 meningococcal strains, including MC58 (labelled in blue), using ClustalW with default settings. The amino acid sequence in MenB sequence strain (MC58) was used as reference sequence. All strains used were MenB strains, except F6124 (MenA), 90/18311 (MenC) and NG P165 (NT). The MenA (strain Z2491) and MenC (strain FAM18) genome sequence strains were also included for comparison (labelled in blue).

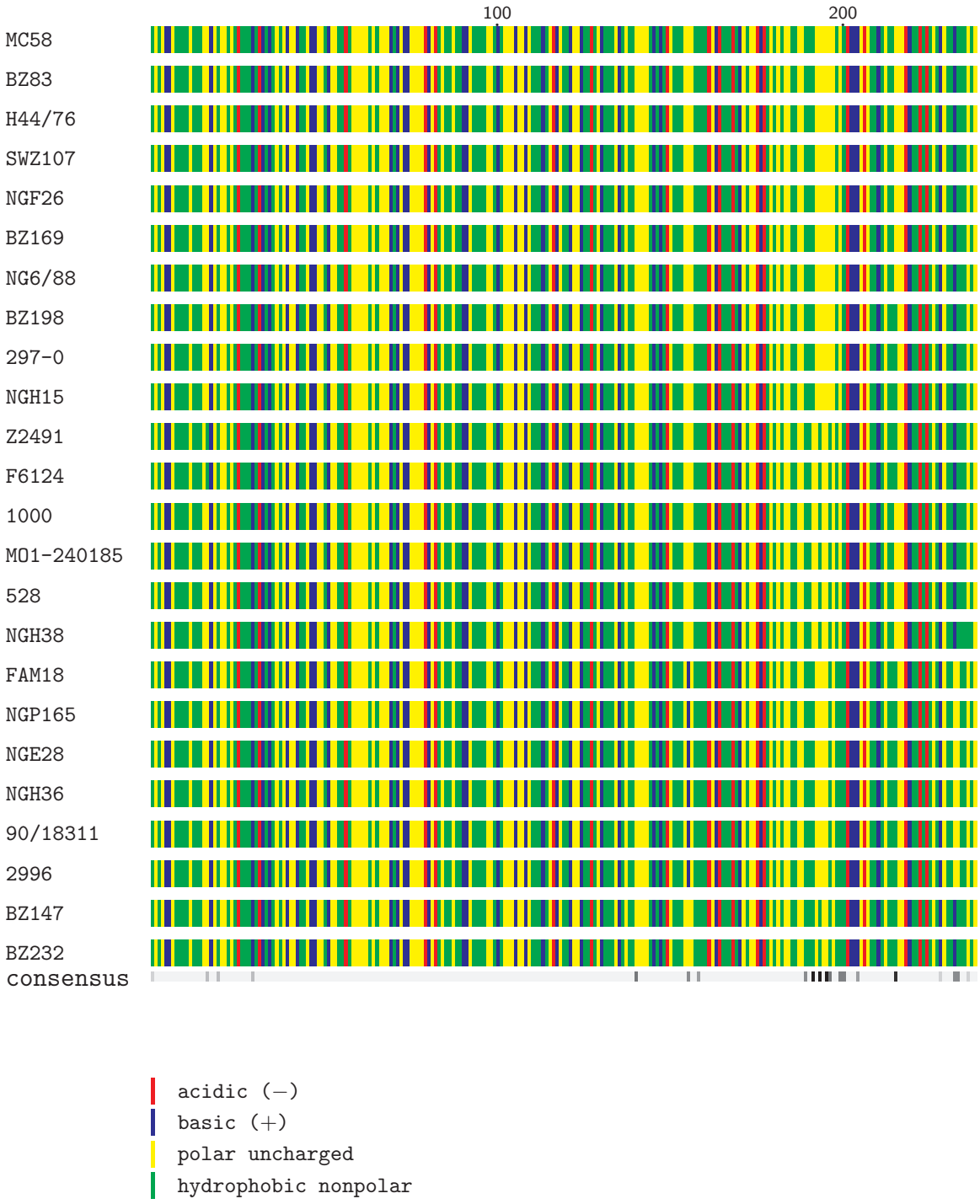


Figure 7.17: Graphical representation of the multiple alignment of protein sequences of NMB0267 (constructed using TEXshade (Beitz, 2000)), visualising regions of charged, hydrophobic or hydrophilic nature. In the consensus line a lighter colour represent similar residues, whereas a darker colour indicates less conserved residues.

7.3 Discussion

Many surface exposed meningococcal proteins, such as PorA, are highly variable among strains. The hypothesis I proposed at the start of this project was that proteins highly expressed during colonisation (modelled by adherence to epithelial cells) may have relatively more variable sequence than proteins more highly expressed on the surface of the bacteria in the course of bloodstream spread. Sequence variability in genes expressed during meningococcal *in vivo* growth, such as colonisation, is likely to be the result of immune selection pressure. In this chapter the sequences of a set of *N. meningitidis* genes encoding putative surface-exposed proteins differentially up-regulated in blood revealed limited sequence variability amongst all the selected strains. Limited sequence variability of these genes may indicate that they are not expressed on the bacterial surface during infection, that they are under selective pressure for attaining functionality, or - in support of my hypothesis - that they are expressed at high levels on the surface only during growth in blood.

Protein NMB1882 is going to be discussed in more detail as it is predicted to contain surface exposed loops, which may be subjected to immune selection pressure. Sequencing of the gene revealed that sequence variants were more common in one of its surface exposed loops, which suggest that it may be of antigenic significance. Sequence variability of the surface exposed regions was compared to that of surface exposed loops of the vaccine candidates PorA and NspA. The PorA protein has three highly variable surface exposed immunogenic regions (VR1, VR2 and VR3). In contrast NspA has been found to have very limited sequence variability in external loops (Moe *et al.*, 1999). The limited sequence variability of outer membrane loops of this protein has been suggested to be due to limited exposure to the environment - the loops potentially being masked by the exopolysaccharide. In clinical trials recombinant NspA protein did not induce protective antibodies, which support the hypothesis that the protein is not accessible to the extracellular milieu, although negative results from clinical trials could also result from incorrect tertiary structure of antigenic epitopes in the recombinant proteins used. While sequence variability in the predicted surface

exposed regions of NMB1882 is limited in comparison to variation of the PorA VR1 and VR2 regions (Supplementary figure D.5), the protein was found to be slightly more variable than NspA.

Future work Variability of meningococcal surface proteins can also occur due to other processes (discussed in detail in Section 1.4.1.2), mainly phase variation switching on or off expression or other processes that regulate protein abundance (such as different strength promoters or regulatory elements). The influence of these processes on the expression of the studied proteins will be explored in future experiments. It would be of value to assess relative protein abundance of these proteins in the 23 strains, and also on their cellular location upon growth in plasma or blood. However, within the time frame of this project the necessary antibodies were unavailable and further studies on these proteins will be the aim of a future follow up study. Work will include assessing both expression of transcripts and proteins among meningococcal strains. First, potential differences in transcription of the genes among meningococcal strains are going to be analysed using standard RT-PCR or TaqMan analysis. Second, protein abundance is going to be assessed (using western blotting and flow cytometry) under normal culturing conditions in broth and during growth in plasma and blood.

Chapter 8

General conclusions and future work

Meningococci commonly colonise the nasopharyngeal mucosa, and occasionally invade to cause life-threatening septicaemia and/or meningitis. Many steps in this disease progression are currently unknown. Greater understanding into pathogenicity of this devastating pathogen is therefore of great importance. Vaccines are the most effective preventive measure for meningococcal disease. However, currently no broad-range protective vaccine is available for serogroup B meningococci, the most common serogroup in Europe.

The work presented here was primarily motivated by the need for new serogroup B vaccine candidates, focusing on antigenic OMPs. It was the aim of this study to identify genes up-regulated under bacterial growth in the bloodstream, encoding surface exposed proteins that may be suitable as vaccine targets. I hypothesized that their candidacy would be strengthened in the case of genes down-regulated or expressed at low levels in the nasopharynx. Using a global approach to characterise changes in the bacterial gene expression in different *ex vivo* models of septicaemia, I identified candidates and confirmed relatively reduced variability in genes with this pattern of expression.

8.1 Transcriptional profiling of meningococci in *ex vivo* models of infection

In order to better understand the genetic response of *N. meningitidis* during growth in the bloodstream on a genome-wide scale, DNA microarray analysis was utilised to quantify transcriptional changes in among all known genes in the genome of *N. meningitidis* strain MC58 in response to culturing in whole human non-bactericidal plasma or blood (Chapters 5 and 6). To my knowledge this is first study to describe the transcriptional response of meningococci to growth in blood. Expression profiling of meningococci in blood or plasma showed that growth under these conditions results in the differential expression of a large proportion of the genome. Growth in blood to 240 min resulted in a significant shift in transcription of 1135 genes (in relation to time-point 0 min). Comparing the transcriptional responses for meningococci incubated in plasma and blood at corresponding time-points, there was a greater response to growth in blood. This likely derives from the more complex environment, as well as the presence of host cells in blood samples. Similar trends in expression patterns were, however, observed in these two growth conditions. In both these experiments a majority of the up-regulated genes encode proteins of unknown function, reflecting the currently limited functional annotation of meningococcal genes. As detailed in Chapter 5 and 6, incubation of meningococci in human blood or plasma samples resulted in an up-regulation of genes involved in metabolic adaptations to the host environment. Transcriptional responses suggested a need for both *de novo* synthesis, as well as transport of molecules from the extracellular milieu. I also observed significant differential regulation of genes involved in bacterial stress responses and cell detoxification, such as efflux proteins, particularly during growth in blood, indicating a potential response to innate immune responses and host cell attacks.

The hypothesis defined in Section 1.5.1 were tested by determining the sequence variability of seven of the genes up-regulated in plasma and/or blood (Chapter 7). Limited sequence variability of these genes was observed for the tested strains, suggesting that they are indeed comparatively invariant, in support of my hypothesis.

It is however possible that they may differ in transcript levels or protein abundance in between meningococcal strains as a result of for example phase-variation. This is going to be examined in future work.

8.2 Conclusions and improvements to the study

In this thesis I have described methods and results of the meningococcal genome-wide transcriptional profile in *ex vivo* models of infection. Some of the transcriptional responses in blood and plasma were comparable to those previously observed in similar studies in other pathogens, such as group A Streptococcus and *P. multocida*. The *ex vivo* whole blood or plasma models have here been used with the objective to find new antigens, and as such should be suitable for studies to find new targets of interest for vaccines. I propose that the *ex vivo* whole blood model can be used to model some responses of the bacteria during septicaemia, although not completely replicating the complex environment encountered by the bacteria during infections. The work presented here may, besides being used to find vaccine targets, have implications for identifying targets for other therapeutic interventions, such as novel anti-bacterial drugs, and contribute to our basic understanding of behaviour of meningococci in the bloodstream. As such, results from studies presented here can be built on to study the importance of specific processes during invasive infection.

For better characterisation of the differences in meningococcal gene expression in the bloodstream to that during colonisation, some improvements can be made to *in vitro* models of both these bacterial growth habitats. Explorations of meningococcal transcriptional profiles during colonisation have relied on models utilising epithelial cells, or other cell types. A microarray data set of bacterial gene expression during attachment to epithelial cells (for up to 3 hr) (Grifantini *et al.*, 2002b) was used to approximate the transcriptional profile of bacteria during nasopharyngeal colonisation, and used in all comparisons in this thesis. Attachment of bacteria to epithelial cells for a limited period of time does not model meningococcal colonisation on the mucosal membrane, which in the host can last for days or weeks. Furthermore, the

environment on the nasopharyngeal mucosal surface is inhabited by other species of bacteria, which is likely to affect growth as well as gene and protein expression of the meningococci. Such systems of complex microbial ecology would however be extremely difficult to model *in vitro*. Better models of meningococcal nasopharyngeal colonisation, such as organ cultures (using explants), have been used to highlight the importance of specific genes during colonisation (Exley *et al.*, 2009). The difficulty in isolating sufficient amounts of RNA from the limited number of bacteria that can be collected from such models has so far limited the possibility to study the genome-wide transcriptional response of bacteria to growth in such systems. Other possible models of colonisation could include longer length incubation of the bacteria with human cells or biofilm-like growth on an abiotic surface, from which isolation of bacterial RNA should be less problematic.

Solutions that could be envisioned for the problem of isolating sufficient amounts of RNA include amplification of the RNA species prior to analysis on microarrays. This strategy has already been used to explore, for example, the gene expression of *M. tuberculosis* in sputum (Garton *et al.*, 2008). During this project I evaluated the use of RNA amplification (using a poly-A based system developed by Ambion) as a way of increasing the amount of RNA and found that the method was reliable, although considerable variability was observed in comparison to unamplified RNA samples, making amplification a less attractive solution. Bacterial RNA isolated from a mixture of bacteria and host cells may contain small amounts of eukaryote RNA. Using a poly-A based system contaminating host RNA may be disproportionally amplified. Specific priming of bacterial transcripts (in a mixture with host material), as described by Lawson & Johnston (2006) and Waddell *et al.* (2008), could be used to avoid this potential short-coming of the poly-A based system.

During this project I have also considered improvements to the models of bloodstream infection presented in this thesis. Potential improvements include better control of nutrients (using for example a flow system), better modelling of the environment in blood vessels by using a system containing endothelial cells, and lower initial

loads of bacteria to closer resemble the scenario in early stages of invasive infection. The use of an animal model could also have been an alternative approach to those explored here. However, as humans are the only natural host for meningococci many of the proteins that are important for establishing disease are lacking (or divergent) in other animals. Therefore, to model meningococcal disease it is advantageous to use material from humans rather than animals.

Developments of new technologies for exploring the complete RNA profile of an organism using high-throughput sequencing methods (454 sequencing or Solexa/Illumina deep sequencing), so-called 'RNA-Seq' (Wang *et al.*, 2009) may also lead to significant improvements to studies of the bacterial transcriptome during infection, although so far such transcriptome studies have only been performed in eukaryote systems (Mortazavi *et al.*, 2008; Vera *et al.*, 2008). They have however been used for studying the bacterial metatranscriptome (Gilbert *et al.*, 2008) and for deduction of RNA interactions with protein Hfq in Salmonella (Sittka *et al.*, 2008). Developments of these methods, as well as improved strategies for RNA isolation and amplification, may ultimately facilitate studies of meningococcal gene expression in the natural host by recovery of bacterial RNA from patient material, such as the nasopharynx or blood.

8.3 Future work

Data derived from transcriptional profiling experiments in this work are currently being used to examine specific genes of interest as vaccine targets. Experiments following from work in this thesis will primarily focus on answering the following questions: are the genes expressed (mRNA and protein) in 'all' meningococcal strains (1); does up-regulation of these genes correlate with increased quantities of protein (2); are these proteins expressed on the surface of meningococci during incubation in blood or plasma (3); do antibodies to these proteins have bactericidal activity against meningococci (4); and do these proteins (either recombinant proteins or expressed on OMVs) protect mice against experimental infection when given as a vaccine (5)?

Potential differences in transcription of the genes among meningococcal strains are

being analysed using TaqMan analysis and protein abundance assessed (using western blotting and flow cytometry) under normal culturing conditions in broth and during growth in plasma and blood. To confirm their cellular location on meningococci under growth in plasma and blood, flow cytometry will be used.

A subset of the genes identified in this project will be over-expressed in commensal Neisseria (*N. flavescens*), using the pMIDG system developed in our laboratory by O'Dwyer *et al.* (2004). Surface expression will be confirmed using flow cytometry. Finally, to test their ability to induce protective bactericidal antibodies OMVs isolated from such strains or *E. coli* recombinant proteins will be used to vaccinate mice, to determine if they protect against experimental meningococcal infection.

In addition, deletion mutants of metabolic genes found to be important for growth in models used in this work will be used to determine their respective contribution to bacterial survival and resistance in infection.

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Appendix A

Supplementary data to Chapter 4

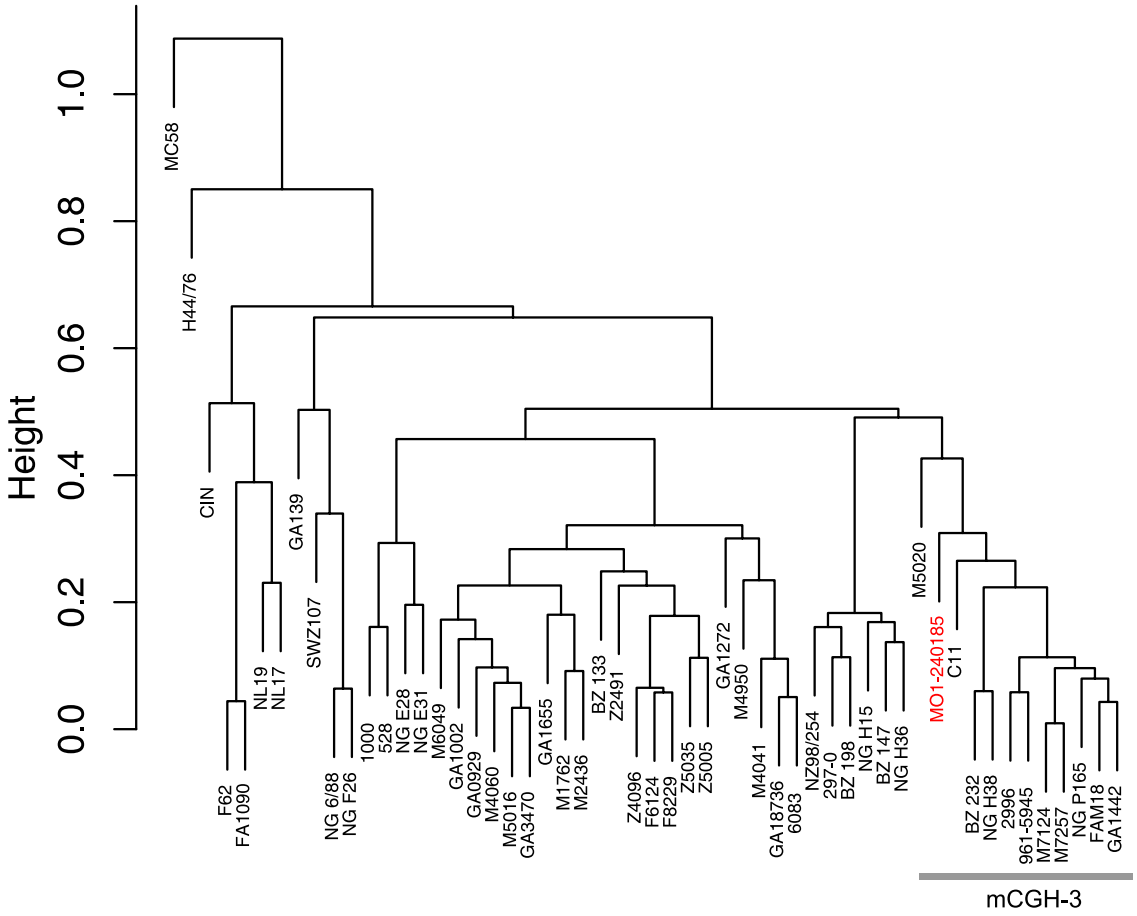


Figure A.1: Hierarchical clustering of Hotopp *et al.*, 2006 data and genomotyping data (strain name in red) from this thesis. The hierarchical clustering was performed using the hclust function available under R, the distance metric was based on (1–correlation) (Pearson) and an average linkage method was used.

Appendix B

Supplementary data to Chapter 5

Table B.1: Genes up-regulated at 60 min during growth in FFP 1 in comparison to meningococci incubated in medium. Genes are divided into functional categories according to TIGR designations.

ID	Name	Annotation	$\log_2$ ratio	P-value*
Amino acid biosynthesis				
NMB0358	*aroE*	shikimate 5-dehydrogenase	0.716	2.24E-02
NMB0763	*cysK*	cysteine synthase	1.306	1.22E-03
NMB1530	*dapE*	succinyl-diaminopimelate desuccinylase	0.888	1.75E-02
Biosynthesis of cofactors, prosthetic groups, and carriers				
NMB0392	*nadB*	l-aspartate oxidase	0.877	3.73E-03
NMB0394	*nadA*	quinolinate synthetase A	0.782	6.25E-03
NMB0396	*nadC*	nicotinate-nucleotide pyrophosphorylase	0.876	3.12E-02
NMB0539	*hemC*	porphobilinogen deaminase	0.644	2.13E-02
NMB1156	*cysG-1*	siroheme synthase	1.580	1.03E-03
NMB1194	*cysG-2*	siroheme synthase	1.626	2.25E-04
Cell envelope				
NMB0285		putative O-antigen acetylase (frameshift)	0.586	4.49E-02
NMB0357	*mtgA*	monofunctional biosynthetic peptidoglycan transglycosylase	1.164	4.26E-03
NMB1820	*pglB*	pilin glycosylation protein	0.929	4.38E-02
NMA0772		pilin gene inverting protein homolog PivNG	2.273	2.48E-02
Cellular processes				
NMB0005	*arsC*	arsenate reductase	0.695	4.58E-02
NMB0277	*mviN*	virulence factor	0.786	4.29E-02
NMB1820	*pglB*	pilin glycosylation protein	0.929	4.38E-02
Central intermediary metabolism				
NMB1151	*cysI-1*	sulphite reductase hemoprotein, β component	1.585	2.83E-03
NMB1152	*cysJ-1*	sulphite reductase (NADPH) flavoprotein, α component	1.162	2.20E-02
NMB1153	*cysN-1*	sulphate adenylyltransferase, subunit 1	1.735	1.49E-03
NMB1154	*cysD-1*	sulphate adenylyltransferase, subunit 2	1.580	8.65E-04
NMB1155	*cysH-1*	phosphoadenosine phosphosulphate reductase	1.428	1.22E-03
NMB1189	*cysI-2*	sulphite reductase hemoprotein, β component	1.686	2.59E-03
NMB1190	*cysJ-2*	sulphite reductase (NADPH) flavoprotein, α component	1.398	2.88E-03
NMB1191	*cysN-2*	sulphate adenylyltransferase, subunit 1	1.745	5.77E-04
NMB1192	*cysD-2*	sulphate adenylyltransferase, subunit 2	1.653	7.12E-04
NMB1193	*cysH-2*	phosphoadenosine phosphosulphate reductase	1.404	3.00E-03

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Table B.1 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
DNA metabolism				
NMB0533	*nth*	endonuclease III	0.675	7.15E-03
NMB0835		putative type I restriction enzyme EcoR124II R protein	0.938	2.98E-03
NMB1233	*recD*	exodeoxyribonuclease V, α subunit	0.945	1.22E-03
NMB1673	*tag*	DNA-3-methyladenine glycosylase I	0.855	4.89E-03
Energy metabolism				
NMB0530		β-hexosaminidase	0.689	2.60E-02
NMA1934	*gcvP*	glycine dehydrogenase	0.856	4.76E-03
Fatty acid and phospholipid metabolism				
NMB1674		GDSL lipase family protein	0.904	4.89E-03
NMB1696	*acp-2*	acyl carrier protein	0.717	4.95E-02
Hypothetical or unassigned proteins				
NMB0108		hypothetical protein	0.598	4.11E-02
NMB0260		hypothetical protein	0.591	4.38E-02
NMB0305		hypothetical protein	0.697	3.02E-02
NMB0395		conserved hypothetical protein	1.191	1.22E-03
NMB0417		hypothetical protein	0.768	2.98E-03
NMB0534		conserved hypothetical protein	0.662	4.95E-02
NMB0793		hypothetical protein	0.939	3.00E-03
NMB0939		conserved hypothetical protein	0.604	3.26E-02
NMB1195		hypothetical protein	0.835	3.77E-02
NMB1316		hypothetical protein	0.671	2.31E-02
NMB1531		conserved hypothetical protein	0.702	3.51E-03
NMB1532		conserved hypothetical protein	0.788	4.36E-03
NMB1672		conserved hypothetical protein	0.655	2.17E-02
NMB1675		hypothetical protein	0.835	1.48E-02
NMB1971		conserved hypothetical protein	0.595	3.30E-02
NMB2099		conserved hypothetical protein	0.588	2.39E-02
NMC0876		hypothetical protein (fragment)	0.604	2.69E-02
Mobile and extrachromosomal element functions				
NMC0866		putative phage-related protein	1.295	1.73E-02
NMC0867		putative phage-related protein	1.050	1.34E-02
Protein fate				
NMB0836	*clpA*	ATP-dependent Clp protease, ATP-binding subunit	0.673	1.34E-02
NMB1820	*pglB*	pilin glycosylation protein	0.929	4.38E-02
Protein synthesis				
NMB0112	*rsmB*	16S RNA methyltransferase	1.763	4.72E-03
NMB0795	*pth*	peptidyl-tRNA hydrolase	0.689	1.75E-02
NMB1859	*queA*	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	0.624	1.54E-02
Regulatory functions				
NMB0437	*nsrR*	nitric oxide sensitive repressor	0.746	2.59E-02
NMB1792		sensor histidine kinase	0.786	3.46E-02
Transcription				
NMB0689	*greB*	transcription elongation factor	0.785	4.28E-03
Transport and binding proteins				
NMB0034		high-affinity iron transporter	0.663	3.55E-02
NMB0209	*kefB*	glutathione-regulated potassium-efflux system protein	0.635	3.22E-02
NMB0787		amino acid ABC transporter, periplasmic substrate-binding protein	1.804	2.25E-04
NMB0788		amino acid ABC transporter, permease protein	1.365	1.03E-03
NMB0789		amino acid ABC transporter, ATP-binding protein	1.121	1.22E-03

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Table B.1 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB1362		putative oxalate/formate antiporter	0.632	4.37E-02
NMB1459		putative drug/metabolite transporter	0.770	2.31E-02
NMB1719	*mtrF*	efflux pump component	0.943	3.91E-02
Unknown function				
NMB0004		EpiH/GdmH-related protein	0.812	4.37E-02
NMB0994		acyl-CoA dehydrogenase family protein	1.305	1.41E-02
NMB0995		macrophage infectivity potentiator-related protein	2.094	2.25E-04
NMB1501		HesA/MoeB/ThiF family protein	0.596	2.39E-02
NMB1521		phytoene synthase-related protein	0.619	2.23E-02
NMB1670		PqiA family protein	0.635	6.23E-03
NMB1692		chorismate mutase-related protein	0.686	4.14E-02

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table B.2: Genes down-regulated at 60 min during growth in FFP 1 in comparison to meningococci incubated in medium. Genes are divided into functional categories according to TIGR designations.

ID	Name	Annotation	$\log_2$ ratio	P-value*
Amino acid biosynthesis				
NMB0678	*trpA*	tryptophan synthase, α subunit	-0.610	1.26E-02
NMB1031	*leuB*	3-isopropylmalate dehydrogenase	-0.755	6.25E-03
NMB1576	*ilvH*	acetolactate synthase III, small subunit	-1.149	6.88E-03
NMB1577	*ilvI*	acetolactate synthase III, large subunit	-1.033	2.04E-02
NMB1579	*hisG*	ATP phosphoribosyltransferase	-0.755	4.72E-03
Biosynthesis of cofactors, prosthetic groups, and carriers				
NMA1255	*ggt*	γ-glutamyltranspeptidase	-0.928	1.33E-02
Cell envelope				
NMB0068	*siaC*	polysialic acid capsule biosynthesis protein	-1.144	1.29E-02
NMB0069	*siaB*	polysialic acid capsule biosynthesis protein	-1.111	3.00E-03
NMB0070	*siaA*	polysialic acid capsule biosynthesis protein	-1.058	1.88E-03
NMB0088		putative outer membrane protein P1	-0.638	2.23E-02
NMB0828	*rfaD*	ADP-L-glycero-D-mannoheptose-6-epimerase	-0.711	4.95E-02
NMA0319	*mafB′*	putative MAFB alternative C-terminus	-0.964	1.22E-03
NMA0325	*mafA*	adhesin	-0.668	2.33E-02
Cellular processes				
NMB0170	*minC*	septum site-determining protein	-0.893	3.08E-03
NMB0172	*minE*	cell division topological specificity factor	-0.760	3.06E-02
NMB0364		FrpC operon protein	-0.820	3.65E-03
NMB0584		FrpC operon protein	-0.802	8.98E-03
NMB0585		putative iron-regulated protein FrpA	-1.066	1.22E-03
NMB0855		putative bacteriocin resistance protein	-0.878	5.50E-03
NMB1412		FrpC operon protein	-0.833	4.36E-03
NMB1414		FrpC operon protein	-0.852	2.83E-03
NMB1985	*iga2*	adhesion and penetration protein	-0.645	3.09E-02
Central intermediary metabolism				
NMB1305	*esd*	putative esterase	-0.606	3.72E-02
DNA metabolism				
NMB0076		methyltransferase HphIm(C) (authentic frameshift)	-0.729	4.54E-02
NMB0829	*hsdM*	type I restriction enzyme EcoR124II M protein	-0.615	1.29E-02
NMB0962	*uvrA*	excinuclease ABC, subunit A	-0.927	2.08E-02
NMB1032	*nlaIVR*	type II restriction enzyme NlaIV	-1.342	2.25E-04
NMB1033		modification methylase NlaIV	-0.754	3.09E-02
NMB1655		N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase	-0.624	3.46E-02

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Table B.2 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
Energy metabolism				
NMB0089	*pykA*	pyruvate kinase II	-0.858	5.52E-03
NMB0948	*sdhC*	succinate dehydrogenase, cytochrome b556 subunit	-0.655	1.84E-02
NMB0949	*sdhD*	succinate dehydrogenase, hydrophobic membrane anchor protein	-0.744	6.23E-03
NMB0950	*sdhA*	succinate dehydrogenase, flavoprotein subunit	-0.714	3.76E-02
NMB0951	*sdhB*	succinate dehydrogenase, iron-sulphur protein	-0.798	1.30E-02
NMB1723	*fixP*	cytochrome c oxidase, subunit III	-0.736	2.14E-02
NMB1724	*fixO*	cytochrome c oxidase, subunit II	-1.057	2.08E-02
NMB1725	*fixN*	cytochrome c oxidase, subunit I	-1.299	4.26E-03
NMB2154	*etfA*	electron transfer flavoprotein, α subunit	-1.089	9.44E-03
NMB2155	*etfB*	electron transfer flavoprotein, β subunit	-1.017	1.37E-02
Hypothetical or unassigned proteins				
NMB0065		hypothetical protein	-0.813	1.19E-02
NMB0091		hypothetical protein	-1.055	3.46E-02
NMB0092		hypothetical protein	-0.798	2.08E-02
NMB0119		hypothetical protein	-0.973	2.31E-02
NMB0120		hypothetical protein	-0.919	7.86E-03
NMB0317		conserved hypothetical protein	-0.701	6.54E-03
NMB0366		hypothetical protein	-1.000	2.90E-04
NMB0367		hypothetical protein	-0.974	2.31E-02
NMB0368		hypothetical protein	-0.985	1.96E-02
NMB0370		hypothetical protein	-0.958	1.34E-02
NMB0371		hypothetical protein	-0.931	2.15E-02
NMB0501		hypothetical protein	-0.910	3.28E-02
NMB0507		hypothetical protein	-0.723	9.44E-03
NMB0515		hypothetical protein	-0.793	2.05E-02
NMB0516		hypothetical protein	-0.732	2.08E-02
NMB0555		hypothetical protein	-0.762	2.42E-02
NMB0644		hypothetical protein	-0.939	1.22E-03
NMB0647		hypothetical protein	-0.793	1.10E-02
NMB0650		hypothetical protein	-0.883	2.98E-03
NMB0651		hypothetical protein	-0.776	5.19E-03
NMB0654		hypothetical protein	-0.843	9.81E-03
NMB0660		hypothetical protein	-0.673	1.70E-02
NMB0856		hypothetical protein	-0.924	3.51E-03
NMB0857		hypothetical protein	-0.769	3.46E-02
NMB0859		hypothetical protein	-0.836	1.14E-02
NMB0888		putative membrane protein	-0.946	4.72E-04
NMB0889		hypothetical protein	-0.838	3.76E-02
NMB0945		hypothetical protein	-0.947	4.11E-02
NMB1056		hypothetical protein	-0.793	4.29E-02
NMB1125		hypothetical protein	-0.849	2.31E-02
NMB1345		hypothetical protein	-0.764	1.10E-02
NMB1402		hypothetical protein	-0.884	1.03E-03
NMB1404		hypothetical protein	-0.963	1.49E-03
NMB1406		hypothetical protein	-0.626	4.14E-02
NMB1436		conserved hypothetical protein	-0.699	3.17E-02
NMB1575		conserved hypothetical protein	-1.162	5.50E-03
NMB1578		conserved hypothetical protein	-0.784	1.65E-02
NMB1633		hypothetical protein	-1.003	4.25E-02
NMB1667		hypothetical protein	-0.647	5.19E-03
NMB1784		hypothetical protein	-0.830	4.40E-02
NMB2085		hypothetical protein	-0.678	4.29E-02
NMB2109		hypothetical protein	-0.830	1.84E-02

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Table B.2 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB2110		hypothetical protein	-0.893	6.46E-03
NMB2112		hypothetical protein	-0.990	1.49E-03
NMB2116		hypothetical protein	-0.927	6.12E-03
NMB2118		hypothetical protein	-1.085	8.83E-04
NMB2123		hypothetical protein	-0.866	1.70E-02
NMB2124		hypothetical protein	-0.621	4.99E-02
NMB2125		hypothetical protein	-0.637	2.39E-02
NMB2146		hypothetical protein	-0.739	2.87E-03
NMB2147		hypothetical protein	-0.611	1.54E-02
NMB2152		hypothetical protein	-0.674	2.54E-02
NMB2153		conserved hypothetical protein	-0.864	1.14E-02
NMA0315	*mafB′*	putative MAFB alternative C-terminus	-0.969	1.22E-03
NMA0318		hypothetical protein	-0.984	8.65E-04
NMA0426a		hypothetical protein	-0.710	2.90E-02
NMA0606		hypothetical protein	-0.682	9.44E-03
NMA0695		conserved hypothetical protein	-1.079	1.62E-03
NMA1288		conserved hypothetical protein	-0.735	3.26E-02
NMA1435		conserved hypothetical protein	-0.681	3.39E-02
NMA1866		putative integral membrane protein	-2.672	4.76E-03
NMA1978		conserved hypothetical protein	-1.002	1.84E-02
NMA2123		conserved hypothetical protein	-0.912	3.08E-03
NMC0082		conserved hypothetical protein	-0.789	1.70E-02
NMC1345		putative iron-regulated protein FrpC outer membrane (pseudogene)	-1.043	2.87E-03
NMC1797		conserved hypothetical protein	-0.990	2.91E-02
NMC2091		hypothetical protein	-0.908	3.51E-03
NMC2086		putative adhesin (pseudogene)	-0.869	5.19E-03
Mobile and extrachromosomal element functions				
NMB0556		putative repressor protein	-0.777	3.00E-03
Protein fate				
NMB0214	*prlC*	oligopeptidase A	-0.915	4.26E-02
NMB0554	*dnaK*	dnaK protein	-1.552	4.72E-03
NMB0561	*grpE*	grpE protein	-0.824	3.96E-02
Protein synthesis				
NMB0854	*hisS*	histidyl-tRNA synthetase	-0.847	4.25E-02
NMB0942	*rpmE2*	putative 50S ribosomal protein L31	-0.947	4.38E-02
Purines, pyrimidines, nucleosides, and nucleotides				
NMB2151	*purD*	phosphoribosylamine-glycine ligase	-0.647	4.29E-02
Regulatory functions				
NMB0173	*oxyR*	transcriptional regulator, LysR family	-0.707	2.80E-02
NMB0556		putative repressor protein	-0.777	3.00E-03
Transcription				
NMB0132	*rpoB*	DNA-directed RNA polymerase, β subunit	-0.847	2.17E-02
NMB2144		putative sigma factor	-0.642	7.05E-03
Transport and binding proteins				
NMB0071	*ctrA*	capsule polysaccharide export, outer membrane protein	-0.791	1.84E-02
NMB0073	*ctrC*	capsule polysaccharide export, inner membrane protein	-0.730	4.91E-02
NMB0098		ABC transporter, ATP-binding protein (authentic frameshift)	-0.719	2.49E-02
NMB0586		putative zinc/manganese transporter	-0.858	1.62E-03
NMB1497		TonB-dependent receptor	-0.588	4.22E-02
NMB1732		putative transporter	-0.788	6.65E-03
NMB1783		putative secretion protein	-0.929	3.88E-02
NMC1691	*tbpB*	transferrin-binding protein B	-1.097	9.41E-03

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Table B.2 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
Unknown function				
NMB0827	*nmgI*	type II restriction enzyme-related protein	-1.273	2.83E-03
NMB0852	*engA*	essential GTPase	-0.852	3.37E-02
NMB1280		very long chain acyl-CoA dehydrogenase-related protein	-0.590	1.19E-02
NMB1381		HesB/YadR/YfhF family protein	-0.712	4.06E-02
NMB1405		FrpA/C-related protein	-0.906	4.83E-03
NMB1409		FrpA/C-related protein	-0.759	3.39E-02
NMB2105	*mafB*	mafB protein	-0.761	6.77E-03
NMB2122	*mafB′*	MafB-related protein	-0.843	8.36E-03

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table B.3: Genes up-regulated at 240 min during growth in FFP 1 in comparison to meningococci incubated in medium. Genes are divided into functional categories according to TIGR designations.

ID	Name	Annotation	$\log_2$ ratio	P-value*
Amino acid biosynthesis				
NMB0307	*aroG*	phospho-2-dehydro-3-deoxyheptonate aldolase	1.141	1.79E-07
NMB0337	*ilvE*	putative branched-chain amino acid aminotransferase	1.387	7.36E-07
NMB1036	*leuC*	3-isopropylmalate dehydratase, large subunit	0.722	3.67E-02
NMB1069	*proB*	glutamate 5-kinase	0.842	4.39E-03
NMB1150	*ilvD-1*	dihydroxy-acid dehydratase	0.836	5.64E-03
NMB1188	*ilvD-2*	dihydroxy-acid dehydratase	0.830	5.64E-03
NMB1371	*argD*	bifunctional N-succinyldiaminopimelate--aminotransferase/acetylornithine transaminase protein	0.585	1.79E-02
NMB1573	*argF*	ornithine carbamoyltransferase, catabolic	1.052	6.43E-06
NMB1710	*gdhA*	glutamate dehydrogenase, NADP-specific	1.935	1.76E-05
Cell envelope				
NMB0663	*nspA*	outer membrane protein	2.234	5.50E-13
NMB1623	*aniA*	nitrite reductase, major anaerobically induced outer membrane protein	1.107	4.96E-04
NMB1994	*nadA*	putative adhesin/invasin	1.196	7.27E-05
Cellular processes				
NMB1994	*nadA*	putative adhesin/invasin	1.196	7.27E-05
Central intermediary metabolism				
NMB0031	*glmS*	glucosamine–fructose-6-phosphate aminotransferase	0.883	1.65E-03
NMB1154	*cysD-1*	sulphate adenylyltransferase, subunit 2	0.978	1.24E-02
NMB1584		3-hydroxyacid dehydrogenase	0.712	1.42E-02
DNA metabolism				
NMB0831		type I restriction enzyme S protein (degenerate)	0.622	2.53E-03
NMB0962	*uvrA*	excinuclease ABC, subunit A	0.883	6.92E-03
Energy metabolism				
NMB0241	*nuoA*	NADH dehydrogenase I, chain A	1.281	1.71E-04
NMB0242	*nuoB*	NADH dehydrogenase I, chain B	1.433	8.31E-08
NMB0243	*nuoC*	NADH dehydrogenase I, chain C	1.253	3.36E-06
NMB0244	*nuoD*	NADH dehydrogenase I, chain D	1.478	9.95E-08
NMB0245	*nuoE*	NADH dehydrogenase I, chain E	1.286	2.74E-05
NMB0246	*nuoF*	NADH dehydrogenase I, chain F	0.965	8.49E-04
NMB0249	*nuoG*	NADH dehydrogenase I, chain G	1.133	1.73E-04
NMB0250	*nuoH*	NADH dehydrogenase I, chain H	1.083	5.17E-05

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Table B.3 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB0251	*nuoI*	NADH dehydrogenase I, chain I	1.377	1.03E-06
NMB0253	*nuoJ*	NADH dehydrogenase I, chain J	0.866	2.33E-02
NMB0254	*nuoK*	NADH dehydrogenase I, chain K	0.946	8.97E-03
NMB0257	*nuoL*	NADH dehydrogenase I, chain L	0.882	3.05E-03
NMB0258	*nuoM*	NADH dehydrogenase I, chain M	0.809	1.64E-02
NMB0340	*gloA*	lactoylglutathione lyase	1.537	6.25E-08
NMB0390	*mapA*	maltose phosphorylase	0.599	1.64E-02
NMB0401	*putA*	proline dehydrogenase	0.958	7.47E-03
NMB0433	*acnA*	aconitate hydratase 1	1.813	8.58E-04
NMB0631		putative phosphate acyltransferase	1.052	8.54E-03
NMB0920	*icd*	isocitrate dehydrogenase	1.192	8.78E-05
NMB0923		cytochrome c	0.754	3.65E-04
NMB0948	*sdhC*	succinate dehydrogenase, cytochrome b556 subunit	0.639	1.44E-02
NMB0949	*sdhD*	succinate dehydrogenase, hydrophobic membrane anchor protein	0.737	4.04E-03
NMB0950	*sdhA*	succinate dehydrogenase, flavoprotein subunit	0.752	8.76E-03
NMB0951	*sdhB*	succinate dehydrogenase, iron-sulphur protein	0.860	1.29E-03
NMB0954	*gltA*	citrate synthase	0.983	3.12E-03
NMB0980	*pntA*	NAD(P) transhydrogenase, α subunit	0.808	1.38E-02
NMB1244	*rpe*	ribulose-phosphate 3-epimerase	0.607	2.31E-02
NMB1388	*pgi-1*	glucose-6-phosphate isomerase	0.598	3.41E-02
NMB1393	*edd*	phosphogluconate dehydratase	0.886	8.16E-03
NMB1474		putative 4-oxalocrotonate tautomerase	0.718	7.45E-03
NMB1572	*acnB*	aconitate hydratase 2	2.493	5.64E-12
NMB1613	*fumA*	fumarate hydratase, class I	1.534	2.31E-09
NMB1677		cytochrome c5	1.616	3.73E-07
NMB1690		putative phosphoglucomutase/phosphomannomutase	0.636	3.01E-02
NMB1723	*fixP*	cytochrome c oxidase, subunit III	1.471	4.22E-07
NMB1724	*fixO*	cytochrome c oxidase, subunit II	1.097	1.00E-02
NMB1805	*cyc*	cytochrome c4	0.662	2.31E-02
NMB2051	*petC*	ubiquinol–cytochrome c reductase, cytochrome c1	0.936	2.20E-03
NMB2052	*petB*	ubiquinol–cytochrome c reductase, cytochrome b	0.768	1.08E-02
NMB2053	*petA*	ubiquinol–cytochrome c reductase, iron-sulphur subunit	0.963	1.57E-03
NMB2096	*mqo*	malate:quinone oxidoreductase	1.306	6.25E-08
NMB1371	*argD*	bifunctional N-succinyldiaminopimelate--aminotransferase/acetylornithine transaminase protein	0.585	1.79E-02
NMB1573	*argF*	ornithine carbamoyltransferase, catabolic	1.052	6.43E-06
NMA2094	*mapA′*	putative maltose phosphorylase (fragment)	0.943	8.44E-04
Hypothetical or unassigned proteins				
NMB0100		hypothetical protein (frameshift)	0.819	5.58E-03
NMB0228		conserved hypothetical protein	1.093	1.82E-03
NMB0230		conserved hypothetical protein	1.012	3.88E-04
NMB0252		hypothetical protein	1.014	1.08E-03
NMB0311		hypothetical protein	0.979	4.79E-03
NMB0500		hypothetical protein	0.912	4.27E-02
NMB0516		hypothetical protein	0.637	3.79E-02
NMB0709		putative membrane protein	1.706	2.24E-08
NMB0710		putative membrane protein	0.888	5.58E-03
NMB0711		conserved hypothetical protein	0.643	2.41E-03
NMB0776		putative secreted protein	0.785	3.04E-02

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Table B.3 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB0921		hypothetical protein	0.737	7.68E-03
NMB0952		conserved hypothetical protein	0.861	1.39E-02
NMB0968		hypothetical protein	1.071	2.10E-03
NMB1035		putative lipoprotein	0.735	4.29E-03
NMB1064		conserved hypothetical protein	0.695	1.96E-03
NMB1144		putative periplasmic protein	0.816	2.74E-03
NMB1365	*dnrN*	conserved hypothetical protein	0.898	2.11E-05
NMB1427		hypothetical protein	0.786	3.81E-02
NMB1431		hypothetical protein	2.983	2.17E-04
NMB1436		conserved hypothetical protein	0.940	1.54E-03
NMB1437		conserved hypothetical protein	1.153	2.80E-03
NMB1438		conserved hypothetical iron-sulphur protein	1.178	1.34E-04
NMB1456		hypothetical protein	0.840	1.67E-03
NMB1571		conserved hypothetical (integral membrane protein)	0.790	5.20E-03
NMB1590		conserved hypothetical protein	0.719	2.63E-04
NMB1598		hypothetical protein	0.746	7.68E-03
NMB1620		conserved hypothetical protein	0.913	3.87E-03
NMB1624		hypothetical protein	1.060	1.73E-04
NMB1656		hypothetical protein	0.998	2.10E-04
NMB1667		hypothetical protein	0.661	2.34E-03
NMB1718		hypothetical protein	0.970	3.17E-03
NMB1824		conserved hypothetical protein	0.993	6.58E-04
NMB2050		putative periplasmic protein	0.594	3.97E-02
NMB2064		conserved hypothetical protein	1.000	7.97E-03
NMB2089		conserved hypothetical protein	0.759	6.00E-03
NMB2097		hypothetical protein	1.346	9.90E-04
NMA0041		conserved hypothetical protein	0.829	6.32E-03
NMA1157		conserved hypothetical protein	2.927	2.63E-03
NMA1214		conserved hypothetical protein	1.561	4.40E-02
NMA1220		conserved hypothetical protein	0.627	2.26E-02
NMA1290		conserved hypothetical protein	4.896	1.84E-07
NMA1293		putative phage anti-repressor protein; AntB	4.558	3.96E-06
NMA1298		conserved hypothetical protein	2.331	6.07E-03
NMA1311		conserved hypothetical protein	3.041	2.24E-05
NMA1461		putative dnaJ-family protein	0.629	1.01E-02
NMA1976		putative cytochrome (pseudogene)	1.265	3.40E-04
NMA1978		conserved hypothetical protein	1.039	8.60E-03
Mobile and extrachromosomal element functions				
NMA1851		Mu-like phage gp29	0.925	4.03E-04
Protein fate				
NMB1649	*dsbB*	disulfide bond formation protein B	0.730	4.18E-03
NMB1998	*mspA*	serine-type peptidase	0.831	1.44E-02
Purines, pyrimidines, nucleosides, and nucleotides				
NMB1849	*carA*	carbamoyl phosphate synthase, small subunit	0.762	3.47E-02
Regulatory functions				
NMB1711	*gdhR*	GntR-family transcriptional regulator	0.655	1.76E-02
Transcription				
NMC0940		putative ATP-dependent helicase	2.017	1.39E-02
Transport and binding proteins				
NMB0041		putative thiamin-binding periplasmic protein	0.757	2.54E-02
NMB0085	*gltS*	sodium/glutamate symport carrier protein	0.674	1.76E-02
NMB0318	*farA*	fatty acid efflux system protein	0.719	4.36E-02
NMB0402	*putP*	sodium/proline symporter	1.320	5.90E-05
NMB0615	*amtB*	putative ammonium transporter	1.413	1.38E-05
NMB1207	*bfrA*	bacterioferritin A	0.759	4.04E-02

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Table B.3 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB1719	*mtrF*	efflux pump component	0.967	2.56E-02
Unknown function				
NMB0255		cell filamentation protein Fic-related protein	1.224	7.37E-04
NMB1220		stomatin/Mec-2 family protein	0.598	3.92E-02
NMB1891		helix-turn-helix family protein	0.696	1.61E-02
NMB1924		inositol monophosphatase family protein	0.931	9.64E-03
NMA0029		urea amidolyase-related protein	1.242	3.88E-04

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table B.4: Genes down-regulated at 240 min during growth in FFP 1 in comparison to meningococci incubated in medium. Genes are divided into functional categories according to TIGR designations.

ID	Name	Annotation	$\log_2$ ratio	P-value*
Amino acid biosynthesis				
NMB0637	*argH*	argininosuccinate lyase	-0.701	3.04E-02
NMB1228	*metM*	homoserine dehydrogenase	-0.604	4.02E-02
Biosynthesis of cofactors, prosthetic groups, and carriers				
NMB0684	*ribE*	riboflavin synthase, β subunit	-0.759	1.44E-02
Cell envelope				
NMB0011	*murA*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	-0.907	8.04E-04
NMB0038	*glmU*	UDP-N-acetylglucosamine pyrophosphorylase	-0.682	1.97E-04
NMB0088		putative outer membrane protein P1	-0.979	1.10E-04
NMB0181		putative outer membrane protein OmpH	-0.644	2.61E-02
NMB0414	*murE*	UDP-N-acetylmuramoylalanyl-D-glutamate–2,6-diaminopimelate ligase	-0.639	6.30E-03
NMB0790	*pgm*	phosphoglucomutase	-0.625	2.34E-03
NMB1279	*mltB*	putative membrane-bound lytic murein transglycosylase B	-1.095	2.11E-05
NMB1716	*mtrC*	membrane fusion protein	-0.731	5.86E-03
Cellular processes				
NMB0364		FrpC operon protein	-1.513	2.04E-08
NMB0584		FrpC operon protein	-1.563	6.25E-08
NMB0585		putative iron-regulated protein FrpA	-1.187	6.67E-05
NMB0799	*ftsJ*	cell division protein	-0.608	3.12E-03
NMB0855		putative bacteriocin resistance protein	-0.623	4.89E-02
NMB1412		FrpC operon protein	-1.557	2.24E-08
NMB1414		FrpC operon protein	-1.525	1.59E-08
NMB1714	*mtrE*	multidrug efflux pump channel protein	-0.916	2.11E-04
Central intermediary metabolism				
NMB0641	*ppa*	inorganic pyrophosphatase	-0.825	4.16E-02
NMB0804		putative NAD(P)H nitroreductase	-0.598	3.75E-02
NMB1799	*metK*	S-adenosylmethionine synthetase	-0.746	3.79E-03
DNA metabolism				
NMB1322	*priB*	putative primosomal replication protein N	-1.374	2.17E-02
NMB1568	*holC*	putative DNA polymerase holoenzyme, chi subunit	-0.643	1.01E-02
Energy metabolism				
NMB0010	*pgk*	phosphoglycerate kinase	-0.890	1.20E-03
NMB0671	*maeA*	malate oxidoreductase (NAD)	-1.142	2.11E-05
NMB0790	*pgm*	phosphoglucomutase	-0.625	2.34E-03
NMB0947	*lpdA2*	putative lipoamide dehydrogenase	-0.779	3.46E-02
NMB1044	*fpr-1*	ferredoxin–NADP reductase	-0.781	1.76E-02
NMB1377	*lldA*	L-lactate dehydrogenase	-2.176	2.72E-07

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Table B.4 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB1934	*atpD*	ATP synthase F1, β subunit	-1.134	4.14E-02
NMB1935	*atpG*	ATP synthase F1, γ subunit	-1.323	1.30E-02
NMB1936	*atpA*	ATP synthase F1, α subunit	-1.211	3.72E-02
NMB2154	*etfA*	electron transfer flavoprotein, α subunit	-0.985	1.40E-02
NMB2155	*etfB*	electron transfer flavoprotein, β subunit	-0.962	1.40E-02
NMA1688		phosphoglycolate phosphatase	-0.902	4.73E-03
NMA1934	*gcvP*	glycine dehydrogenase	-0.655	2.73E-02
Fatty acid and phospholipid metabolism				
NMB0179	*fabZ*	(3R)-hydroxymyristoyl-(acyl carrier protein) dehydratase	-0.609	1.19E-02
NMB0180	*lpxD*	UDP-3-O-(3-hydroxymyristoyl)-glucosamine N-acyltransferase	-0.664	2.95E-02
NMB0219	*fabF*	3-oxoacyl-(acyl-carrier-protein) synthase II	-1.396	2.47E-05
Hypothetical or unassigned proteins				
NMB0035		putative lipoprotein	-1.622	1.52E-06
NMB0036		possible periplasmic protein	-1.161	1.86E-04
NMB0313		conserved hypothetical protein	-0.730	2.54E-03
NMB0347		conserved hypothetical protein	-0.837	1.44E-02
NMB0593		conserved hypothetical protein	-0.814	3.22E-05
NMB0596		possible integral membrane protein	-0.692	2.11E-05
NMB0597		hypothetical protein	-0.700	1.03E-03
NMB0730		putative membrane protein	-0.920	1.73E-04
NMB0744		hypothetical protein	-1.959	4.01E-05
NMB0803		conserved hypothetical protein	-0.642	3.32E-04
NMB0847		hypothetical protein	-0.632	7.79E-04
NMB0850		putative periplasmic protein	-1.470	6.44E-04
NMB0856		hypothetical protein	-0.689	2.49E-02
NMB0864		hypothetical protein	-1.068	8.32E-03
NMB0866		putative periplasmic protein	-0.641	1.48E-02
NMB0905		hypothetical protein	-0.938	4.51E-04
NMB1327		conserved hypothetical protein	-0.664	7.84E-04
NMB1336		conserved hypothetical protein	-0.619	1.44E-02
NMB1564		conserved hypothetical protein	-0.832	3.96E-03
NMB1565		hypothetical protein	-0.719	4.29E-03
NMB1648		conserved hypothetical protein	-0.658	1.69E-02
NMB1959		conserved hypothetical protein	-0.717	3.14E-02
NMB2085		putative ATP-binding protein	-0.738	1.86E-02
NMB2147		putative lipoprotein	-0.614	9.09E-03
NMB2153		conserved hypothetical protein	-0.861	7.17E-03
NMC0806		putative integral membrane protein	-1.155	2.80E-03
NMC1345		pseudogene	-1.632	3.39E-08
NMA2123		conserved hypothetical protein	-1.199	2.24E-05
Protein fate				
NMA1758	*pepA*	aminopeptidase A	-1.004	1.82E-03
NMB0059	*dnaJ*	DnaJ protein	-0.727	2.34E-03
NMB0214	*prlC*	oligopeptidase A	-1.448	3.57E-04
NMB0294	*dsbA-2*	thiol:disulfide interchange protein	-0.858	4.29E-03
NMB0550	*dsbC*	thiol:disulfide interchange protein	-1.137	5.02E-04
NMB0554	*dnaK*	putative chaperone protein	-1.473	4.29E-03
NMB0561	*grpE*	probable heat shock protein	-1.523	2.24E-05
NMB0700	*iga*	IgA-specific serine endopeptidase	-1.132	8.04E-03
NMB0791	*ppiB*	peptidyl-prolyl cis-trans isomerase B	-1.471	9.37E-04
NMB1231	*lon*	ATP-dependent protease La	-0.704	4.29E-02
NMB1519	*dsbD*	thiol:disulfide interchange protein	-0.595	4.21E-02
NMB1664		putative protease	-0.620	4.33E-03
NMB1972	*groEL*	chaperonin, 60 kDa	-1.409	2.95E-02

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Table B.4 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB1973	*groES*	chaperonin, 10 kDa	-1.866	6.06E-04
Protein synthesis				
NMB0128	*rplA*	50S ribosomal protein L1	-1.329	1.47E-02
NMB0145	*rplB*	50S ribosomal protein L2	-1.257	4.72E-02
NMB0146	*rpsS*	30S ribosomal protein S19	-1.320	4.77E-02
NMB0147	*rplV*	50S ribosomal protein L22	-1.479	3.75E-02
NMB0152	*rplN*	50S ribosomal protein L14	-1.508	3.34E-02
NMB0154	*rplE*	50S ribosomal protein L5	-1.868	7.69E-03
NMB0155	*rpsN*	30S ribosomal protein S14	-1.602	2.50E-02
NMB0156	*rpsH*	30S ribosomal protein S8	-1.531	3.32E-02
NMB0169	*rplQ*	50S ribosomal protein L17	-1.237	2.49E-02
NMB0174	*valS*	valyl-tRNA synthetase	-0.673	1.58E-02
NMB0626	*prfC*	peptide chain release factor 3	-0.742	3.96E-06
NMB0721	*infC*	translation initiation factor 3	-1.017	2.57E-02
NMB1301	*rpsA*	30S ribosomal protein S1	-1.112	4.26E-02
NMB1321	*rpsR*	30S ribosomal protein S18	-1.314	4.33E-02
NMB1323	*rpsF*	30S ribosomal protein S6	-1.254	4.49E-02
NMB1358	*gatB*	Glu-tRNA(Gln) amidotransferase, subunit B	-0.910	1.27E-04
NMB1425	*lysS*	lysyl-tRNA synthetase, heat inducible	-0.743	8.17E-03
NMB1911	*rpmF*	50S ribosomal protein L32	-1.145	5.02E-04
Purines, pyrimidines, nucleosides, and nucleotides				
NMB0849		putative deoxycytidine triphosphate deaminase	-1.168	1.97E-04
NMB1252	*purM*	putative phosphoribosylformylglycinamidine cyclo-ligase	-0.835	4.85E-03
NMB1566	*purN*	phosphoribosylglycinamide formyltransferase	-0.687	2.92E-03
Transcription				
NMB0132	*rpoB*	DNA-directed RNA polymerase, β subunit	-0.983	1.23E-03
NMB0133	*rpoC*	DNA-directed RNA polymerase, β' subunit	-0.816	8.45E-03
NMB0168	*rpoA*	DNA-directed RNA polymerase, α subunit	-1.251	4.29E-02
NMB0617	*rho*	transcription termination factor Rho	-0.814	5.12E-03
NMB0683	*nusB*	N utilization substance protein B	-0.758	4.73E-03
NMB1538	*rpoD*	RNA polymerase sigma factor RpoD	-0.717	2.46E-02
Transport and binding proteins				
NMB0034		high-affinity iron transporter	-1.129	7.09E-05
NMB0293		putative TonB-dependent receptor	-1.424	8.81E-08
NMB0378		putative phosphate permease	-1.262	1.21E-03
NMB0460	*tbpB*	transferrin-binding protein 2	-2.071	1.03E-08
NMB0461	*tbpA*	transferrin-binding protein 1	-2.271	1.83E-12
NMB1497		TonB-dependent receptor	-0.810	2.03E-03
NMB1540	*lbpA*	lactoferrin-binding protein A	-1.376	1.71E-04
NMB1541	*lbpB*	lactoferrin-binding protein B	-1.325	1.34E-04
NMB1714	*mtrE*	multidrug efflux pump channel protein	-0.916	2.11E-04
NMB1715	*mtrD*	multiple transferable resistance system protein	-0.926	1.66E-04
NMB1716	*mtrC*	membrane fusion protein	-0.731	5.86E-03
NMB1728	*exbD*	biopolymer transport protein	-1.412	2.03E-03
NMB1729	*exbB*	biopolymer transport protein	-1.150	7.18E-03
NMB1730	*tonB*	TonB protein	-0.925	1.16E-02
NMB1988	*fetA*	iron-regulated outer membrane protein FrpB	-2.518	2.31E-09
NMB1989	*fetB*	iron(III) ABC transporter, periplasmic substrate-binding protein	-0.987	2.19E-06
NMA1740	*lbpB*	lactoferrin-binding protein B	-1.520	1.08E-05
NMA2025	*tbpB*	transferrin-binding protein 2	-2.371	8.31E-08
NMC1469	*lbpB*	lactoferrin-binding protein B	-0.941	2.24E-02
NMC1690	*tbpA*	transferrin-binding protein 1	-1.060	1.86E-03
NMC1691	*tbpB*	transferrin-binding protein B	-0.700	4.00E-02
NMC1946	*hpuA*	haemoglobin-haptoglobin-utilization protein	-1.872	2.34E-03

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Table B.4 – continued from previous page

ID	Name	Annotation	log_2 ratio	P-value*
Unknown function				
NMB0598		Maf/YceF/YhdE family protein	-0.641	1.57E-03
NMB0766	*lepA*	GTP-binding protein	-0.675	8.32E-03
NMB0852		essential GTPase	-0.816	3.47E-02
NMB1199	*typA*	GTP-binding protein	-0.981	5.28E-03
NMB1280		very long chain acyl-CoA dehydrogenase-related protein	-1.060	8.18E-07
NMB1638		YhbX/YhjW/YijP/YjdB family protein	-1.115	7.72E-07

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table B.5: Genes down-regulated at both 60 and 240 min during growth in FFP 1 in comparison to meningococci incubated in medium.

ID	Name	Annotation	60 min log_2 ratio	240 min log_2 ratio
NMB0088		putative outer membrane protein P1	-0.638	-0.979
NMB0132	*rpoB*	DNA-directed RNA polymerase, subunit β	-0.847	-0.983
NMB0214	*prlC*	oligopeptidase A	-0.915	-1.448
NMB0554	*dnaK*	molecular chaperone	-1.552	-1.473
NMB0561	*grpE*		-0.824	-1.523
NMB0584		FrpC operon protein	-0.802	-1.563
NMB0585		putative iron-regulated protein FrpA	-1.066	-1.187
NMB0852	*engA*	GTP-binding protein	-0.852	-0.816
NMB0855		putative bacteriocin resistance protein	-0.878	-0.623
NMB0856		hypothetical protein	-0.924	-0.689
NMB1280		very long chain acyl-CoA dehydrogenase-related protein	-0.590	-1.060
NMB1412		FrpC operon protein	-0.833	-1.557
NMB1414		FrpC operon protein	-0.852	-1.525
NMB1497		TonB-dependent receptor	-0.588	-0.810
NMB2085		hypothetical protein	-0.678	-0.738
NMB2147		hypothetical protein	-0.611	-0.614
NMB2153		hypothetical protein	-0.864	-0.861
NMB2154	*etfA*	electron transfer flavoprotein, α subunit	-1.089	-0.985
NMB2155	*etfB*	electron transfer flavoprotein, β subunit	-1.017	-0.962
NMA2123		hypothetical protein	-0.912	-1.199
NMC1345		pseudogene	-1.043	-1.632
NMC1691	*tbpB*	transferrin-binding protein B	-1.097	-0.700

Table B.6: Genes up-regulated at 60 min during growth in FFP 2 in comparison to t = 0 min (meningococci on solid agar medium). Genes are divided into functional categories according to TIGR designations.

ID	Name	Annotation	log_2 ratio	P-value*
Amino acid biosynthesis				
NMB0760	*dapF*	diaminopimelate epimerase	0.688	1.05E-02
NMB0802	*metB*	cystathionine γ-synthase	0.633	1.28E-02
NMB0943	*metF*	5,10-methylenetetrahydrofolate reductase	0.740	3.53E-03
NMB0981	*serB*	phosphoserine phosphatase	0.759	4.39E-03
NMB1021	*trpE*	anthranilate synthase, component I	0.857	3.10E-03
NMB1031	*leuB*	3-isopropylmalate dehydrogenase	0.750	7.82E-03
NMB1034	*leuD*	isopropylmalate isomerase, small subunit	1.604	8.86E-10
NMB1036	*leuC*	isopropylmalate isomerase, large subunit	2.168	3.49E-09

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Table B.6 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB1070	*leuA*	2-isopropylmalate synthase	1.378	1.06E-06
NMB1150	*ilvD-1*	dihydroxy-acid dehydratase	0.749	2.55E-02
NMB1188	*ilvD-2*	dihydroxy-acid dehydratase	0.747	2.48E-02
NMB1446	*aroD*	3-dehydroquinate dehydratase	0.669	1.85E-02
NMB1498	*lysC*	aspartate kinase	0.929	2.94E-03
NMB1680	*aroC*	chorismate synthase	0.742	1.01E-03
Biosynthesis of cofactors, prosthetic groups, and carriers				
NMB0392	*nadB*	L-aspartate oxidase	1.117	2.04E-04
NMB0394	*nadA*	quinolinate synthetase	1.141	6.97E-05
NMB0396	*nadC*	nicotinate-nucleotide pyrophosphorylase	1.159	3.89E-03
NMB0527		putative 6-pyruvoyl tetrahydrobiopterin synthase	0.713	1.40E-02
NMB0539	*hemC*	porphobilinogen deaminase	0.752	2.00E-03
NMB0563	*apbE*	thiamine biosynthesis lipoprotein	0.642	1.39E-02
NMB0777	*hemD*	uroporphyrinogen-III synthase	0.706	2.05E-02
NMB1037	*gshA*	glutamate–cysteine ligase	0.975	2.12E-04
NMB1130		putative phytoene synthase	1.275	2.16E-08
NMB1156	*cysG-1*	siroheme synthase	1.212	1.04E-02
NMB1168		putative phytoene synthase	1.486	1.64E-10
NMB1194	*cysG-2*	siroheme synthase	1.438	5.80E-04
NMB1254	*ribA*	GTP cyclohydrolase II	0.627	3.10E-02
Cell envelope				
NMB0011	*murA*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	0.795	7.54E-03
NMB1273		putative alginate O-acetylation protein AlgI	0.715	1.93E-02
NMB1297	*mltD*	membrane-bound lytic murein transglycosylase D	0.708	3.70E-02
NMB1533		H.8 outer membrane protein	0.845	1.76E-02
NMB1704	*lgtF*	β-1,4-glucosyltransferase	1.036	1.35E-04
NMB1705	*rfaK*	α-1,2-N-acetylglucosamine transferase	0.790	1.91E-03
NMB1929	*lgtA*	lacto-N-neotetraose biosynthesis glycosyl transferase	0.820	7.04E-03
Cellular processes				
NMB0191		ParA family protein	0.602	9.50E-03
NMB0525		putative aluminum resistance protein	1.192	1.67E-03
NMB0582		putative bacteriocin resistance protein	1.803	3.54E-06
NMB1621	*gpxA*	glutathione peroxidase	0.590	3.65E-02
NMA0860	*apaH*	bis(5-nucleosyl)-tetraphosphatase (symmetrical)	0.804	1.01E-03
Central intermediary metabolism				
NMB0468	*speA*	arginine decarboxylase	0.730	2.97E-04
NMB0998		putative oxidoreductase	1.308	2.57E-06
NMB1151	*cysI-1*	sulphite reductase hemoprotein, β component	1.341	1.24E-02
NMB1152	*cysJ-1*	sulphite reductase (NADPH) flavoprotein, α component	1.176	2.34E-02
NMB1153	*cysN-1*	sulphate adenylyltransferase, subunit 1	1.421	1.00E-02
NMB1154	*cysD-1*	sulphate adenylyltransferase subunit 2	1.315	3.95E-03
NMB1189	*cysI-2*	sulphite reductase hemoprotein, β component	1.405	1.28E-02
NMB1190	*cysJ-2*	sulphite reductase (NADPH) flavoprotein, α component	1.393	2.71E-03
NMB1191	*cysN-2*	sulphate adenylyltransferase, subunit 1	1.636	6.46E-04
NMB1192	*cysD-2*	sulphate adenylyltransferase, subunit 2	1.447	1.80E-03
NMB1338		putative isomerase	0.627	1.23E-03
NMB1799	*metK*	S-adenosylmethionine synthetase	1.174	1.67E-05
DNA metabolism				
NMB0533	*nth*	endonuclease III	0.599	2.29E-02
NMB1982	*polA*	DNA polymerase I	0.870	1.42E-03

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Table B.6 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMA0059		pseudogene (modification methylase)	0.826	8.33E-03
Energy metabolism				
NMB0010	*pgk*	phosphoglycerate kinase	0.790	8.71E-03
NMB1044	*fpr-1*	ferredoxin–NADP reductase	0.792	6.79E-03
NMB1613	*fumA*	fumarate hydratase, class I, anaerobic	0.726	3.96E-03
NMB2053	*petA*	ubiquinol–cytochrome c reductase, iron-sulphur subunit	0.924	1.28E-02
Fatty acid and phospholipid metabolism				
NMB1913	*plsX*	fatty acid/phospholipid synthesis protein	0.723	3.01E-02
Hypothetical or unassigned proteins				
NMB0263		putative ribosome-associated GTPase	0.770	1.94E-02
NMB0266		hypothetical protein	0.914	1.58E-03
NMB0267		putative rare lipoprotein A	1.616	1.44E-06
NMB0292		hypothetical protein	0.715	2.97E-04
NMB0316		hypothetical protein	1.232	1.79E-07
NMB0317		putative 7-cyano-7-deazaguanine reductase	1.152	8.12E-06
NMB0320		hypothetical protein	1.225	6.79E-03
NMB0395		hypothetical protein	1.829	3.63E-07
NMB0438		hypothetical protein	0.986	8.61E-04
NMB0531		hypothetical protein	0.749	7.76E-05
NMB0545		hypothetical protein	0.591	2.89E-02
NMB0596		hypothetical protein	0.587	8.38E-04
NMB0597		hypothetical protein	0.688	2.89E-03
NMB0742		hypothetical protein	1.000	1.48E-03
NMB0762		hypothetical protein	0.692	2.56E-02
NMB0775		hypothetical protein	0.608	1.94E-02
NMB0819		hypothetical protein	0.805	1.67E-05
NMB0820		hypothetical protein	0.634	1.04E-02
NMB0821		hypothetical protein	1.115	3.03E-07
NMB0840		hypothetical protein	0.831	5.94E-03
NMB0861		hypothetical protein	0.949	4.08E-04
NMB0862		hypothetical protein	0.696	1.14E-02
NMB0865		hypothetical protein	0.869	3.82E-04
NMB0866		hypothetical protein	0.675	1.81E-02
NMB0872		hypothetical protein	0.618	3.89E-03
NMB1018		hypothetical protein	0.625	2.89E-02
NMB1020		hypothetical protein	0.649	9.35E-03
NMB1035		hypothetical protein	1.000	7.68E-03
NMB1040		hypothetical protein	0.713	6.79E-03
NMB1073		hypothetical protein	0.780	1.04E-02
NMB1142		hypothetical protein	0.681	1.41E-02
NMB1180		hypothetical protein	0.665	2.22E-02
NMB1253		hypothetical protein	0.666	1.66E-02
NMB1270		hypothetical protein	0.903	2.22E-06
NMB1286		hypothetical protein	0.962	4.19E-03
NMB1308		hypothetical protein	0.630	2.09E-02
NMB1336		hypothetical protein	0.897	6.69E-04
NMB1337		Holliday junction resolvase-like protein	0.750	9.40E-04
NMB1349		hypothetical protein	0.874	1.86E-04
NMB1426		hypothetical protein	0.778	3.87E-03
NMB1427		hypothetical protein	1.268	2.05E-04
NMB1531		hypothetical protein	0.669	5.93E-03
NMB1534		hypothetical protein	0.621	1.38E-02
NMB1565		hypothetical protein	0.747	5.90E-03
NMB1641		hypothetical protein	1.030	4.67E-05
NMB1800		hypothetical protein	1.139	2.56E-05

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Table B.6 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB1866		hypothetical protein	0.895	3.42E-02
NMB1906		hypothetical protein	0.978	1.65E-04
NMB1912		hypothetical protein	0.985	1.16E-03
NMB1977		hypothetical protein	0.854	9.73E-04
NMA0380		hypothetical protein	0.833	2.76E-04
NMA2069		pseudogene	0.889	4.85E-04
Protein fate				
NMB0059	*dnaJ*	molecular chaperone DnaJ	1.435	4.15E-08
NMB0214	*prlC*	oligopeptidase A	0.936	4.60E-02
NMB0554	*dnaK*	molecular chaperone DnaK	1.221	3.55E-02
NMB0561	*grpE*	molecular chaperone GrpE	1.201	2.09E-03
NMB0822	*htpX*	heat shock protein HtpX	0.787	3.89E-03
NMB0922	*lst*	α-2,3-sialyltransferase	1.101	1.28E-03
NMB1231	*lon*	ATP-dependent Lon protease	1.226	1.59E-03
NMB1372	*clpX*	ATP-dependent protease, ATP-binding subunit	0.816	2.17E-03
NMB2000	*hslO*	Hsp33-like chaperonin	0.809	7.09E-03
Protein synthesis				
NMB0142	*rplC*	50S ribosomal protein L3	1.503	1.70E-02
NMB0174	*valS*	valyl-tRNA synthetase	0.743	1.30E-02
NMB0268		RNA methyltransferase, TrmH family	1.355	4.89E-07
NMB0626	*prfC*	peptide chain release factor 3	1.041	7.65E-09
NMB0719	*tgt*	queuine tRNA-ribosyltransferase	0.819	3.34E-07
NMB0720	*thrS*	threonyl-tRNA synthetase	0.755	1.39E-02
NMB1348		putative RNA methylase	1.050	6.46E-04
NMB1425	*lysS*	lysyl-tRNA synthetase	0.878	4.15E-04
NMB1643	*infB*	translation initiation factor IF-2	0.825	2.71E-03
NMB1679	*trmA*	tRNA (uracil-5-)-methyltransferase	1.096	3.92E-07
NMA1588	*truB*	tRNA pseudouridine synthase B	1.239	1.44E-06
Purines, pyrimidines, nucleosides, and nucleotides				
NMB0774	*upp*	uracil phosphoribosyltransferase	1.000	1.18E-03
NMB0815	*purA*	adenylosuccinate synthetase	0.954	1.59E-03
NMB1019	*purK*	phosphoribosylaminoimidazole carboxylase, ATPase subunit	0.815	1.28E-02
NMB1252	*purM*	phosphoribosylaminoimidazole synthetase	0.866	6.93E-03
NMB1709	*thyA*	thymidylate synthase	0.664	1.01E-03
Regulatory functions				
NMB0594		sensor histidine kinase	0.651	1.27E-04
NMB1561	*glpR*	transcriptional regulator, DeoR family	0.740	2.00E-03
NMB2055	*metR*	transcriptional regulator, LysR family	0.796	1.91E-02
Transcription				
NMB0075		putative transcriptional accessory protein Tex	0.795	2.74E-02
NMB1373	*rbfA*	ribosome-binding factor A	0.875	5.09E-05
NMB1538	*rpoD*	RNA polymerase sigma factor RpoD	0.798	1.91E-02
NMB1642	*nusA*	transcription elongation factor NusA	1.078	7.65E-03
NMB1905	*rnpA*	ribonuclease P	0.816	4.10E-03
Transport and binding proteins				
NMB0098		ABC transporter, ATP-binding protein (authentic frameshift)	0.882	5.90E-03
NMB0393		multidrug resistance protein	0.790	4.46E-02
NMB0611	*potB*	spermidine/putrescine ABC transporter, permease protein	0.786	7.11E-04
NMB0612	*potC*	spermidine/putrescine ABC transporter, permease protein	0.774	3.65E-02
NMB0787		amino acid ABC transporter, periplasmic substrate-binding protein	1.547	4.08E-04
NMB0788		amino acid ABC transporter, permease protein	1.751	1.21E-05

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Table B.6 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB0789		amino acid ABC transporter, ATP-binding protein	1.576	2.50E-06
NMB1614	*trkA*	potassium transporter, peripheral membrane component	0.763	2.17E-03
NMB1962		L-glutamate ABC transporter, putative NTP-binding protein	0.679	4.78E-03
NMB1966		L-glutamate ABC transporter, putative ATP-binding protein	0.801	7.09E-03
Unknown function				
NMB0282		ribonuclease II-related protein	0.727	4.38E-04
NMB0995		macrophage infectivity potentiator-related protein	1.466	1.86E-03
NMB1335	*creA*	creA protein	0.643	2.28E-03
NMB1347	*suhB*	extragenic suppressor protein	1.172	7.59E-04
NMB1421		nifR3 protein	0.667	1.31E-02
NMB1961		VacJ-related protein	0.798	6.38E-04
NMA0271	*pilS7*	truncated pilin, pseudogene	0.698	4.08E-04
NMA0971		lipoprotein	0.749	4.34E-04

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table B.7: Genes down-regulated at 60 min during growth in FFP 2 in comparison to t = 0 min (meningococci on solid agar medium). Genes are divided into functional categories according to TIGR designations.

ID	Name	Annotation	$\log_2$ ratio	P-value*
Amino acid biosynthesis				
NMB0878	*ilvA*	threonine dehydratase	-1.092	2.09E-07
NMB1046	*thrC*	threonine synthase	-0.670	4.33E-02
NMB1055	*glyA*	serine hydroxymethyltransferase	-0.756	4.46E-02
NMB1476	*gluD*	glutamate dehydrogenase, NAD-specific	-1.716	6.40E-09
NMB1579	*hisG*	ATP phosphoribosyltransferase, catalytic subunit	-1.036	9.78E-05
NMB1581	*hisD*	histidinol dehydrogenase	-0.920	4.95E-03
NMB2005	*argJ*	bifunctional ornithine acetyltransferase/N-acetylglutamate synthase protein	-1.369	7.53E-04
Biosynthesis of cofactors, prosthetic groups, and carriers				
NMB1055	*glyA*	serine hydroxymethyltransferase	-0.756	4.46E-02
NMB1364	*nadE*	putative NH(3)-dependent NAD+ synthetase	-0.769	3.65E-02
Cell envelope				
NMB0756	*rfbD*	putative dTDP-L-rhamnose synthase	-0.778	1.01E-05
NMB1279	*mltB*	putative membrane-bound lytic murein transglycosylase B	-1.093	8.13E-05
NMB1716	*mtrC*	membrane fusion protein	-1.340	1.98E-06
NMB1994	*nadA*	putative adhesin/invasin	-0.974	2.29E-02
NMB2019	*kdtB*	phosphopantetheine adenylyltransferase	-0.675	7.69E-05
NMA0325	*mafA*	adhesin	-0.686	2.34E-02
NMC1731	*ddpX*	putative D-alanyl-D-alanine dipeptidase	-3.104	1.13E-10
Cellular processes				
NMB0216	*katA*	catalase	-1.904	2.80E-03
NMB0585		putative iron-regulated protein FrpA	-0.856	9.41E-03
NMB0712	*rpoH*	RNA polymerase factor sigma-32	-1.054	1.75E-02
NMB0768	*pilT-2*	twitching motility protein	-0.597	5.93E-03
NMB0784		putative phage shock protein E precursor	-0.975	7.93E-05
NMB0851	*rdgC*	recombination associated protein	-1.809	2.93E-02
NMB0855		putative bacteriocin resistance protein	-1.302	3.92E-05

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Table B.7 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB0977		putative modulator of drug activity B	-2.535	1.36E-12
NMB1398	*sodC*	Cu-Zn-superoxide dismutase	-0.896	1.50E-02
NMB1646		putative hemolysin	-0.775	1.66E-02
NMB1714	*mtrE*	multidrug efflux pump channel protein	-1.621	6.09E-09
NMB1791		cytoplasmic axial filament protein	-0.687	1.13E-02
NMB1857	*mdaB*	modulator of drug activity B	-2.464	4.64E-05
NMB1994	*nadA*	putative adhesin/invasin	-0.974	2.29E-02
Central intermediary metabolism				
NMB1305	*esd*	putative esterase	-0.726	1.28E-02
NMB1584		3-hydroxyacid dehydrogenase	-1.824	6.40E-09
DNA metabolism				
NMB0851	*rdgC*	recombination associated protein	-1.809	2.93E-02
NMB1655		N5-glutamine S-adenosyl-L-methionine--dependent methyltransferase	-0.835	4.19E-03
Energy metabolism				
NMB0089	*pykA*	pyruvate kinase II	-0.880	5.13E-03
NMB0176	*dadA*	D-amino acid dehydrogenase, small subunit	-1.764	1.32E-04
NMB0207	*gapA*	glyceraldehyde-3-phosphate dehydrogenase	-1.071	5.05E-03
NMB0334	*pgi-1*	glucose-6-phosphate isomerase	-0.919	9.32E-03
NMB0401	*putA*	bifunctional proline dehydrogenase/pyrroline-5-carboxylate dehydrogenase	-1.121	3.26E-03
NMB0431	*prpC*	methylcitrate synthase	-5.745	8.54E-14
NMB0433	*acnA*	aconitate hydratase	-2.146	7.76E-03
NMB0434		putative AcnD-accessory protein PrpF	-2.515	4.17E-06
NMB0435	*ackA-2*	acetate kinase	-1.693	2.36E-07
NMB0546	*adhA*	alcohol dehydrogenase	-1.507	1.86E-03
NMB0574	*gcvT*	glycine cleavage system aminomethyltransferase T	-3.552	3.95E-24
NMB0575	*gcvH*	glycine cleavage system protein H	-2.338	1.91E-06
NMB0618	*ppsA*	phosphoenolpyruvate synthase	-1.076	7.69E-05
NMB0631		putative phosphate acetyltransferase Pta	-1.535	2.76E-04
NMB0920	*icd*	isocitrate dehydrogenase, NADP-dependent, monomeric type	-1.318	2.89E-04
NMB0948	*sdhC*	succinate dehydrogenase, cytochrome b556 subunit	-0.996	2.60E-04
NMB0949	*sdhD*	succinate dehydrogenase, hydrophobic membrane anchor protein	-1.060	9.70E-05
NMB0950	*sdhA*	succinate dehydrogenase, flavoprotein subunit	-1.430	1.80E-05
NMB0951	*sdhB*	succinate dehydrogenase, iron-sulphur subunit	-1.361	1.68E-05
NMB0954	*gltA*	type II citrate synthase	-2.525	6.86E-11
NMB0955	*sucA*	α-ketoglutarate decarboxylase	-1.517	6.34E-06
NMB0956	*sucB*	dihydrolipoamide acetyltransferase	-1.175	2.10E-04
NMB0957	*lpdA3*	dihydrolipoamide dehydrogenase	-0.772	4.59E-02
NMB0959	*sucC*	succinyl-CoA synthetase, subunit β	-1.808	3.94E-04
NMB0960	*sucD*	succinyl-CoA synthetase, subunit α	-1.887	7.98E-08
NMB0978	*pntB*	pyridine nucleotide transhydrogenase	-0.789	2.09E-02
NMB1244	*rpe*	ribulose-phosphate 3-epimerase	-1.064	1.08E-04
NMB1304	*adhC*	alcohol dehydrogenase, class III	-0.975	7.87E-03
NMB1353		aldehyde dehydrogenase family protein	-0.868	2.09E-03
NMB1393	*edd*	phosphogluconate dehydratase	-0.935	9.77E-03
NMB1395		alcohol dehydrogenase, zinc-containing	-2.252	1.55E-11
NMB1493	*cstA*	carbon starvation protein A	-2.650	2.91E-12
NMB1572	*acnB*	bifunctional aconitate hydratase 2/2-methylisocitrate dehydratase	-1.345	1.53E-04
NMB1604	*gpm*	phosphoglyceromutase	-0.877	2.28E-03
NMB1609	*metZ*	trans-sulphuration enzyme family protein	-0.946	1.53E-03

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Table B.7 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB1734	*grxB*	glutaredoxin 2	-0.889	1.44E-03
NMB1842		putative 4-hydroxyphenylacetate 3-hydroxylase, small subunit	-0.711	4.29E-02
NMB1968	*aldA*	aldehyde dehydrogenase A	-2.076	3.49E-09
NMB2096	*mqo*	malate:quinone oxidoreductase	-1.803	8.29E-11
Fatty acid and phospholipid metabolism				
NMB0679	*accD*	acetyl-CoA carboxylase, subunit β	-0.645	2.88E-02
Hypothetical or unassigned proteins				
NMB0035		hypothetical protein	-1.365	2.40E-05
NMB0036		hypothetical protein	-1.319	8.64E-05
NMB0039		hypothetical protein	-0.596	2.10E-02
NMB0042		hypothetical protein	-0.811	7.74E-04
NMB0060		hypothetical protein	-1.605	4.08E-08
NMB0183		hypothetical protein	-0.765	1.44E-03
NMB0227		hypothetical protein	-1.839	5.90E-08
NMB0228		hypothetical protein	-1.487	7.42E-05
NMB0286		hypothetical protein	-0.654	1.63E-03
NMB0366		hypothetical protein	-0.940	1.23E-03
NMB0377	*anmK*	putative anhydro-N-acetylmuramic acid kinase	-0.724	1.26E-02
NMB0429		hypothetical protein	-2.440	4.00E-09
NMB0507		hypothetical protein	-0.608	3.93E-02
NMB0514		hypothetical protein	-0.841	1.66E-02
NMB0515		hypothetical protein	-1.044	1.80E-03
NMB0516		hypothetical protein	-1.273	3.92E-05
NMB0542		hypothetical protein	-0.740	1.59E-02
NMB0572		hypothetical protein	-0.879	5.09E-04
NMB0619		hypothetical protein	-0.705	3.69E-03
NMB0625		hypothetical protein	-0.652	1.81E-02
NMB0650		hypothetical protein	-0.674	2.89E-02
NMB0651		hypothetical protein	-0.674	1.97E-02
NMB0706		hypothetical protein	-2.273	2.55E-02
NMB0711		hypothetical protein	-1.210	1.65E-07
NMB0753		hypothetical protein	-0.797	1.31E-02
NMB0754		hypothetical protein	-1.133	7.12E-04
NMB0755		hypothetical protein	-0.752	1.94E-02
NMB0783		hypothetical protein	-0.629	2.87E-02
NMB0793		hypothetical protein	-1.203	1.01E-03
NMB0850		hypothetical protein	-1.193	1.27E-02
NMB0853		hypothetical protein	-1.778	3.63E-07
NMB0856		hypothetical protein	-1.017	1.23E-03
NMB0864		hypothetical protein	-1.648	4.78E-04
NMB0915		hypothetical protein	-0.855	2.55E-02
NMB0952		hypothetical protein	-1.951	1.61E-07
NMB0953		hypothetical protein	-1.706	1.43E-07
NMB0958		hypothetical protein	-1.298	8.72E-04
NMB1047		hypothetical protein	-0.832	9.31E-04
NMB1048		hypothetical protein	-2.708	6.94E-09
NMB1064		hypothetical protein	-0.839	1.58E-03
NMB1124		hypothetical protein	-1.724	1.32E-05
NMB1125		hypothetical protein	-1.415	4.82E-04
NMB1162		hypothetical protein	-1.733	1.01E-05
NMB1163		hypothetical protein	-1.402	3.14E-04
NMB1221		hypothetical protein	-0.892	9.08E-03
NMB1245		hypothetical protein	-0.906	3.60E-03
NMB1354		hypothetical protein	-0.851	6.30E-04
NMB1361		hypothetical protein	-0.616	3.60E-03

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Table B.7 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB1402		hypothetical protein	-0.872	7.42E-04
NMB1404		hypothetical protein	-0.944	1.63E-03
NMB1452		hypothetical protein	-2.230	4.98E-10
NMB1453		hypothetical protein	-0.728	5.82E-03
NMB1469		hypothetical protein	-1.185	2.62E-02
NMB1477		hypothetical protein	-0.747	1.81E-02
NMB1485		hypothetical protein	-0.744	2.29E-02
NMB1490		hypothetical protein	-0.831	1.70E-02
NMB1491		hypothetical protein	-1.955	2.77E-10
NMB1492		hypothetical protein	-1.906	4.98E-10
NMB1494		hypothetical protein	-2.107	4.75E-10
NMB1495		hypothetical protein	-0.637	4.16E-02
NMB1500		hypothetical protein	-1.310	1.84E-04
NMB1557		hypothetical protein	-0.624	2.10E-02
NMB1578		hypothetical protein	-1.147	3.46E-04
NMB1580		hypothetical protein	-1.548	6.81E-07
NMB1590		hypothetical protein	-0.764	3.40E-04
NMB1631		hypothetical protein	-0.775	4.49E-02
NMB1667		hypothetical protein	-1.013	1.23E-05
NMB1695		hypothetical protein	-0.965	9.34E-04
NMB1784		hypothetical protein	-1.260	1.78E-03
NMB1806		putative GTPase EngB	-0.758	1.28E-02
NMB1840		hypothetical protein	-0.731	2.91E-02
NMB1858		hypothetical protein	-0.773	7.03E-03
NMB1983		hypothetical protein	-0.625	1.18E-02
NMB2004		hypothetical protein	-0.990	8.43E-03
NMB2018		hypothetical protein	-0.834	6.79E-03
NMB2050		hypothetical protein	-0.705	2.00E-02
NMB2064		hypothetical protein	-0.894	3.37E-02
NMB2097		hypothetical protein	-1.105	1.08E-04
NMA0131		conserved hypothetical protein	-4.154	2.26E-21
NMA0607		hypothetical protein	-0.985	1.94E-02
NMA0695		conserved hypothetical protein	-0.810	4.29E-02
NMA1078		conserved hypothetical protein	-1.802	1.43E-04
NMA1194		hypothetical protein	-1.724	4.55E-06
NMA1220		conserved hypothetical protein	-1.715	6.09E-09
NMA1227		conserved hypothetical protein	-0.785	3.17E-02
NMA1291	*gam*	host-nuclease inhibitor protein	-0.737	2.68E-02
NMA1613		dehydrogenase fragment	-1.477	4.96E-07
NMA1645		NLP/P60 family domain protein	-0.763	6.38E-04
NMA1858		hypothetical protein	-1.874	1.98E-06
NMA1987		hypothetical protein	-0.751	1.02E-04
NMA2123		conserved hypothetical protein	-0.763	1.66E-02
NMC0806		putative integral membrane protein	-1.998	1.44E-06
NMC0893		putative ParB protein	-0.926	3.27E-02
NMC1053		hypothetical protein	-0.948	2.94E-04
NMC1345		pseudogene	-0.988	4.41E-03
NMC1730		putative integral membrane protein	-2.634	2.08E-08
Mobile and extrachromosomal element functions				
NMB0583		IS1016C2 transposase	-0.869	3.64E-04
NMB0917		death-on-curing protein	-0.585	3.65E-02
NMB1401		IS1016C2 transposase	-0.829	4.85E-04
NMB1411		IS1016C2 transposase	-0.642	3.98E-02
Protein fate				
NMB0027	*fbp*	FKBP-type peptidyl-prolyl cis-trans isomerase	-0.668	2.09E-03
NMB0281		peptidyl-prolyl cis-trans isomerase	-0.730	7.09E-03

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Table B.7 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB1428		putative aminopeptidase	-0.909	4.66E-02
NMB1519	*dsbD*	thiol:disulfide interchange protein precursor	-0.779	9.92E-03
NMB1737	*tolC*	putative secretion protein	-0.834	2.72E-02
Protein synthesis				
NMB0111	*fmt*	methionyl-tRNA formyltransferase	-0.728	2.27E-02
NMB0854	*hisS*	histidyl-tRNA synthetase	-1.489	2.37E-04
Purines, pyrimidines, nucleosides, and nucleotides				
NMB0849		deoxycytidine triphosphate deaminase	-0.786	2.78E-02
Regulatory functions				
NMB1049		putative transcriptional regulator	-0.749	7.03E-03
NMB1303		transcriptional regulator, MerR family	-0.592	3.23E-02
NMB1843		transcriptional regulator, MarR family	-0.852	2.43E-02
Transcription				
NMB1368		putative ATP-dependent RNA helicase	-3.255	2.16E-06
NMB0712	*rpoH*	RNA polymerase factor sigma-32	-1.054	1.75E-02
Transport and binding proteins				
NMB0034		high-affinity iron transporter	-0.903	3.66E-03
NMB0177		putative sodium/alanine symporter	-1.905	3.63E-07
NMB0194		putative amino acid symporter	-0.791	3.96E-02
NMB0378		putative phosphate permease	-1.806	1.55E-05
NMB0402	*putP*	sodium/proline symporter	-2.275	1.40E-09
NMB0543	*lctP*	putative L-lactate permease	-1.713	4.96E-07
NMB0610	*potA-1*	spermidine/putrescine ABC transporter, ATP-binding protein	-1.660	5.47E-04
NMB0615	*amtB*	putative ammonium transporter AmtB	-1.333	1.42E-04
NMB0792		transporter, NadC family	-2.430	1.35E-13
NMB0879	*cysA*	sulphate ABC transporter, ATP-binding protein	-0.796	1.35E-04
NMB1277		transporter, BCCT family	-0.782	1.31E-03
NMB1400		ABC transporter family protein	-1.091	7.59E-03
NMB1540	*lbpA*	lactoferrin-binding protein A	-2.601	5.78E-10
NMB1541	*lbpB*	lactoferrin-binding protein B	-2.307	4.00E-09
NMB1668	*hmbR*	hemoglobin receptor	-1.277	8.41E-07
NMB1714	*mtrE*	multidrug efflux pump channel protein	-1.621	6.09E-09
NMB1715	*mtrD*	multiple transferable resistance system protein MtrD	-1.389	2.53E-07
NMB1716	*mtrC*	membrane fusion protein	-1.340	1.98E-06
NMB1732		putative transporter	-0.949	1.10E-03
NMB1738	*hlyD*	putative secretion protein	-0.727	4.46E-02
NMB1988	*fetA*	iron-regulated outer membrane protein FrpB	-1.137	2.21E-02
NMA1083		periplasmic protein	-1.528	2.80E-03
NMA1740	*lbpB*	lactoferrin binding protein B	-2.280	4.83E-09
NMC1469	*lbpB*	lactoferrin binding protein B	-2.404	3.92E-07
NMC1691	*tbpB*	transferrin binding protein B	-1.735	4.89E-07
Unknown function				
NMB0006		thioredoxin-related protein	-1.143	4.14E-06
NMB0852		GTP-binding protein EngA	-1.797	4.42E-06
NMB1220		stomatin/Mec-2 family protein	-1.339	4.56E-06
NMB1280		very long chain acyl-CoA dehydrogenase-related protein	-1.120	1.54E-06
NMB1403		FrpA/C-related protein	-1.016	1.32E-05
NMB1405		FrpA/C-related protein	-0.915	4.97E-03
NMB1526	*smpB*	SsrA-binding protein	-0.756	4.12E-02
NMB1669	*hemO*	iron-starvation protein PigA	-2.178	2.44E-08
NMA0029		urea amidolyase-related protein	-1.337	4.08E-04

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table B.8: Genes up-regulated at 240 min during growth in FFP 2 in comparison to t = 0 min (meningococci on solid agar medium). Genes are divided into functional categories according to TIGR designations.

ID	Name	Annotation	$\log_2$ ratio	P-value*
Amino acid biosynthesis				
NMB0337	*ilvE*	branched-chain amino acid aminotransferase	0.783	2.11E-02
NMB0540	*aspC*	aromatic amino acid aminotransferase	0.904	1.32E-03
NMB0678	*trpA*	tryptophan synthase, subunit α	0.624	3.35E-02
NMB0763	*cysK*	cysteine synthase	1.480	4.64E-04
NMB0802		cystathionine γ-synthase	0.844	1.43E-03
NMB1034	*leuD*	isopropylmalate isomerase, small subunit	0.612	3.13E-02
NMB1036	*leuC*	isopropylmalate isomerase, large subunit	1.227	9.56E-04
NMB1069	*proB*	γ-glutamyl kinase	0.929	1.68E-02
NMB1074	*argB*	acetylglutamate kinase	0.715	5.12E-03
NMB1371	*argD*	bifunctional N-succinyldiaminopimelate--aminotransferase/acetylornithine transaminase protein	1.165	1.48E-05
NMB1678	*tyrB*	aromatic amino acid aminotransferase	0.588	3.74E-02
NMB2129	*argG*	argininosuccinate synthase	0.801	2.15E-03
Biosynthesis of cofactors, prosthetic groups, and carriers				
NMB0394	*nadA*	quinolinate synthetase	0.845	7.39E-03
NMB1037	*gshA*	glutamate–cysteine ligase	0.725	1.33E-02
NMB1156	*cysG-1*	siroheme synthase	2.168	7.25E-06
NMB1168		putative phytoene synthase	0.650	6.00E-03
NMB1194	*cysG-2*	siroheme synthase	2.526	1.47E-08
NMB2040	*thiC*	thiamine biosynthesis protein	1.879	2.00E-06
NMB2067	*cytX*	putative hydroxymethylpyrimidine transporter	2.396	1.99E-07
NMB2068	*thiO*	glycine oxidase	2.397	5.61E-04
NMB2069	*thiE*	thiamin-phosphate pyrophosphorylase	1.978	2.52E-06
NMB2071	*thiG*	thiazole synthase	0.871	6.00E-03
Cell envelope				
NMB0749		penicillin-binding protein 4	1.059	1.45E-06
NMB0790	*pgm*	phosphoglucomutase	0.717	2.50E-03
NMB1297	*mltD*	membrane-bound lytic murein transglycosylase D	1.066	1.98E-03
NMB1898	*mlp*	lipoprotein	0.929	8.32E-03
Cellular processes				
NMB0191		ParA family protein	0.655	8.81E-03
NMB1067	*ftsK-1*	cell division protein	0.629	1.68E-02
Central intermediary metabolism				
NMB0031	*glmS*	D-fructose-6-phosphate amidotransferase	0.965	3.08E-03
NMB0468	*speA*	arginine decarboxylase	0.637	3.75E-03
NMB1151	*cysI-1*	sulphite reductase hemoprotein, β component	2.237	4.49E-05
NMB1152	*cysJ-1*	sulphite reductase (NADPH) flavoprotein, α component	1.996	1.43E-04
NMB1153	*cysN-1*	sulphate adenylyltransferase, subunit 1	2.629	3.03E-06
NMB1154	*cysD-1*	sulphate adenylyltransferase, subunit 2	2.338	8.78E-07
NMB1155	*cysH-1*	phosphoadenosine phosphosulphate reductase	1.201	1.29E-02
NMB1189	*cysI-2*	sulphite reductase hemoprotein, β component	2.301	6.26E-05
NMB1190	*cysJ-2*	sulphite reductase (NADPH) flavoprotein, α component	2.367	1.08E-06
NMB1191	*cysN-2*	sulphate adenylyltransferase, subunit 1	2.793	4.41E-08
NMB1192	*cysD-2*	sulphate adenylyltransferase, subunit 2	2.514	2.60E-07

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Table B.8 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB1193	*cysH-2*	phosphoadenosine phosphosulphate reductase	1.473	4.02E-03
NMB1799	*metK*	S-adenosylmethionine synthetase	0.758	4.22E-03
DNA metabolism				
NMB0835		putative type I restriction enzyme EcoR124II R protein	0.841	1.56E-02
NMB1442	*mutL*	DNA mismatch repair protein	0.612	2.57E-02
Energy metabolism				
NMB0010	*pgk*	phosphoglycerate kinase	0.685	4.91E-02
NMB0389	*galM*	aldose 1-epimerase	0.746	1.50E-02
NMB0390	*mapA*	maltose phosphorylase	1.127	3.78E-05
NMB0391	*pgmB*	β-phosphoglucomutase	1.104	4.75E-05
NMB0790	*pgm*	phosphoglucomutase	0.717	2.50E-03
NMB1371	*argD*	bifunctional N-succinyldiaminopimelate--aminotransferase/acetylornithine transaminase protein	1.165	1.48E-05
NMB1390	*glk*	glucokinase	1.122	1.90E-02
NMB2053	*petA*	ubiquinol–cytochrome c reductase, iron-sulphur subunit	1.309	6.47E-04
NMA2094	*mapA′*	possible maltose phosphorylase (fragment)	0.982	8.09E-03
Hypothetical or unassigned proteins				
NMB0086		hypothetical protein	0.635	3.71E-02
NMB0213		hypothetical protein	0.707	9.96E-03
NMB0438		hypothetical protein	1.138	2.81E-04
NMB0762		hypothetical protein	1.085	5.61E-04
NMB0786		hypothetical protein	0.713	6.80E-03
NMB0821		hypothetical protein	0.748	1.05E-03
NMB1024		hypothetical protein	0.703	4.02E-03
NMB1040		hypothetical protein	0.687	1.75E-02
NMB1073		hypothetical protein	0.975	2.41E-03
NMB1270		hypothetical protein	0.831	3.55E-05
NMB1286		hypothetical protein	0.797	3.93E-02
NMB1365	*dnrN*	hypothetical protein	0.884	2.54E-04
NMB1436		hypothetical protein	0.801	2.66E-02
NMB1437		hypothetical protein	1.138	1.29E-02
NMB1438		hypothetical protein	1.065	3.29E-03
NMB1531		hypothetical protein	0.722	6.00E-03
NMB1800		hypothetical protein	0.820	5.93E-03
NMB1886		hypothetical protein	0.631	1.40E-02
NMB2072		hypothetical protein	1.050	1.62E-02
Protein fate				
NMB1969	*aspA/nalP*	putative serotype-1-specific antigen	0.876	3.71E-02
Protein synthesis				
NMB0268		RNA methyltransferase, TrmH family	0.715	1.68E-02
NMB0876		50S ribosomal protein L25/general stress protein Ctc	0.611	1.29E-02
Purines, pyrimidines, nucleosides, and nucleotides				
NMB0284	*purB*	adenylosuccinate lyase	0.591	3.71E-02
Regulatory functions				
NMB1735	*relA*	GTP pyrophosphokinase	0.972	2.54E-04
Transport and binding proteins				
NMB0085	*gltS*	sodium/glutamate symporter	0.717	3.56E-02
NMB0098		ABC transporter, ATP-binding protein (authentic frameshift)	0.932	7.10E-03
NMB0293		putative TonB-dependent receptor	0.823	8.32E-03
NMB0388		putative sugar transporter	1.050	7.13E-04

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Table B.8 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB0470		C4-dicarboxylate transporter	0.807	1.11E-05
NMB0535	*gluP*	glucose/galactose transporter	1.072	2.37E-04
NMB0787		amino acid ABC transporter, periplasmic substrate-binding protein	1.279	8.09E-03
NMB0788		amino acid ABC transporter, permease protein	1.126	1.10E-02
NMB0789		amino acid ABC transporter, ATP-binding protein	1.066	3.31E-03
NMB1017	*sbp*	sulphate ABC transporter, periplasmic substrate-binding protein	1.491	1.45E-02
NMB2067	*cytX*	probable hydroxymethylpyrimidine transporter	2.396	1.99E-07
Unknown function				
NMB0995		macrophage infectivity potentiator-related protein	1.869	9.04E-04
NMB1389		RpiR/YebK/YfhH family protein	0.915	2.25E-02

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table B.9: Genes down-regulated at 240 min during growth in FFP 2 in comparison to t = 0 min (meningococci on solid agar medium). Genes are divided into functional categories according to TIGR designations.

ID	Name	Annotation	$\log_2$ ratio	P-value*
Amino acid biosynthesis				
NMB0878	*ilvA*	threonine dehydratase	-0.685	2.12E-03
NMC0399a	*pglB-2*	carbamoyl-phosphate synthase	-1.317	1.95E-02
Cell envelope				
NMB0178	*lpxA*	UDP-N-acetylglucosamine acyltransferase	-0.601	2.42E-02
NMB1279	*mltB*	putative membrane-bound lytic murein transglycosylase B	-2.055	2.12E-11
NMB2019	*kdtB*	phosphopantetheine adenylyltransferase	-0.723	5.62E-05
Cellular processes				
NMB0216	*katA*	catalase	-1.625	2.33E-02
NMB0585		putative iron-regulated protein FrpA	-0.770	3.97E-02
NMB0851	*rdgC*	recombination associated protein	-1.226	2.10E-06
NMB0855		putative bacteriocin resistance protein	-0.756	4.02E-02
NMB0977		putative modulator of drug activity B	-2.851	2.55E-14
NMB1857	*mdaB*	modulator of drug activity B	-2.750	1.48E-05
NMB1714	*mtrE*	multidrug efflux pump channel protein	-1.128	6.20E-05
NMA0688		Possible hemagglutinin (DUF637) family	-1.626	3.31E-03
Central intermediary metabolism				
NMB1305	*esd*	putative esterase	-1.053	4.64E-04
DNA metabolism				
NMB0399	*xthA*	exodeoxyribonuclease III	-0.865	2.25E-02
NMB1655		N5-glutamine S-adenosyl-L-methionine--dependent methyltransferase	-0.894	4.55E-03
NMB0851	*rdgC*	recombination associated protein	-1.226	2.10E-06
NMB0831		type I restriction enzyme S protein (degenerate)	-0.615	1.14E-02
Energy metabolism				
NMB0207	*gapA*	glyceraldehyde-3-phosphate dehydrogenase	-1.108	7.62E-03
NMB0431	*prpC*	methylcitrate synthase	-5.724	9.96E-14
NMB0434		putative AcnD-accessory protein PrpF	-2.174	1.71E-04
NMB0435	*ackA-2*	acetate kinase	-1.317	1.04E-04
NMB0567	*nqrC*	Na(+)-translocating NADH-quinone reductase, subunit C	-0.709	1.50E-02

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Table B.9 – continued from previous page

ID	Name	Annotation	$\log_2$ ratio	P-value*
NMB0574	*gcvT*	glycine cleavage system aminomethyltransferase T	-1.885	4.91E-12
NMB0955	*sucA*	α-ketoglutarate decarboxylase	-0.808	3.84E-02
NMB0960	*sucD*	succinyl-CoA synthetase, subunit α	-1.257	5.62E-04
NMB1304	*adhC*	alcohol dehydrogenase, class III	-1.284	7.98E-04
NMB1393	*edd*	phosphogluconate dehydratase	-1.252	8.62E-04
NMB1395		alcohol dehydrogenase, zinc-containing	-0.801	1.75E-02
NMB1842		putative 4-hydroxyphenylacetate 3-hydroxylase, small subunit	-0.902	1.33E-02
NMB1968	*aldA*	aldehyde dehydrogenase A	-0.958	9.31E-03
NMB2096	*mqo*	malate:quinone oxidoreductase	-0.738	8.49E-03
Fatty acid and phospholipid metabolism				
NMC0398		putative hydrolase, haloacid dehalogenase-like hydrolase	-0.928	1.75E-02
Hypothetical or unassigned proteins				
NMB0183		conserved hypothetical protein	-0.865	6.93E-04
NMB0226		conserved hypothetical protein	-0.666	1.14E-02
NMB0227		conserved hypothetical protein	-1.052	3.42E-03
NMB0272		hypothetical protein	-0.705	2.44E-02
NMB0366		hypothetical protein	-0.897	4.76E-03
NMB0429		hypothetical protein	-2.118	3.70E-07
NMB0572		hypothetical protein	-0.981	2.54E-04
NMB0741		conserved hypothetical protein	-0.699	4.97E-02
NMB0753		conserved hypothetical protein	-1.059	1.57E-03
NMB0754		hypothetical protein	-1.193	8.13E-04
NMB0755		hypothetical protein	-0.818	1.80E-02
NMB0853		conserved hypothetical protein	-1.034	6.30E-03
NMB0864		hypothetical protein	-1.059	1.70E-02
NMB0915		hypothetical protein	-0.931	2.42E-02
NMB0953		hypothetical protein	-0.974	8.32E-03
NMB1048		hypothetical protein	-1.539	1.43E-03
NMB1345		hypothetical protein	-0.728	3.36E-02
NMB1361		conserved hypothetical protein	-0.748	7.98E-04
NMB1452		conserved hypothetical protein	-2.257	7.46E-09
NMB1485		conserved hypothetical protein	-1.332	5.18E-05
NMB1580		hypothetical protein	-0.927	6.30E-03
NMB1773		hypothetical protein	-1.397	4.97E-02
NMB1858		hypothetical protein	-0.882	4.02E-03
NMB2018		conserved hypothetical protein	-0.959	3.43E-03
NMA0131		conserved hypothetical protein	-3.822	2.92E-19
NMA0432		conserved hypothetical protein	-1.744	3.36E-02
NMA0607		hypothetical protein	-1.157	9.43E-03
NMA0779		integral membrane protein	-0.753	2.04E-02
NMA1194		hypothetical protein	-1.354	7.13E-04
NMA1227		conserved hypothetical protein	-1.027	2.02E-03
NMA1725		virulence associated protein	-1.141	3.19E-02
NMA1858		hypothetical protein	-1.485	3.63E-04
NMA2123		conserved hypothetical protein	-0.727	4.23E-02
NMC0876		hypothetical protein (fragment)	-0.778	3.97E-02
NMC0209		conserved hypothetical protein	-1.344	9.31E-03
NMC0806		putative integral membrane protein	-1.069	2.14E-02
NMC1053		hypothetical protein	-0.909	5.93E-03
NMC1345		putative iron-regulated protein FrpC outer membrane (pseudogene)	-0.957	1.18E-02
NMC1730		putative integral membrane protein	-2.994	1.03E-09

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Table B.9 – continued from previous page

ID	Name	Annotation	log_2 ratio	P-value*
Mobile and extrachromosomal element functions				
NMB0583		IS1016C2 transposase	-0.601	3.19E-02
NMB1401		IS1016C2 transposase	-0.608	2.40E-02
Protein fate				
NMB1519	*dsbD*	thiol:disulfide interchange protein precursor	-0.747	2.58E-02
NMB1587		predicted inner membrane peptidase	-1.140	8.32E-03
Protein synthesis				
NMB0721	*infC*	translation initiation factor IF-3	-1.816	2.54E-04
NMB0937	*efp*	elongation factor P	-1.419	1.27E-02
NMB0942	*rpmE2*	50S ribosomal protein L31 type B	-1.493	2.60E-03
NMB2102	*tsf*	elongation factor Ts	-1.176	4.44E-02
Purines, pyrimidines, nucleosides, and nucleotides				
NMB2151	*purD*	phosphoribosylamine–glycine ligase	-1.200	2.54E-04
Regulatory functions				
NMB0398		transcriptional regulator, ArsR family	-1.626	7.76E-03
NMB1650	*lrp*	leucine-responsive regulatory protein	-0.827	3.14E-02
Transcription				
NMB0617	*rho*	transcription termination factor Rho	-1.174	3.14E-04
NMB0683	*nusB*	transcription antitermination protein	-0.757	1.75E-02
NMB0758	*pnp*	polynucleotide phosphorylase/polyadenylase	-0.899	3.14E-02
NMB1368		putative ATP-dependent RNA helicase	-5.538	3.71E-11
NMB1863	*orn*	oligoribonuclease	-0.878	9.96E-03
Transport and binding proteins				
NMB0177		putative sodium/alanine symporter	-0.969	1.95E-02
NMB0402	*putP*	sodium/proline symporter	-0.883	3.08E-02
NMB0610	*potA-1*	spermidine/putrescine ABC transporter, ATP-binding protein	-1.557	2.82E-03
NMB0792		transporter, NadC family	-1.051	5.28E-04
NMB1714	*mtrE*	multidrug efflux pump channel protein	-1.128	6.20E-05
NMB1715	*mtrD*	multiple transferable resistance system protein MtrD	-0.788	6.80E-03
NMB1732		putative transporter	-0.855	7.62E-03
NMC1690	*tbpA*	transferrin-binding protein 1	-1.050	6.93E-05
NMC1691	*tbpB*	transferrin-binding protein B	-2.501	1.85E-10
Unknown function				
NMB0006		thioredoxin-related protein	-0.698	1.10E-02
NMB0643	*mafB3*	MafB-related protein	-1.087	9.01E-03
NMB0852	*engA*	GTP-binding protein EngA	-1.136	8.32E-03
NMB1280		very long chain acyl-CoA dehydrogenase-related protein	-1.811	3.85E-12
NMB1638		YhbX/YhjW/YijP/YjdB family protein	-0.793	2.59E-03
NMB1669	*hemO*	iron-starvation protein PigA	-1.228	2.80E-03
NMB2063	*slyX*	hypothetical protein	-0.769	6.38E-03
NMC1731	*ddpX*	putative D-alanyl-D-alanine dipeptidase	-3.487	3.85E-12

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table B.10: Differentially expressed genes at both 60 and 240 min during growth in FFP 2 in comparison to t = 0 min (meningococci on solid agar medium).

ID	Name	Annotation	60 min log_2 ratio	240 min log_2 ratio
Up-regulated genes				
NMB0010	*pgk*	phosphoglycerate kinase	0.790	0.685
NMB0098		ABC transporter, ATP-binding protein (authentic frameshift)	0.882	0.932
NMB0191		ParA family protein	0.602	0.655

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Table B.10 – continued from previous page

ID	Name	Annotation	60 min $\log_2$ ratio	240 min $\log_2$ ratio
NMB0268		RNA methyltransferase, TrmH family	1.355	0.715
NMB0292		hypothetical protein	0.715	0.823
NMB0394	*nadA*	quinolinate synthetase	1.141	0.845
NMB0438		hypothetical protein	0.986	1.138
NMB0468	*speA*	arginine decarboxylase	0.730	0.637
NMB0762		hypothetical protein	0.692	1.085
NMB0787		amino acid ABC transporter, periplasmic substrate-binding protein	1.547	1.279
NMB0788		amino acid ABC transporter, permease protein	1.751	1.126
NMB0789		amino acid ABC transporter, ATP-binding protein	1.576	1.066
NMB0802	*metB*	cystathionine γ-synthase	0.633	0.844
NMB0821		hypothetical protein	1.115	0.748
NMB0995		macrophage infectivity potentiator-related protein	1.466	1.869
NMB1034	*leuD*	isopropylmalate isomerase, small subunit	1.604	0.612
NMB1036	*leuC*	isopropylmalate isomerase, large subunit	2.168	1.227
NMB1037	*gshA*	glutamate–cysteine ligase	0.975	0.725
NMB1040		hypothetical protein	0.713	0.687
NMB1073		hypothetical protein	0.780	0.975
NMB1151	*cysI-1*	sulphite reductase hemoprotein, β component	1.341	2.237
NMB1152	*cysJ-1*	sulphite reductase (NADPH) flavoprotein, α component	1.176	1.996
NMB1153	*cysN-1*	sulphate adenylyltransferase, subunit 1	1.421	2.629
NMB1154	*cysD-1*	sulphate adenylyltransferase, subunit 2	1.315	2.338
NMB1156	*cysG-1*	siroheme synthase	1.212	2.168
NMB1168		putative phytoene synthase	1.486	0.650
NMB1189	*cysI-2*	sulphite reductase hemoprotein, β component	1.405	2.301
NMB1190	*cysJ-2*	sulphite reductase (NADPH) flavoprotein, α component	1.393	2.367
NMB1191	*cysN-2*	sulphate adenylyltransferase, subunit 1	1.636	2.793
NMB1192	*cysD-2*	sulphate adenylyltransferase, subunit 2	1.447	2.514
NMB1194	*cysG-2*	siroheme synthase	1.438	2.526
NMB1270		hypothetical protein	0.903	0.831
NMB1286		hypothetical protein	0.962	0.797
NMB1297	*mltD*	membrane-bound lytic murein transglycosylase D	0.708	1.066
NMB1531		hypothetical protein	0.669	0.722
NMB1799	*metK*	S-adenosylmethionine synthetase	1.174	0.758
NMB1800		hypothetical protein	1.139	0.820
NMB1866		hypothetical protein	0.895	0.631
NMB2053	*petA*	ubiquinol–cytochrome c reductase, iron-sulphur subunit	0.924	1.309
Down-regulated genes				
NMB0006		thioredoxin-related protein	-1.143	-0.698
NMB0177		putative sodium/alanine symporter	-1.905	-0.969
NMB0183		hypothetical protein	-0.765	-0.865
NMB0207	*gapA*	glyceraldehyde-3-phosphate dehydrogenase	-1.071	-1.108
NMB0216	*katA*	catalase	-1.904	-1.625
NMB0227		hypothetical protein	-1.839	-1.052
NMB0366		hypothetical protein	-0.940	-0.897
NMB0402	*putP*	sodium/proline symporter	-2.275	-0.883
NMB0429		hypothetical protein	-2.440	-2.118
NMB0431	*prpC*	methylcitrate synthase	-5.745	-5.724
NMB0434		putative AcnD-accessory protein PrpF	-2.515	-2.174

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Table B.10 – continued from previous page

ID	Name	Annotation	60 min $\log_2$ ratio	240 min $\log_2$ ratio
NMB0435	*ackA2*	acetate kinase	-1.693	-1.317
NMB0572		hypothetical protein	-0.879	-0.981
NMB0574	*gcvT*	glycine cleavage system aminomethyltransferase T	-3.552	-1.885
NMB0583		IS1016C2 transposase	-0.869	-0.601
NMB0585		putative iron-regulated protein FrpA	-0.856	-0.770
NMB0610	*potA-1*	spermidine/putrescine ABC transporter, ATP-binding protein	-1.660	-1.557
NMB0753		hypothetical protein	-0.797	-1.059
NMB0754		hypothetical protein	-1.133	-1.193
NMB0755		hypothetical protein	-0.752	-0.818
NMB0792		transporter, NadC family	-2.430	-1.051
NMB0851	*rdgC*	recombination associated protein	-1.809	-1.226
NMB0852		GTP-binding protein EngA	-1.797	-1.136
NMB0853		hypothetical protein	-1.778	-1.034
NMB0855		putative bacteriocin resistance protein	-1.302	-0.756
NMB0864		hypothetical protein	-1.648	-1.059
NMB0878	*ilvA*	threonine dehydratase	-1.092	-0.685
NMB0915		hypothetical protein	-0.855	-0.931
NMB0953		hypothetical protein	-1.706	-0.974
NMB0955	*sucA*	α-ketoglutarate decarboxylase	-1.517	-0.808
NMB0960	*sucD*	succinyl-CoA synthetase, subunit α	-1.887	-1.257
NMB0977		putative modulator of drug activity B	-2.535	-2.851
NMB1048		hypothetical protein	-2.708	-1.539
NMB1279	*mltB*	putative membrane-bound lytic murein transglycosylase B	-1.093	-2.055
NMB1280		very long chain acyl-CoA dehydrogenase-related protein	-1.120	-1.811
NMB1304	*adhC*	alcohol dehydrogenase, class III	-0.975	-1.284
NMB1305	*esd*	putative esterase	-0.726	-1.053
NMB1361		hypothetical protein	-0.616	-0.748
NMB1368		putative ATP-dependent RNA helicase	-3.255	-5.538
NMB1393	*edd*	phosphogluconate dehydratase	-0.935	-1.252
NMB1395		alcohol dehydrogenase, zinc-containing	-2.252	-0.801
NMB1401		IS1016C2 transposase	-0.829	-0.608
NMB1452		hypothetical protein	-2.230	-2.257
NMB1485		hypothetical protein	-0.744	-1.332
NMB1519	*dsbD*	thiol:disulfide interchange protein precursor	-0.779	-0.747
NMB1580		hypothetical protein	-1.548	-0.927
NMB1655		N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase	-0.835	-0.894
NMB1669	*hemO*	iron-starvation protein	-2.178	-1.228
NMB1714	*mtrE*	multidrug efflux pump channel protein	-1.621	-1.128
NMB1715	*mtrD*	multiple transferable resistance system protein MtrD	-1.389	-0.788
NMB1732		putative transporter	-0.949	-0.855
NMB1857	*mdaB*	modulator of drug activity B	-2.464	-2.750
NMB1858		hypothetical protein	-0.773	-0.882
NMB1968	*aldA*	aldehyde dehydrogenase A	-2.076	-0.958
NMB2018		hypothetical protein	-0.834	-0.959
NMB2019	*kdtB*	phosphopantetheine adenylyltransferase	-0.675	-0.723
NMB2096	*mqo*	malate:quinone oxidoreductase	-1.803	-0.738
NMA0131		conserved hypothetical protein	-4.154	-3.822
NMA0607		hypothetical protein	-0.985	-1.157
NMA1194		hypothetical protein	-1.724	-1.354

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Table B.10 – continued from previous page

ID	Name	Annotation	60 min $\log_2$ ratio	240 min $\log_2$ ratio
NMA1227		conserved hypothetical protein	-0.785	-1.027
NMA1858		hypothetical protein	-1.874	-1.485
NMA2123		conserved hypothetical protein	-0.763	-0.727
NMC0806		putative integral membrane protein	-1.998	-1.069
NMC1053		hypothetical protein	-0.948	-0.909
NMC1345		pseudogene	-0.988	-0.957
NMC1691	*tbpB*	transferrin binding protein B	-1.735	-2.501
NMC1730		putative integral membrane protein	-2.634	-2.994
NMC1731	*ddpX*	putative D-alanyl-D-alanine dipeptidase	-3.104	-3.487

Table B.11: Progressively up-regulated genes during meningococcal growth in plasma (240 min versus 60 min). Expression data from experiments in medium are also included for those genes that were significantly differentially expressed under this growth condition (between 60 and 240 min). Genes that were up-regulated during growth in both FFP 1 and FFP 2, but not in media-grown meningococci, are shaded in grey.

			FFP 1		FFP 2		Medium	
ID	**Name**	**Annotation**	**$\log_2$ ratio**	**P-value***	**$\log_2$ ratio**	**P-value***	**$\log_2$ ratio**	**P-value***
Amino acid biosynthesis								
Aromatic amino acid family								
NMB0307	*aroG*	phospho-2-dehydro-3-heoxyheptonate aldolase	0.796	5.58E-04				
NMB0358	*aroE*	shikimate 5-dehydrogenase			0.694	4.03E-02		
Aspartate family								
NMB0540	*aspC*	aromatic amino acid aminotransferase	0.695	6.80E-03	0.779	4.68E-03	1.160	2.65E-05
Glutamate family								
NMB0359	*glnA*	glutamate–ammonia ligase	1.962	2.40E-08	0.972	1.66E-02	1.033	4.74E-03
NMB1069	*proB*	γ-glutamyl kinase	0.605	2.86E-02				
NMB1074	*argB*	acetylglutamate kinase	0.960	3.73E-06				
NMB1371	*argD*	bifunctional N-succinyldiaminopimelate-aminotransferase/acetylornithine transaminase protein	0.839	1.84E-04	0.668	1.42E-02		
NMB1476	*gluD*	glutamate dehydrogenase, NAD-specific			1.049	3.38E-04		
NMB1573	*argF*	ornithine carbamoyltransferase, catabolic	0.892	5.35E-05				
NMB1710	*gdhA*	glutamate dehydrogenase	3.715	1.66E-13			1.276	6.43E-03
NMB1787	*argC*	N-acetyl-gamma-glutamyl-phosphate reductase			0.606	4.88E-02		
NMB1995	*glnB*	nitrogen regulatory protein P-II	1.020	1.91E-06			0.779	5.42E-04
NMB2005	*argJ*	bifunctional ornithine acetyltransferase/N-acetylglutamate synthase protein	1.709	2.46E-06	1.169	6.16E-03	0.901	2.44E-02
Histidine family								
NMB0603	*hisE*	phosphoribosyl-ATP pyrophosphatase	0.856	4.37E-05			1.153	7.25E-07
NMB1579	*hisG*	ATP phosphoribosyltransferase, catalytic subunit	1.011	1.61E-05	0.611	3.57E-02		
NMB1581	*hisD*	histidinol dehydrogenase	0.901	1.31E-03				
NMB1582	*hisC*	histidinol-phosphate aminotransferase	0.793	2.15E-02				
NMB1583	*hisB*	imidazoleglycerol-phosphate dehydratase	0.951	1.30E-02				
Pyruvate family								
NMB0337	*ilvE*	branched-chain amino acid aminotransferase	1.670	3.62E-09	0.911	3.79E-03		
NMB1031	*leuB*	3-isopropylmalate dehydrogenase	0.656	6.37E-03				
NMB1574	*ilvC*	ketol-acid reductoisomerase	0.767	4.85E-02				
Serine family								
NMB0381	*cysB*	transcriptional regulator CysB-like protein	0.603	5.01E-04				
NMB1055	*glyA*	serine hydroxymethyltransferase	0.738	1.65E-02	1.005	6.41E-03		
Biosynthesis of cofactors, prosthetic groups, and carriers								
Folic acid								
NMB1055	*glyA*	serine hydroxymethyltransferase	0.738	1.65E-02	1.005	6.41E-03		
NMB1063	*folB*	dihydroneopterin aldolase	1.033	1.31E-06	0.888	3.11E-04		
Heme, porphyrin, and cobalamin								
NMB0801	*hemB*	delta-aminolevulinic acid dehydratase	0.712	5.66E-04	0.993	6.21E-05	0.824	2.21E-04
NMB1194	*cysG-2*	siroheme synthase			1.089	1.42E-02		

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Table B.11 – continued from previous page

ID	Name	Annotation	FFP 1		FFP 2		Medium	
			log_2 ratio	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***
Menaquinone and ubiquinone								
NMB0323		hypothetical protein	0.940	3.83E-04			0.972	6.67E-04
Pyridine nucleotides								
NMB1364	*nadE*	putative NH(3)-dependent NAD+ synthetase			1.414	7.98E-05		
Pyridoxine								
NMB1360	*pdxH*	pyridoxamine 5'-phosphate oxidase	0.671	2.86E-02	0.785	4.07E-02	1.145	4.82E-04
Riboflavin, FMN, and FAD								
NMB1247	*ribC*	riboflavin synthase, subunit α	0.587	1.09E-02				
Thiamine								
NMB2040	*thiC*	thiamine biosynthesis protein	1.145	4.80E-04	2.005	5.64E-07	0.824	1.25E-02
NMB2068	*thiO*	glycine oxidase	2.351	4.04E-05	2.332	5.08E-04	1.866	7.77E-04
NMB2069	*thiE*	thiamin-phosphate pyrophosphorylase	1.815	5.33E-07	2.144	5.64E-07	2.001	3.60E-07
NMB2071	*thiG*	thiazole synthase	0.658	8.83E-03	1.154	1.11E-04	1.264	3.82E-06
NMB2067	*cytX*	probable hydroxymethylpyrimidine transporter	2.138	7.98E-08	2.467	1.27E-07	1.929	3.07E-06
Cell envelope								
Biosynthesis and degradation of murein sacculus and peptidoglycan								
NMB0749		penicillin-binding protein 4	0.908	1.33E-06				
Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides								
NMB0756	*rfbD*	putative dTDP-L-rhamnose synthase	0.664	1.90E-05				
NMB2090	*lpcA*	phosphoheptose isomerase	0.791	1.43E-02				
Other								
NMB0182	*omp85*	outer membrane protein Omp85	0.936	1.78E-03			0.696	4.44E-02
NMB0345		putative cell-binding factor	0.627	4.48E-02				
NMB0382	*rmpM*	outer membrane protein class 4	1.186	1.42E-02				
NMB0663	*nspA*	outer membrane protein	2.478	1.66E-13				
NMB1483		putative lipoprotein NlpD			1.001	1.33E-02		
NMB1716	*mtrC*	membrane fusion protein			0.835	4.42E-03	0.690	1.09E-02
NMB1898	*mlp*	lipoprotein	1.095	9.01E-05	0.935	5.15E-03	0.712	1.07E-02
Surface structures								
NMB0333	*pilG*	pilus assembly protein	0.693	1.62E-02			0.900	4.26E-03
NMB0886		fimbrial protein FimT	1.007	1.16E-02				
NMB0887		putative type IV pilus assembly protein PilV	0.821	5.72E-06				
NMB1808	*pilM*	pilM protein	0.820	3.52E-02				
NMB1810	*pilO*	pilO protein	0.807	4.76E-02				
NMB1994	*nadA*	putative adhesin/invasin	2.261	2.67E-10				
Cellular processes								
Adaptations to atypical conditions								
NMB0784		putative phage shock protein E precursor	0.765	1.51E-03	1.117	1.45E-05	1.010	1.10E-04
NMB1953	*regF*	stringent starvation protein A	1.253	7.78E-06			0.991	3.03E-03
Cell division								
NMB0171	*minD*	septum site-determining protein	0.845	2.02E-02				
NMB0172	*minE*	cell division topological specificity factor	0.740	2.63E-02				

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Table B.11 – continued from previous page

			FFP 1		FFP 2		Medium	
ID	**Name**	**Annotation**	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***
NMB1791	*cafA*	cytoplasmic axial filament protein			0.619	3.11E-02		
Chemotaxis and motility								
NMB0051	*pilU*	twitching motility protein	0.856	9.84E-03				
NMB0768	*pilT-2*	twitching motility protein	0.901	2.26E-06				
Detoxification								
NMB0884	*sodB*	superoxide dismutase	1.415	3.00E-03				
NMB1398	*sodC*	Cu-Zn-superoxide dismutase	2.636	4.74E-13	1.327	3.16E-04	1.671	9.26E-07
DNA transformation								
NMB0692	*tpc*	tpc protein	0.911	2.14E-05	0.680	8.13E-03	0.791	5.49E-04
Pathogenesis								
NMB0333	*pilG*	pilus assembly protein	0.693	1.62E-02			0.900	4.26E-03
NMB0585		putative iron-regulated protein FrpA	0.709	1.16E-02			0.830	7.50E-03
NMB0887		putative type IV pilus assembly protein PilV	0.821	5.72E-06				
NMB1415	*frpC*	iron-regulated protein FrpC	0.894	6.58E-03				
NMB1750	*pivNM-2*	pilin gene inverting protein	1.415	8.94E-03				
NMB1985	*iga2*	adhesion and penetration protein	0.590	2.13E-02				
NMB1994	*nadA*	putative adhesin/invasin	2.261	2.67E-10				
Pathogenesis and DNA transformation								
NMB1808	*pilM*	pilM protein	0.820	3.52E-02				
NMB1810	*pilO*	pilO protein	0.807	4.76E-02				
Pathogenesis and toxin production and resistance								
NMB0493		hemagglutinin/hemolysin-related protein	1.574	1.07E-02				
NMB0497		hemagglutinin/hemolysin-related protein	0.720	3.90E-02				
Toxin production and resistance								
NMB0691		putative colicin V production protein	0.717	3.83E-04				
NMB0697	*ksgA*	dimethyladenosine transferase	0.719	2.96E-04				
NMB0855		putative bacteriocin resistance protein	0.585	3.66E-02				
NMB1763		putative toxin-activating protein	1.229	4.34E-02				
NMB2091		putative hemolysin	1.160	2.84E-04	0.995	1.00E-02		
Central intermediary metabolism								
Other								
NMB0924		oxidoreductase, short-chain dehydrogenase/reductase family	1.113	8.40E-08	0.727	2.12E-03		
NMB1127		hypothetical protein	0.752	2.21E-03	0.786	8.51E-03		
NMB1165		short chain dehydrogenase	0.729	4.18E-03	0.805	8.91E-03		
NMB1391	*pgl*	oxidoreductase, Sol/DevB family	0.742	1.92E-03				
NMB1584		3-hydroxyacid dehydrogenase	1.382	4.13E-07	1.238	7.94E-05		
Phosphorus compounds								
NMB1900	*ppk*	polyphosphate kinase	0.952	7.05E-04	0.756	3.17E-02		
Sulfur metabolism								
NMB1153	*cysN-1*	sulfate adenylyltransferase, subunit 1			1.208	4.14E-02		
NMB1154	*cysD-1*	sulfate adenylyltransferase, subunit 2			1.023	3.97E-02		
NMB1191	*cysN-2*	sulfate adenylyltransferase, subunit 1			1.156	2.61E-02		

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ID	Name	Annotation	FFP 1		FFP 2		Medium	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB1192	*cysD-2*	sulfate adenylyltransferase, subunit 2			1.067	3.49E-02		
DNA metabolism								
DNA replication, recombination, and repair								
NMB0826	*nmgII*	C-5 cytosine-specific DNA methylase	0.759	4.85E-02				
NMB0962	*uvrA*	excinuclease ABC, subunit A	1.424	3.56E-06				
NMB1295	*fpg*	formamidopyrimidine-DNA glycosylase			0.655	2.76E-02		
NMB1445	*recA*	recombinase A	0.673	2.46E-02			0.984	2.48E-03
NMB1750	*pivNM-2*	pilin gene inverting protein	1.415	8.94E-03				
NMB1902	*dnaN*	DNA polymerase III, subunit β			0.593	1.17E-02	0.763	2.66E-04
Restriction modification								
NMB0726		type II restriction enzyme HgaI	0.959	3.30E-02				
NMB1032		type II restriction enzyme NlaIV	1.166	7.64E-06				
NMB1290		C-5 cytosine-specific DNA-methylase	0.784	3.20E-02				
Energy metabolism								
Amino acids and amines								
NMB0176	*dadA*	D-amino acid dehydrogenase, small subunit			1.095	2.83E-02		
NMB0401	*putA*	bifunctional proline dehydrogenase/pyrroline-5-carboxylate dehydrogenase	1.048	1.39E-03	0.980	1.44E-02		
NMB0574	*gcvT*	glycine cleavage system aminomethyltransferase T			1.667	8.90E-12		
NMB0575	*gcvH*	glycine cleavage system protein H			1.257	1.53E-02		
NMB1371	*argD*	bifunctional N-succinyldiaminopimelate-aminotransferase/acetylornithine transaminase protein	0.839	1.84E-04	0.668	1.42E-02		
NMB1573	*argF*	ornithine carbamoyltransferase, catabolic	0.892	5.35E-05				
NMB1609	*metZ*	trans-sulfuration enzyme family protein	0.955	1.03E-03	1.102	3.35E-04	0.966	2.52E-03
Electron transport								
NMB0241	*nuoA*	NADH dehydrogenase I, subunit A	0.704	2.97E-02				
NMB0242	*nuoB*	NADH dehydrogenase I, subunit B	0.638	1.01E-02			-0.793	3.53E-03
NMB0244	*nuoD*	NADH dehydrogenase I, subunit D	0.727	4.70E-03			-0.710	1.37E-02
NMB0245	*nuoE*	NADH dehydrogenase I, subunit E	0.803	5.43E-03				
NMB0246	*nuoF*	NADH dehydrogenase I, subunit F	0.643	1.87E-02				
NMB0249	*nuoG*	NADH dehydrogenase I, subunit G	0.685	1.68E-02				
NMB0251	*nuoI*	NADH dehydrogenase I, subunit I	0.808	5.70E-03			-0.785	7.50E-03
NMB0253	*nuoJ*	NADH dehydrogenase I, subunit J	0.780	2.40E-02				
NMB0254	*nuoK*	NADH dehydrogenase I, subunit K	0.754	2.46E-02				
NMB0257	*nuoL*	NADH dehydrogenase I, subunit L	0.817	3.05E-03				
NMB0978	*pntB*	pyridine nucleotide transhydrogenase	1.323	5.49E-06	1.129	8.70E-04		
NMB0980	*pntA*	NAD(P) transhydrogenase, subunit α	1.519	7.95E-07	1.254	3.55E-04		
NMB1450	*fpr-2*	ferredoxin–NADP reductase	0.743	4.70E-03				
NMB1677		cytochrome c5	2.069	2.92E-10				
NMB1723	*fixP*	cytochrome c oxidase, subunit III	2.120	1.01E-11	0.816	1.39E-02		
NMB1724	*fixO*	cytochrome c oxidase, subunit II	2.145	1.33E-07				
NMB1805	*cyc*	cytochrome c4	0.804	2.00E-03				
NMB2051	*petC*	ubiquinol–cytochrome c reductase, cytochrome c1	1.109	1.01E-04				

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Table B.11 – continued from previous page

			FFP 1		FFP 2		Medium	
ID	**Name**	**Annotation**	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***
NMB2052	*petB*	ubiquinol–cytochrome c reductase, cytochrome b	1.044	1.61E-04				
NMB2053	*petA*	ubiquinol–cytochrome c reductase, iron-sulfur subunit	1.405	8.40E-08				
NMB2155	*etfB*	electron transfer flavoprotein, β subunit	0.730	4.31E-02				
Entner-Doudoroff								
NMB1393	*edd*	phosphogluconate dehydratase	0.919	2.77E-03				
NMB1394	*eda*	keto-hydroxyglutarate-aldolase/keto-deoxy-phosphogluconate aldolase	1.024	8.79E-04				
Fermentation								
NMB0546	*adhA*	alcohol dehydrogenase, propanol-preferring	3.160	2.77E-11	1.774	3.55E-04	2.086	5.11E-06
NMB0604		alcohol dehydrogenase, zinc-containing	3.450	1.53E-16	1.230	1.68E-03	3.028	5.30E-14
NMB0631		putative phosphate acetyltransferase Pta	2.412	1.54E-09	1.567	3.26E-04		
NMB1395		alcohol dehydrogenase, zinc-containing	1.043	8.46E-04	1.451	1.45E-08	0.733	4.14E-02
NMB1968	*aldA*	aldehyde dehydrogenase A			1.118	1.32E-03		
Glycolysis/gluconeogenesis								
NMB0089	*pykA*	pyruvate kinase II	1.852	4.51E-10			0.641	3.88E-02
NMB0618	*ppsA*	phosphoenolpyruvate synthase	0.921	5.36E-04	0.797	5.10E-03	0.955	9.12E-04
NMB0997	*dld*	D-lactate dehydrogenase			0.972	2.74E-03		
NMB1285	*eno*	phosphopyruvate hydratase	0.694	4.69E-02				
NMB1388	*pgi1*	glucose-6-phosphate isomerase	0.726	3.48E-03				
NMB1390	*glk*	glucokinase	1.175	1.49E-03				
NMB1604	*gpm*	phosphoglyceromutase	0.950	1.53E-04	0.910	2.30E-03	0.656	1.97E-02
NMB1869	*fba*	fructose-1,6-bisphosphate aldolase	1.471	1.41E-06	0.865	1.68E-02	1.384	8.22E-07
NMB1887	*tpi*	triosephosphate isomerase	0.652	7.81E-03				
NMB2159		glyceraldehyde 3-phosphate dehydrogenase	1.772	7.78E-06				
Other								
NMB0340	*gloA*	lactoylglutathione lyase	0.968	2.25E-04			-0.768	8.03E-03
NMB1493	*cstA*	carbon starvation protein A	2.072	2.63E-10	2.313	1.40E-09	1.024	1.41E-03
NMB2061	*ppc*	phosphoenolpyruvate carboxylase			0.718	3.34E-02		
Pentose phosphate pathway								
NMB1244	*rpe*	ribulose-phosphate 3-epimerase	0.596	1.30E-02	1.016	3.36E-04		
NMB1392	*zwf*	glucose-6-phosphate 1-dehydrogenase	0.769	6.67E-03				
Sugars								
NMB0389	*galM*	aldose 1-epimerase			0.709	1.51E-02		
NMB0390	*mapA*	maltose phosphorylase	1.434	3.73E-09	0.784	3.82E-03	0.947	1.05E-04
NMB0391	*pgmB*	β-phosphoglucomutase	1.423	3.73E-09	0.826	2.05E-03	1.066	1.05E-05
TCA cycle								
NMB0920	*icd*	isocitrate dehydrogenase, NADP-dependent, monomeric type	1.795	4.92E-09	1.501	7.33E-05		
NMB0948	*sdhC*	succinate dehydrogenase, cytochrome b556 subunit	0.874	2.43E-04	1.500	2.03E-08		
NMB0949	*sdhD*	succinate dehydrogenase, hydrophobic membrane anchor protein	1.048	1.23E-05	1.205	2.05E-05		
NMB0950	*sdhA*	succinate dehydrogenase, flavoprotein subunit	0.957	1.01E-03	1.156	8.40E-04		
NMB0951	*sdhB*	succinate dehydrogenase, iron-sulfur subunit	0.869	1.57E-03	1.072	1.14E-03	-0.789	4.11E-03
NMB0954	*gltA*	type II citrate synthase	1.011	1.02E-03	1.714	3.41E-06		
NMB0956	*sucB*	dihydrolipoamide acetyltransferase	0.892	1.21E-03	0.819	1.51E-02		

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Table B.11 – continued from previous page

			FFP 1		FFP 2		Medium	
ID	**Name**	**Annotation**	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***
NMB1572	*acnB*	bifunctional aconitate hydratase 2/2-methylisocitrate dehydratase	2.499	3.09E-12	1.771	2.11E-06		
NMB1613	*fumA*	fumarate hydratase class I, anaerobic	0.764	4.38E-04			-0.747	2.97E-03
NMB2096	*mqo*	malate:quinone oxidoreductase	0.918	4.11E-05	1.065	6.25E-05		
Fatty acid and phospholipid metabolism								
Biosynthesis								
NMB2034	*nlaB*	putative 1-acyl-sn-glycerol-3-phosphate acyltransferase	0.695	2.97E-02				
Hypothetical or unassigned proteins								
Conserved								
NMB0035		hypothetical protein	0.687	3.23E-02	1.261	1.65E-04	2.613	3.32E-12
NMB0036		hypothetical protein			0.787	3.16E-02	1.686	3.60E-07
NMB0050		hypothetical protein	0.720	3.30E-05				
NMB0053		hypothetical protein			0.696	1.68E-03		
NMB0060		hypothetical protein			1.217	3.95E-05		
NMB0121		hypothetical protein	0.803	5.96E-03				
NMB0227		hypothetical protein			0.788	2.78E-02		
NMB0228		hypothetical protein			1.036	8.73E-03		
NMB0230		hypothetical protein	0.607	2.48E-02	0.931	3.78E-03		
NMB0248		hypothetical protein	0.589	3.76E-02				
NMB0286		hypothetical protein			0.598	5.99E-03		
NMB0377		anhydro-N-acetylmuramic acid kinase			0.865	3.22E-03	0.795	9.12E-04
NMB0479		hypothetical protein	0.817	1.72E-02				
NMB0557		hypothetical protein	0.802	4.47E-03				
NMB0619		hypothetical protein			0.595	2.10E-02		
NMB0711		hypothetical protein	1.203	1.01E-08	1.095	3.41E-06		
NMB0776		hypothetical protein	0.683	3.64E-02				
NMB0783		hypothetical protein	0.601	1.16E-02			0.871	6.84E-04
NMB0853		hypothetical protein	1.092	1.29E-03				
NMB0883		hypothetical protein	0.774	3.06E-02				
NMB0952		hypothetical protein	0.940	3.02E-03	1.470	9.94E-05		
NMB1016		hypothetical protein	0.724	5.25E-03				
NMB1030		hypothetical protein	1.047	2.22E-06				
NMB1064		hypothetical protein	1.581	2.48E-11	0.983	3.29E-04		
NMB1286		hypothetical protein	0.762	8.40E-03				
NMB1306		hypothetical protein	0.606	1.60E-02				
NMB1333		hypothetical protein	0.622	5.19E-03			0.595	1.77E-02
NMB1365		hypothetical protein	0.855	2.07E-05	0.746	1.68E-03		
NMB1417		hypothetical protein	0.599	5.56E-03	0.750	3.91E-03		
NMB1436		hypothetical protein	0.625	2.53E-02			-1.015	6.81E-04
NMB1437		hypothetical protein	0.809	2.55E-02			-1.046	8.40E-03
NMB1438		hypothetical protein	0.939	1.17E-03			-0.685	3.95E-02
NMB1494		hypothetical protein	1.223	1.16E-05	1.651	8.27E-07	0.748	2.26E-02
NMB1500		hypothetical protein	1.999	1.56E-09	1.655	5.78E-06	1.550	3.07E-06

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Table B.11 – continued from previous page

			FFP 1		FFP 2		Medium	
ID	**Name**	**Annotation**	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***
NMB1557		hypothetical protein	1.253	1.13E-07	0.667	1.53E-02	0.798	1.09E-03
NMB1578		hypothetical protein	1.392	1.31E-06				
NMB1590		hypothetical protein			0.736	8.55E-04		
NMB1619		hypothetical protein	0.618	3.22E-03				
NMB1620		hypothetical protein	1.317	2.22E-04				
NMB1627		hypothetical protein	0.668	2.81E-02				
NMB1652		hypothetical protein	0.835	1.09E-03			0.621	3.31E-02
NMB1653		hypothetical protein	0.796	1.51E-03				
NMB1665		hypothetical protein	0.908	4.26E-03				
NMB1727		hypothetical protein	0.861	7.95E-05	0.648	1.42E-02		
NMB1749		hypothetical protein	0.724	3.90E-02				
NMB1759		hypothetical protein	1.392	2.33E-02				
NMB1760		hypothetical protein	1.374	1.06E-02				
NMB1761		hypothetical protein	1.303	4.68E-02				
NMB1806	*engB*	GTPase EngB	0.725	4.76E-03				
NMB1824		hypothetical protein	0.836	6.87E-03				
NMB1840		hypothetical protein	0.964	1.29E-03				
NMB1890		hypothetical protein	0.585	1.52E-02				
Unassigned								
NMB0065		hypothetical protein	1.046	1.82E-04				
NMB0086		hypothetical protein	0.670	3.32E-03				
NMB0091		hypothetical protein	2.343	1.28E-07			1.092	2.26E-02
NMB0092		hypothetical protein	2.044	2.92E-10	0.870	5.65E-03	0.773	1.95E-02
NMB0094		hypothetical protein	0.924	4.39E-03				
NMB0095		hypothetical protein	0.700	2.34E-03				
NMB0100		hypothetical protein	1.272	4.30E-05				
NMB0102		hypothetical protein	1.026	1.13E-03				
NMB0119		hypothetical protein	1.548	2.82E-05				
NMB0120		hypothetical protein	0.716	1.81E-02				
NMB0237		hypothetical protein	0.772	2.42E-02				
NMB0240		hypothetical protein	0.688	5.42E-03				
NMB0247		hypothetical protein	0.695	2.42E-02				
NMB0252		hypothetical protein	0.903	1.83E-03				
NMB0256		hypothetical protein	0.791	1.60E-02				
NMB0306		hypothetical protein	0.650	2.01E-03				
NMB0311		hypothetical protein	1.175	2.59E-04				
NMB0366		hypothetical protein	0.653	4.29E-03				
NMB0367		hypothetical protein	0.886	1.62E-02				
NMB0370		hypothetical protein	0.750	2.59E-02				
NMB0371		hypothetical protein	0.906	8.57E-03				
NMB0376		hypothetical protein	0.645	5.25E-03				
NMB0383		hypothetical protein	0.848	5.47E-03				

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			FFP 1		FFP 2		Medium	
ID	**Name**	**Annotation**	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***
NMB0491		hypothetical protein	0.876	3.98E-02				
NMB0500		hypothetical protein	1.039	8.56E-03				
NMB0504		hypothetical protein	0.602	4.17E-02				
NMB0507		hypothetical protein	1.027	2.51E-05	0.672	2.46E-02		
NMB0510		hypothetical protein	0.695	6.58E-03				
NMB0512		hypothetical protein	1.223	9.44E-03				
NMB0515		hypothetical protein	0.866	2.87E-03				
NMB0516		hypothetical protein	1.418	4.06E-07	0.892	6.08E-03		
NMB0519		hypothetical protein	0.679	1.84E-02				
NMB0542		hypothetical protein			0.664	4.14E-02		
NMB0644		hypothetical protein	0.874	3.76E-04				
NMB0648		hypothetical protein	0.952	2.30E-02				
NMB0649		hypothetical protein	1.531	4.94E-06				
NMB0650		hypothetical protein	1.356	3.33E-07			0.680	1.78E-02
NMB0651		hypothetical protein	1.186	1.85E-06			0.616	2.44E-02
NMB0654		hypothetical protein	0.849	2.63E-03	0.786	2.49E-02		
NMB0660		hypothetical protein	0.674	5.05E-03				
NMB0667		hypothetical protein	1.097	3.13E-05			0.639	3.14E-02
NMB0698		hypothetical protein	0.600	2.87E-02				
NMB0709		hypothetical protein	1.714	7.39E-09				
NMB0793		hypothetical protein	-0.748	9.44E-03	1.173	2.05E-03		
NMB0856		hypothetical protein	0.585	3.54E-02				
NMB0857		hypothetical protein	0.729	1.96E-02				
NMB0897		hypothetical protein	1.192	2.05E-02				
NMB0915		hypothetical protein	0.694	3.06E-02				
NMB0916		hypothetical protein	0.902	6.71E-04				
NMB0928		hypothetical protein	0.585	3.88E-02				
NMB0958		hypothetical protein	0.995	2.48E-04				
NMB0979		hypothetical protein	1.523	2.16E-04	1.150	2.34E-02		
NMB1035		hypothetical protein	0.982	6.75E-04				
NMB1048		hypothetical protein	-0.829	3.72E-02	1.169	1.44E-02	-0.973	2.88E-02
NMB1056		hypothetical protein	0.932	4.90E-03				
NMB1100		hypothetical protein	0.914	4.63E-02				
NMB1124		hypothetical protein	2.050	2.10E-08	1.955	2.45E-06	0.932	1.62E-02
NMB1125		hypothetical protein	2.162	6.64E-10	1.787	2.30E-05		
NMB1144		hypothetical protein	1.102	1.71E-05				
NMB1162		hypothetical protein	1.940	6.73E-08	1.971	1.91E-06	0.904	1.88E-02
NMB1163		hypothetical protein	2.064	1.05E-08	1.763	1.44E-05	0.857	2.54E-02
NMB1164		hypothetical protein	1.455	8.65E-07	1.257	2.08E-04	0.679	3.88E-02
NMB1182		hypothetical protein	0.711	4.61E-03				
NMB1205		hypothetical protein	0.948	6.62E-04			0.704	4.90E-02
NMB1211		hypothetical protein	1.797	1.23E-02				

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ID	Name	Annotation	FFP 1		FFP 2		Medium	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1212		hypothetical protein	1.494	4.33E-03				
NMB1221		hypothetical protein	0.842	3.74E-03	1.005	3.96E-03		
NMB1311		hypothetical protein	0.774	2.96E-02				
NMB1387		hypothetical protein	0.757	5.11E-04				
NMB1397		hypothetical protein	0.627	4.57E-03				
NMB1402		hypothetical protein	1.356	1.46E-08	0.944	3.86E-04		
NMB1404		hypothetical protein	1.118	2.14E-05	0.822	9.09E-03		
NMB1406		hypothetical protein	0.832	5.30E-03				
NMB1408		hypothetical protein	1.250	5.24E-04				
NMB1410		hypothetical protein	0.742	2.00E-02				
NMB1424		hypothetical protein	0.914	1.85E-04	0.737	1.30E-02		
NMB1477		hypothetical protein			0.807	1.23E-02	0.783	2.91E-03
NMB1490		hypothetical protein			0.735	4.88E-02		
NMB1546		hypothetical protein	0.945	3.43E-02				
NMB1580		hypothetical protein	0.960	3.76E-04				
NMB1596		hypothetical protein	0.703	1.97E-02				
NMB1656		hypothetical protein	1.152	6.11E-05				
NMB1666		hypothetical protein	1.066	5.64E-05				
NMB1667		hypothetical protein	0.886	1.45E-05	1.148	2.35E-06		
NMB1701		hypothetical protein	0.921	1.84E-02				
NMB1718		hypothetical protein	0.838	4.19E-02				
NMB1739		hypothetical protein			1.060	2.70E-02		
NMB1755		hypothetical protein	1.386	2.26E-02				
NMB1774		hypothetical protein	0.719	3.34E-02				
NMB1784		hypothetical protein	1.071	2.17E-03				
NMB1853		hypothetical protein	1.003	1.45E-03				
NMB1870		hypothetical protein	1.288	1.75E-05	0.783	3.55E-02	0.861	8.58E-03
NMB1941	*atpI*	hypothetical protein	0.713	1.84E-02				
NMB1986		hypothetical protein	0.685	8.25E-03				
NMB2108		hypothetical protein	0.882	4.77E-03			0.606	2.84E-02
NMB2109		hypothetical protein	0.917	2.21E-03				
NMB2110		hypothetical protein	0.614	3.42E-02				
NMB2118		hypothetical protein	0.810	2.83E-03				
NMB2137		hypothetical protein	1.216	6.62E-03				
NMB2141		hypothetical protein	0.927	1.19E-02				
NMB2146		hypothetical protein	0.730	5.66E-04				
Mobile and extrachromosomal element functions								
Plasmid functions								
NMB0495		replication protein	0.851	6.72E-03				
Prophage functions								
NMB0896		integrase (authentic frameshift)	0.849	4.83E-02				
NMB0556		putative repressor protein	0.914	6.11E-05				

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Table B.11 – continued from previous page

ID	Name	Annotation	FFP 1		FFP 2		Medium	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0748	*hfq*	host factor-I	1.168	1.68E-02				
Protein fate								
Degradation of proteins, peptides, and glycopeptides								
NMB1969	*aspA/nalP*	putative serotype-1-specific antigen	1.315	3.61E-05	1.188	1.66E-03	0.854	1.59E-02
NMB1998	*mspA*	serine-type peptidase	0.767	7.40E-03				
Protein and peptide secretion and trafficking								
NMB1789	*secB*	preprotein translocase subunit	0.985	3.37E-03				
Protein folding and stabilization								
NMB0278	*dsbA-1*	thiol:disulfide interchange protein	0.755	3.76E-04			1.154	8.22E-07
Protein modification and repair								
NMB0044	*pilB*	trifunctional thioredoxin/methionine sulfoxide reductase A/B protein			0.832	1.42E-03	0.701	3.45E-03
NMB1649	*dsbB*	disulfide bond formation protein B	0.777	3.50E-03				
Protein synthesis								
tRNA aminoacylation								
NMB0111	*fmt*	methionyl-tRNA formyltransferase	0.738	1.40E-02	0.882	5.91E-03		
NMB0854	*hisS*	histidyl-tRNA synthetase	0.781	2.86E-02				
NMB1471	*trpS*	tryptophanyl-tRNA synthetase	0.761	3.27E-02				
tRNA and rRNA base modification								
NMB0697	*ksgA*	dimethyladenosine transferase	0.719	2.96E-04				
Purines, pyrimidines, nucleosides, and nucleotides								
Purine ribonucleotide biosynthesis								
NMB0690	*purF*	amidophosphoribosyltransferase	1.101	3.64E-04			0.966	4.38E-03
NMB0757	*purC*	phosphoribosylaminoimidazole-succinocarboxamide synthase	0.735	2.92E-04				
NMB0983	*purH*	bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	1.056	1.14E-03			1.232	1.05E-05
Pyrimidine ribonucleotide biosynthesis								
NMB1849	*carA*	carbamoyl phosphate synthase, small subunit	0.957	8.09E-03				
Regulatory functions								
Other								
NMB0381	*cysB*	transcriptional regulator CysB-like protein	0.603	5.01E-04				
NMB0556		putative repressor protein	0.914	6.11E-05				
NMB0748	*hfq*	host factor-I	1.168	1.68E-02				
NMB0910		transcriptional regulator	0.640	4.34E-02				
NMB1049		putative transcriptional regulator			0.700	1.53E-02		
NMB1711		transcriptional regulator, GntR family	1.030	4.19E-05				
NMB1735	*relA*	GTP pyrophosphokinase	1.008	5.88E-06	0.903	4.56E-04	0.655	6.70E-03
NMB1843		transcriptional regulator, MarR family	0.707	2.59E-02				
NMB1995	*glnB*	nitrogen regulatory protein P-II	1.020	1.91E-06			0.779	5.42E-04
Transcription								
RNA processing								
NMB0843	*pcnB*	polyA polymerase			0.708	4.70E-02		

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Table B.11 – continued from previous page

ID	Name	Annotation	FFP 1		FFP 2		Medium	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
Transport and binding proteins								
Amino acids, peptides and amines								
NMB0085	*gltS*	sodium/glutamate symporter	1.627	3.73E-09	1.208	7.94E-05		
NMB0177		putative sodium/alanine symporter			0.936	1.73E-02		
NMB0194		putative amino acid symporter	1.426	7.78E-06			0.903	9.46E-03
NMB0402	*putP*	sodium/proline symporter			1.392	1.56E-04		
NMB1612	*hisJ*	amino acid ABC transporter, periplasmic amino acid-binding protein	0.618	2.07E-02			0.667	2.63E-02
Anions								
NMB0378		putative phosphate permease	0.844	2.16E-02	1.308	2.72E-03	1.920	1.56E-06
NMB0879	*cysA*	sulfate ABC transporter, ATP-binding protein			0.718	8.87E-04		
Carbohydrates, organic alcohols, and acids								
NMB0388		putative sugar transporter	0.833	1.02E-03	0.914	2.64E-03	0.868	1.73E-03
NMB0535	*gluP*	glucose/galactose transporter	0.804	4.64E-03	1.344	6.06E-08		
NMB0543	*lctP*	putative L-lactate permease	0.983	8.16E-04	1.728	1.21E-06		
Cations and iron carrying compounds								
NMB0034		putative high-affinity iron transporter			0.853	8.61E-03	1.212	2.46E-05
NMB0586		putative zinc/manganese ABC transporter, periplasmic binding protein	0.772	9.69E-04				
NMB0615	*amtB*	putative ammonium transporter AmtB	1.326	1.76E-05	0.968	8.87E-03		
NMB0634	*fbpA*	iron(III) ABC transporter, periplasmic binding protein	1.418	3.32E-03			2.131	4.35E-05
NMB1206	*bfrB*	bacterioferritin B	1.030	7.95E-05				
NMB1207	*bfrA*	bacterioferritin A	0.984	2.40E-03				
NMB1540	*lbpA*	lactoferrin-binding protein A			1.966	2.11E-06	1.605	1.36E-05
NMB1541	*lbpB*	lactoferrin-binding protein B			1.674	1.79E-05	1.306	2.06E-04
NMB1668	*hmbR*	hemoglobin receptor			0.878	1.00E-03		
Nucleosides, purines and pyrimidines								
NMB2067	*cytX*	probable hydroxymethylpyrimidine transporter CytX	2.138	7.98E-08	2.467	1.27E-07	1.929	3.07E-06
Other								
NMB1277		transporter, BCCT family			0.739	3.58E-03		
NMB1715	*mtrD*	multiple transferable resistance system protein			0.601	3.93E-02	0.763	2.56E-03
NMB1716	*mtrC*	membrane fusion protein			0.835	4.42E-03	0.690	1.09E-02
NMB1716	*mtrC*	membrane fusion protein			0.835	4.42E-03	0.690	1.09E-02
NMB1719	*mtrF*	efflux pump component			0.993	4.20E-02		
NMB1738	*hlyD*	putative secretion protein	1.012	5.69E-04	0.847	1.88E-02		
Porins								
NMB2039	*porB*	major outer membrane protein PIB	0.692	3.56E-02				
Unknown substrate								
NMB0041		ABC transporter, periplasmic solute-binding protein	1.132	1.88E-04				
NMB0792		transporter, NadC family			1.379	3.41E-06		
NMB1732		putative transporter	0.704	5.31E-03				
Unknown function								
NMB0255		cell filamentation protein Fic-related protein	0.767	4.52E-02			-0.958	1.10E-02
NMB0341	*tspA*	tspA protein	0.785	2.18E-03				

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Table B.11 – continued from previous page

			FFP 1		FFP 2		Medium	
ID	**Name**	**Annotation**	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***
NMB0374	*mafB2*	MafB-related protein	0.947	3.93E-02				
NMB0375	*mafA2*	mafA protein	1.294	1.02E-03				
NMB0490		PspA-related protein	1.047	4.44E-03				
NMB0496		hemolysin activator-related protein	0.751	2.41E-02				
NMB0652	*mafA-2*	mafA protein	1.069	1.71E-06	0.677	1.01E-02		
NMB0653		MafB-related protein	0.809	2.35E-03	0.799	1.41E-02		
NMB0827	*nmgI*	ADP-L-glycero-D-mannoheptose-6-epimerase	0.987	6.40E-03				
NMB1220		stomatin/Mec-2 family protein	1.709	8.17E-10	1.735	4.54E-08	0.786	4.99E-03
NMB1389		RpiR/YebK/YfhH family protein	1.234	7.22E-05				
NMB1405		FrpA/C-related protein	0.991	4.07E-04			0.684	3.14E-02
NMB1409		FrpA/C-related protein	0.774	1.10E-02				
NMB1526	*smpB*	SsrA-binding protein	0.700	2.16E-02				
NMB1657		comE operon protein 1-related protein	0.626	1.73E-02				
NMB1669	*hemO*	iron-starvation protein	0.765	2.16E-02	0.950	1.88E-02		
NMB1747		putative tspB protein	0.645	2.46E-02				
NMB1891		helix-turn-helix family protein	0.856	1.56E-03	0.799	2.16E-02		
NMB1924		inositol monophosphatase family protein	0.765	2.13E-02				

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Appendix C

Supplementary data to Chapter 6

Table C.1: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *amino acid biosynthesis*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Aromatic amino acid family												
NMB0275	*trpC*	indole-3-glycerol phosphate synthase							0.610	2.09E-04		
NMB0307	*aroG*	phospho-2-dehydro-3-deoxyheptonate aldolase, phe-sensitive									1.381	7.82E-07
NMB0678	*trpA*	tryptophan synthase, α subunit			-0.805	2.03E-07	-0.607	1.15E-04	-0.966	2.51E-09	-1.015	1.08E-09
NMB1021	*trpE*	anthranilate synthase, component I	0.851	4.23E-04			0.735	5.14E-03				
NMB1678	*tyrB*	aromatic-amino-acid aminotransferase			0.622	4.34E-06	0.707	9.28E-07	0.598	1.14E-05		
NMB1680	*aroC*	chorismate synthase	0.604	1.81E-06	0.784	1.24E-09	0.712	5.82E-08	0.677	7.36E-08		
Aspartate family												
NMB0203	*dapB*	dihydrodipicolinate reductase									-0.842	6.15E-09
NMB0335	*dapD*	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase									-0.819	2.93E-04
NMB0540	*aspC*	aspartate aminotransferase			0.672	3.41E-03	0.686	4.27E-03			0.860	2.38E-04
NMB0929	*dapA*	dihydrodipicolinate synthase									-0.989	2.27E-04
NMB0943	*metF*	5,10-methylenetetrahydrofolate reductase	1.959	5.36E-14	1.938	2.25E-14	1.232	1.20E-07			0.669	1.65E-03
NMB0944	*metH*	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	1.956	4.58E-11	2.015	5.73E-12	1.497	1.11E-07	0.744	3.60E-03		
NMB1046	*thrC*	threonine synthase									-0.711	2.10E-04
NMB1228	*metM*	homoserine dehydrogenase	1.486	3.81E-10	1.442	1.42E-11	1.438	1.61E-09	0.979	1.43E-06	0.979	1.32E-06
NMB1498	*lysC*	aspartokinase, α and β subunits	1.186	3.01E-06	1.296	1.70E-07	1.307	4.84E-07	0.761	1.39E-03		
NMB1530	*dapE*	succinyl-diaminopimelate desuccinylase									0.820	3.19E-07
Glutamate family												
NMB0055	*proC*	pyrroline-5-carboxylate reductase									0.692	9.01E-04
NMB0359	*glnA*	glutamate ammonia ligase			1.137	5.57E-10	0.831	3.41E-06				
NMB0637	*argH*	argininosuccinate lyase									-1.086	2.06E-08
NMB1069	*proB*	gamma-glutamyl kinase							0.737	7.39E-06		
NMB1710	*gdhA*	glutamate dehydrogenase, NADP-specific	1.204	1.65E-04	1.504	1.75E-06	1.028	1.31E-03				
NMB1876	*argA*	N-acetylglutamate synthase							0.802	5.22E-03		
Histidine family												
NMB0603	*hisE*	phosphoribosyl-ATP pyrophosphatase	0.763	4.60E-06	0.944	1.41E-08	0.669	5.34E-05			0.808	9.74E-07
NMB0628	*hisF*	hisF protein (cyclase)									-0.780	7.24E-08
NMB0629	*hisA*	phosphoribosylformimino-5-aminoimidazole carbox-amide ribotide isomerase	-0.664	1.24E-03							-0.721	3.41E-04
NMB1579	*hisG*	ATP phosphoribosyltransferase	-1.455	2.65E-13	-1.026	5.39E-09	-0.833	2.09E-06	-1.221	2.87E-11		
NMB1581	*hisD*	histidinol dehydrogenase	-1.114	3.51E-05	-0.730	4.42E-03					-1.211	1.23E-06
NMB1582	*hisC*	histidinol-phosphate aminotransferase									-1.543	3.47E-05
NMB2033		putative histidinol-phosphatase	0.856	8.91E-06	0.641	4.25E-04	0.779	5.29E-05	0.711	1.14E-04		

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Table C.1 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Pyruvate family												
NMB0337	*ilvE*	putative branched-chain amino acid aminotransferase									-0.765	2.10E-04
NMB0878	*ilvA*	threonine dehydratase	-1.568	1.84E-10	-1.435	8.84E-10	-1.533	6.00E-10	-1.436	8.33E-10	-1.320	1.56E-08
NMB1034	*leuD*	3-isopropylmalate dehydratase, small subunit	1.073	4.05E-08	1.088	9.85E-09	0.988	3.76E-07				
NMB1036	*leuC*	3-isopropylmalate dehydratase, large subunit	1.102	2.76E-07	1.206	1.12E-08	1.302	6.26E-09	0.945	1.12E-06		
NMB1070	*leuA*	2-isopropylmalate synthase	0.973	2.46E-05	0.974	1.15E-05	0.994	1.93E-05				
NMB1150	*ilvD-1*	dihydroxy-acid dehydratase									-0.636	9.79E-03
NMB1188	*ilvD-2*	dihydroxy-acid dehydratase									-0.708	1.89E-03
NMB1574	*ilvC*	ketol-acid reductoisomerase									-1.446	8.22E-11
NMB1823	*avtA*	valine pyruvate aminotransferase			0.607	4.97E-04						
Serine family												
NMB0763	*cysK*	cysteine synthase	1.373	1.26E-11	1.555	4.49E-14	1.316	1.06E-10	0.869	1.23E-06	-0.591	7.02E-04
NMB0981	*serB*	phosphoserine phosphatase	0.878	1.96E-05			0.768	1.81E-04	0.817	3.86E-05	1.152	4.73E-08
NMB1055	*glyA*	serine hydroxymethyltransferase									-0.797	1.79E-05

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.2: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *biosynthesis of cofactors, prosthetic groups, and carriers*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Biotin												
NMB0474	*bioC*	putative biotin synthesis protein									0.585	6.67E-04
NMB1146	*bioB-1*	biotin synthetase									-0.624	6.10E-04
NMB1184	*bioB-2*	biotin synthetase									-0.601	9.85E-04
NMB2075	*birA*	BirA protein Bvg accessory factor							0.593	5.34E-04		
Folic acid												
NMB0694	*folI*	folI protein									-0.720	6.92E-10
NMB0745	*folK*	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine-pyrophosphokinase	-0.642	1.36E-05	-0.651	5.04E-06	-0.733	1.26E-06			-0.962	8.02E-10
NMB1055	*glyA*	serine hydroxymethyltransferase									-0.797	1.79E-05
NMB1063	*folB*	dihydroneopterin aldolase									0.906	2.40E-07
Glutathione and analogs												
NMB1037	*gshA*	glutamate cysteine ligase	0.895	6.05E-06	0.923	1.43E-06	1.040	3.31E-07	0.654	4.74E-04	0.955	1.12E-06
NMB1559	*gshB*	glutathione synthetase									-0.758	9.80E-05
Heme, porphyrin, and cobalamin												
NMB0539	*hemC*	porphobilinogen deaminase			0.589	1.47E-05	0.585	4.13E-05			0.782	6.36E-08

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Table C.2 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0665		oxygen-independent coprophorphyrinogen III oxidase family protein							0.642	3.60E-03	0.867	1.20E-04
NMB0801	*hemB*	delta-aminolevulinic acid dehydratase					-0.660	7.77E-04	-0.719	1.48E-04	-0.932	2.14E-06
NMB1156	*cysG-1*	siroheme synthase							0.965	7.92E-03		
Lipoate												
NMB1217	*lipB*	lipoate-protein ligase B									0.669	4.26E-03
Menaquinone and ubiquinone												
NMB0261		geranyltranstransferase							0.794	3.64E-08		
NMB0323		UbiH family protein			0.661	2.88E-06						
NMB0326		octaprenyl-diphosphate synthase	0.598	2.92E-08								
NMB0735	*ubiA*	4-hydroxybenzoate, octaprenyltransferase									0.585	1.84E-05
NMB0743	*ubiE*	ubiquinone menaquinone biosynthesis methyl-transferase	0.854	3.60E-07			0.718	1.44E-05				
Other												
NMB0527		putative 6-pyruvoyl tetrahydrobiopterin synthase	0.756	1.17E-04							0.623	1.04E-03
NMB1130		putative phytoene synthase	1.373	7.57E-13	1.081	5.95E-10	1.384	1.15E-12	1.253	5.21E-12	0.691	2.52E-05
NMB1168		putative phytoene synthase	1.492	6.75E-14	1.244	1.27E-11	1.533	4.90E-14	1.273	5.21E-12	0.765	5.50E-06
NMB1379		nifS protein	-1.859	1.35E-10	-0.917	1.07E-04	-0.904	5.80E-04	-0.961	1.50E-04		
Pantothenate and coenzyme A												
NMB0870	*panB*	3-methyl-2-oxobutanoate hydroxy methyltransferase					0.596	1.83E-04				
NMB1282	*panD*	aspartate 1-decarboxylase									-0.702	1.25E-07
Pyridine nucleotides												
NMB0392	*nadB*	l-aspartate oxidase							0.850	1.80E-05		
NMB0394	*nadA*	quinolinate synthetase A							1.129	4.63E-11	1.170	3.37E-11
NMB0396	*nadC*	nicotinate-nucleotide pyrophosphorylase							0.932	7.98E-07	0.963	5.28E-07
NMB1364	*nadE*	putative NH(3)-dependent NAD+ synthetase							0.630	1.14E-03		
Riboflavin, FMN, and FAD												
NMB1247	*ribC*	riboflavin synthase, α subunit	0.669	3.31E-05			0.592	2.31E-04				
NMB1817	*ribD*	riboflavin biosynthesis protein							0.927	3.33E-05		
NMB1834	*ribF*	riboflavin kinase FMN adenylyltransferase							0.796	5.64E-03		
Thiamine												
NMB0563	*apbE*	thiamine biosynthesis lipoprotein	0.648	2.65E-06	0.799	7.67E-09	0.596	1.47E-05			0.988	3.34E-11
NMB2040	*thiC*	thiamine biosynthesis protein									1.048	3.66E-05
NMB2067	*cytX*	probable hydroxymethylpyrimidine transporter							0.724	2.93E-03	0.897	2.81E-04
NMB2069	*thiE*	thiamin-phosphate pyrophosphorylase							0.827	2.48E-03	1.652	3.30E-09
NMB2070	*thiS*	thiamine biosynthesis protein									1.221	8.73E-04
NMB2071	*thiG*	thiG protein							0.911	2.04E-05	1.154	9.00E-07

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.3: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into TIGR role category *cell envelope*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Biosynthesis and degradation of murein sacculus and peptidoglycan												
NMB0011	*murA*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	0.739	4.34E-06	0.618	4.72E-05	0.597	1.69E-04	1.081	1.14E-10		
NMB0038	*glmU*	UDP-N-acetylglucosamine pyrophosphorylase					0.609	7.27E-05			0.764	6.76E-07
NMB0357	*mtgA*	monofunctional biosynthetic peptidoglycan transglycosylase							0.762	5.24E-05		
NMB0422	*murG*	UDP-N-acetylglucosamine–N-acetylmuramyl--(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase									0.839	9.69E-06
NMB0423	*murC*	UDP-N-acetylmuramate alanine ligase									-0.669	1.38E-06
NMB0811	*murB*	UDP-N-acetylpyruvoylglucosamine reductase									1.210	4.28E-03
NMB0877		penicillin-binding protein									-3.375	5.14E-08
NMB1085		putative N-acetylmuramoyl-L-alanine amidase									1.169	4.98E-06
NMB1797		penicillin-binding protein 3									-0.780	1.17E-06
NMB1807	*ponA*	penicillin-binding protein 1			-0.808	1.22E-03						
Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides												
NMB0014	*kdtA*	3-deoxy-D-manno-octulosonic-acid transferase									0.851	2.75E-04
NMB0038	*glmU*	UDP-N-acetylglucosamine pyrophosphorylase					0.609	7.27E-05			0.764	6.76E-07
NMB0062	*rfbA-1*	glucose-1-phosphate thymidylyltransferase									0.707	1.68E-05
NMB0063	*rfbB-1*	dTDP-D-glucose 4,6-dehydratase									0.930	5.69E-10
NMB0069	*siaB*	polysialic acid capsule biosynthesis protein									-0.721	1.68E-03
NMB0070	*siaA*	polysialic acid capsule biosynthesis protein							-0.997	7.54E-06	-0.862	1.00E-04
NMB0079	*rfbB-2*	dTDP-D-glucose 4,6-dehydratase									1.004	2.49E-08
NMB0080	*rfbA-2*	glucose-1-phosphate thymidylyltransferase									0.619	1.49E-04
NMB0199	*lpxB*	lipid-A-disaccharide synthase							0.691	2.77E-04	0.657	6.10E-04
NMB0544		UDP-2,3-diacylglucosamine hydrolase							0.619	8.75E-05		
NMB0756	*rfbD*	putative dTDP-L-rhamnose synthase					-0.639	5.58E-07			-1.098	2.69E-14
NMB0790	*pgm*	phosphoglucomutase			1.229	1.76E-13						
NMB0825	*rfaE*	putative ADP-heptose synthase							-0.868	1.68E-06	-1.401	7.34E-12
NMB0828	*rfaD*	ADP-L-glycero-D-mannoheptose-6-epimerase							-0.870	4.61E-05		
NMB1279	*mltB*	putative membrane-bound lytic murein transglycosylase B	-0.717	5.22E-04	-1.109	9.82E-08	-0.838	6.77E-05	-0.796	7.82E-05	-1.606	5.70E-12
NMB1359		putative CDP-6-deoxy-delta-3,4-glucoseen reductase							0.604	3.31E-04		
NMB1704	*lgtF*	β-1,4-glucosyltransferase	0.960	3.57E-05	0.844	1.38E-04	0.884	1.45E-04	0.760	6.18E-04	0.806	3.33E-04
NMB1705	*rfaK*	α-1,2-N-acetylglucosamine transferase			0.654	1.69E-06					1.041	1.58E-11
NMB1926	*lgtE*	lacto-N-neotetraose biosynthesis glycosyl transferase									0.725	1.32E-04
NMB1928	*lgtB*	lacto-N-neotetraose biosynthesis glycosyl transferase									0.600	9.38E-03
NMB1929	*lgtA*	lacto-N-neotetraose biosynthesis glycosyl transferase	0.726	9.58E-03			0.904	5.27E-04	0.779	2.71E-03	0.974	3.67E-04

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Table C.3 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB2019	*kdtB*	lipopolysaccharide core biosynthesis protein					-0.645	3.15E-03				
NMB2156	*rfaC*	heptosyltransferase I							-0.793	4.59E-05		
Other												
NMB0345		putative cell-binding factor	-1.090	5.24E-05					-0.999	1.23E-04	-1.388	3.79E-07
NMB0382	*rmpM*	outer membrane protein class 4									-1.657	2.18E-11
NMB0663	*nspA*	outer membrane protein	2.854	2.55E-23	1.732	2.22E-14	1.192	1.58E-08	0.715	1.64E-04		
NMB0703	*comL*	competence lipoprotein									-1.252	4.96E-15
NMB0946		peroxiredoxin 2 family protein glutaredoxin			1.436	1.95E-07	0.811	3.50E-03			-2.851	1.32E-16
NMB1053	*opcA*	class 5 outer membrane protein	1.124	2.51E-04	1.068	2.64E-04						
NMB1072		prolipoprotein diacylglyceryl transferase					-1.017	3.32E-03	-1.502	1.00E-05		
NMB1213		putative lipoprotein							0.698	9.68E-05	0.589	9.85E-04
NMB1533		H.8 outer membrane protein	1.005	2.72E-05	1.284	7.57E-08	1.051	1.45E-05			-1.067	6.62E-06
NMB1592		putative lipoprotein							-0.927	7.81E-07	-0.864	4.07E-06
NMB1716	*mtrC*	membrane fusion protein	-1.644	1.04E-09	-1.342	7.68E-08	-1.136	1.69E-06	-1.144	4.08E-07		
NMB1907	*yidC*	putative inner membrane protein, translocase component	0.650	1.43E-04								
NMB1946		outer membrane lipoprotein									-0.953	7.01E-05
Surface structures												
NMB0018	*pilE*	pilin	0.837	4.69E-04	0.714	1.89E-03	0.723	2.71E-03				
NMB0333	*pilG*	pilus assembly protein										
NMB0770		putative type IV pilus assembly protein PilZ							-0.844	8.03E-04	-1.389	2.22E-07
NMB0886		fimbrial protein FimT									0.623	3.01E-04
NMB0888	*pilW*	type IV pilus assembly protein					-0.654	1.57E-03	-0.992	1.40E-06	-0.879	1.77E-05
NMB0889	*pilX*	type IV pilus assembly protein							-0.817	6.02E-03	-1.116	7.51E-04
NMB0992	*nhhA*	adhesin			-1.129	6.82E-03						
NMB1811	*pilP*	pilP protein			0.659	4.35E-03						
NMB1812	*pilQ*	pilQ protein			0.894	9.88E-04					-0.823	6.61E-03
NMB1847		pilC1 protein (authentic frameshift)									0.742	4.90E-05
NMB1994	*nadA*	putative adhesin/invasin NadA	-0.812	3.09E-03	-0.743	4.98E-03	-0.737	7.73E-03	-0.698	8.43E-03		

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.4: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *cellular processes*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Adaptations to atypical conditions												
NMB0712	*rpoH*	RNA polymerase sigma-32 factor	-0.945	7.15E-06	-0.822	3.47E-05	-0.659	1.47E-03	-1.068	2.57E-07	-1.161	5.91E-08
NMB0838		cold-shock domain family protein			-0.800	2.61E-06	-0.912	4.84E-07	-0.737	1.48E-05		
Cell division												
NMB0170	*minC*	septum site-determining protein									-0.608	4.72E-04
NMB0171	*minD*	septum site-determining protein							-0.783	1.58E-03	-1.270	1.16E-06
NMB0172	*minE*	cell division topological specificity factor							-0.657	1.43E-04	-1.487	4.61E-13
NMB0191		ParA family protein									0.661	3.21E-03
NMB0342	*ispA*	intracellular septation protein A	-1.221	9.06E-08	-0.783	1.86E-04	-0.812	2.22E-04	-1.158	1.57E-07	-1.594	3.72E-11
NMB0427	*ftsZ*	cell division protein							-0.688	4.54E-04	-1.022	7.61E-07
NMB0799	*ftsJ*	cell division protein			0.711	3.51E-08					0.992	4.57E-12
NMB1791		cytoplasmic axial filament protein	-0.879	7.58E-09	-0.660	2.44E-06	-0.647	9.26E-06				
Chemotaxis and motility												
NMB0051	*pilU*	twitching motility protein	0.618	2.27E-07	0.603	1.53E-07					-0.602	2.82E-07
NMB0052	*pilT-1*	twitching motility protein									-0.641	1.55E-04
NMB0768	*pilT-2*	twitching motility protein	-0.735	4.75E-06	-0.759	1.06E-06	-0.702	1.32E-05	-1.043	3.59E-10	-1.499	3.18E-15
Detoxification												
NMB0005	*arsC*	arsenate reductase	-0.701	1.03E-03								
NMB0216	*katA*	catalase			2.656	9.29E-11	2.750	1.19E-10	3.301	5.91E-14	2.483	9.33E-10
NMB0280		putative organic solvent tolerance protein									-0.705	9.85E-08
NMB0525		putative aluminum resistance protein	1.244	3.19E-13					0.636	1.06E-05	1.190	1.14E-12
NMB0884	*sodB*	superoxide dismutase					-0.764	2.34E-06				
NMB1398	*sodC*	Cu-Zn-superoxide dismutase							-1.092	1.10E-05	-1.360	1.55E-07
NMB1621	*gpxA*	glutathione peroxidase	0.960	3.60E-07	1.077	8.31E-09	1.139	7.75E-09	0.774	1.49E-05		
NMB1714	*mtrE*	multidrug efflux pump channel protein	-1.704	3.84E-14	-1.563	2.62E-14	-1.259	1.46E-09	-1.094	1.93E-08	-1.007	2.03E-07
NMB1955	*cad*	cadmium resistance protein									-0.855	4.34E-03
DNA transformation												
NMB0116	*dprA*	DNA processing chain A							0.671	8.13E-05		
NMB0702	*comA*	competence protein							0.694	7.01E-03	0.979	1.94E-04
NMB0703	*comL*	competence lipoprotein									-1.252	4.96E-15
Pathogenesis												
NMB0018	*pilE*	pilin	0.837	4.69E-04	0.714	1.89E-03	0.723	2.71E-03				
NMB0277	*mviN*	virulence factor									0.698	6.84E-06
NMB0333	*pilG*	pilus assembly protein									-0.641	4.87E-05
NMB0364		FrpC operon protein	-1.388	1.78E-10	-1.399	4.56E-11	-1.404	2.14E-10	-1.845	3.08E-15	-1.278	1.30E-09
NMB0584		FrpC operon protein	-1.104	6.77E-07	-0.936	8.22E-06	-1.046	2.44E-06	-1.422	3.90E-10	-0.856	5.53E-05
NMB0585		putative iron-regulated protein FrpA	-1.242	3.77E-08	-1.166	6.45E-08	-1.391	2.90E-09	-1.856	5.29E-14	-1.455	3.37E-10

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Table C.4 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0770		putative type IV pilus assembly protein PilZ							-0.844	8.03E-04	-1.389	2.22E-07
NMB0926		opacity protein (authentic frameshift)			0.673	1.75E-03					-0.884	6.54E-05
NMB0992	*nhhA*	adhesin			-1.129	6.82E-03						
NMB1412		FrpC operon protein	-1.219	3.63E-08	-1.200	1.91E-08	-1.203	6.84E-08	-1.661	1.15E-12	-1.020	1.42E-06
NMB1414		FrpC operon protein	-0.963	6.36E-06	-0.904	9.20E-06	-0.945	1.02E-05	-1.370	4.91E-10	-0.732	3.31E-04
NMB1415		iron-regulated protein FrpC	-1.164	1.70E-07	-1.183	4.25E-08	-1.306	1.35E-08	-1.546	2.84E-12	-1.348	2.73E-09
NMB1552	*pivNM-1A*	pilin gene inverting protein									0.711	2.95E-04
NMB1567		macrophage infectivity potentiator									-1.331	5.65E-06
NMB1625	*pivNM-1B*	pilin gene inverting protein									0.686	2.29E-03
NMB1811	*pilP*	pilP protein			0.659	4.35E-03						
NMB1812	*pilQ*	pilQ protein			0.894	9.88E-04					-0.823	6.61E-03
NMB1847		pilC1 protein (authentic frameshift)									0.742	4.90E-05
NMB1985	*iga2*	adhesion and penetration protein							-1.138	1.93E-08	-1.535	4.57E-12
NMB1994	*nadA*	adhesin/ invasin NadA	-0.812	3.09E-03	-0.743	4.98E-03	-0.737	7.73E-03	-0.698	8.43E-03		
NMB2095		putative adhesin complex protein									-1.404	9.16E-08
Toxin production and resistance												
NMB0103		putative bacteriocin resistance protein					-0.624	6.30E-04	-0.985	1.04E-07	-1.014	7.13E-08
NMB0493		hemagglutinin/hemolysin-related protein	-0.823	2.47E-06			-0.708	4.44E-05	-0.933	7.81E-08		
NMB0497		hemagglutinin/hemolysin-related protein	-0.777	4.05E-04			-0.590	7.61E-03			1.133	4.47E-07
NMB0582		putative bacteriocin resistance protein									1.875	6.32E-05
NMB0697	*ksgA*	dimethyladenosine transferase									0.654	2.61E-04
NMB0839		pmbA protein	1.096	1.45E-08	0.757	1.84E-05	0.970	3.76E-07			-2.475	8.75E-22
NMB0855		putative bacteriocin resistance protein	-1.102	1.15E-05	-0.888	1.90E-04	-1.044	3.47E-05	-1.606	6.91E-10	-0.629	8.57E-03
NMB0977		putative modulator of drug activity B	-2.987	1.69E-19	-2.940	8.76E-20	-3.215	1.91E-20	-3.291	6.38E-22	-3.017	9.01E-20
NMB1210		putative toxin-activating protein	-0.935	8.58E-06	-0.823	1.36E-05	-1.161	1.17E-07	-1.009	1.03E-06		
NMB1214		hemagglutinin/hemolysin-related protein	-0.737	6.86E-04			-0.936	2.53E-05	-1.171	1.24E-07		
NMB1603		putative tellurite resistance protein									1.229	2.90E-03
NMB1646		putative hemolysin	-1.001	6.06E-04	-0.962	6.16E-04	-1.019	1.97E-04				
NMB1763		putative toxin-activating protein							-0.814	1.07E-03		
NMB1779		hemagglutinin/hemolysin-related protein									1.057	5.87E-06
NMB1780		putative hemolysin activation protein HecB	-1.033	1.04E-04	-0.991	1.04E-04	-1.191	1.16E-05	-1.227	3.09E-06	-0.801	1.82E-03
NMB1857		modulator of drug activity B	-2.913	1.40E-17	-3.036	4.12E-19	-2.993	6.58E-18	-3.106	2.04E-19	-2.951	5.24E-18
NMB2091		putative hemolysin									-1.150	2.38E-07

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.5: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *central intermediary metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Amino sugars												
NMB0031	*glmS*	glucosamine fructose-6-phosphate aminotransferase (isomerizing)					0.859	2.74E-08	0.645	5.81E-06	0.709	1.13E-06
Other												
NMB0029		glycerate dehydrogenase									-0.804	1.38E-06
NMB0614		putative oxidoreductase	0.751	1.96E-05							0.622	2.48E-04
NMB0894		aminotransferase, class I	-0.942	1.63E-06	-1.181	2.81E-09	-1.288	1.12E-09	-1.494	1.85E-12	-1.330	1.67E-10
NMB0998		putative oxidoreductase	0.894	8.00E-08	1.018	7.82E-09	1.017	3.07E-08	0.842	9.23E-07		
NMB1305	*esd*	putative esterase	-0.892	5.09E-09	-0.863	4.56E-09	-1.010	2.36E-10	-1.232	5.50E-14	-1.103	5.70E-12
NMB1473		aminotransferase, class I									-0.839	6.33E-04
NMB1584		3-hydroxyacid dehydrogenase	-1.463	4.49E-11	-0.875	2.07E-06	-0.984	1.69E-06	-1.376	9.45E-11	-1.477	2.23E-11
NMB1799	*metK*	S-adenosylmethionine synthetase	1.202	1.05E-11	1.468	1.98E-15	1.519	4.08E-15	1.232	1.30E-12		
Phosphorus compounds												
NMB0641	*ppa*	inorganic pyrophosphatase							-0.608	5.32E-04	-1.297	8.22E-11
Polyamine biosynthesis												
NMB0468	*speA*	biosynthetic arginine decarboxylase			0.742	1.61E-07	0.916	3.31E-09				
NMB0469	*speB*	agmatinase			0.684	9.09E-08	0.668	5.52E-07				
Sulfur metabolism												
NMB1151	*cysI-1*	sulfite reductase hemoprotein, β component			0.808	8.17E-04			0.656	6.72E-03	0.674	5.66E-03
NMB1152	*cysJ-1*	sulfite reductase (NADPH) flavoprotein, α component							1.180	2.01E-04	1.178	7.30E-04
NMB1154	*cysD-1*	sulfate adenylyltransferase, subunit 2									0.917	4.92E-03
NMB1189	*cysI-2*	sulfite reductase hemoprotein, β component			0.658	4.98E-03			0.669	4.32E-03		
NMB1191	*cysN-2*	sulfate adenylyltransferase, subunit 1							0.815	3.61E-03		
NMB1192	*cysD-2*	sulfate adenylyltransferase, subunit 2									0.856	7.97E-03

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.6: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *DNA metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Chromosome-associated proteins												
NMB1230	*hupB*	DNA-binding protein HU-β									-0.816	2.29E-04

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ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Degradation of DNA												
NMB0262	*xseB*	exodeoxyribonuclease, small subunit					0.791	3.93E-04			0.653	4.85E-03
NMB1363	*xseA*	exodeoxyribonuclease, large subunit	0.850	2.55E-07	0.894	2.82E-08					1.077	3.46E-10
DNA replication, recombination, and repair												
NMB0056	*dksA*	DnaK suppressor protein	0.982	1.81E-07	0.855	1.55E-06	0.794	1.63E-05				
NMB0118	*topA*	DNA topoisomerase I			0.669	3.06E-06	0.703	2.97E-06				
NMB0212	*gyrB*	DNA gyrase, subunit B			-0.650	3.50E-06			-0.804	3.85E-08	-1.241	1.00E-13
NMB0232	*uvrD*	DNA helicase II			0.599	2.76E-04	0.644	1.99E-04	0.895	2.49E-07		
NMB0265	*ruvA*	Holliday junction DNA helicase	0.685	9.15E-06	0.737	9.42E-07	0.789	6.49E-07	1.118	1.33E-11	0.606	5.38E-05
NMB0287	*dinG*	probable ATP-dependent helicase							0.724	1.12E-05	1.033	5.23E-09
NMB0399	*xthA*	exodeoxyribonuclease III	-0.679	1.71E-06	-0.682	1.54E-07	-0.750	2.59E-07	-0.740	1.32E-07		
NMB0447	*recO*	DNA repair protein	-0.634	9.70E-05			-0.630	1.25E-04				
NMB0533	*nth*	endonuclease III									0.955	1.97E-08
NMB0551	*priA*	primosomal protein n′									0.830	8.43E-03
NMB0729	*himA*	integration host factor, α subunit							-0.585	2.49E-03	-2.228	7.19E-18
NMB0751	*xerD*	integrase recombinase	-0.655	2.36E-03							0.848	6.99E-05
NMB0769	*holB*	putative DNA polymerase III, δ ′ subunit			-0.625	2.34E-05	-0.609	8.06E-05	-0.743	1.03E-06	-1.087	7.93E-11
NMB0785	*recB*	exodeoxyribonuclease V 135 KD polypeptide									0.681	1.57E-04
NMB0826	*nmgII*	C-5 cytosine-specific DNA methylase							-0.938	1.43E-05	-1.471	5.64E-10
NMB0842	*recJ*	single-stranded-DNA-specific exonuclease									-0.822	9.80E-07
NMB0885	*dnaB*	replicative DNA helicase									1.107	6.95E-03
NMB1038	*radC*	DNA repair protein							0.585	1.05E-04	0.832	1.65E-07
NMB1222	*ung*	uracil-DNA glycosylase							0.883	5.58E-03		
NMB1233	*recD*	exodeoxyribonuclease V, α subunit									-1.003	1.67E-03
NMB1243	*ruvB*	Holliday junction DNA helicase	0.627	2.28E-06								
NMB1295	*fpg*	formamidopyrimidine-DNA glycosylase									0.689	2.89E-03
NMB1302	*hip*	integration host factor, β subunit	0.726	2.31E-03	0.669	3.56E-03	0.627	9.00E-03			-1.100	4.39E-06
NMB1322	*priB*	putative primosomal replication protein n′	1.769	4.30E-11	1.433	6.73E-09	1.466	1.39E-08	0.692	2.69E-03	-1.248	3.37E-07
NMB1384	*gyrA*	DNA gyrase, subunit A			-0.847	5.14E-08	-0.640	4.24E-05	-0.987	9.05E-10	-1.074	1.50E-10
NMB1396	*mutY*	A/G-specific adenine glycosylase									0.859	1.52E-03
NMB1442	*mutL*	mismatch repair protein							0.700	3.59E-04		
NMB1445	*recA*	recA protein									-1.246	1.71E-08
NMB1447	*rep*	ATP-dependent DNA helicase									1.208	1.70E-04
NMB1460	*ssb*	single-strand binding protein									-1.321	1.87E-03
NMB1552	*pivNM-1A*	pilin gene inverting protein									0.711	2.95E-04
NMB1625	*pivNM-1B*	pilin gene inverting protein									0.686	2.29E-03
NMB1682	*parE*	topoisomerase IV, subunit B									1.123	7.03E-09
NMB1683		dinucleoside polyphosphate hydrolase									0.707	9.68E-03
NMB2160	*mutS*	DNA mismatch repair protein									0.594	6.12E-05
Restriction modification												
NMB0076		methyltransferase HphIm(C) (frameshift)									-0.827	1.52E-03

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Table C.6 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0725		modification methylase HgaI-1	1.168	8.84E-09								
NMB0726		type II restriction enzyme HgaI	1.288	2.70E-08								
NMB0727		N-6 adenine-specific DNA methylase	1.097	1.50E-06								
NMB0829		type I restriction enzyme EcoR124II M protein					-0.626	1.58E-03	-1.096	5.64E-08	-0.983	9.07E-07
NMB0835		putative type I restriction enzyme EcoR124II R protein									0.643	3.57E-03
NMB1033		modification methylase NlaIV							-0.784	3.04E-04	-0.731	8.11E-04
NMB1289		putative type II restriction enzyme									-1.623	1.57E-12
NMB1655		putative adenine specific methylase	-0.964	6.76E-06	-1.017	9.77E-07	-1.174	1.33E-07	-1.534	1.67E-11	-1.448	2.28E-10
NMB2065	*hemK*	hemK protein									0.768	7.52E-03

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.7: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *energy metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Aerobic												
NMB0434		putative AcnD-accessory protein PrpF	-2.110	3.06E-15	-1.868	7.27E-14	-1.930	1.52E-13	-1.588	1.94E-11	-1.015	3.60E-06
Amino acids and amines												
NMB0176	*dadA*	D-amino acid dehydrogenase, small subunit	-1.125	5.38E-08	-0.747	7.98E-05	-0.955	5.33E-07	-0.747	9.25E-05		
NMB0206	*aat*	leucyl phenylalanyl-tRNA protein transferase									0.672	6.36E-08
NMB0401	*putA*	proline dehydrogenase									-0.586	7.00E-04
NMB0574	*gcvT*	glycine cleavage system T protein	-2.337	2.04E-24	-1.758	2.86E-19	-1.877	1.97E-19	-1.837	5.36E-20	-1.618	3.07E-17
NMB0575	*gcvH*	glycine cleavage system H protein	-2.058	3.99E-14	-1.403	3.25E-09	-1.527	1.41E-09	-1.833	7.79E-13	-2.176	3.29E-15
NMB1609	*metZ*	trans-sulfuration enzyme family protein									0.768	1.46E-03
Anaerobic												
NMB1622	*norB*	nitric oxide reductase	-0.882	7.36E-04	-1.286	7.34E-07	-1.313	1.34E-06				
ATP-proton motive force interconversion												
NMB1933	*atpC*	ATP synthase F1, ϵ subunit	0.977	1.54E-06	0.842	1.20E-05	0.616	1.87E-03			-0.823	2.63E-05
NMB1934	*atpD*	ATP synthase F1, β subunit	1.226	7.49E-06	0.669	5.93E-03	0.736	6.36E-03				
NMB1935	*atpG*	ATP synthase F1, γ subunit	0.908	2.18E-03							-1.369	4.29E-06
NMB1939	*atpE*	ATP synthase F0, C subunit			0.847	8.74E-03					-1.087	8.53E-04
Biosynthesis and degradation of polysaccharides												
NMB0530		glycosyl hydrolase, family 3							0.78			
Electron transport												
NMB0123		ferredoxin, 4Fe-4S bacterial type	1.087	5.54E-06	0.768	6.25E-04					0.676	2.89E-03

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Table C.7 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0208		ferredoxin, 4Fe-4S bacterial type					1.099	1.89E-04	0.846	3.05E-03	1.026	3.12E-04
NMB0241	*nuoA*	NADH dehydrogenase I, A subunit	0.923	8.56E-08							1.216	2.50E-11
NMB0242	*nuoB*	NADH dehydrogenase I, B subunit							0.719	3.46E-05		
NMB0243	*nuoC*	NADH dehydrogenase I, C subunit	1.359	5.23E-09	0.918	1.36E-05	0.664	2.29E-03			1.392	1.80E-09
NMB0244	*nuoD*	NADH dehydrogenase I, D subunit	1.126	2.15E-10	0.657	2.53E-05			0.643	4.28E-05	1.209	1.63E-11
NMB0245	*nuoE*	NADH dehydrogenase I, E subunit	1.045	3.98E-07	0.587	2.05E-03					1.904	2.73E-15
NMB0246	*nuoF*	NADH dehydrogenase I, F subunit	0.843	2.65E-08							1.028	6.75E-11
NMB0249	*nuoG*	NADH dehydrogenase I, G subunit									1.067	1.88E-09
NMB0250	*nuoH*	NADH dehydrogenase I, H subunit									0.738	6.56E-03
NMB0251	*nuoI*	NADH dehydrogenase I, I subunit	0.802	1.15E-03							0.721	2.65E-03
NMB0254	*nuoK*	NADH dehydrogenase I, K subunit									0.921	1.02E-03
NMB0257	*nuoL*	NADH dehydrogenase I, L subunit	0.622	8.86E-06							0.617	7.16E-06
NMB0258	*nuoM*	NADH dehydrogenase I, M subunit									1.304	2.69E-07
NMB0581		electron transfer flavoprotein-ubiquinone oxidoreductase									0.624	1.42E-05
NMB0564	*nqrF*	Na(+)-translocating NADH-quinone reductase, subunit F	0.814	2.47E-04								
NMB0565	*nqrE*	Na(+)-translocating NADH-quinone reductase, subunit E	0.829	2.71E-04								
NMB0566	*nqrD*	Na(+)-translocating NADH-quinone reductase, subunit D	0.674	3.05E-03								
NMB0567	*nqrC*	Na(+)-translocating NADH-quinone reductase, subunit C	0.750	1.80E-03							-0.636	6.35E-03
NMB0569	*nqrA*	Na(+)-translocating NADH-quinone reductase, subunit A	0.713	1.01E-03								
NMB0717		putative cytochrome									0.938	1.16E-06
NMB0923		cytochrome c	0.881	4.51E-07	1.014	5.72E-09	1.047	9.34E-09				
NMB0978	*pntB*	NAD(P) transhydrogenase, β subunit	-0.822	3.93E-03								
NMB0980	*pntA*	NAD(P) transhydrogenase, α subunit	-0.802	1.27E-03								
NMB0993		rubredoxin									0.673	9.97E-03
NMB1044	*fpr-1*	ferredoxin NADP reductase	1.035	4.91E-09	1.121	1.77E-10	1.052	4.82E-09				
NMB1134	*fdx-1*	ferredoxin, 2Fe-2S type									-0.857	1.34E-05
NMB1172	*fdx-2*	ferredoxin, 2Fe-2S type									-0.853	1.10E-05
NMB1287		putative ferredoxin			-0.710	7.06E-03						
NMB1366	*trxA*	thioredoxin			1.364	1.90E-10	1.003	1.47E-06	0.815	3.45E-05		
NMB1677		cytochrome c5	1.405	6.64E-06	1.055	3.25E-04	0.967	1.62E-03				
NMB1723	*fixP*	cytochrome c oxidase, subunit III	1.522	4.79E-09	0.977	3.04E-05	0.721	3.03E-03			-1.209	8.51E-07
NMB1724	*fixO*	cytochrome c oxidase, subunit II	1.465	1.06E-06	0.802	4.09E-03						
NMB1734	*grxB*	glutaredoxin	-1.505	4.12E-11	-1.349	3.82E-10	-1.306	4.29E-09	-1.416	8.33E-11	-0.901	9.00E-06
NMB1790		glutaredoxin 3	-1.325	2.77E-05							-1.398	7.47E-06
NMB1805	*cyc*	cytochrome c4	0.959	2.11E-08	0.882	6.08E-08	0.875	2.71E-07				

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Table C.7 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB1958		putative thioredoxin	0.594	3.30E-03								
NMB2051	*petC*	ubiquinol cytochrome c reductase, cytochrome c1	1.492	6.24E-11	1.061	2.03E-07	1.006	1.96E-06				
NMB2052	*petB*	ubiquinol cytochrome c reductase, cytochrome b	1.902	1.37E-10	1.669	2.12E-09	1.359	1.02E-06				
NMB2053	*petA*	ubiquinol cytochrome c reductase, iron-sulfur subunit	1.777	2.83E-10	1.455	2.42E-08	1.285	1.34E-06	0.816	9.20E-04		
NMB2155	*etfB*	electron transfer flavoprotein, β subunit									-0.713	4.59E-03
Entner-Doudoroff												
NMB1393	*edd*	phosphogluconate dehydratase	-1.372	9.90E-17	-1.106	1.01E-13	-0.927	2.34E-10	-1.100	1.33E-13	-1.879	7.41E-23
NMB1394	*eda*	4-hydroxy-2-oxoglutarate aldolase/2-dehydro-3-deoxyphosphogluconate aldolase	-0.611	5.48E-05							-1.046	1.75E-10
Fermentation												
NMB0435	*ackA-2*	acetate kinase	-1.514	1.66E-11	-1.465	1.51E-11	-1.609	3.89E-12	-1.116	4.65E-08	-1.124	5.54E-08
NMB0546	*adhA*	alcohol dehydrogenase, propanol-preferring	-1.404	8.14E-07	-1.455	1.38E-07	-1.708	9.95E-09	-2.083	5.44E-12	-2.443	4.95E-14
NMB0604		alcohol dehydrogenase, zinc-containing							-0.774	2.97E-04	-1.148	3.86E-07
NMB0631		putative phosphate acetyltransferase Pta	-1.725	7.96E-16	-1.128	5.95E-10	-1.093	5.93E-09	-1.414	4.11E-13	-2.993	1.92E-26
NMB1304	*adhC*	alcohol dehydrogenase, class III	-1.575	2.69E-13	-1.599	4.34E-14	-1.668	6.27E-14	-1.625	3.25E-14	-2.011	1.23E-17
NMB1353		aldehyde dehydrogenase family protein	-0.825	3.88E-04	-1.121	2.80E-07	-0.901	1.32E-04				
NMB1395		alcohol dehydrogenase, zinc-containing	-1.911	1.86E-16	-1.261	1.49E-10	-1.427	1.72E-11	-1.211	4.77E-10	-1.719	7.13E-15
NMB1518	*ackA-1*	acetate kinase									-1.988	1.05E-17
NMB1968	*aldA*	aldehyde dehydrogenase A	-1.557	8.91E-06	-1.220	7.54E-05	-0.949	1.07E-03	-1.179	6.30E-04	-1.325	3.98E-05
Glycolysis/gluconeogenesis												
NMB0010	*pgk*	phosphoglycerate kinase	0.854	4.34E-06	1.178	7.41E-10	0.905	3.22E-07	0.975	1.32E-07	0.963	2.34E-07
NMB0089	*pykA*	pyruvate kinase II					-0.673	5.49E-04	-0.814	1.85E-05	-0.942	1.44E-06
NMB0334	*pgi-2*	glucose-6-phosphate isomerase	-0.970	8.52E-06	-0.701	6.37E-04	-0.977	8.74E-06				
NMB0618	*ppsA*	phosphoenolpyruvate synthase	-0.658	3.45E-03							-0.857	1.17E-04
NMB1285	*eno*	enolase	1.249	2.78E-06	1.420	6.33E-08	1.233	4.11E-06				
NMB1377	*lldA*	L-lactate dehydrogenase	-3.708	5.09E-26	-2.508	9.76E-19	-2.152	5.91E-15	-1.802	6.89E-13	-1.854	5.69E-13
NMB1388	*pgi-1*	glucose-6-phosphate, isomerase									-0.604	7.24E-06
NMB1390	*glk*	glucokinase									-0.751	1.07E-07
NMB1604	*gpm*	phosphoglycerate mutase									-1.290	1.24E-10
NMB2159		glyceraldehyde 3-phosphate dehydrogenase									-1.995	5.93E-17
Other												
NMB0340	*gloA*	lactoylglutathione lyase									-1.675	1.27E-12
NMB1488	*gabD*	succinate-semialdehyde dehydrogenase (NADP+)			-0.812	1.41E-07	-0.689	1.41E-05	-0.617	4.03E-05		
NMB1493		carbon starvation protein A	1.697	2.22E-10								
Pentose phosphate pathway												
NMB0015	*gnd*	6-phosphogluconate dehydrogenase, decarboxylating							0.621	1.79E-04		
NMB0351	*tal*	transaldolase	-0.732	4.73E-07								
NMB1392	*zwf*	glucose-6-phosphate 1-dehydrogenase							-0.671	2.76E-06	-1.135	3.45E-12
NMB1457	*tkt*	transketolase			0.635	1.73E-03	0.845	1.97E-04				
NMB1511	*rpiA*	ribose 5-phosphate isomerase A	0.673	3.83E-03							0.762	7.94E-04

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Table C.7 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
Pyruvate dehydrogenase												
NMB1341	*aceE*	pyruvate dehydrogenase, E1 component									-1.764	7.97E-13
NMB1342	*aceF*	dihydrolipoamide acetyltransferase (pyruvate dehydrogenase, E2 component)									-1.970	1.41E-15
NMB1344	*lpdA*	lipoamide dehydrogenase (pyruvate dehydrogenase, E3 component)									-1.148	9.00E-07
Sugars												
NMB0390	*mapA*	maltose phosphorylase	0.602	8.46E-05	0.692	3.58E-06						
NMB0391	*pgmB*	β-phosphoglucomutase									-0.733	9.51E-07
NMB0790	*pgm*	phosphoglucomutase			1.229	1.76E-13						
TCA cycle												
NMB0431	*prpC*	methylcitrate synthase citrate synthase 2	-3.932	3.37E-26	-3.977	1.64E-27	-4.129	2.29E-27	-3.506	1.60E-24	-2.241	2.09E-15
NMB0433	*acnA*	aconitate hydratase 1	-1.143	3.30E-03	-1.642	3.02E-05						
NMB0920	*icd*	isocitrate dehydrogenase, NADP-dependent (monomeric type)									-1.433	3.28E-08
NMB0947	*lpdA2*	lipoamide dehydrogenase (2-oxoglutarate dehydrogenase, E3 component)			1.214	3.59E-07					-0.858	2.57E-04
NMB0948	*sdhC*	succinate dehydrogenase, cytochrome b556 subunit									-1.172	2.59E-06
NMB0949	*sdhD*	succinate dehydrogenase, hydrophobic membrane anchor protein							-0.777	9.15E-04	-1.089	7.65E-06
NMB0950	*sdhA*	succinate dehydrogenase, flavoprotein subunit							-0.795	3.27E-03		
NMB0951	*sdhB*	succinate dehydrogenase, iron-sulfur protein			-0.675	2.73E-03	-0.821	5.08E-04	-1.307	5.30E-08	-1.363	2.59E-08
NMB0954	*gltA*	citrate synthase	-1.547	2.39E-08	-1.305	5.07E-07	-1.559	2.76E-08	-2.154	4.11E-14	-2.634	3.57E-16
NMB0955	*sucA*	2-oxoglutarate dehydrogenase, E1 component	-1.093	6.56E-04	-1.132	2.49E-04	-1.096	7.07E-04	-1.538	1.61E-06	-1.638	5.71E-07
NMB0956	*sucB*	dihydrolipoamide succinyltransferase (2-oxoglutarate dehydrogenase, E2 component)	-1.052	2.38E-04	-1.015	2.24E-04	-1.018	4.10E-04	-1.420	7.53E-07	-1.709	1.29E-08
NMB0957	*lpdA3*	lipoamide dehydrogenase (2-oxoglutarate dehydrogenase, E3 component)			-0.807	1.51E-05	-0.619	1.31E-03	-1.133	1.12E-08		
NMB0959	*sucC*	succinyl-CoA synthetase, β subunit	-1.011	5.89E-03					-1.531	2.25E-05	-1.464	5.69E-05
NMB0960	*sucD*	succinyl-CoA synthetase, α subunit							-1.359	1.85E-05	-1.280	6.18E-05
NMB1458	*fumC*	fumarate hydratase, class II, aerobic	-1.434	7.82E-05	-1.421	4.28E-04	-0.821	9.60E-03	-1.013	5.07E-03	-0.920	8.39E-03
NMB1572	*acnB*	aconitate hydratase 2									-1.267	2.07E-08
NMB1613	*fumA*	fumarate hydratase, class I, anaerobic	1.000	4.14E-03			1.032	3.36E-03	0.828	8.90E-03		
NMB2096	*mqo*	malate:quinone oxidoreductase	-1.226	3.48E-10	-0.764	1.05E-05	-0.705	9.39E-05	-0.594	5.41E-04	-0.696	7.31E-05

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.8: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *fatty acid and phospholipid metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Biosynthesis												
NMB0179	*fabZ*	(3R)-hydroxymyristoyl-(acyl-carrier-protein) dehydratase					0.596	1.01E-03			0.614	4.77E-04
NMB0219	*fabF*	3-oxoacyl-(acyl-carrier-protein) synthase II	1.417	2.34E-10	1.553	4.35E-12	1.441	2.40E-10	0.986	1.18E-06		
NMB0220	*acpP*	acyl carrier protein									-2.314	6.21E-14
NMB0336	*fabI*	enoyl-(acyl-carrier-protein) reductase									-1.039	4.64E-13
NMB0679	*accD*	acetyl-CoA carboxylase carboxyltransferase, β subunit							-0.691	4.47E-06	-1.397	1.31E-14
NMB1139	*accA-1*	acetyl-CoA carboxylase carboxyltransferase, α subunit							-0.872	4.15E-04	-1.280	8.24E-07
NMB1177	*accA-2*	acetyl-CoA carboxylase carboxyltransferase, α subunit							-0.904	2.60E-04	-1.366	2.01E-07
NMB1294	*plsC*	1-acyl-sn-glycerol-3-phosphate acyltransferase	-0.665	2.71E-03			-0.641	4.15E-03				
NMB1558	*dgk*	diacylglycerol kinase							-0.629	4.09E-05	-0.602	9.82E-05
NMB1697		putative acyl carrier protein									0.788	7.23E-04
NMB1860	*accB*	acetyl-CoA carboxylase, biotin carboxyl carrier protein									-1.203	1.74E-04
NMB1861	*accC*	acetyl-CoA carboxylase, biotin carboxylase					1.015	9.28E-07				
NMB1913	*plsX*	fatty acid phospholipid synthesis protein	1.067	5.40E-08	0.882	5.99E-06	0.769	3.10E-04	0.661	5.68E-04		
NMB2034	*nlaB*	putative 1-acyl-sn-glycerol-3-phosphate acyltransferase	0.650	9.94E-03								
Degradation												
NMB0464		putative phospholipase A1	1.144	1.04E-10	0.661	1.97E-05	0.588	2.56E-04				
NMB1555	*fadD*	long-chain-fatty-acid CoA ligase			0.634	2.95E-03					0.763	1.86E-03

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.9: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into TIGR role category *hypothetical proteins* or unassigned. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Conserved												
NMB0035		conserved hypothetical protein	-3.265	4.09E-23	-2.568	7.78E-19	-2.095	3.10E-14	-2.078	7.96E-15	-3.016	2.18E-21
NMB0036		conserved hypothetical protein	-1.766	1.10E-08	-1.557	9.11E-08	-1.436	1.69E-06	-0.784	4.90E-03	-1.107	1.03E-04
NMB0042		conserved hypothetical protein	-0.679	1.36E-04							-0.607	4.60E-04

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0060		conserved hypothetical protein	-0.966	4.67E-07	-0.860	2.34E-06	-0.939	1.05E-06	-0.672	1.77E-04		
NMB0109		conserved hypothetical protein	0.637	7.96E-04	0.618	6.50E-04						
NMB0121		conserved hypothetical protein					-0.631	2.98E-03			0.586	4.14E-03
NMB0122		conserved hypothetical protein	-0.667	2.77E-05	-0.638	2.90E-05	-0.732	6.04E-06				
NMB0135		conserved hypothetical protein									0.737	6.56E-03
NMB0188		conserved hypothetical protein							0.740	3.69E-05		
NMB0215		conserved hypothetical protein			0.633	7.32E-05			0.759	3.66E-06		
NMB0227		conserved hypothetical protein	-1.265	5.78E-10	-1.011	8.03E-08	-1.003	3.31E-07				
NMB0228		conserved hypothetical protein	-1.310	3.35E-08	-0.850	8.38E-05	-0.835	2.26E-04				
NMB0230		conserved hypothetical protein	-1.241	1.06E-09	-0.864	2.73E-06	-0.666	4.10E-04				
NMB0248		conserved hypothetical protein	0.818	3.72E-04							0.690	1.94E-03
NMB0263		conserved hypothetical protein	0.620	9.73E-03			0.626	9.37E-03	0.875	1.65E-04	1.277	2.03E-07
NMB0266		conserved hypothetical protein			0.833	3.51E-04					0.686	3.55E-03
NMB0267		conserved hypothetical protein	0.803	4.44E-05	1.050	8.89E-08	1.053	3.02E-07	0.854	9.28E-06	0.922	2.63E-06
NMB0276		conserved hypothetical protein							0.701	9.00E-04		
NMB0279		conserved hypothetical protein									-0.720	9.04E-07
NMB0291		conserved hypothetical protein			0.701	2.31E-04	0.670	1.51E-03	0.752	2.23E-04	0.824	7.20E-05
NMB0310		conserved hypothetical protein									0.662	3.46E-03
NMB0313		conserved hypothetical protein	-0.857	5.21E-04							-0.817	6.89E-04
NMB0315		conserved hypothetical protein							0.747	2.09E-04	0.654	1.20E-03
NMB0316		conserved hypothetical protein	1.508	1.86E-09	0.651	3.50E-03			0.982	1.65E-05	0.961	2.95E-05
NMB0317		conserved hypothetical protein	1.900	3.26E-15	1.182	6.22E-09			0.594	1.69E-03	0.738	1.39E-04
NMB0339		conserved hypothetical protein									-0.665	5.42E-08
NMB0343		conserved hypothetical protein	-1.377	1.77E-05					-0.904	3.01E-03	-1.742	9.86E-08
NMB0353		conserved hypothetical protein	-0.812	2.05E-08	-0.615	4.23E-06	-0.621	9.12E-06	-0.681	6.43E-07	-0.913	5.57E-10
NMB0355		conserved hypothetical protein	-0.596	4.70E-05							-0.609	2.38E-05
NMB0377		conserved hypothetical protein							0.616	3.51E-03		
NMB0395		conserved hypothetical protein					0.954	3.31E-07	1.462	3.43E-13		
NMB0404		conserved hypothetical protein									-1.079	9.32E-09
NMB0409		conserved hypothetical protein	-0.629	8.05E-03	-0.662	3.69E-03	-0.896	1.89E-04				
NMB0410		conserved hypothetical protein	-1.124	3.60E-07	-0.942	6.04E-06	-0.842	9.63E-05	-1.084	4.32E-07	-0.703	6.89E-04
NMB0411		conserved hypothetical protein	-0.688	2.05E-04								
NMB0444		conserved hypothetical protein									0.831	1.10E-04
NMB0455		conserved hypothetical protein									0.637	6.32E-05
NMB0465		conserved hypothetical protein	0.887	3.28E-06								
NMB0479		conserved hypothetical protein							-0.922	2.50E-04	-1.429	1.05E-07
NMB0529		conserved hypothetical protein							0.623	8.79E-04		
NMB0531		conserved hypothetical protein	1.015	5.75E-10	0.851	2.46E-08	0.892	2.94E-08	0.662	7.84E-06	0.807	1.67E-07
NMB0534		conserved hypothetical protein									0.889	1.18E-04
NMB0545		conserved hypothetical protein							0.710	1.10E-05	1.089	6.84E-10
NMB0557		conserved hypothetical protein			0.639	4.94E-04					-0.935	1.30E-06

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0562		conserved hypothetical protein			0.641	4.43E-05						
NMB0571		conserved hypothetical protein	-1.387	2.61E-09	-1.126	1.83E-07	-1.053	2.34E-06	-0.717	5.69E-04		
NMB0606		conserved hypothetical protein							-0.871	4.89E-06	-2.260	6.58E-19
NMB0619		conserved hypothetical protein	-1.302	1.37E-09	-1.151	1.36E-08	-1.013	9.28E-07	-0.701	2.52E-04	-1.289	1.23E-09
NMB0621		conserved hypothetical protein									0.942	5.21E-05
NMB0625		conserved hypothetical protein					-0.627	1.40E-04	-0.643	5.15E-05	-0.977	1.10E-08
NMB0681		conserved hypothetical protein									1.089	8.24E-06
NMB0711		conserved hypothetical protein	-0.669	6.94E-06	-0.781	1.03E-07	-0.717	2.01E-06	-1.007	1.08E-10	-1.116	9.46E-12
NMB0714		conserved hypothetical protein									0.881	3.19E-05
NMB0742		conserved hypothetical protein	1.363	5.86E-10	0.820	2.75E-05	0.952	4.55E-06			0.622	1.48E-03
NMB0746		conserved hypothetical protein	-0.597	7.54E-04			-0.660	2.33E-04			-0.637	2.50E-04
NMB0753		conserved hypothetical protein	-1.749	4.13E-10	-1.417	4.45E-08	-1.569	1.72E-09	-1.938	6.99E-13	-1.493	2.25E-08
NMB0759		conserved hypothetical protein	0.721	6.10E-08							0.920	4.61E-11
NMB0771		conserved hypothetical protein									-0.917	2.33E-09
NMB0773		conserved hypothetical protein									-1.365	6.45E-11
NMB0776		conserved hypothetical protein			0.723	5.05E-06						
NMB0797		conserved hypothetical protein			0.758	2.12E-06						
NMB0800		conserved hypothetical protein							-0.725	2.44E-05		
NMB0803		conserved hypothetical protein					0.637	1.92E-06				
NMB0840		conserved hypothetical protein	1.477	1.03E-12	0.933	1.97E-07	1.119	8.92E-09				
NMB0853		conserved hypothetical protein	-1.118	1.01E-03							-0.984	3.84E-03
NMB0874		conserved hypothetical protein									-0.870	5.00E-09
NMB0883		conserved hypothetical protein					-0.652	5.27E-04	-0.900	1.40E-06		
NMB0952		conserved hypothetical protein	-1.019	2.81E-04	-0.990	2.39E-04	-1.107	9.99E-05	-1.817	7.32E-10	-1.692	9.92E-09
NMB1009		conserved hypothetical protein			-0.835	7.67E-04	-1.073	8.47E-05	-1.136	1.69E-05		
NMB1016		conserved hypothetical protein									0.632	4.94E-05
NMB1018		conserved hypothetical protein									0.597	4.11E-05
NMB1023		conserved hypothetical protein					0.593	5.23E-03	0.675	4.51E-04		
NMB1026		conserved hypothetical protein									-0.948	1.21E-08
NMB1039		conserved hypothetical protein	0.870	4.80E-06	0.626	4.54E-04	0.920	1.77E-06				
NMB1071		conserved hypothetical protein	0.954	5.34E-08	0.755	3.73E-06	0.843	1.11E-06				
NMB1073		conserved hypothetical protein	0.676	1.17E-04	0.795	3.57E-06	0.898	8.31E-07	0.822	2.20E-06		
NMB1095		conserved hypothetical protein									1.421	2.97E-04
NMB1119		conserved hypothetical protein							0.677	6.12E-03		
NMB1128		conserved hypothetical protein							0.655	9.14E-03		
NMB1138		conserved hypothetical protein							-0.701	6.75E-05		
NMB1176		conserved hypothetical protein							-0.767	5.73E-06		
NMB1218		conserved hypothetical protein									-0.605	6.14E-03
NMB1264		conserved hypothetical protein							-0.588	2.00E-03		
NMB1268		conserved hypothetical protein							-0.683	4.25E-05	-1.070	3.59E-09
NMB1286		conserved hypothetical protein	0.907	2.67E-07	1.245	1.51E-11	0.865	9.52E-07				

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1327		conserved hypothetical protein							0.606	9.88E-03		
NMB1336		conserved hypothetical protein			1.100	2.81E-09	0.663	2.22E-04				
NMB1354		conserved hypothetical protein	-0.894	1.12E-06	-1.123	1.61E-09	-0.953	3.53E-07				
NMB1365		conserved hypothetical protein					-0.849	5.74E-06	-0.690	9.68E-05	-0.626	4.27E-04
NMB1378		conserved hypothetical protein	-1.849	4.53E-17	-1.107	9.74E-10	-0.932	2.95E-07	-0.814	2.13E-06		
NMB1382		conserved hypothetical protein									-0.884	3.98E-07
NMB1436		conserved hypothetical protein	0.866	1.68E-04	1.017	6.04E-06	1.259	2.15E-07	0.642	3.67E-03		
NMB1437		conserved hypothetical protein	1.324	4.70E-09	1.309	2.33E-09	1.626	1.76E-11	1.025	1.09E-06	0.896	1.79E-05
NMB1438		conserved hypothetical protein	1.254	2.09E-08	1.344	9.64E-10	1.487	2.87E-10	1.118	1.43E-07		
NMB1444		conserved hypothetical protein	0.904	1.41E-07	0.985	6.22E-09	1.029	7.59E-09	0.724	8.11E-06		
NMB1464		conserved hypothetical protein	-0.613	6.20E-03			-0.628	5.33E-03				
NMB1475		conserved hypothetical protein			2.355	1.99E-10	2.258	3.06E-09	1.437	2.46E-05	1.237	2.72E-04
NMB1496		conserved hypothetical protein									0.617	1.61E-03
NMB1500		conserved hypothetical protein	-0.963	4.17E-04					-0.799	2.36E-03	-1.256	4.48E-06
NMB1531		conserved hypothetical protein			0.674	6.11E-05	0.591	7.47E-04				
NMB1532		conserved hypothetical protein									0.675	6.97E-07
NMB1543		conserved hypothetical protein					-1.045	3.57E-03				
NMB1557		conserved hypothetical protein	-0.976	1.32E-08			-0.620	4.05E-04	-0.858	8.59E-07	-1.676	2.26E-15
NMB1564		conserved hypothetical protein			1.028	8.88E-09					-0.592	4.84E-04
NMB1575		conserved hypothetical protein							-0.617	3.41E-03	-1.232	5.75E-08
NMB1578		conserved hypothetical protein	-1.914	3.26E-15	-1.586	1.35E-11	-1.277	2.94E-08	-1.710	9.72E-13	-0.833	8.28E-05
NMB1590		conserved hypothetical protein	-1.127	2.45E-07	-1.251	7.17E-09	-1.238	3.47E-08	-1.544	1.33E-11	-2.134	2.35E-16
NMB1634		conserved hypothetical protein					-0.879	6.97E-03				
NMB1641		conserved hypothetical protein	1.439	2.34E-10	0.709	3.41E-04	1.042	1.15E-06	0.716	3.27E-04		
NMB1648		conserved hypothetical protein	1.108	4.56E-05	0.969	1.88E-04	1.244	7.29E-06	0.902	5.32E-04		
NMB1652		conserved hypothetical protein	0.725	6.93E-03	1.008	1.01E-04						
NMB1653		conserved hypothetical protein	0.793	8.25E-04	1.048	2.97E-05	0.714	6.41E-03			-0.787	6.89E-04
NMB1654		conserved hypothetical protein	-0.765	3.97E-05	-0.586	4.36E-04	-0.822	1.39E-05	-0.806	2.93E-06		
NMB1663		conserved hypothetical protein									-0.751	1.43E-05
NMB1665		conserved hypothetical protein			-0.858	6.66E-04	-0.654	7.05E-03	-0.688	6.50E-03	-3.398	9.70E-22
NMB1694		conserved hypothetical protein	-0.774	6.93E-03								
NMB1721		conserved hypothetical protein			-0.750	1.25E-03	-0.753	5.21E-03				
NMB1727		conserved hypothetical protein	-0.852	5.95E-07							-1.081	1.03E-09
NMB1731		conserved hypothetical protein									-0.607	1.17E-04
NMB1749		conserved hypothetical protein									2.185	1.71E-11
NMB1759		conserved hypothetical protein									-0.667	1.09E-03
NMB1760		conserved hypothetical protein					-0.715	6.17E-05	-0.963	7.70E-08	-1.073	6.80E-09
NMB1761		conserved hypothetical protein					-0.758	1.37E-04	-0.827	1.78E-05		
NMB1796		conserved hypothetical protein	-1.806	2.72E-14	-0.927	2.07E-06	-0.909	7.96E-06	-1.270	9.83E-10	-3.146	1.11E-24
NMB1806		conserved hypothetical protein					-0.703	2.81E-05	-0.919	4.86E-08		
NMB1826		conserved hypothetical protein									0.709	2.47E-04

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB1866		conserved hypothetical protein	1.186	3.20E-06	0.738	2.90E-03	0.872	4.92E-04	0.954	8.10E-05		
NMB1884		conserved hypothetical protein							0.744	3.60E-03		
NMB1886		conserved hypothetical protein									1.087	8.98E-06
NMB1906		conserved hypothetical protein	1.262	6.43E-06	0.888	7.17E-04	0.794	3.95E-03	0.746	4.62E-03		
NMB1908		conserved hypothetical protein									0.948	2.78E-07
NMB1910		conserved hypothetical protein	1.811	4.58E-11	1.303	1.29E-07	1.560	5.79E-09	0.943	8.13E-05		
NMB1912		conserved hypothetical protein	1.240	2.32E-12	1.088	5.74E-11	0.945	1.35E-08	0.633	2.79E-05		
NMB1951		conserved hypothetical protein			0.645	5.55E-04	0.595	1.01E-03				
NMB1980		conserved hypothetical protein									-0.713	5.35E-06
NMB2000		conserved hypothetical protein			1.109	4.56E-11	0.785	1.25E-06	1.213	2.25E-12		
NMB2003		conserved hypothetical protein									0.773	8.44E-03
NMB2018		conserved hypothetical protein	-0.651	1.82E-03			-0.687	1.10E-03	-0.645	1.35E-03	-0.585	3.96E-03
NMB2020		conserved hypothetical protein									-1.339	9.44E-11
NMB2021		conserved hypothetical protein			0.813	3.76E-03						
NMB2024		conserved hypothetical protein							0.660	3.59E-04	0.740	8.71E-05
NMB2025		conserved hypothetical protein									0.689	4.53E-04
NMB2035		conserved hypothetical protein							0.803	1.05E-04		
NMB2050		conserved hypothetical protein			-0.602	6.29E-03						
NMB2054		conserved hypothetical protein	0.661	3.49E-06	0.594	4.43E-05						
NMB2078		conserved hypothetical protein	-0.832	6.70E-08							-1.416	2.09E-15
NMB2139		conserved hypothetical protein									1.164	3.05E-06
NMB2142		conserved hypothetical protein							0.641	9.81E-03		
NMB2143		conserved hypothetical protein							0.639	1.90E-06	0.963	8.09E-11
NMB2153		conserved hypothetical protein	0.585	4.36E-03							0.889	1.31E-05
Unassigned												
NMB0028		hypothetical protein									-0.658	8.69E-03
NMB0046		hypothetical protein									0.822	5.27E-09
NMB0086		hypothetical protein							0.605	1.19E-06		
NMB0091		hypothetical protein			0.644	2.17E-03					-0.768	3.32E-04
NMB0092		hypothetical protein									-0.861	4.91E-03
NMB0096		hypothetical protein									-0.694	5.50E-03
NMB0099		hypothetical protein					-0.862	8.31E-03				
NMB0102		hypothetical protein					-0.606	3.40E-04	-0.585	3.23E-04		
NMB0104		hypothetical protein			-1.013	1.37E-10	-1.284	2.63E-13	-1.319	2.09E-14	-1.872	1.94E-20
NMB0113		hypothetical protein	-0.907	1.53E-06	-0.950	2.03E-07	-0.903	1.91E-06	-0.749	3.00E-05	-0.909	9.80E-07
NMB0129		hypothetical protein	1.580	1.80E-06	1.334	1.97E-05	1.198	2.26E-04			0.872	5.10E-03
NMB0189		hypothetical protein							0.718	1.35E-03		
NMB0197		hypothetical protein									-0.660	1.46E-04
NMB0200		hypothetical protein	-0.712	4.89E-03	-0.796	1.05E-03	-0.783	2.12E-03				
NMB0213		hypothetical protein							0.958	8.14E-08	0.862	1.15E-06
NMB0237		hypothetical protein							-0.805	7.29E-03		

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0239		hypothetical protein	1.269	2.47E-08	0.924	1.03E-05	0.685	1.47E-03			1.725	1.76E-12
NMB0240		hypothetical protein	0.914	1.35E-07	0.641	6.17E-05						
NMB0247		hypothetical protein	1.051	1.71E-04								
NMB0252		hypothetical protein	0.842	2.80E-04					-0.698	1.72E-03		
NMB0260		hypothetical protein									0.684	1.24E-06
NMB0271		hypothetical protein							0.656	9.15E-04		
NMB0273		hypothetical protein	-0.723	2.46E-05	-0.748	6.23E-06	-0.633	2.11E-04	-0.668	5.23E-05	-0.608	2.40E-04
NMB0297		hypothetical protein	0.676	1.15E-03								
NMB0298		hypothetical protein			0.863	8.05E-04	0.763	4.73E-03				
NMB0305		hypothetical protein							-0.832	2.66E-04		
NMB0311		hypothetical protein	-0.758	2.40E-03			-0.816	1.19E-03	-1.247	7.19E-07	-0.720	3.01E-03
NMB0320		hypothetical protein	1.796	1.41E-09	1.811	4.08E-10	1.537	1.20E-07	1.061	6.87E-05		
NMB0338		hypothetical protein									-0.720	1.61E-06
NMB0346		hypothetical protein	-1.043	2.94E-07					-0.717	1.59E-04	-0.678	3.89E-04
NMB0354		hypothetical protein	-0.806	6.35E-09							-0.838	1.47E-09
NMB0363		hypothetical protein			-0.851	2.73E-04	-0.839	6.06E-04	-0.938	7.61E-05		
NMB0367		hypothetical protein									0.663	9.35E-05
NMB0368		hypothetical protein							-0.642	1.35E-03	0.653	1.26E-03
NMB0369		hypothetical protein									0.721	1.38E-04
NMB0370		hypothetical protein									0.631	1.07E-04
NMB0371		hypothetical protein									0.781	1.26E-05
NMB0372		hypothetical protein			-0.611	6.75E-04	-0.833	1.42E-05	-0.824	8.67E-06		
NMB0373		hypothetical protein							-0.607	8.90E-03		
NMB0376		hypothetical protein									0.907	1.20E-03
NMB0384		hypothetical protein									-0.725	6.36E-05
NMB0429		hypothetical protein	-1.080	2.90E-04	-1.301	6.42E-06	-1.124	2.88E-04	-0.885	3.06E-03		
NMB0449		hypothetical protein					-0.728	4.46E-04	-0.715	8.59E-04	-1.734	5.10E-12
NMB0450		hypothetical protein			-0.637	1.55E-03	-0.751	3.89E-04	-0.891	1.58E-05	-1.510	6.70E-11
NMB0451		hypothetical protein							-0.764	4.39E-03	-1.156	2.89E-05
NMB0475		hypothetical protein							-0.646	6.52E-03		
NMB0476		hypothetical protein									0.921	2.62E-04
NMB0478		hypothetical protein									-1.239	1.48E-08
NMB0481		hypothetical protein							-0.645	6.37E-05		
NMB0485		hypothetical protein									1.271	1.56E-04
NMB0488		hypothetical protein									-0.597	5.08E-05
NMB0491		hypothetical protein							-0.671	1.40E-03		
NMB0492		hypothetical protein					-0.620	4.14E-03	-0.843	5.94E-05		
NMB0499		hypothetical protein	-0.888	4.89E-03			-0.864	6.51E-03				
NMB0500		hypothetical protein									-1.348	2.60E-09
NMB0501		hypothetical protein			-0.742	7.34E-03			-0.760	5.98E-03	-1.161	4.56E-05
NMB0502		hypothetical protein	-0.946	3.78E-05	-1.026	4.23E-06	-1.007	1.51E-05				

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0503		hypothetical protein									-0.945	3.43E-05
NMB0507		hypothetical protein	-1.082	2.07E-07	-1.227	3.22E-09	-1.019	9.62E-07	-1.334	2.95E-10	-3.078	3.58E-24
NMB0508		hypothetical protein	-1.130	1.67E-04	-0.799	5.50E-03	-0.868	1.80E-03	-0.954	9.26E-04		
NMB0509		hypothetical protein	-1.000	1.23E-05	-0.755	4.77E-04	-0.909	7.15E-05				
NMB0510		hypothetical protein	-1.002	3.88E-05	-0.843	2.80E-04	-0.929	1.44E-04	-0.796	6.32E-04		
NMB0511		hypothetical protein	-0.887	2.66E-03	-0.848	2.85E-03	-0.819	5.91E-03				
NMB0512		hypothetical protein	-0.835	1.20E-04	-0.776	1.93E-04	-0.737	7.18E-04	-0.872	3.78E-05	-0.894	3.12E-05
NMB0515		hypothetical protein	-0.766	8.70E-05			-0.627	1.28E-03				
NMB0516		hypothetical protein	-1.518	1.21E-11	-0.966	8.52E-07	-1.185	2.57E-08	-1.142	2.13E-08	-1.217	6.02E-09
NMB0518		hypothetical protein	-1.088	3.70E-03	-1.003	4.50E-03			-1.107	2.64E-03	-1.319	8.48E-04
NMB0519		hypothetical protein	-1.224	1.32E-08	-1.191	9.46E-09	-1.301	3.99E-09	-1.015	1.49E-07	-1.894	5.95E-15
NMB0521		hypothetical protein	-0.961	3.44E-05	-0.862	9.84E-05	-0.935	6.32E-05	-1.148	1.67E-07	-1.387	9.90E-09
NMB0526		hypothetical protein	0.807	7.15E-06					0.670	9.37E-05	1.157	1.01E-09
NMB0541		hypothetical protein									1.235	3.57E-15
NMB0552		hypothetical protein									0.733	1.01E-03
NMB0570		hypothetical protein	-0.618	7.19E-05								
NMB0572		hypothetical protein	-1.692	2.72E-14	-1.290	1.38E-11	-1.210	2.88E-09	-0.768	2.44E-05	-1.084	2.25E-08
NMB0596		hypothetical protein									0.720	4.74E-07
NMB0600		hypothetical protein			0.667	3.51E-06						
NMB0613		hypothetical protein	0.814	5.96E-03								
NMB0647		hypothetical protein			-0.738	1.91E-05	-0.833	5.21E-06				
NMB0648		hypothetical protein									-1.140	3.14E-08
NMB0649		hypothetical protein									-0.792	2.08E-03
NMB0650		hypothetical protein					-0.732	1.49E-03	-0.823	2.17E-04	-1.083	2.73E-06
NMB0651		hypothetical protein							-1.009	3.53E-05	-0.883	6.19E-04
NMB0654		hypothetical protein			-0.872	3.84E-05	-1.288	3.73E-08	-1.486	1.79E-10	-0.590	5.25E-03
NMB0655		hypothetical protein			-0.673	8.39E-05	-0.904	1.00E-06	-1.002	3.79E-08		
NMB0656		hypothetical protein			-0.837	1.15E-05	-0.737	4.34E-04	-0.676	7.43E-04		
NMB0660		hypothetical protein									-0.627	4.65E-04
NMB0664		hypothetical protein									1.662	6.84E-06
NMB0673		hypothetical protein	-0.609	6.29E-05	-0.588	5.64E-05						
NMB0695		hypothetical protein									1.336	3.82E-03
NMB0701		hypothetical protein									0.980	4.72E-05
NMB0715		hypothetical protein									1.872	1.05E-09
NMB0716		hypothetical protein									0.727	1.38E-03
NMB0730		hypothetical protein									1.139	9.53E-07
NMB0731		hypothetical protein									0.925	2.40E-07
NMB0734		hypothetical protein									0.713	1.00E-05
NMB0744		hypothetical protein	-3.549	7.39E-24	-2.663	9.00E-19	-2.585	2.82E-17	-2.436	6.48E-17	-3.396	7.41E-23
NMB0761		hypothetical protein									-1.320	9.44E-05
NMB0762		hypothetical protein	1.012	1.81E-06	1.061	2.52E-07	0.965	5.50E-06	0.715	3.35E-04		

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0813		hypothetical protein							1.000	6.37E-03		
NMB0816		hypothetical protein	0.614	1.21E-03	0.673	2.31E-04						
NMB0818		hypothetical protein									-1.033	4.72E-05
NMB0819		hypothetical protein	-0.602	1.36E-03	-0.743	4.67E-05	-0.746	9.79E-05				
NMB0820		hypothetical protein	0.978	2.63E-07	1.131	2.09E-09	1.089	2.74E-08				
NMB0821		hypothetical protein	0.940	1.67E-10	1.061	9.69E-13	1.100	1.63E-12	0.665	6.42E-07	1.496	3.74E-18
NMB0841		hypothetical protein	0.917	5.10E-08	0.736	2.73E-06	0.798	1.43E-06	0.921	2.11E-08		
NMB0850		hypothetical protein	-2.849	5.07E-14	-1.918	5.65E-09	-2.106	1.81E-09	-2.277	3.30E-11	-2.249	1.08E-10
NMB0856		hypothetical protein	-1.028	5.30E-05	-0.902	2.04E-04	-0.969	1.48E-04	-1.580	9.93E-11		
NMB0857		hypothetical protein	-1.198	6.39E-06	-1.112	1.66E-06	-1.244	7.37E-07	-1.300	1.62E-07	-0.844	8.39E-04
NMB0858		hypothetical protein	-1.144	2.24E-03	-1.129	1.70E-03	-1.086	4.00E-03	-1.223	7.22E-04	-0.987	6.53E-03
NMB0859		hypothetical protein	-0.634	8.03E-03	-0.690	2.45E-03	-0.622	9.79E-03	-0.819	3.55E-04	-0.927	8.44E-05
NMB0860		hypothetical protein			-0.672	2.54E-05	-0.691	3.84E-05				
NMB0861		hypothetical protein	0.737	7.67E-06			0.766	4.31E-06			0.799	1.09E-06
NMB0862		hypothetical protein	1.059	1.13E-06	0.898	1.24E-05	1.135	3.22E-07	0.644	1.47E-03	0.717	4.90E-04
NMB0863		hypothetical protein	1.038	3.23E-09	0.674	2.00E-05	0.744	8.49E-06			1.530	5.31E-15
NMB0864		hypothetical protein	-2.899	2.02E-14	-2.424	3.79E-12	-2.647	1.05E-12	-2.708	7.40E-14	-2.061	1.08E-09
NMB0865		hypothetical protein	0.747	4.89E-03			0.768	4.12E-03			1.130	1.89E-05
NMB0866		hypothetical protein	0.620	6.89E-05								
NMB0882		hypothetical protein									-0.667	3.10E-03
NMB0897		hypothetical protein									-0.649	6.03E-03
NMB0898		hypothetical protein									-1.189	7.08E-07
NMB0899		hypothetical protein									-1.217	7.13E-08
NMB0900		hypothetical protein							-0.587	5.57E-04	-0.887	7.61E-07
NMB0902		hypothetical protein							-0.863	3.38E-03	-1.062	6.17E-04
NMB0907		hypothetical protein									1.008	1.47E-06
NMB0908		hypothetical protein									1.076	3.12E-04
NMB0915		hypothetical protein							-1.099	2.80E-04		
NMB0928		hypothetical protein									-1.378	1.58E-14
NMB0945		hypothetical protein	1.911	2.02E-09	1.726	1.06E-10	1.842	1.09E-07	1.329	5.01E-04	0.981	1.28E-04
NMB0958		hypothetical protein							-0.748	1.24E-03	-0.634	6.51E-03
NMB0965		hypothetical protein									0.987	1.50E-04
NMB0979		hypothetical protein	-0.977	2.28E-03								
NMB0986		hypothetical protein	0.828	4.07E-05								
NMB0989		hypothetical protein									1.104	2.80E-03
NMB1003		hypothetical protein			-0.883	5.31E-03						
NMB1006		hypothetical protein	-1.543	7.26E-03	-1.591	9.36E-03	-2.100	3.00E-04	-2.262	1.07E-04		
NMB1008		hypothetical protein	-1.114	3.46E-05	-1.562	1.07E-08	-1.939	1.19E-10	-2.331	4.11E-14	-1.166	1.12E-05
NMB1010		hypothetical protein			-0.663	4.66E-03	-1.249	6.24E-07	-1.285	1.66E-06		
NMB1011		hypothetical protein							-0.997	1.24E-03	-1.266	6.48E-05
NMB1020		hypothetical protein	0.805	1.52E-05	0.911	5.68E-07	0.689	2.03E-04				

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1035		hypothetical protein			0.909	1.16E-05	0.825	1.30E-04				
NMB1047		hypothetical protein			-0.600	1.03E-05			-0.667	5.44E-06		
NMB1048		hypothetical protein	-2.224	4.90E-15	-2.729	1.64E-20	-2.446	1.79E-16	-2.575	3.83E-18	-2.137	1.48E-14
NMB1079		hypothetical protein	-1.489	1.52E-05	-1.697	1.30E-07	-1.693	4.60E-07	-1.666	2.72E-05	-1.022	3.48E-03
NMB1084		hypothetical protein							-0.939	1.28E-03	-1.310	1.41E-05
NMB1086		hypothetical protein									1.251	2.62E-04
NMB1089		hypothetical protein									-1.093	1.06E-03
NMB1092		hypothetical protein					-1.033	4.60E-03				
NMB1093		hypothetical protein	-0.881	3.49E-03	-1.038	3.56E-04	-1.113	2.69E-04	-0.969	8.95E-04	-1.074	2.84E-04
NMB1100		hypothetical protein									0.827	2.21E-04
NMB1107		hypothetical protein	-0.833	1.06E-03	-1.330	1.85E-07	-0.952	2.21E-04	-0.952	1.24E-04	-2.397	5.55E-15
NMB1124		hypothetical protein									-1.348	8.56E-08
NMB1129		hypothetical protein					0.978	3.27E-03				
NMB1132		hypothetical protein	-0.758	1.61E-04	-0.742	1.18E-04	-0.691	6.06E-04	-0.661	6.21E-04		
NMB1143		hypothetical protein									-0.639	3.55E-03
NMB1144		hypothetical protein									-0.906	2.14E-04
NMB1161		hypothetical protein									0.587	4.81E-03
NMB1162		hypothetical protein									-1.226	2.95E-06
NMB1164		hypothetical protein	1.061	2.06E-04	0.781	4.38E-03	0.940	1.05E-03			0.703	5.96E-03
NMB1170		hypothetical protein	-0.808	2.64E-05	-0.731	6.79E-05	-0.686	3.46E-04	-0.624	6.55E-04		
NMB1181		hypothetical protein									-0.688	1.75E-03
NMB1182		hypothetical protein									-1.031	5.84E-10
NMB1187		hypothetical protein									-0.884	1.95E-03
NMB1195		hypothetical protein							0.715	5.90E-03		
NMB1202		hypothetical protein									0.628	1.12E-03
NMB1205		hypothetical protein	0.888	1.39E-03								
NMB1208		hypothetical protein							-1.909	4.27E-03		
NMB1209		hypothetical protein					-1.101	6.63E-03				
NMB1211		hypothetical protein					-1.049	4.12E-03	-1.594	9.10E-06		
NMB1212		hypothetical protein					-1.027	2.35E-03	-1.429	1.53E-05		
NMB1229		hypothetical protein	1.176	6.64E-06	0.756	4.07E-03	0.761	5.09E-03				
NMB1236		hypothetical protein	-0.785	2.47E-06	-0.714	1.43E-06	-0.656	7.02E-05	-0.822	4.20E-07	-1.135	1.52E-10
NMB1245		hypothetical protein	-1.012	1.14E-08	-0.629	8.56E-05	-0.747	1.15E-05	-1.018	4.08E-09	-0.875	2.69E-07
NMB1272		hypothetical protein									1.153	2.84E-03
NMB1274		hypothetical protein							0.783	1.34E-08	0.803	1.05E-08
NMB1293		hypothetical protein	-0.899	1.92E-04	-0.747	1.19E-03	-0.851	4.42E-04			-0.638	6.10E-03
NMB1334		hypothetical protein			0.863	2.34E-06						
NMB1340		hypothetical protein									-2.495	8.26E-17
NMB1343		hypothetical protein									-1.400	2.44E-08
NMB1345		hypothetical protein							-0.787	5.77E-06		
NMB1350		hypothetical protein							-0.948	2.78E-06	-1.124	9.26E-08

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1370		hypothetical protein	0.788	1.12E-05	0.686	5.81E-05	0.631	3.93E-04	0.651	1.46E-04	0.822	3.60E-06
NMB1387		hypothetical protein	-0.737	2.10E-07	-0.860	1.59E-09	-0.914	1.28E-09	-1.023	6.71E-12	-1.590	7.94E-19
NMB1402		hypothetical protein	-1.074	9.03E-09	-1.723	3.07E-16	-1.726	2.38E-15	-1.855	1.86E-17	-0.992	4.70E-08
NMB1404		hypothetical protein			-1.280	1.47E-08	-1.298	4.00E-08	-1.386	3.30E-10	-1.548	1.47E-10
NMB1408		hypothetical protein			-0.887	1.74E-04	-1.087	1.49E-05	-1.326	1.11E-07	-2.161	6.42E-14
NMB1410		hypothetical protein			-0.816	1.94E-05	-1.019	6.90E-07	-1.135	2.08E-08	-0.642	7.86E-04
NMB1426		hypothetical protein	0.976	1.54E-06	0.615	4.29E-04	0.940	3.83E-06	1.007	3.47E-07	1.021	4.03E-07
NMB1453		hypothetical protein	-0.730	4.89E-03	-0.811	1.75E-03						
NMB1468		hypothetical protein							-0.741	6.64E-03	-3.239	2.26E-18
NMB1469		hypothetical protein									-2.260	1.57E-04
NMB1486		hypothetical protein									-1.176	2.40E-09
NMB1489		hypothetical protein	-0.622	3.50E-05					-0.681	3.94E-06		
NMB1490		hypothetical protein	-1.067	5.53E-04					-1.054	4.27E-04	-0.903	2.62E-03
NMB1507		hypothetical protein					0.585	2.26E-04			0.880	6.39E-08
NMB1508		hypothetical protein					0.638	9.84E-03				
NMB1517		hypothetical protein									0.774	1.83E-05
NMB1523		hypothetical protein	0.655	3.00E-03	0.646	2.35E-03	0.716	1.28E-03			-1.067	1.79E-06
NMB1534		hypothetical protein	0.959	2.03E-05	1.302	1.08E-08	0.943	3.17E-05	0.726	7.01E-04		
NMB1535		hypothetical protein					-0.748	1.12E-05				
NMB1544		hypothetical protein	-0.748	2.90E-04	-0.673	6.74E-04	-0.742	3.65E-04	-0.621	1.74E-03		
NMB1547		hypothetical protein	-0.804	3.64E-04	-0.725	8.05E-04	-0.870	1.41E-04				
NMB1580		hypothetical protein	-1.482	1.86E-09	-0.960	1.51E-05	-0.967	3.17E-05	-1.273	1.02E-08		
NMB1589		hypothetical protein	-0.783	3.40E-03	-1.039	6.08E-05	-1.105	5.17E-05	-0.918	1.78E-04		
NMB1596		hypothetical protein					-0.748	4.01E-03				
NMB1597		hypothetical protein							-0.818	6.68E-03	-0.888	3.48E-03
NMB1599		hypothetical protein			-0.694	2.46E-03	-0.870	3.05E-04	-0.870	7.18E-05	-0.966	4.40E-05
NMB1631		hypothetical protein									0.951	1.76E-03
NMB1633		hypothetical protein	-0.850	6.34E-05	-0.663	1.03E-03	-0.919	2.03E-05	-0.644	1.50E-03		
NMB1644		hypothetical protein							0.768	2.57E-03		
NMB1666		hypothetical protein									-2.284	1.28E-06
NMB1667		hypothetical protein							-0.630	8.70E-03	-1.305	3.16E-07
NMB1675		hypothetical protein									0.787	1.43E-03
NMB1681		hypothetical protein	0.761	1.22E-04	0.765	6.05E-05	0.766	1.30E-04			-0.676	4.53E-04
NMB1693		hypothetical protein	-0.645	2.46E-05			-0.609	7.18E-05				
NMB1695		hypothetical protein	-0.939	8.06E-05	-1.024	3.55E-05	-1.039	6.43E-05				
NMB1699		hypothetical protein									0.808	1.99E-06
NMB1700		hypothetical protein									0.906	1.44E-05
NMB1701		hypothetical protein	0.943	2.12E-12			0.663	9.88E-08	0.768	4.91E-10		
NMB1739		hypothetical protein									0.963	7.01E-04
NMB1740		hypothetical protein									0.717	8.13E-03
NMB1742		hypothetical protein									1.378	3.63E-04

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1743		hypothetical protein									1.263	6.49E-04
NMB1755		hypothetical protein	-0.757	2.90E-04	-0.794	8.12E-05	-0.887	3.17E-05	-0.982	2.25E-06	-0.970	3.68E-06
NMB1756		hypothetical protein									-1.041	9.93E-03
NMB1757		hypothetical protein							-0.886	2.96E-03		
NMB1758		hypothetical protein							-0.845	1.84E-04		
NMB1764		hypothetical protein							-0.692	1.30E-03	-0.736	2.81E-04
NMB1765		hypothetical protein									-0.776	6.63E-04
NMB1766		hypothetical protein	-0.643	1.13E-03	-0.702	2.26E-04	-0.847	2.75E-05				
NMB1771		hypothetical protein	-0.619	1.27E-03	-0.596	1.24E-03	-0.619	1.39E-03				
NMB1772		hypothetical protein									0.927	2.79E-03
NMB1773		hypothetical protein			-0.675	7.97E-03			-0.698	6.02E-03	-1.159	1.08E-05
NMB1774		hypothetical protein									-1.458	2.57E-09
NMB1775		hypothetical protein	-0.901	4.20E-03			-0.914	3.99E-03			0.840	5.96E-03
NMB1776		hypothetical protein							1.013	1.33E-05		
NMB1777		hypothetical protein	-1.016	1.33E-06	-0.747	1.42E-04	-0.801	3.17E-05			-1.778	9.36E-14
NMB1781		hypothetical protein					-1.262	3.86E-03				
NMB1782		hypothetical protein	-0.694	9.30E-04			-0.673	1.45E-03				
NMB1784		hypothetical protein	-1.398	6.06E-09	-1.090	9.20E-07	-1.500	1.38E-09	-1.138	4.56E-07		
NMB1786		hypothetical protein	-0.686	1.39E-03			-0.888	5.19E-05				
NMB1800		hypothetical protein	1.182	1.24E-08	1.056	7.57E-08	0.951	2.30E-06			1.197	6.00E-09
NMB1858		hypothetical protein			-0.897	2.73E-03						
NMB1865		hypothetical protein							0.645	6.02E-03		
NMB1870		hypothetical protein			0.642	3.68E-04						
NMB1875		hypothetical protein					0.707	4.40E-03	0.654	3.45E-03		
NMB1931		hypothetical protein									1.111	1.69E-07
NMB1941	*atpI*	hypothetical protein					-0.904	2.27E-05	-1.143	8.14E-08	-0.863	3.12E-05
NMB1942		hypothetical protein							-0.940	4.27E-04	-0.658	6.99E-03
NMB1977		hypothetical protein					0.754	2.79E-03	0.998	4.63E-05	2.034	1.27E-12
NMB1986		hypothetical protein	-0.594	9.38E-03	-0.636	3.77E-03			-0.814	2.29E-04	-1.073	2.96E-06
NMB2013		hypothetical protein									-1.713	9.32E-10
NMB2015		hypothetical protein									-1.478	1.51E-10
NMB2037		hypothetical protein	-0.817	2.90E-04	-0.668	2.02E-03	-0.606	7.53E-03			-0.973	1.46E-05
NMB2073		hypothetical protein									1.165	5.22E-03
NMB2074		hypothetical protein									0.995	1.43E-07
NMB2081		hypothetical protein									0.674	7.69E-03
NMB2085		hypothetical protein							-0.700	3.29E-04		
NMB2094		hypothetical protein	-0.782	2.00E-03					-0.905	2.28E-04		
NMB2100		hypothetical protein									0.806	1.95E-04
NMB2109		hypothetical protein			-0.641	2.52E-03	-0.930	9.66E-06	-0.611	4.14E-03	-0.815	1.76E-04
NMB2110		hypothetical protein					-0.702	1.19E-03	-0.759	2.71E-04		
NMB2113		hypothetical protein									-1.215	2.08E-03

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Table C.9 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB2116		hypothetical protein									-0.903	2.24E-04
NMB2118		hypothetical protein									-0.718	1.27E-03
NMB2120		hypothetical protein									-0.708	1.50E-04
NMB2121		hypothetical protein									-0.761	2.62E-04
NMB2123		hypothetical protein									-0.859	1.09E-06
NMB2125		hypothetical protein									-1.144	9.00E-07
NMB2130		hypothetical protein									-0.986	4.57E-04
NMB2137		hypothetical protein									-3.883	6.45E-17
NMB2141		hypothetical protein									-2.254	2.08E-14
NMB2147		hypothetical protein							-0.688	2.96E-06		
NMB2149		hypothetical protein									0.639	3.40E-04
NMB2152		hypothetical protein									0.657	3.01E-04

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.10: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *mobile and extra chromosomal element functions*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
Plasmid functions												
NMB0495		replication protein	-1.288	2.57E-13	-1.171	1.74E-12	-1.430	9.19E-15	-1.113	8.44E-12	-0.938	3.90E-09
Prophage functions												
NMB0556		putative repressor protein	-0.697	6.73E-04					-0.657	4.32E-04	-0.770	1.38E-04
NMB0748*	*hfq*	host factor-I							-0.893	4.06E-03	-1.923	1.38E-08
NMB0896		integrase (authentic frameshift)							-0.713	6.75E-05	-0.669	2.00E-04
NMB0961		funZ protein									-0.964	4.05E-07
NMB1104		phage sheath protein							0.832	4.35E-03		
NMB1110		tail protein, 43 kDa							0.780	9.94E-03		
Transposon functions												
NMB0225		transposase, IS30 family									0.847	1.79E-06
NMB0583		IS1016C2 transposase			-0.651	8.10E-04	-0.887	1.88E-05	-0.871	1.31E-05		
NMB0635		transposase, IS30 family									0.649	6.40E-04
NMB0805		transposase, IS30 family									1.286	3.89E-10
NMB0911		transposase, IS30 family									0.663	4.88E-03
NMB0991		IS1106 transposase									1.117	9.92E-09
NMB1022		transposase, IS30 family									0.668	6.30E-04
NMB1081		bacteriophage transposase									1.047	9.95E-04

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Table C.10 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1083		putative bacteriophage, DNA transposition protein B									0.746	4.02E-03
NMB1099		transposase, IS30 family									0.713	7.66E-05
NMB1251		transposase, IS30 family									0.638	4.70E-03
NMB1259		transposase, IS30 family									0.660	3.96E-03
NMB1401		IS1016C2 transposase	-0.648	1.78E-04	-0.771	5.06E-06	-0.979	9.78E-08	-0.899	2.57E-07		
NMB1411		IS1016C2 transposase			-0.799	1.89E-05	-0.977	1.05E-06	-0.966	5.83E-07		
NMB1602		putative transposase									1.599	5.14E-19
NMB1713		transposase, IS30 family									1.151	1.58E-08
NMB1770		transposase, IS30 family									0.724	9.78E-05
NMB2148		transposase, IS30 family									1.177	5.58E-09

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.11: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *protein fate*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Degradation of proteins, peptides, and glycopeptides												
NMB0214	*prlC*	oligopeptidase A			1.795	1.01E-12	1.384	1.28E-08	0.864	8.12E-05		
NMB0700	*iga*	IgA-specific serine endopeptidase							-1.118	6.30E-05	-1.699	1.54E-08
NMB1231	*lon*	ATP-dependent protease La			1.968	6.97E-11	1.629	5.27E-08	1.006	2.11E-04	-0.747	2.97E-03
NMB1312	*clpP*	ATP-dependent Clp protease, proteolytic subunit	0.961	1.08E-05	0.794	1.24E-04	0.715	9.35E-04			1.472	2.79E-10
NMB1332	*prc*	carboxy-terminal peptidase									1.217	2.22E-08
NMB1428	*clpX*	putative aminopeptidase	0.782	2.02E-05	0.627	3.10E-04	0.823	9.46E-06				
NMB1472	*clpB*	clpB protein	-0.913	6.48E-06	1.145	1.54E-08	0.643	1.23E-03				
NMB1587		predicted inner membrane peptidase	0.834	1.60E-05								
NMB1664		putative protease	0.850	3.67E-10								
Protein and peptide secretion and trafficking												
NMB0125	*secE*	preprotein translocase, SecE subunit	0.911	2.23E-04								
NMB0608	*secF*	protein-export membrane protein SecF									-1.085	3.24E-08
NMB1536	*secA*	preprotein translocase, SecA subunit			0.743	9.21E-05						
NMB1789	*secB*	protein-export protein SecB	-1.269	2.45E-07							-1.765	1.90E-11
Protein folding and stabilization												
NMB0059	*dnaJ*	dnaJ protein			1.598	2.09E-18	1.180	2.88E-12	1.060	1.61E-11		
NMB0278	*dsbA-1*	thiol:disulfide interchange protein			0.588	1.86E-05						
NMB0281		peptidyl-prolyl cis-trans isomerase							-0.648	4.39E-06	-1.135	2.35E-12
NMB0294	*dsbA-2*	thiol:disulfide interchange protein									-1.297	7.65E-11

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Table C.11 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0550	*dsbC*	thiol:disulfide interchange protein			0.698	5.56E-07	0.609	2.18E-05				
NMB0554	*dnaK*	dnaK protein			1.785	1.59E-09	1.507	3.82E-07	0.986	3.09E-04		
NMB0561	*grpE*	grpE protein	0.864	5.21E-04	2.397	8.22E-16	1.828	1.06E-10	1.316	1.91E-07	1.564	3.49E-09
NMB0791	*ppiB*	peptidyl-prolyl cis-trans isomerase			1.366	2.84E-10	0.861	3.47E-05			-1.338	1.00E-09
NMB0822	*htpX*	heat shock protein	1.034	6.69E-09	0.991	7.55E-09	1.064	4.82E-09	0.712	1.40E-05	0.734	9.72E-06
NMB1131	*hscA-1*	chaperone protein									-0.730	8.83E-05
NMB1169	*hscA-2*	chaperone protein									-0.693	1.33E-04
NMB1262	*ppiA*	peptidyl-prolyl cis-trans isomerase									0.810	3.60E-06
NMB1383	*hscB*	chaperone protein	-1.226	8.11E-13	-1.100	1.14E-11	-1.032	4.19E-10	-0.995	2.46E-10	-0.980	7.78E-10
NMB1519	*dsbD*	thiol:disulfide interchange protein	-1.006	1.05E-06	-0.597	1.82E-03	-0.660	1.01E-03	-0.854	1.40E-05	-1.192	1.31E-08
NMB1522	*slyD*	FKBP-type peptidyl-prolyl cis-trans isomerase	-0.627	2.49E-03	-0.680	6.50E-04	-0.697	8.61E-04	-0.949	4.10E-06	-1.639	3.45E-12
NMB1972	*groEL*	chaperonin, 60 kDa			2.462	1.18E-10	2.260	5.93E-09	1.516	1.54E-05	1.316	1.72E-04
NMB1973	*groES*	chaperonin, 10 kDa			2.554	6.34E-10	2.263	6.03E-08	1.435	1.60E-04		
Protein modification and repair												
NMB0044	*pilB*	trifunctional thioredoxin/methionine sulfoxide reductase A/B protein			1.045	4.08E-05			0.629	8.36E-03	0.891	5.20E-04
NMB0218	*pglA*	glycosyltransferase							0.640	2.70E-05		
NMB0922	*lst*	α-2,3-sialyltransferase	1.159	1.22E-04	0.809	5.08E-03	0.913	2.48E-03			0.686	8.59E-03
NMB1885	*pcm*	protein-L-isoaspartate O-methyltransferase			0.733	3.03E-06	0.830	2.97E-07	0.585	8.30E-05		

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.12: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *protein synthesis*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Other												
NMB0795	*pth*	peptidyl-tRNA hydrolase			0.736	9.36E-09	0.623	1.47E-06				
Ribosomal proteins: synthesis and modification												
NMB0127	*rplK*	50S ribosomal protein L11	0.946	1.41E-04								
NMB0128	*rplA*	50S ribosomal protein L1	0.919	1.02E-04								
NMB0130	*rplJ*	50S ribosomal protein L10	1.083	2.65E-03							-1.132	1.26E-03
NMB0131	*rplL*	50S ribosomal protein L7 L12	1.243	2.24E-07	1.061	2.73E-06	1.055	8.01E-06			-1.708	2.38E-11
NMB0136	*rpsL*	30S ribosomal protein S12	1.087	3.02E-05	0.793	1.31E-03	0.841	1.17E-03			-0.696	5.22E-03
NMB0137	*rpsG*	30S ribosomal protein S7	0.747	1.95E-03			0.661	6.41E-03			-0.880	2.04E-04
NMB0140	*rpsJ*	30S ribosomal protein S10	1.230	9.04E-07	0.943	5.67E-05	0.871	3.63E-04			-0.653	5.34E-03
NMB0142	*rplC*	50S ribosomal protein L3	0.778	1.61E-03								

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Table C.12 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0144	*rplW*	50S ribosomal protein L23	1.503	1.18E-06	1.248	1.87E-05	1.086	3.33E-04				
NMB0145	*rplB*	50S ribosomal protein L2	0.962	1.12E-03	0.773	6.65E-03						
NMB0146	*rpsS*	30S ribosomal protein S19	1.431	7.15E-06	1.265	3.00E-05	0.926	3.23E-03				
NMB0147	*rplV*	50S ribosomal protein L22	1.008	5.07E-05	0.792	8.05E-04	0.808	1.12E-03				
NMB0148	*rpsC*	30S ribosomal protein S3	0.983	1.69E-04	0.666	7.97E-03						
NMB0149	*rplP*	50S ribosomal protein L16	0.751	1.76E-03	0.598	9.89E-03						
NMB0150	*rpmC*	50S ribosomal protein L29	1.560	4.05E-05	1.360	1.78E-04	0.994	8.68E-03				
NMB0151	*rpsQ*	30S ribosomal protein S17	1.514	2.79E-05	1.464	2.42E-05	1.152	1.33E-03				
NMB0152	*rplN*	50S ribosomal protein L14	0.742	7.37E-04							-1.043	2.52E-06
NMB0153	*rplX*	50S ribosomal protein L24	0.701	1.95E-03							-0.851	1.35E-04
NMB0156	*rpsH*	30S ribosomal protein S8	0.815	6.12E-04								
NMB0157	*rplF*	50S ribosomal protein L6	0.731	1.35E-03								
NMB0158	*rplR*	50S ribosomal protein L18	0.660	6.62E-03								
NMB0160	*rpmD*	50S ribosomal protein L30	1.274	1.52E-04	1.041	1.19E-03						
NMB0161	*rplO*	50S ribosomal protein L15	0.841	9.54E-04								
NMB0164	*rpmJ*	50S ribosomal protein L36	1.359	5.89E-04	1.188	1.77E-03						
NMB0165	*rpsM*	30S ribosomal protein S13	0.844	1.16E-03							-0.707	4.98E-03
NMB0167	*rpsD*	30S ribosomal protein S4									-1.016	1.01E-03
NMB0169	*rplQ*	50S ribosomal protein L17	1.160	2.05E-04							-1.668	1.92E-07
NMB0321	*rpmB*	50S ribosomal protein L28									-1.911	2.77E-10
NMB0322	*rpmG*	50S ribosomal protein L33	0.729	9.73E-03							-0.874	1.38E-03
NMB0324	*rpmA*	50S ribosomal protein L27	1.164	1.04E-04	1.029	3.30E-04	0.915	2.27E-03			-1.504	7.37E-07
NMB0325	*rplU*	50S ribosomal protein L21	0.918	7.74E-04	0.709	7.11E-03					-1.179	1.50E-05
NMB0589	*rplS*	50s ribosomal protein L19	1.844	6.25E-13	1.359	3.20E-09	1.339	1.82E-08				
NMB0592	*rpsP*	30S ribosomal protein S16	1.532	5.24E-08	1.194	5.05E-06	1.107	4.72E-05			-0.732	4.64E-03
NMB0609	*rpsO*	30s ribosomal protein S15	0.793	3.29E-04							-1.563	1.06E-10
NMB0722	*rpmI*	50S ribosomal protein L35	0.785	2.51E-03							-2.175	4.86E-13
NMB0723	*rplT*	50S ribosomal protein L20	0.726	3.29E-03							-1.908	7.38E-12
NMB0941	*rpmJ2*	50S ribosomal protein L36			1.133	1.38E-04	1.173	3.89E-04				
NMB0942	*rpmE2*	putative 50S ribosomal protein L31			1.664	7.34E-08	1.262	5.18E-05			-1.536	8.09E-07
NMB1301	*rpsA*	30S ribosomal protein S1	0.973	9.21E-05	0.781	9.88E-04	0.819	1.00E-03				
NMB1320	*rplI*	50S ribosomal protein L9	1.521	3.04E-08	1.253	1.06E-06	1.245	3.30E-06	0.855	6.30E-04	-0.863	6.35E-04
NMB1321	*rpsR*	30S ribosomal protein S18	1.818	1.10E-12	1.540	9.34E-11	1.534	5.13E-10	0.789	2.34E-04	-1.729	4.81E-12
NMB1323	*rpsF*	30S ribosomal protein S6	1.718	2.85E-07	1.413	7.10E-06	1.479	1.66E-06	0.937	1.30E-03	-2.008	4.02E-09
NMB1911	*rpmF*	50S ribosomal protein L32	1.201	6.89E-05	0.886	2.07E-03	0.835	5.54E-03				
NMB1950	*rpsU*	30S ribosomal protein S21	1.027	2.71E-04							-1.719	7.42E-09
NMB1956	*rpmE*	50S ribosomal protein L31	1.214	5.18E-03	1.354	7.46E-05	0.888	7.55E-03			-1.592	4.76E-06
NMB2056	*rpsI*	30S ribosomal protein S9	1.244	2.49E-08	0.984	2.13E-06	1.073	1.00E-06			-0.940	7.79E-06
NMB2057	*rplM*	50S ribosomal protein L13	1.779	8.07E-11	1.476	6.22E-09	1.560	5.79E-09	1.033	1.86E-05		
NMB2101	*rpsB*	30S ribosomal protein S2	1.773	7.26E-10	1.376	1.85E-07	1.473	1.37E-07	0.655	9.92E-03	-1.257	2.25E-06

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Table C.12 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Translation factors												
NMB0124	*tufA2*	translation elongation factor Tu	0.676	9.85E-03								
NMB0139	*tufA1*	translation elongation factor Tu	0.863	1.28E-03	0.699	6.98E-03	0.749	5.57E-03				
NMB0163	*infA*	translation initiation factor IF-1	1.243	1.39E-03							1.113	3.25E-03
NMB0626	*prfC*	peptide chain release factor 3	1.280	2.30E-13	0.900	5.15E-09	1.073	1.59E-10	0.751	5.23E-07	1.386	7.50E-15
NMB0937	*efp*	elongation factor P (EF-P)	1.535	1.35E-07	1.103	4.14E-05	1.231	1.51E-05			1.231	9.10E-06
NMB1643	*infB*	translation initiation factor IF-2	1.078	4.13E-08			0.675	3.22E-04			-0.825	9.04E-06
NMB1686	*prfA*	peptide chain release factor 1					0.701	5.32E-05				
NMB2138	*prfB*	peptide chain release factor 2	0.968	9.22E-07	0.752	4.63E-05	0.836	1.85E-05	0.653	1.79E-04	0.952	9.00E-07
tRNA aminoacylation												
NMB0003	*gltX*	glutamyl-tRNA synthetase									0.871	1.83E-06
NMB0174	*valS*	valyl-tRNA synthetase	0.717	1.69E-06	0.733	4.10E-07	0.746	9.05E-07				
NMB0466	*aspS*	aspartyl-tRNA synthetase	0.894	1.14E-05	0.686	3.62E-04	0.808	7.18E-05			1.066	2.18E-07
NMB0719	*tgt*	queuine tRNA-ribosyltransferase	0.706	3.05E-07							0.694	3.13E-07
NMB0720	*thrS*	threonyl-tRNA synthetase	1.357	4.13E-10	1.068	8.89E-08	1.108	1.35E-07			1.347	3.65E-10
NMB0724	*pheS*	phenylalanyl-tRNA synthetase, α chain	1.447	7.47E-10	0.883	2.46E-05	0.642	3.08E-03				
NMB0728	*pheT*	phenylalanyl-tRNA synthetase, β chain	0.850	3.19E-06								
NMB0854	*hisS*	histidyl-tRNA synthetase	-1.985	4.12E-11	-1.538	1.90E-08	-1.700	5.89E-09	-2.071	2.75E-12	-1.582	1.77E-08
NMB1339	*proS*	prolyl-tRNA synthetase			0.683	2.11E-04						
NMB1355	*gatC*	putative glutamyl-tRNA (Gln) amidotransferase, subunit C									-0.810	3.12E-05
NMB1356	*gatA*	Glu-tRNA(Gln) amidotransferase, subunit A									-1.164	8.22E-11
NMB1425	*lysS*	lysyl-tRNA synthetase, heat inducible	1.568	3.67E-12	1.324	2.76E-10	1.457	8.94E-11	1.120	6.53E-09	0.931	2.73E-06
NMB1506	*argS*	arginyl-tRNA synthetase	0.613	2.28E-03			0.619	9.67E-04				
NMB1595	*alaS*	alanyl-tRNA synthetase					0.593	9.35E-04				
NMB1835	*tyrS*	tyrosyl-tRNA synthetase	0.884	6.61E-04			0.762	3.57E-03				
tRNA and rRNA base modification												
NMB0268		RNA methyltransferase, TrmH family			0.768	6.87E-04	0.692	3.52E-03				
NMB0590	*trmD*	tRNA (guanine-N1)-methyltransferase	1.540	3.93E-10	1.139	3.47E-07	1.255	1.34E-07				
NMB0697	*ksgA*	dimethyladenosine transferase									0.654	2.61E-04
NMB0935	*miaA*	tRNA $\delta(2)$-isopentenylpyrophosphate transferase							0.602	7.47E-03	0.689	1.59E-03
NMB1679	*trmA*	tRNA (uracil-5-)-methyltransferase					0.675	4.51E-03			0.834	3.09E-04

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.13: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *purines, pyrimidines, nucleosides and nucleotides biosynthesis*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
2'-Deoxyribonucleotide metabolism												
NMB0893	*dut*	deoxyuridine 5'-triphosphate nucleotidohydrolase									-0.618	7.31E-03
NMB1709	*thyA*	thymidylate synthase	1.043	4.07E-07	1.186	6.99E-09	1.153	5.34E-08	0.986	7.54E-07	0.811	3.72E-05
Nucleotide and nucleoside interconversions												
NMB0642	*ntpA*	dATP pyrophosphohydrolase									0.898	8.28E-11
NMB0849		putative deoxycytidine triphosphate deaminase	-2.116	3.84E-14	-1.226	1.72E-07	-1.228	5.67E-07	-1.220	2.62E-07	-0.766	7.86E-04
NMB1661	*gmk*	guanylate kinase			-0.644	9.00E-04	-0.681	8.25E-04	-0.732	1.93E-04	-0.956	2.66E-06
NMB2103	*pyrH*	uridylate kinase									0.725	1.27E-05
Purine ribonucleotide biosynthesis												
NMB0284	*purB*	adenylosuccinate lyase			0.767	7.66E-06	0.614	5.05E-04				
NMB0690	*purF*	amidophosphoribosyltransferase	0.689	2.68E-04	1.074	2.39E-08						
NMB0757	*purC*	phosphoribosylaminoimidazole-succinocarboxamide synthase							-0.763	1.61E-03		
NMB0983	*purH*	phosphoribosylaminoimidazole carboxamide formyl-transferase/IMP cyclohydrolase	1.431	2.14E-04	1.996	9.75E-09	1.377	3.96E-04			1.022	6.20E-03
NMB1019	*purK*	phosphoribosylaminoimidazole carboxylase, ATPase subunit	0.753	3.93E-04	0.622	7.41E-04	0.760	3.93E-04			0.843	6.07E-05
NMB1201	*guaB*	IMP dehydrogenase									-0.916	1.17E-06
NMB1252	*purM*	phosphoribosylformylglycinamidine cyclo-ligase	1.576	3.19E-13	1.401	6.16E-12	1.170	6.22E-09	0.620	5.24E-04		
NMB1439	*purE*	phosphoribosylaminoimidazole carboxylase, catalytic subunit									-0.640	3.85E-03
NMB1920	*guaA*	GMP synthase			0.587	4.61E-03	0.762	9.56E-04			1.937	3.20E-13
NMB1996	*purL*	phosphoribosylformylglycinamidine synthase									0.743	1.80E-04
Pyrimidine ribonucleotide biosynthesis												
NMB0106	*pyrB*	aspartate carbamoyltransferase, catalytic subunit									-0.658	1.38E-07
NMB0682	*pyrC*	dihydroorotase							0.632	3.82E-05		
NMB0824	*pyrF*	orotidine 5'-phosphate decarboxylase									1.033	1.83E-08
NMB1554	*pyrG*	CTP synthase	1.098	1.89E-08	0.961	1.70E-07	1.084	3.52E-08				
NMB1849	*carA*	carbamoyl-phosphate synthase, small subunit			0.648	2.74E-05	0.848	3.13E-07	0.772	1.21E-06	0.852	1.49E-07
Salvage of nucleosides and nucleotides												
NMB0774	*upp*	uracil phosphoribosyltransferase	1.075	2.64E-07	1.079	9.01E-08	1.097	2.24E-07	0.651	7.51E-04		
Sugar-nucleotide biosynthesis and conversions												
NMB0638	*galU*	UTP glucose-1-phosphate uridylyltransferase									-0.939	2.19E-06

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.14: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *regulatory functions*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

			20 min		40 min		60 min		90 min		240 min	
ID	**Name**	**Annotation**	**$\log_2$ ratio**	**P-value***	**$\log_2$ ratio**	**P-value***	**$\log_2$ ratio**	**P-value***	**$\log_2$ ratio**	**P-value***	**$\log_2$ ratio**	**P-value***
DNA interactions												
NMB1816		transcriptional regulator, NrdR family					0.663	3.82E-03	0.811	2.34E-04		
Other												
NMB0115	*ntrX*	nitrogen assimilation regulatory protein							0.659	6.24E-05	0.744	9.72E-06
NMB0173	*oxyR*	transcriptional regulator, LysR family									-1.251	6.03E-13
NMB0205	*fur*	ferric uptake regulation protein	-0.835	4.13E-06					-0.592	5.34E-04	-1.899	2.72E-17
NMB0290		putative transcriptional regulator	-0.673	4.66E-03								
NMB0380	*fnr*	transcriptional regulator, Crp/Fnr family							-0.709	4.93E-07		
NMB0398		transcriptional regulator, ArsR family	-1.254	1.69E-09	-1.397	1.66E-11	-1.447	4.53E-11	-0.919	1.38E-06	-1.448	1.58E-11
NMB0556		putative repressor protein	-0.697	6.73E-04					-0.657	4.32E-04	-0.770	1.38E-04
NMB0573		transcriptional regulator, AsnC family	-1.640	1.40E-14	-1.359	3.79E-12	-1.212	6.77E-10	-1.272	2.79E-11		
NMB0595		DNA-binding response regulator									-1.053	1.91E-07
NMB0729	*himA*	ribonuclease inhibitor barstar							-0.585	2.49E-03	-2.228	7.19E-18
NMB0737		putative HPr kinase phosphatase	0.727	1.18E-05			0.611	2.12E-04			0.770	2.78E-06
NMB0748	*hfq*	host factor-I							-0.893	4.06E-03	-1.923	1.38E-08
NMB0810		transcriptional regulator, TetR family									1.246	7.29E-06
NMB1007		transcriptional regulator	-1.345	1.41E-09	-1.894	4.08E-15	-2.328	6.58E-18	-2.709	8.80E-22	-0.740	2.28E-04
NMB1204		transcriptional regulator	0.953	5.67E-07							0.723	6.82E-05
NMB1249	*narX*	putative nitrate/nitrite sensory protein NarX	-1.281	7.34E-15	-1.403	4.22E-17	-1.193	1.91E-13	-1.163	8.05E-14		
NMB1250		transcriptional regulator, LuxR family	-0.869	3.19E-07	-1.029	1.75E-09	-0.968	3.39E-08	-0.833	4.34E-07		
NMB1302	*hip*	integration host factor, β subunit	0.726	2.31E-03	0.669	3.56E-03	0.627	9.00E-03			-1.100	4.39E-06
NMB1303		transcriptional regulator, MerR family	-0.674	3.96E-04	-0.871	3.31E-06	-1.117	4.18E-08	-1.615	1.19E-13	-1.400	3.36E-11
NMB1563		transcriptional regulator, GntR family			0.831	8.04E-05						
NMB1585		transcriptional regulator, MarR family					-0.733	3.12E-03				
NMB1591	*mtrA*	transcriptional regulator MtrA							-0.608	8.59E-04		
NMB1650	*lrp*	leucine-responsive regulatory protein			-0.600	9.45E-03	-0.838	1.08E-03	-1.183	8.74E-07	-0.706	4.22E-03
NMB1659	*spoT*	guanosine-3',5'-bis(diphosphate) 3'-pyrophospho-hydrolase					-0.794	6.06E-07	-1.215	9.62E-13	-0.672	9.56E-06
NMB1843		transcriptional regulator, MarR family	-0.846	4.67E-04	-1.005	6.52E-06			-0.627	7.05E-03	-0.874	2.28E-04
NMB1967		transcriptional regulator, AraC family									1.173	8.65E-06
NMB1995	*glnB*	nitrogen regulatory protein P-II	1.021	1.45E-05	1.153	5.66E-07	0.800	6.09E-04				
NMB2012		transcriptional regulator, HTH 3 family	0.657	1.28E-04							-1.139	6.60E-10
NMB2055	*metR*	transcriptional regulator, LysR family									0.621	2.36E-04
NMB2066	*tldD*	tldD protein							0.674	1.35E-03		
NMB2075	*birA*	BirA protein Bvg accessory factor							0.593	5.34E-04		

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.15: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into functional group *transcription*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

			20 min		40 min		60 min		90 min		240 min	
ID	**Name**	**Annotation**	**$\log_2$ ratio**	**P-value***	**$\log_2$ ratio**	**P-value***	**$\log_2$ ratio**	**P-value***	**$\log_2$ ratio**	**P-value***	**$\log_2$ ratio**	**P-value***
Degradation of RNA												
NMB0192	*rnhB*	ribonuclease HII	0.815	1.15E-07	0.637	8.65E-06	0.619	3.47E-05	0.784	1.42E-07	1.169	2.35E-12
NMB0662		putative ribonuclease	0.840	1.06E-05	0.753	3.39E-05	0.815	2.07E-05	0.743	5.07E-05		
NMB0758	*pnp*	polyribonucleotide nucleotidyltransferase	0.644	5.12E-03								
NMB1200	*vacB*	ribonuclease II family protein									-0.602	4.07E-03
DNA-dependent RNA polymerase												
NMB0132	*rpoB*	DNA-directed RNA polymerase, β subunit	0.912	8.55E-04								
NMB0133	*rpoC*	DNA-directed RNA polymerase, β' subunit	1.102	1.32E-05	0.957	6.86E-05	0.999	7.90E-05				
NMB0168	*rpoA*	DNA-directed RNA polymerase, α subunit	0.929	8.85E-05	0.599	8.31E-03					-1.098	3.50E-06
RNA processing												
NMB0196	*rne*	ribonuclease E									-0.971	2.45E-08
NMB0591	*rimM*	16S rRNA processing protein	1.119	3.66E-05	0.807	1.73E-03	0.864	1.36E-03				
NMB0832		anticodon nuclease									-0.839	5.86E-06
NMB1373	*rbfA*	ribosome-binding factor A	0.871	9.35E-08	0.715	2.92E-06	0.789	1.06E-06				
NMB1499	*rph*	ribonuclease PH					0.689	7.72E-03			0.829	9.30E-04
NMB1905	*rnpA*	ribonuclease P, protein component	1.386	3.81E-10	0.857	1.28E-05	0.926	7.96E-06	0.790	6.06E-05		
Transcription factors												
NMB0075		putative transcriptional accessory protein Tex									1.953	1.13E-15
NMB0126	*nusG*	transcription antitermination protein									-0.678	8.55E-03
NMB0617	*rho*	transcription termination factor	0.835	2.77E-07			0.625	8.21E-05				
NMB0689	*greB*	transcription elongation factor	0.846	8.24E-04	0.789	1.80E-03					1.025	4.47E-05
NMB0712	*rpoH*	RNA polymerase sigma-32 factor	-0.945	7.15E-06	-0.822	3.47E-05	-0.659	1.47E-03	-1.068	2.57E-07	-1.161	5.91E-08
NMB1368		putative ATP-dependent RNA helicase	-2.458	1.27E-15	-3.449	4.21E-24	-2.986	7.83E-19	-2.668	1.31E-17	-2.752	7.78E-18
NMB1430	*greA*	transcription elongation factor	1.739	8.90E-12	1.065	1.58E-06	1.123	1.44E-06	0.770	3.88E-04		
NMB1538	*rpoD*	RNA polymerase sigma factor			1.240	4.44E-08	1.009	1.09E-05			1.096	1.25E-06
NMB1642	*nusA*	N utilization substance protein A	0.779	8.17E-04			0.660	4.86E-03				

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.16: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point categorised into TIGR role category *transport and binding proteins*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Amino acids, peptides and amines												
NMB0085	*gltS*	sodium/glutamate symporter	1.061	3.51E-05								
NMB0177		putative sodium/alanine symporter	-0.992	2.41E-03			-0.903	6.12E-03				
NMB0402	*putP*	sodium/proline symporter			-0.668	2.28E-04						
NMB0611	*potB*	spermidine/putrescine ABC transporter, permease protein	0.915	6.20E-07	0.765	9.95E-06						
NMB0612	*potC*	spermidine/putrescine ABC transporter, permease protein	1.064	6.67E-11	0.706	5.05E-06	0.614	1.25E-04				
NMB0623	*potD-2*	spermidine/putrescine ABC transporter, periplasmic substrate-binding protein	0.857	3.97E-05	0.815	4.63E-05	0.840	6.50E-05				
NMB0787		amino acid ABC transporter, periplasmic substrate-binding protein	1.940	4.24E-15	1.655	4.42E-13	1.478	1.19E-10	0.863	1.49E-05		
NMB0788		amino acid ABC transporter, permease protein	1.710	4.53E-17	1.192	9.37E-12	0.987	1.06E-08	0.594	1.26E-04		
NMB0789		amino acid ABC transporter, ATP-binding protein	1.128	7.33E-13	0.918	2.10E-10	0.594	1.88E-05			0.951	1.44E-10
NMB1594	*potD-3*	spermidine/putrescine ABC transporter, periplasmic substrate-binding protein	0.802	1.71E-04	0.752	2.41E-04	0.697	1.13E-03			-0.782	1.84E-04
NMB1612	*hisJ*	amino acid ABC transporter, periplasmic substrate-binding protein			0.606	2.49E-03					-0.649	3.68E-03
NMB1962		L-glutamate ABC transporter, putative NTP-binding protein			0.670	8.02E-03	0.756	6.96E-03				
NMB1963		L-glutamate ABC transporter, putative periplasmic transport protein	0.592	7.65E-04	0.655	1.16E-04	0.682	1.38E-04				
NMB1964		L-glutamate ABC transporter, putative outer membrane substrate-binding protein					0.631	5.54E-03				
NMB1965		L-glutamate ABC transporter, putative inner membrane permease					0.733	5.02E-03			1.492	3.59E-09
NMB1966		L-glutamate ABC transporter, putative, ATP-binding protein	0.714	2.22E-04	0.627	1.11E-03	0.662	2.42E-04				
NMB2031	*mtr*	tryptophan transporter							0.781	1.48E-03	0.785	2.89E-03
Anions												
NMB0378		putative phosphate permease	1.095	1.87E-03	1.762	5.14E-07						
NMB0879	*cysA*	sulfate ABC transporter, ATP-binding protein	-1.242	4.60E-12	-0.926	9.75E-09	-0.981	8.92E-09	-0.898	2.91E-08		
NMB0880	*cysW*	sulfate ABC transporter, permease protein									1.650	9.51E-07
NMB1017	*sbp*	sulfate ABC transporter, periplasmic sulfate-binding protein									0.728	1.04E-03

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Table C.16 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
Carbohydrates, organic alcohols, and acids												
NMB0071	*ctrA*	capsule polysaccharide export, outer membrane protein							-0.592	9.00E-04	-0.724	7.34E-05
NMB0072	*ctrB*	capsule polysaccharide export, inner membrane protein									-0.622	6.25E-04
NMB0543	*lctP*	putative L-lactate permease	-0.689	6.78E-03							-0.993	2.31E-04
NMB1362		putative oxalate/ formate antiporter	1.462	2.85E-05	1.439	1.84E-05						
NMB1794		citrate transporter									0.635	6.99E-03
Cations and iron carrying compounds												
NMB0034		putative high-affinity iron transporter	-1.823	1.84E-10	-1.470	2.46E-08	-1.245	3.19E-06	-0.651	8.92E-03	-0.925	2.62E-04
NMB0209	*kefB*	glutathione-regulated potassium-efflux system protein							1.076	1.32E-11	0.880	9.92E-09
NMB0293		putative TonB-dependent receptor	-1.279	8.53E-09	-0.971	2.12E-06	-0.986	4.11E-06				
NMB0460	*tbpB*	transferrin-binding protein 2	-2.920	1.42E-21	-1.945	1.59E-14	-1.569	1.61E-10	-1.685	2.62E-12	-1.181	1.42E-07
NMB0461	*tbpA*	transferrin-binding protein 1	-2.608	5.09E-26	-2.064	1.04E-21	-1.790	5.75E-18	-1.509	1.04E-15	-1.796	1.83E-18
NMB0579	*nosF*	copper ABC transporter, ATP-binding protein									0.818	2.01E-03
NMB0586		putative zinc/manganese transporter			0.649	1.54E-04						
NMB0634	*fbpA*	iron(III) ABC transporter, periplasmic substrate-binding protein	-3.400	2.72E-23	-2.259	7.04E-16	-1.937	1.63E-12	-2.278	7.30E-16	-3.504	1.27E-23
NMB0752		putative bacterioferritin-associated ferredoxin	-1.107	9.07E-06					-0.827	5.84E-04	-1.030	2.36E-05
NMB0964		TonB-dependent receptor					0.871	4.78E-03	0.767	9.39E-03		
NMB1206	*bfrB*	bacterioferritin B	2.482	5.29E-18	1.193	7.57E-08	0.633	4.17E-03			1.001	5.50E-06
NMB1207	*bfrA*	bacterioferritin A	2.186	7.46E-16	0.969	5.80E-06					1.164	2.03E-07
NMB1271		putative mercury transport, periplasmic protein									0.762	1.32E-04
NMB1540	*lbpA*	lactoferrin-binding protein A	-3.180	6.77E-16	-2.917	4.33E-15	-2.807	1.41E-13	-2.203	8.33E-11	-2.601	7.97E-13
NMB1541	*lbpB*	lactoferrin-binding protein B	-4.191	7.29E-34	-3.576	1.50E-30	-3.652	1.65E-28	-3.325	2.74E-27	-2.702	2.75E-22
NMB1614	*trkA*	Trk system potassium uptake protein	1.012	5.85E-10	0.889	7.88E-09	1.000	1.42E-09	0.893	8.70E-09		
NMB1668	*hmbR*	hemoglobin receptor	-1.952	9.86E-24	-1.689	8.76E-20	-1.621	5.75E-18	-1.257	2.66E-14	-1.815	1.25E-20
NMB1730	*tonB*	TonB protein	-1.325	1.81E-10	-0.741	4.60E-05					-1.095	2.38E-08
NMB1882		TonB-dependent receptor							0.866	1.67E-04		
NMB1988	*fetA*	iron-regulated outer membrane protein FrpB	-2.989	5.47E-20	-2.486	2.06E-16	-2.435	2.82E-17	-2.337	7.30E-16	-1.819	1.30E-11
NMB1989	*fetB*	iron(III) ABC transporter, periplasmic substrate-binding protein	-0.872	2.62E-04	-0.840	1.04E-04	-0.687	1.95E-03	-0.633	3.45E-03		
Nucleosides, purines and pyrimidines												
NMB2067	*cytX*	probable hydroxymethylpyrimidine transporter							0.724	2.93E-03	0.897	2.81E-04
Other												
NMB0097		putative secretion protein (degenerate)					-0.667	1.60E-04	-0.683	6.18E-05	-0.939	1.71E-07
NMB0318	*farA*	fatty acid efflux system protein							0.618	3.26E-03	1.414	1.35E-09
NMB0393		multidrug resistance protein							0.816	2.80E-06		
NMB0812	*norM*	multidrug efflux protein									0.637	8.58E-03
NMB1277		transporter, BCCT family			-0.635	4.94E-04	-0.673	4.34E-04				
NMB1714	*mtrE*	multidrug efflux pump channel protein	-1.704	3.84E-14	-1.563	2.62E-14	-1.259	1.46E-09	-1.094	1.93E-08	-1.007	2.03E-07

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Table C.16 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1715	*mtrD*	multiple transferable resistance system protein	-1.445	2.24E-07	-1.329	1.41E-07	-1.025	1.53E-04	-0.841	1.08E-03	-0.876	7.66E-04
NMB1716	*mtrC*	membrane fusion protein	-1.644	1.04E-09	-1.342	7.68E-08	-1.136	1.69E-06	-1.144	4.08E-07		
NMB1719	*mtrF*	efflux pump component	0.743	1.10E-04								
NMB1728	*exbD*	biopolymer transport protein	-1.618	3.59E-13	-1.136	7.55E-09	-0.758	9.14E-05	-0.979	3.72E-07	-2.545	2.06E-21
NMB1729	*exbB*	biopolymer transport protein	-1.499	5.54E-06	-1.025	3.25E-03					-1.905	1.21E-07
NMB1783		putative secretion protein (authentic frameshift)					-0.669	5.77E-03				
Porins												
NMB1429	*porA*	outer membrane protein PorA									-2.007	9.22E-10
NMB2039	*porB*	major outer membrane protein PIB									-2.560	3.57E-13
Unknown substrate												
NMB0041	*tbpA*	ABC transporter, periplasmic solute-binding protein	-0.803	6.89E-06	-0.624	2.08E-04					-0.851	1.44E-06
NMB0098		ABC transporter, ATP-binding protein (frameshift)					-0.612	2.05E-03	-0.770	6.50E-05	-0.871	9.94E-06
NMB0264		ABC transporter, ATP-binding protein							0.748	1.52E-05		
NMB0387		ABC transporter, ATP-binding protein									-0.852	1.54E-09
NMB0549		ABC transporter, ATP-binding protein									0.974	2.63E-09
NMB1051	*abcZ*	ABC transporter, ATP-binding protein	0.617	6.63E-04							0.799	1.05E-05
NMB1240		ABC transporter, ATP-binding protein									-0.653	5.60E-04
NMB1400		ABC transporter family protein					-0.663	4.17E-03				
NMB1707		sodium- and chloride-dependent transporter									0.827	3.97E-05
NMB1732		putative transporter	-1.286	1.49E-07	-1.288	5.37E-08	-1.004	6.51E-06	-1.237	1.84E-07	-1.755	1.74E-11
NMB1880	*fetB2*	putative ABC transporter, periplasmic solute-binding protein									1.233	6.19E-04
NMB1975		sodium- and chloride-dependent transporter									0.586	9.50E-04
NMB2026		ABC transporter, permease protein							0.977	7.50E-05		

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.17: Differentially expressed genes in blood at 20, 40, 60, 90 and/or 240 min in comparison to the 0 min time-point with unknown function. Only genes with a fold change of ≥ 1.5 and P-value ≤ 0.01 are listed in the table.

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0006		thioredoxin-related protein	-1.136	5.08E-13	-0.968	3.74E-11	-1.065	9.79E-12	-1.048	2.53E-12	-1.127	5.03E-13
NMB0009		BolA/ YrbA family protein	1.199	3.25E-05	1.053	5.03E-05	0.985	6.04E-04				
NMB0105		PhnO-related protein	-0.665	5.13E-08	-0.646	3.87E-08	-0.626	2.92E-07	-0.762	4.73E-10	-0.641	8.61E-08
NMB0117		putative smg protein	0.599	6.89E-05	0.868	1.38E-08	1.091	1.19E-10	0.652	1.02E-05	0.956	2.09E-09
NMB0193	*gidA*	tRNA uridine 5-carboxymethyl aminomethyl modification enzyme	0.735	1.71E-06			0.595	8.60E-05	0.780	2.49E-07	1.351	2.30E-14
NMB0255		cell filamentation protein Fic-related protein	0.725	3.38E-05							0.823	2.33E-06

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Table C.17 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0282		ribonuclease II-related protein	0.777	4.62E-09	0.697	2.73E-08	0.836	9.68E-10	0.734	9.44E-09	0.927	1.63E-11
NMB0296		CcsA-related protein	-0.727	9.02E-06	-0.698	9.08E-06	-0.841	6.06E-07	-0.789	9.82E-07	-0.947	1.92E-08
NMB0344		BolA/ YrbA family protein	-1.167	9.16E-05					-0.890	9.26E-04	-1.518	5.28E-07
NMB0374	*mafB2′*	MafB-related protein					-0.598	1.08E-04			0.754	1.05E-06
NMB0375	*mafA-1*	mafA protein									0.729	1.44E-03
NMB0412		cell division protein FtsL-related protein	-0.678	1.51E-04								
NMB0480		TspB-related protein									-0.623	4.70E-05
NMB0490		PspA-related protein									1.095	3.91E-08
NMB0496		hemolysin activator-related protein	-1.310	1.05E-07	-0.957	2.67E-05	-1.296	1.85E-07	-1.040	7.54E-06		
NMB0577		NosR-related protein			-0.768	7.67E-04	-0.915	1.48E-04				
NMB0598		Maf/YceF/YhdE family protein	0.690	7.37E-09							0.768	2.35E-10
NMB0602	*hitA*	hitA protein	0.802	9.87E-06	0.993	4.11E-08	0.771	2.36E-05			0.664	1.52E-04
NMB0653		MafB-related protein					-0.739	4.91E-03				
NMB0680	*cnp1*	cryptic protein	0.694	3.41E-05	0.878	1.29E-07	0.868	5.67E-07	0.655	5.15E-05	0.650	6.96E-05
NMB0827	*nmgI*	type II restriction enzyme-related protein (authentic frameshift)							-1.078	1.99E-06	-0.729	1.87E-03
NMB0852	*engA*	essential GTPase	-1.775	1.84E-10	-1.585	1.75E-09	-1.764	3.73E-10	-2.080	3.25E-13	-1.809	7.10E-11
NMB0867		YabO/YceC/SfhB family protein					-0.585	4.81E-05				
NMB0890		type IV pilin-related protein					-0.666	7.28E-04	-0.599	1.46E-03		
NMB0901		D-lactate dehydrogenase-related protein			-0.627	2.74E-03			-0.729	2.44E-04	-1.212	8.05E-08
NMB0946		peroxiredoxin 2 family protein/glutaredoxin			1.436	1.95E-07	0.811	3.50E-03			-2.851	1.32E-16
NMB0982		chloride channel protein-related protein									1.282	5.54E-03
NMB0985		E16-related protein									1.775	3.27E-20
NMB0995		macrophage infectivity potentiator-related protein	1.337	2.41E-06	0.958	3.01E-04	0.741	7.53E-03	0.734	5.73E-03	0.729	6.36E-03
NMB1052	*dedA*	dedA protein	0.846	8.06E-05								
NMB1076		DnaA-related protein	-0.729	2.90E-06	-0.857	2.84E-08	-0.762	1.34E-06	-0.790	2.70E-07		
NMB1220		stomatin/Mec-2 family protein	-0.798	9.16E-05					-0.744	1.60E-04	-0.930	4.87E-06
NMB1238		peptidyl-prolyl cis-trans isomerase-related protein					0.689	3.13E-05				
NMB1280		very long chain acyl-CoA dehydrogenase-related protein	-0.821	5.12E-05	-1.052	1.72E-07	-0.776	1.39E-04	-0.946	2.25E-06	-1.051	3.19E-07
NMB1335	*creA*	creA protein			1.228	4.74E-13	0.732	2.57E-06				
NMB1347	*suhB*	extragenic suppressor protein SuhB	1.703	5.60E-16	1.280	5.73E-12	1.209	1.86E-10	0.623	1.42E-04		
NMB1380		nifU protein	-1.654	5.34E-12	-0.927	6.78E-06	-0.856	6.43E-05	-1.050	6.83E-07	-1.059	7.68E-07
NMB1381		HesB/YadR/YfhF family protein	-1.572	1.02E-13	-0.984	5.39E-08	-0.981	2.15E-07	-1.005	4.29E-08	-1.959	1.12E-17
NMB1405		FrpA/C-related protein	-1.182	8.60E-08	-1.360	8.14E-10	-1.535	1.19E-10	-1.711	5.10E-13	-1.621	7.25E-12
NMB1409		FrpA/C-related protein			-0.784	7.76E-06	-0.902	1.33E-06	-1.203	2.76E-10	-1.496	4.98E-13
NMB1421		nifR3 protein	0.669	2.09E-07			0.647	5.58E-07			0.671	1.35E-07
NMB1448	*dinP*	DNA-damage-inducible protein P			-0.852	7.10E-03						
NMB1501		HesA/MoeB/ThiF family protein									0.635	7.29E-03
NMB1516	*fixS*	fixS protein									0.659	8.42E-05
NMB1526	*smpB*	small major protein B	-0.654	6.03E-03	-0.747	1.08E-03	-0.810	7.36E-04	-1.125	2.15E-06	-1.284	1.69E-07

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Table C.17 – continued from previous page

ID	Name	Annotation	20 min		40 min		60 min		90 min		240 min	
			log_2 ratio	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***
NMB1638		YhbX/YhjW/YijP/YjdB family protein									-0.616	9.43E-06
NMB1669	*hemO*	iron-starvation protein PigA			-0.892	6.74E-04	-1.184	1.71E-04	-1.202	2.84E-05	-1.657	2.15E-07
NMB1689		putative dedA protein									-1.048	2.29E-09
NMB1692		chorismate mutase-related protein							0.592	9.34E-03		
NMB1747		putative tspB protein									0.651	8.49E-03
NMB1753		VapD-related protein	-1.071	2.48E-05	-0.828	5.82E-04	-0.878	5.08E-04	-1.280	4.16E-07	-1.791	8.21E-11
NMB1891		helix-turn-helix family protein									-0.651	3.07E-03
NMB1924		inositol monophosphatase family protein							0.827	8.90E-03		
NMB1952	*sspB*	ClpXP protease specificity-enhancing factor			0.661	1.56E-03			0.614	3.41E-03		
NMB1961		VacJ-related protein	0.742	6.10E-04	0.691	8.05E-04	0.721	9.56E-04	0.634	2.21E-03		
NMB2105	*mafB*	mafB protein					-0.649	1.42E-05				
NMB2122	*mafB′*	MafB-related protein							-0.593	3.41E-03		
NMB2132		transferrin-binding protein-related protein	0.661	3.25E-05								

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.18: Genes up-regulated at any of the five time-points in blood compared to the 0 min time-point divided into cellular locations based on predictions and/or published experimental data. Only those genes which are up-regulated at any of the five time-points in blood (indicated by 'Up') and which encode proteins that have a known or putative location in the periplasm or the outer membrane are listed in the table.

		Time-point (min)						PSORTb	PSLpred	CBS	
ID	**Name**	**20**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB0003	*gltX*					Up	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0010	*pgk*	Up	Up	Up	Up	Up	OMVs (Vaughan *et al.*, 2006)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0018	*pilE*	Up	Up	Up			OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	Fimbria	√
NMB0044	*pilB*		Up		Up	Up	Surface located gonococci (Skaar *et al.*, 2002); OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	LipoP	√
NMB0046						Up		Unknown	Periplasmic	Cytoplasmic	
NMB0051	*pilU*	Up	Up			Down	OMVs (Ferrari *et al.*, 2006)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0086					Up		OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Multiple locations	Cytoplasmic	Cytoplasmic	
NMB0091			Up			Down		Unknown	Periplasmic	Cytoplasmic	
NMB0109		Up	Up				OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Inner membrane	Cytoplasmic	
NMB0118	*topA*		Up	Up				Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0123		Up	Up			Up		Cytoplasmic	Extracellular	Cytoplasmic	
NMB0124	*tufA2*	Up					Surface located (Kolberg *et al.*, 2008); OM and OMVs (Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0125	*secE*	Up					OMVs (Ferrari *et al.*, 2006)	Unknown	Inner membrane	TMHMM	
NMB0127	*rplK*	Up						Unknown	Periplasmic	SecretomeP	
NMB0128	*rplA*	Up					OM and OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	SecretomeP	
NMB0130	*rplJ*	Up				Down	OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0132	*rpoB*	Up					OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0133	*rpoC*	Up	Up	Up			OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0135						Up		Unknown	Periplasmic	Cytoplasmic	
NMB0136	*rpsL*	Up	Up	Up		Down		Unknown	Periplasmic	Cytoplasmic	
NMB0137	*rpsG*	Up		Up		Down	OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0139	*tufA1*	Up	Up	Up			OM and OMVs (Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0140	*rpsJ*	Up	Up	Up		Down	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0142	*rplC*	Up					OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Periplasmic	SecretomeP	
NMB0144	*rplW*	Up	Up	Up			OM and OMVs (Williams *et al.*, 2007)	Unknown	Periplasmic	Cytoplasmic	
NMB0145	*rplB*	Up	Up				OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0146	*rpsS*	Up	Up	Up			OM (Williams *et al.*, 2007)	Unknown	Periplasmic	Cytoplasmic	
NMB0147	*rplV*	Up	Up	Up			OM (Williams *et al.*, 2007)	Unknown	Outer membrane	Cytoplasmic	
NMB0149	*rplP*	Up	Up				OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0150	*rpmC*	Up	Up	Up			OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0151	*rpsQ*	Up	Up	Up			OMVs (Williams *et al.*, 2007)	Unknown	Periplasmic	Cytoplasmic	
NMB0152	*rplN*	Up				Down	OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	

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Table C.18 – continued from previous page

		Time-point (min)						PSORTb	PSLpred	CBS	
ID	**Name**	**20**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB0153	*rplX*	Up				Down	OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0157	*rplF*	Up						Unknown	Cytoplasmic	SecretomeP	
NMB0158	*rplR*	Up						Cytoplasmic	Outer membrane	Cytoplasmic	
NMB0160	*rpmD*	Up	Up				OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0161	*rplO*	Up					OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0165	*rpsM*	Up				Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0168	*rpoA*	Up	Up			Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0169	*rplQ*	Up				Down	OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0174	*valS*	Up	Up	Up			OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0179	*fabZ*			Up		Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0208				Up	Up	Up		Unknown	Periplasmic	Cytoplasmic	
NMB0214	*prlC*		Up	Up	Up		Surface located (Grifantini *et al.*, 2002a); OM and OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0216	*katA*		Up	Up	Up	Up		Periplasmic	Periplasmic	Cytoplasmic	
NMB0219	*fabF-1*	Up	Up	Up	Up		OM and OMVs (Vipond *et al.*, 2006; Williams *et al.*, 2007)	Inner membrane	Cytoplasmic	Cytoplasmic	
NMB0225						Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0265	*ruvA*	Up	Up	Up	Up	Up	OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0266			Up			Up		Multiple locations	Cytoplasmic	SignalP	✓
NMB0267		Up	Up	Up	Up	Up		Unknown	Periplasmic	SignalP	✓
NMB0278	*dsbA-1*		Up				OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	LipoP	✓
NMB0284	*purB*		Up	Up			OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0315					Up	Up	Surface located (Grifantini *et al.*, 2002a)	Unknown	Cytoplasmic	SignalP	✓
NMB0317		Up	Up		Up	Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0318	*farA*				Up	Up		Unknown	Outer membrane	SignalP	✓
NMB0322	*rpmG*	Up				Down		Unknown	Periplasmic	SecretomeP	
NMB0324	*rpmA*	Up	Up	Up		Down	OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0359	*glnA*		Up	Up			OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0369						Up		Unknown	Cytoplasmic	SecretomeP	
NMB0374	*mafB2*			Down		Up		Unknown	Outer membrane	SignalP	✓
NMB0375	*mafA-1*					Up	OM and OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Extracellular	LipoP	✓
NMB0376						Up		Unknown	Periplasmic	Cytoplasmic	
NMB0390	*mapA*	Up	Up				OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Cytoplasmic	Cytoplasmic	
NMB0392	*nadB*				Up			Periplasmic	Periplasmic	Cytoplasmic	
NMB0464		Up	Up	Up			OMVs (Vipond *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SecretomeP	
NMB0468	*speA*		Up	Up				Multiple locations	Periplasmic	Cytoplasmic	
NMB0469	*speB*		Up	Up			OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0476						Up		Unknown	Periplasmic	Cytoplasmic	
NMB0490						Up		Unknown	Outer membrane	SecretomeP	
NMB0497		Down		Down		Up		Outer membrane	Extracellular	SecretomeP	
NMB0540	*aspC*		Up	Up		Up	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0545					Up	Up		Cytoplasmic	Periplasmic	Cytoplasmic	

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Table C.18 – continued from previous page

		Time-point (min)						PSORTb	PSLpred	CBS	
ID	**Name**	**20**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB0549	*ybjZ*					Up	OM (Williams *et al.*, 2007)	Inner membrane	Inner membrane	TMHMM	
NMB0550	*dsbC*		Up	Up			OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Periplasmic	Periplasmic	LipoP	√
NMB0554	*dnaK*		Up	Up	Up		Plasminogen binding (Knaust *et al.*, 2007); OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	Cytoplasmic	
NMB0561	*grpE*	Up	Up	Up	Up	Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0563	*apbE*	Up	Up	Up		Up		Unknown	Extracellular	LipoP	√
NMB0569	*nqrA*	Up					OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0586			Up				OMVs (Vipond *et al.*, 2006)	Inner membrane	Periplasmic	SignalP	√
NMB0589	*rplS*	Up	Up	Up			OM and OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0592	*rpsP*	Up	Up	Up		Down	OM and OMVs (Williams *et al.*, 2007)	Unknown	Outer membrane	Cytoplasmic	
NMB0598		Up				Up		Unknown	Periplasmic	Cytoplasmic	
NMB0609	*rpsO*	Up				Down	OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0613		Up						Unknown	Periplasmic	Cytoplasmic	
NMB0623	*potD-2*	Up	Up	Up			OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Periplasmic	Periplasmic	LipoP	√
NMB0635						Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0663	*nspA*	Up	Up	Up	Up		OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	√
NMB0664						Up		Unknown	Periplasmic	SignalP	√
NMB0680	*cnp1*	Up	Up	Up	Up	Up		Unknown	Cytoplasmic	SignalP	√
NMB0681						Up		Unknown	Periplasmic	Cytoplasmic	
NMB0701						Up		Unknown	Extracellular	Cytoplasmic	
NMB0717						Up		Unknown	Periplasmic	SignalP	√
NMB0720	*thrS*	Up	Up	Up		Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0722	*rpmI*	Up				Down		Unknown	Periplasmic	SignalP	√
NMB0723	*rplT*	Up				Down	OM and OMVs (Williams *et al.*, 2007)	Unknown	Outer membrane	Cytoplasmic	
NMB0727		Up						Unknown	Extracellular	Cytoplasmic	
NMB0758	*pnp*	Up					OM and OMVs (Vipond *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0763	*cysK*	Up	Up	Up	Up	Down	OM and OMVs (Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0776			Up					Unknown	Periplasmic	SignalP	√
NMB0787		Up	Up	Up	Up		Surface located (Grifantini *et al.*, 2002a); OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Periplasmic	Periplasmic	LipoP	√
NMB0791	*ppiB*		Up	Up		Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0805						Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0820		Up	Up	Up				Unknown	Periplasmic	SecretomeP	
NMB0821		Up	Up	Up	Up	Up		Unknown	Outer membrane	TMHMM	
NMB0840		Up	Up	Up				Unknown	Periplasmic	Cytoplasmic	
NMB0841		Up	Up	Up	Up			Unknown	Periplasmic	LipoP	√
NMB0866		Up						Outer membrane	Extracellular	SignalP	√
NMB0886	*fimT*					Up		Unknown	Periplasmic	Fimbria	
NMB0907						Up		Unknown	Extracellular	SecretomeP	

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Table C.18 – continued from previous page

		Time-point (min)						PSORTb	PSLpred	CBS	
ID	**Name**	**20**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB0922	*lst*	Up	Up	Up		Up	Surface located (Shell *et al.*, 2002); OMVs (Vipond *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0923		Up	Up	Up				Periplasmic	Periplasmic	LipoP	√
NMB0942	*rpmE2*		Up	Up		Down		Unknown	Periplasmic	SecretomeP	
NMB0944	*metE*	Up	Up	Up	Up		OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0946			Up	Up		Down	Plasminogen binding (Knaust *et al.*, 2007); OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	SecretomeP	
NMB0947	*lpdA2*		Up			Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0964				Up	Up		OMVs (Vipond *et al.*, 2006)	Unknown	Outer membrane	SignalP	√
NMB0965						Up		Unknown	Periplasmic	Cytoplasmic	
NMB0983	*purH*	Up	Up	Up		Up	OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0995		Up	Up	Up	Up	Up	Surface located (Grifantini *et al.*, 2002a)	Unknown	Cytoplasmic	Cytoplasmic	
NMB1016						Up		Unknown	Periplasmic	SecretomeP	
NMB1017	*sbp*					Up		Periplasmic	Periplasmic	LipoP	√
NMB1020		Up	Up	Up				Cytoplasmic	Extracellular	Cytoplasmic	
NMB1022						Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1035			Up	Up			OM (Williams *et al.*, 2007)	Unknown	Periplasmic	LipoP	√
NMB1037	*gshA*	Up	Up	Up	Up	Up	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1038	*radC*					Up	OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB1040				Up	Up		OMVs (Ferrari *et al.*, 2006)	Cytoplasmic	Outer membrane	SignalP	√
NMB1044	*fpr-1*	Up	Up	Up			OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB1053	*opcA*	Up	Up				OM and OMVs (Vipond *et al.*, 2006; Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	√
NMB1070	*leuA*	Up	Up	Up			OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1073		Up	Up	Up	Up		OM and OMVs (Williams *et al.*, 2007)	Unknown	Outer membrane	Cytoplasmic	
NMB1081						Up		Unknown	Periplasmic	Cytoplasmic	
NMB1099						Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1100						Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1119					Up		Surface located (Grifantini *et al.*, 2002a)	Unknown	Periplasmic	Cytoplasmic	
NMB1128					Up		OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	SignalP	√
NMB1164		Up	Up	Up		Up	OM and OMVs (Vipond *et al.*, 2006; Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Outer membrane	LipoP	√
NMB1195					Up			Unknown	Outer membrane	Cytoplasmic	
NMB1204		Up				Up		Unknown	Periplasmic	Cytoplasmic	
NMB1205		Up						Unknown	Extracellular	SignalP	√
NMB1206	*bfrB*	Up	Up	Up		Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1207	*bfrA*	Up	Up			Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1228	*metM*	Up	Up	Up	Up	Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1229		Up	Up	Up				Unknown	Periplasmic	Cytoplasmic	
NMB1238				Up				Unknown	Periplasmic	Cytoplasmic	
NMB1251						Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1252	*purM*	Up	Up	Up	Up		OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	

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Table C.18 – continued from previous page

		Time-point (min)						PSORTb	PSLpred	CBS	
ID	**Name**	**20**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB1259						Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1262	*ppiA*					Up		Periplasmic	Periplasmic	SignalP	√
NMB1274					Up	Up		Unknown	Periplasmic	SignalP	√
NMB1285	*eno*	Up	Up	Up			Plasminogen binding (Knaust *et al.*, 2007); OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1301	*rpsA*	Up	Up	Up			OM and OMVs (Ferrari *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1312	*clpP*	Up	Up	Up		Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1327					Up		OMVs (Vipond *et al.*, 2006)	Extracellular	Periplasmic	Cytoplasmic	
NMB1332	*prc*					Up	OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	SignalP	√
NMB1334			Up					Unknown	Periplasmic	Cytoplasmic	
NMB1339	*proS*		Up				OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1347	*suhB*	Up	Up	Up	Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1364	*nadE*				Up		OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1366	*trxA*		Up	Up	Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1437		Up	Up	Up	Up	Up	OMVs (Vipond *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasmic	
NMB1444		Up	Up	Up	Up			Unknown	Periplasmic	Cytoplasmic	
NMB1457	*tktA*		Up	Up			OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasmic	
NMB1472	*clpB*	Down	Up	Up			OM and OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1475			Up	Up	Up	Up		Unknown	Periplasmic	SignalP	√
NMB1506	*argS*	Up		Up			OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1507				Up		Up		Unknown	Periplasmic	SecretomeP	
NMB1523		Up	Up	Up		Down		Multiple locations	Extracellular	LipoP	√
NMB1533		Up	Up	Up		Down	OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Periplasmic	Periplasmic	LipoP	√
NMB1538	*rpoD*		Up	Up		Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1554	*pyrG*	Up	Up	Up			OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	TMHMM	
NMB1594	*potD-3*	Up	Up	Up		Down		Periplasmic	Periplasmic	LipoP	√
NMB1602						Up		Unknown	Periplasmic	Cytoplasmic	
NMB1609	*metZ*					Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Inner-membrane	Cytoplasmic	
NMB1612	*hisJ*		Up			Down	OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Periplasmic	Periplasmic	LipoP	√
NMB1643	*infB*	Up		Up		Down	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	SecretomeP	
NMB1648		Up	Up	Up	Up			Cytoplasmic	Outer-membrane	Cytoplasmic	
NMB1652		Up	Up				OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB1677		Up	Up	Up				Unknown	Periplasmic	SecretomeP	
NMB1680	*aroC*	Up	Up	Up	Up		OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1681		Up	Up	Up		Down	OM (Williams *et al.*, 2007)	Unknown	Periplasmic	Cytoplasmic	
NMB1683						Up		Cytoplasmic	Outer-membrane	Cytoplasmic	
NMB1700						Up		Unknown	Periplasmic	Cytoplasmic	

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Table C.18 – continued from previous page

		Time-point (min)						PSORTb	PSLpred	CBS	
ID	**Name**	**20**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB1710	*gdhA*	Up	Up	Up			OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1723	*fixP*	Up	Up	Up		Down		Unknown	Periplasmic	TMHMM	√
NMB1747						Up		Outer membrane	Periplasmic	TMHMM	
NMB1749						Up		Unknown	Periplasmic	TMHMM	
NMB1772						Up		Unknown	Periplasmic	TMHMM	
NMB1775		Down		Down		Up		Outer membrane	Periplasmic	TMHMM	√
NMB1776					Up			Unknown	Extracellular	SignalP	√
NMB1779						Up		Outer membrane	Extracellular	SecretomeP	
NMB1800		Up	Up	Up		Up		Unknown	Extracellular	SecretomeP	
NMB1805	*cyc*	Up	Up	Up			OM (Williams *et al.*, 2007)	Periplasmic	Periplasmic	SignalP	√
NMB1811	*pilP*		Up					Unknown	Extracellular	Cytoplasmic	
NMB1812	*pilQ*		Up			Down	OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	√
NMB1816				Up	Up			Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1849	*carA*		Up	Up	Up	Up	OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB1861	*accC*			Up			OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1870			Up				Surface located (Masignani *et al.*, 2003); OM (Williams *et al.*, 2007); OMVs (Ferrari *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasmic	
NMB1875				Up	Up		Surface located (Grifantini *et al.*, 2002a)	Unknown	Cytoplasmic	TMHMM	
NMB1876	*argA*				Up		Surface located (Grifantini *et al.*, 2002a)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1880	*fetB2*					Up		Unknown	Periplasmic	LipoP	√
NMB1882					Up			Outer membrane	Outer membrane	SignalP	√
NMB1905	*rnpA*	Up	Up	Up	Up			Unknown	Periplasmic	Cytoplasmic	
NMB1911	*rpmF*	Up	Up	Up				Unknown	Periplasmic	SecretomeP	
NMB1934	*atpD*	Up	Up	Up			OM and OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1935	*atpG*	Up				Down	OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB1939	*atpE*		Up			Down	OM (Williams *et al.*, 2007)	Inner membrane	Inner membrane	TMHMM	
NMB1950	*rpsU*	Up				Down	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1951			Up	Up				Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1956	*rpmE*	Up	Up	Up		Down		Unknown	Periplasmic	SecretomeP	
NMB1958		Up						Unknown	Periplasmic	SignalP	√
NMB1961		Up	Up	Up	Up		OM (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	√
NMB1963		Up	Up	Up			OM (Williams *et al.*, 2007)	Unknown	Periplasmic	SignalP	√
NMB1964				Up				Unknown	Cytoplasmic	SignalP	√
NMB1972	*groEL*		Up	Up	Up	Up	OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1973	*groES*		Up	Up	Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1977				Up	Up	Up	OM (Williams *et al.*, 2007)	Unknown	Outer membrane	LipoP	√
NMB1996	*purL*					Up	OMVs (Vaughan *et al.*, 2006)	Outer membrane	Cytoplasmic	Cytoplasmic	
NMB2000	*hslO*		Up	Up	Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB2021			Up					Unknown	Periplasmic	Cytoplasmic	

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Table C.18 – continued from previous page

		Time-point (min)						PSORTb	PSLpred	CBS	
ID	**Name**	**20**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB2051	*petC*	Up	Up	Up				Unknown	Periplasmic	TMHMM	√
NMB2053	*petA*	Up	Up	Up	Up		OM (Williams *et al.*, 2007)	Inner membrane	Periplasmic	SecretomeP	
NMB2056	*rpsI*	Up	Up	Up		Down	OM and OMVs (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB2057	*rplM*	Up	Up	Up	Up			Unknown	Periplasmic	Cytoplasmic	
NMB2073						Up		Unknown	Periplasmic	Cytoplasmic	
NMB2074						Up		Unknown	Periplasmic	Cytoplasmic	
NMB2101	*rpsB*	Up	Up	Up	Up	Down	OMVs (Vaughan *et al.*, 2006)	Unknown	Cytoplasmic	Cytoplasmic	
NMB2132		Up					Surface located (Pizza *et al.*, 2000)	Unknown	Outer membrane	LipoP	√
NMB2139						Up	OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	LipoP	√
NMB2148						Up		Cytoplasmic	Periplasmic	Cytoplasmic	

OM or OMVs in the table indicate that the protein has been detected in outer membrane or outer membrane vesicles preparations, respectively. For predictions the algorithms PSORTb, PSLpred, and tools at CBS were used. Denominations from CBS: SignalP, the protein encodes a signal peptide; TMHMM, the protein encodes transmembrane helices; LipoP, the proteins is a lipoprotein and SecretomeP, the protein is secreted through a non-classical i.e. not signal peptide triggered protein secretion.

Table C.19: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *amino acid biosynthesis*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Aromatic amino acid family										
NMB0307	*aroG*	phospho-2-dehydro-3-deoxyheptonate aldolase, phe-sensitive							0.989	7.84E-05
NMB1446	*aroD*	3-dehydroquinate dehydratase							-0.786	4.46E-03
NMB1678	*tyrB*	aromatic-amino-acid aminotransferase							-0.739	1.17E-07
Aspartate family										
NMB0203	*dapB*	dihydrodipicolinate reductase							-1.080	1.70E-12
NMB0540	*aspC*	aspartate aminotransferase							0.618	7.01E-03
NMB0929	*dapA*	dihydrodipicolinate synthase							-0.808	2.11E-03
NMB0943	*metF*	5,10-methylenetetrahydrofolate reductase					-0.775	2.11E-03		
NMB0944	*metH*	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase							-0.922	2.97E-04
NMB1498	*lysC*	aspartokinase, α and β subunits							-0.921	1.13E-04
Glutamate family										
NMB0055	*proC*	pyrroline-5-carboxylate reductase							0.973	4.12E-06
NMB0224	*glnE*	glutamate-ammonia-ligase adenylyltransferase					0.585	5.40E-03		
NMB0359	*glnA*	glutamate ammonia ligase	0.591	1.75E-03			-0.604	3.21E-03		
NMB0637	*argH*	argininosuccinate lyase							-0.593	8.30E-04
NMB1710	*gdhA*	glutamate dehydrogenase, NADP-specific							-0.998	1.06E-03
NMB1787	*argC*	N-acetyl-gamma-glutamyl-phosphate reductase							-0.829	5.04E-04
NMB1876	*argA*	N-acetylglutamate synthase							-0.849	4.93E-03
Histidine family										
NMB0603	*hisE*	phosphoribosyl-ATP pyrophosphatase							0.668	2.49E-05
NMB0627	*hisI*	phosphoribosyl-AMP cyclohydrolase							-1.355	8.56E-04
NMB1579	*hisG*	ATP phosphoribosyltransferase							1.129	3.61E-10
NMB1581	*hisD*	histidinol dehydrogenase							-0.745	6.47E-04
NMB1582	*hisC*	histidinol-phosphate aminotransferase							-1.025	2.03E-03
Pyruvate family										
NMB0337	*ilvE*	putative branched-chain amino acid aminotransferase	0.748	1.11E-03					-0.896	1.37E-05
NMB1150	*ilvD-1*	dihydroxy-acid dehydratase							-0.741	2.36E-03
NMB1188	*ilvD-2*	dihydroxy-acid dehydratase							-0.764	6.93E-04
NMB1574	*ilvC*	ketol-acid reductoisomerase					-0.673	4.24E-03	-1.196	1.02E-08
Serine family										
NMB0763	*cysK*	cysteine synthase							-1.460	5.65E-13

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.20: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *biosynthesis of co-factors, prosthetic groups and carriers*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Biotin										
NMB0474	*bioC*	putative biotin synthesis protein							1.161	4.70E-10
NMB1146	*bioB-1*	biotin synthetase							-0.969	2.25E-07
NMB1184	*bioB-2*	biotin synthetase							-0.997	1.36E-07
NMB2075	*birA*	BirA protein Bvg accessory factor							-0.647	1.52E-04
Folic acid										
NMB1063	*folB*	dihydroneopterin aldolase							1.239	2.71E-11
NMB1691	*dhpS*	dihydropteroate synthase							-0.957	1.73E-05
Menaquinone and ubiquinone										
NMB0261		geranyltranstransferase							-0.655	1.19E-05
NMB1945		3-octaprenyl-4-hydroxybenzoate carboxy-lyase							-1.045	7.71E-03
Other										
NMB1379		nifS protein	0.943	4.14E-04						
Pyridine nucleotides										
NMB0392	*nadB*	l-aspartate oxidase					0.747	2.11E-03	-0.709	2.52E-04
NMB0394	*nadA*	quinolinate synthetase A					0.706	3.55E-04		
NMB0396	*nadC*	nicotinate-nucleotide pyrophosphorylase	0.742	2.73E-04						
Riboflavin, FMN, and FAD										
NMB1817	*ribD*	riboflavin biosynthesis protein							-1.042	3.65E-06
Thiamine										
NMB2040	*thiC*	thiamine biosynthesis protein							0.748	2.27E-03
NMB2069	*thiE*	thiamin-phosphate pyrophosphorylase							0.825	9.40E-04
Thiamine/ Pyridoxine										
NMB0184	*dxr*	1-deoxy-D-xylulose 5-phosphate reductoisomerase							-0.627	8.28E-07

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.21: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into TIGR role category *cell envelope*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Biosynthesis and degradation of murein sacculus and peptidoglycan										
NMB0011	*murA*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase							-0.892	2.71E-08

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Table C.21 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0357	*mtgA*	monofunctional biosynthetic peptidoglycan transglycosylase					0.635	4.28E-03		
NMB0414	*murE*	UDP-N-acetylmuramoylalanyl-D-glutamate 2,6-diaminopimelate ligase							-0.765	3.22E-08
NMB0877		penicillin-binding protein							-2.797	2.18E-06
NMB1085		putative N-acetylmuramoyl-L-alanine amidase							1.121	7.85E-06
NMB1183		UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase							-0.644	3.24E-04
Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides										
NMB0062	*rfbA-1*	glucose-1-phosphate thymidylyltransferase							0.873	1.60E-07
NMB0063	*rfbB-1*	dTDP-D-glucose 4,6-dehydratase							1.227	3.31E-14
NMB0064	*galE-1*	UDP-glucose 4-epimerase					0.673	4.77E-03		
NMB0069	*siaB*	polysialic acid capsule biosynthesis protein					-0.794	4.17E-03		
NMB0070	*siaA*	polysialic acid capsule biosynthesis protein					-0.724	4.60E-03		
NMB0078	*galE-2*	UDP-glucose 4-epimerase (truncation)					0.635	3.21E-03		
NMB0079	*rfbB-2*	dTDP-D-glucose 4,6-dehydratase							1.151	3.30E-10
NMB0080	*rfbA-2*	glucose-1-phosphate thymidylyltransferase							0.749	4.68E-06
NMB0082	*lipA*	capsule polysaccharide modification protein					0.722	4.32E-03		
NMB0199	*lpxB*	lipid-A-disaccharide synthase					0.704	2.81E-03		
NMB0544		UDP-2,3-diacylglucosamine hydrolase					0.589	2.52E-03		
NMB0790	*pgm*	phosphoglucomutase	0.688	1.40E-05	-0.671	3.71E-04				
NMB0828	*rfaD*	ADP-L-glycero-D-mannoheptose-6-epimerase							1.116	3.36E-07
NMB1279	*mltB*	putative membrane-bound lytic murein transglycosylase B							-0.810	5.27E-05
Other										
NMB0345		putative cell-binding factor	0.709	9.37E-03			-0.829	6.06E-03		
NMB0382	*rmpM*	outer membrane protein class 4							-1.199	1.00E-07
NMB0663	*nspA*	outer membrane protein	-1.122	1.30E-07					-1.058	9.17E-08
NMB0946		peroxiredoxin 2 family protein/glutaredoxin	1.062	4.02E-04					-3.110	2.54E-18
NMB1213		putative lipoprotein							1.388	3.33E-05
NMB1533		H.8 outer membrane protein							-1.644	9.33E-11
NMB1716	*mtrC*	membrane fusion protein							0.802	1.96E-04
NMB1946		outer membrane lipoprotein							-0.857	8.51E-05
Surface structures										
NMB0018	*pilE*	pilin							-1.022	1.31E-05
NMB0333	*pilG*	pilus assembly protein							-0.619	6.23E-05
NMB1812	*pilQ*	pilQ protein							-0.820	3.66E-03
NMB1847		pilC1 protein (authentic frameshift)							0.770	1.94E-05

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.22: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *cellular processes*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Cell division										
NMB0172	*minE*	cell division topological specificity factor					-0.599	4.14E-03	-0.830	2.39E-06
NMB0798	*ftsH*	cell division protein	0.672	1.88E-03						
NMB0799	*ftsJ*	cell division protein							0.774	4.26E-09
Chemotaxis and motility										
NMB0051	*pilU*	twitching motility protein							-0.658	2.08E-08
Detoxification										
NMB0216	*katA*	catalase	1.831	5.31E-05						
NMB0525		putative aluminum resistance protein	-0.682	1.53E-05						
NMB0884	*sodB*	superoxide dismutase							0.605	6.51E-05
NMB1621	*gpxA*	glutathione peroxidase							-0.949	2.02E-07
DNA transformation										
NMB0703	*comL*	competence lipoprotein							-0.679	3.31E-07
Other										
NMB1981	*luxS*	S-ribosylhomocysteinase	0.756	6.54E-04						
Pathogenesis										
NMB0018	*pilE*	pilin							-1.022	1.31E-05
NMB0333	*pilG*	pilus assembly protein							-0.619	6.23E-05
NMB0926		opacity protein (authentic frameshift)							-0.814	1.60E-04
NMB1412		FrpC operon protein							0.641	1.37E-03
NMB1414		FrpC operon protein							0.638	1.39E-03
NMB1552	*pivNM-1A*	pilin gene inverting protein							0.916	3.81E-06
NMB1567		macrophage infectivity potentiator							-1.190	2.98E-05
NMB1625	*pivNM-1B*	pilin gene inverting protein							0.826	2.14E-04
NMB1812	*pilQ*	pilQ protein							-0.820	3.66E-03
NMB1847		pilC1 protein (authentic frameshift)							0.770	1.94E-05
NMB2095		putative adhesin complex protein							-1.075	1.51E-05
Toxin production and resistance										
NMB0408		bacitracin resistance protein							0.686	1.67E-06
NMB0493		hemagglutinin/hemolysin-related protein							1.196	6.29E-11
NMB0497		hemagglutinin/hemolysin-related protein					0.952	4.68E-04	0.771	2.61E-04
NMB0582		putative bacteriocin resistance protein							1.472	8.94E-04
NMB0697	*ksgA*	dimethyladenosine transferase					-0.614	2.81E-03	1.051	4.15E-09
NMB0839	*pmbA*	pmbA protein							-2.921	1.10E-25
NMB0855		putative bacteriocin resistance protein							0.978	4.59E-05
NMB1210		putative toxin-activating protein							0.810	5.04E-05
NMB1214		hemagglutinin/hemolysin-related protein							1.378	1.46E-09

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Table C.22 – continued from previous page

ID	Name	Annotation	20→40 log$_2$ ratio	20→40 P-value*	40→60 log$_2$ ratio	40→60 P-value*	60→90 log$_2$ ratio	60→90 P-value*	90→240 log$_2$ ratio	90→240 P-value*
NMB1762		putative hemolysin activation protein HecB							0.883	3.04E-05
NMB1779		hemagglutinin/hemolysin-related protein					0.865	2.11E-03	0.585	8.99E-03
NMB2091		putative hemolysin							-1.024	1.99E-06

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.23: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *central intermediary metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40 log$_2$ ratio	20→40 P-value*	40→60 log$_2$ ratio	40→60 P-value*	60→90 log$_2$ ratio	60→90 P-value*	90→240 log$_2$ ratio	90→240 P-value*
Other										
NMB0029		glycerate dehydrogenase							-0.738	5.34E-06
NMB1338		putative isomerase							-0.599	1.15E-04
NMB1473		aminotransferase, class I							-0.835	5.16E-04
NMB1584		3-hydroxyacid dehydrogenase	0.589	4.49E-03						
NMB1799	*metK*	S-adenosylmethionine synthetase							-0.808	2.40E-07
Phosphorus compounds										
NMB0641	*ppa*	inorganic pyrophosphatase							-0.689	8.56E-05
Polyamine biosynthesis										
NMB0469	*speB*	agmatinase							-0.593	2.43E-06

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.24: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *DNA metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40 log$_2$ ratio	20→40 P-value*	40→60 log$_2$ ratio	40→60 P-value*	60→90 log$_2$ ratio	60→90 P-value*	90→240 log$_2$ ratio	90→240 P-value*
Chromosome-associated proteins										
NMB1230	*hupB*	DNA-binding protein HU-β							-0.717	5.16E-03
Degradation of DNA										
NMB1363	*xseA*	exodeoxyribonuclease, large subunit			-0.589	7.71E-03			0.741	2.25E-06
DNA replication, recombination, and repair										
NMB0056	*dksA*	DnaK suppressor protein							-0.621	3.27E-04

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Table C.24 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0729	*himA*	integration host factor, α subunit							-1.643	4.94E-13
NMB0751	*xerD*	integrase recombinase							0.851	4.84E-05
NMB0842	*recJ*	single-stranded-DNA-specific exonuclease							-0.620	1.04E-04
NMB1233	*recD*	exodeoxyribonuclease V, α subunit							-1.152	4.62E-05
NMB1322	*priB*	putative primosomal replication protein n					-0.774	4.54E-03	-1.940	6.59E-13
NMB1326	*uvrC*	excinuclease ABC, subunit C							1.055	8.26E-03
NMB1396	*mutY*	A/G-specific adenine glycosylase							0.929	5.21E-04
NMB1445	*recA*	recA protein							-1.280	3.60E-08
NMB1552	*pivNM-1A*	pilin gene inverting protein							0.916	3.81E-06
NMB1625	*pivNM-1B*	pilin gene inverting protein							0.826	2.14E-04
NMB1673	*tag*	DNA-3-methyladenine glycosylase I							0.853	1.71E-03
NMB1682	*parE*	topoisomerase IV, subunit B							0.588	8.30E-04
NMB1683		MutT nudix family protein							0.886	1.06E-03
Restriction modification										
NMB0725		modification methylase HgaI-1	-0.778	1.44E-04					0.883	2.57E-06
NMB0726		type II restriction enzyme HgaI	-0.936	6.71E-05						
NMB0727		N-6 adenine-specific DNA methylase	-0.708	3.85E-03						
NMB0835		putative type I restriction enzyme EcoR124II R protein					0.709	5.98E-03		
NMB1032		type II restriction enzyme NlaIV							0.866	1.41E-04
NMB1260		type III restriction-modification system EcoPI enzyme, subunit res							1.050	2.44E-03
NMB1289		putative type II restriction enzyme							-1.451	2.95E-11
NMB0831		type I restriction enzyme S protein (degenerate)							-0.650	8.53E-04

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.25: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *energy metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Amino acids and amines										
NMB0176	*dadA*	D-amino acid dehydrogenase, small subunit							0.873	6.17E-06
NMB0401	*putA*	proline dehydrogenase							-0.674	8.57E-05
NMB1609	*metZ*	trans-sulfuration enzyme family protein							0.785	4.76E-04
Anaerobic										
NMB1622	*norB*	nitric oxide reductase					0.976	2.11E-03		
ATP-proton motive force interconversion										
NMB1933	*atpC*	ATP synthase F1, ϵ subunit							-0.886	5.11E-06

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Table C.25 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB1934	*atpD*	ATP synthase F1, β subunit					-0.800	8.07E-03		
NMB1935	*atpG*	ATP synthase F1, γ subunit							-0.984	5.43E-04
Electron transport										
NMB0123		ferredoxin, 4Fe-4S bacterial type					-0.688	8.96E-03	0.834	2.09E-04
NMB0241	*nuoA*	NADH dehydrogenase I, A subunit							0.901	6.31E-08
NMB0242	*nuoB*	NADH dehydrogenase I, B subunit					0.655	2.11E-03		
NMB0243	*nuoC*	NADH dehydrogenase I, C subunit							0.867	3.82E-05
NMB0245	*nuoE*	NADH dehydrogenase I, E subunit							1.518	4.83E-12
NMB0246	*nuoF*	NADH dehydrogenase I, F subunit							0.610	4.22E-06
NMB0249	*nuoG*	NADH dehydrogenase I, G subunit							0.804	1.20E-06
NMB0250	*nuoH*	NADH dehydrogenase I, H subunit							0.713	3.31E-03
NMB0251	*nuoI*	NADH dehydrogenase I, I subunit							1.181	1.56E-06
NMB0254	*nuoK*	NADH dehydrogenase I, K subunit							1.555	2.28E-08
NMB0258	*nuoM*	NADH dehydrogenase I, M subunit							1.218	1.28E-07
NMB0717		putative cytochrome							1.404	1.57E-11
NMB0923		cytochrome c							-0.748	6.17E-06
NMB1044	*fpr-1*	ferredoxin NADP reductase							-0.822	5.83E-07
NMB1366	*trxA*	thioredoxin	1.305	5.23E-09					-1.295	8.67E-10
NMB1723		cytochrome c oxidase, subunit III							-1.335	5.62E-08
NMB1790		glutaredoxin 3	1.088	1.38E-03						
NMB2051	*petC*	ubiquinol cytochrome c reductase, cytochrome c1					-0.592	9.79E-03	-0.767	9.98E-05
Entner-Doudoroff										
NMB1393	*edd*	phosphogluconate dehydratase							-0.778	7.40E-09
NMB1394	*eda*	4-hydroxy-2-oxoglutarate aldolase/2-dehydro-3-deoxyphosphogluconate aldolase							-0.807	1.11E-07
Fermentation										
NMB0631		putative phosphate acetyltransferase Pta	0.597	1.48E-03					-1.580	7.96E-15
NMB1395		alcohol dehydrogenase, zinc-containing	0.650	1.17E-03						
NMB1518	*ackA-1*	acetate kinase							-1.952	2.19E-18
NMB0431	*prpC*	methylcitrate synthase citrate synthase 2							1.265	7.58E-08
Glycolysis/gluconeogenesis										
NMB0334	*pgi-1*	glucose-6-phosphate isomerase					0.708	4.25E-03		
NMB0618	*ppsA*	phosphoenolpyruvate synthase	0.668	8.84E-03						
NMB1285	*eno*	enolase							-1.081	2.00E-05
NMB1377	*lldA*	L-lactate dehydrogenase	1.201	6.45E-07						
NMB1388	*pgi*	glucose-6-phosphate isomerase							-0.612	4.03E-06
NMB1390	*glk*	glucokinase							-1.127	4.10E-13
NMB1604	*gpm*	phosphoglycerate mutase							-0.749	9.20E-06
NMB2159		glyceraldehyde 3-phosphate dehydrogenase							-1.577	2.81E-13
Other										
NMB0340	*gloA*	lactoylglutathione lyase							-1.527	1.43E-11

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Table C.25 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1488	*gabD*	succinate-semialdehyde dehydrogenase (NADP+)							0.609	1.54E-04
NMB1493		carbon starvation protein A	-1.393	1.30E-07						
Pentose phosphate pathway										
NMB0351	*tal*	transaldolase	0.638	2.38E-05						
NMB1244	*rpe*	ribulose-phosphate 3-epimerase	-0.871	7.46E-07						
NMB1392	*zwf*	glucose-6-phosphate 1-dehydrogenase					-0.656	2.65E-04		
NMB1457	*tkt*	transketolase							-0.955	2.09E-06
NMB1511	*rpiA*	ribose 5-phosphate isomerase A							0.633	4.60E-03
Pyruvate dehydrogenase										
NMB1341	*aceE*	pyruvate dehydrogenase, E1 component							-1.388	7.77E-10
NMB1342	*aceF*	dihydrolipoamide acetyltransferase (pyruvate dehydrogenase, E2 component)							-1.547	4.46E-12
NMB1344	*lpdA*	lipoamide dehydrogenase (pyruvate dehydrogenase, E3 component)							-1.046	4.03E-06
Sugars										
NMB0064	*galE-1*	UDP-glucose 4-epimerase					0.673	4.77E-03		
NMB0078	*galE-2*	UDP-glucose 4-epimerase (truncation)					0.635	3.21E-03		
NMB0391	*pgmB*	β-phosphoglucomutase							-0.848	2.04E-08
NMB0790	*pgm*	phosphoglucomutase	0.688	1.40E-05	-0.671	3.71E-04				
TCA cycle										
NMB0920	*icd*	isocitrate dehydrogenase, NADP-dependent, monomeric type							-0.851	3.73E-04
NMB0947	*lpdA2*	lipoamide dehydrogenase (putative 2-oxoglutarate dehydrogenase, E3 component)	0.959	2.17E-04					-1.042	9.20E-06
NMB0957	*lpdA3*	lipoamide dehydrogenase (2-oxoglutarate dehydrogenase, E3 component)							0.810	1.52E-05
NMB1572	*acnB*	aconitate hydratase 2							-1.002	2.74E-06

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.26: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *fatty acid and phospholipid metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Biosynthesis										
NMB0219	*fabF-1*	3-oxoacyl-(acyl-carrier-protein) synthase II							-0.900	6.39E-06
NMB0220	*acpP-1*	acyl carrier protein							-2.083	1.38E-12
NMB0336	*fabI*	enoyl-(acyl-carrier-protein) reductase							-1.186	1.90E-15
NMB0679	*accD*	acetyl-CoA carboxylase carboxyl transferase, β subunit							-0.706	2.67E-06
NMB1294	*plsC*	1-acyl-sn-glycerol-3-phosphate acyltransferase					0.662	8.07E-03		
NMB1697		putative acyl carrier protein							0.687	2.69E-03

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Table C.26 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1860	*accB*	acetyl-CoA carboxylase, biotin carboxyl carrier protein							-1.375	1.58E-04
NMB1861	*accC*	acetyl-CoA carboxylase, biotin carboxylase					-0.949	2.11E-03		

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.27: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into TIGR role category *hypothetical proteins* or unassigned. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Conserved										
NMB0035		conserved hypothetical protein	0.696	6.01E-03					-0.938	2.22E-05
NMB0047		conserved hypothetical protein							0.637	3.59E-05
NMB0053		conserved hypothetical protein							0.697	5.37E-04
NMB0109		conserved hypothetical protein							-0.594	2.48E-03
NMB0121		conserved hypothetical protein							0.873	2.19E-05
NMB0230		conserved hypothetical protein							0.630	4.53E-04
NMB0248		conserved hypothetical protein							0.900	5.27E-05
NMB0276		conserved hypothetical protein							-0.646	2.06E-03
NMB0310		conserved hypothetical protein							0.994	1.36E-05
NMB0313		conserved hypothetical protein							-0.978	4.57E-05
NMB0316		conserved hypothetical protein	-0.858	6.56E-04						
NMB0317		conserved hypothetical protein	-0.718	8.13E-04						
NMB0330		conserved hypothetical protein							-0.622	9.85E-05
NMB0343		conserved hypothetical protein							-0.837	5.70E-03
NMB0377		conserved hypothetical protein					0.657	7.70E-03	-0.671	1.37E-03
NMB0395		conserved hypothetical protein	0.689	1.32E-04					-0.938	1.58E-07
NMB0409		conserved hypothetical protein					0.755	5.18E-03		
NMB0455		conserved hypothetical protein							0.605	1.01E-04
NMB0477		conserved hypothetical protein					-0.646	6.73E-03		
NMB0529		conserved hypothetical protein					0.648	4.20E-03		
NMB0534		conserved hypothetical protein							0.737	1.02E-03
NMB0557		conserved hypothetical protein	0.715	5.81E-04					-1.387	2.53E-11
NMB0606		conserved hypothetical protein							-1.389	2.92E-11
NMB0619		conserved hypothetical protein							-0.588	1.91E-03
NMB0621		conserved hypothetical protein							0.766	6.89E-04
NMB0681		conserved hypothetical protein							0.838	4.41E-04
NMB0759		conserved hypothetical protein							0.636	4.84E-07
NMB0773		conserved hypothetical protein							-1.023	2.13E-08

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Table C.27 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0840		conserved hypothetical protein					-0.636	3.04E-03		
NMB0874		conserved hypothetical protein							-0.664	1.91E-06
NMB0883		conserved hypothetical protein							0.984	1.70E-07
NMB1009		conserved hypothetical protein							0.926	3.32E-04
NMB1026		conserved hypothetical protein							-0.635	3.63E-05
NMB1039		conserved hypothetical protein							-0.893	1.36E-06
NMB1166		conserved hypothetical protein							-1.068	1.21E-03
NMB1218		conserved hypothetical protein							-0.844	5.03E-05
NMB1319		conserved hypothetical protein							-0.770	2.65E-03
NMB1336		conserved hypothetical protein	1.357	3.14E-11						
NMB1378		conserved hypothetical protein	0.742	6.47E-05						
NMB1436		conserved hypothetical protein							-0.787	3.56E-04
NMB1438		conserved hypothetical protein							-1.034	1.47E-06
NMB1475		conserved hypothetical protein	2.505	1.85E-10						
NMB1496		conserved hypothetical protein							0.710	2.47E-04
NMB1543		conserved hypothetical protein							0.953	5.21E-03
NMB1557		conserved hypothetical protein							-0.818	2.10E-06
NMB1564		conserved hypothetical protein	1.192	9.59E-10	-0.772	4.58E-04			-0.648	1.10E-04
NMB1575		conserved hypothetical protein					-0.725	4.24E-03	-0.615	3.30E-03
NMB1578		conserved hypothetical protein							0.878	2.64E-05
NMB1590		conserved hypothetical protein							-0.590	3.24E-03
NMB1634		conserved hypothetical protein							1.106	3.79E-04
NMB1641		conserved hypothetical protein	-0.730	1.11E-03						
NMB1653		conserved hypothetical protein							-0.978	2.98E-05
NMB1654		conserved hypothetical protein							1.144	4.57E-10
NMB1665		conserved hypothetical protein							-2.710	3.58E-16
NMB1727		conserved hypothetical protein							-0.628	8.26E-05
NMB1749		conserved hypothetical protein							2.100	2.92E-11
NMB1796		conserved hypothetical protein	0.879	3.66E-05					-1.877	2.27E-15
NMB1886		conserved hypothetical protein							0.676	3.92E-03
NMB1906		conserved hypothetical protein							-0.968	2.33E-04
NMB1908		conserved hypothetical protein							1.062	1.06E-08
NMB1910		conserved hypothetical protein							-1.075	8.25E-06
NMB2000		conserved hypothetical protein	0.833	7.20E-07					-0.943	5.31E-09
NMB2020		conserved hypothetical protein	0.655	1.41E-03					-0.992	1.72E-07
NMB2078		conserved hypothetical protein							-0.851	1.54E-08
NMB2098		conserved hypothetical protein					0.698	7.97E-03		
NMB2140		conserved hypothetical protein							-0.585	4.53E-04
NMB2153		conserved hypothetical protein							1.013	7.24E-07
Unassigned										
NMB0046		hypothetical protein							0.917	1.13E-10

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Table C.27 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0091		hypothetical protein							-0.656	1.74E-03
NMB0092		hypothetical protein							-1.137	6.55E-04
NMB0213		hypothetical protein					0.646	2.11E-03		
NMB0222		hypothetical protein					0.750	9.74E-03		
NMB0239		hypothetical protein							1.422	3.85E-10
NMB0252		hypothetical protein							1.011	8.49E-06
NMB0271		hypothetical protein					0.638	6.06E-03	-0.600	2.27E-03
NMB0298		hypothetical protein							-1.139	1.46E-05
NMB0305		hypothetical protein							1.043	6.79E-05
NMB0320		hypothetical protein							-1.147	1.77E-05
NMB0346		hypothetical protein	0.753	4.02E-04						
NMB0350		hypothetical protein	0.679	1.11E-03					0.681	2.10E-04
NMB0363		hypothetical protein							0.799	6.14E-04
NMB0367		hypothetical protein							1.023	8.83E-09
NMB0368		hypothetical protein							1.295	2.57E-09
NMB0369		hypothetical protein							1.232	1.06E-09
NMB0370		hypothetical protein							0.893	9.67E-08
NMB0371		hypothetical protein							1.148	1.08E-09
NMB0372		hypothetical protein							1.271	1.89E-10
NMB0429		hypothetical protein							0.859	7.58E-03
NMB0449		hypothetical protein							-1.019	3.53E-06
NMB0450		hypothetical protein							-0.619	2.04E-03
NMB0476		hypothetical protein							1.340	2.25E-07
NMB0478		hypothetical protein							-0.745	2.00E-04
NMB0483		hypothetical protein							0.796	1.61E-04
NMB0485		hypothetical protein							1.283	1.00E-04
NMB0492		hypothetical protein							0.807	9.95E-05
NMB0499		hypothetical protein					1.095	3.26E-03		
NMB0500		hypothetical protein							-0.785	1.26E-04
NMB0507		hypothetical protein							-1.744	4.56E-14
NMB0509		hypothetical protein					0.766	3.92E-03		
NMB0519		hypothetical protein							-0.879	2.86E-06
NMB0541		hypothetical protein							0.696	1.15E-07
NMB0552		hypothetical protein					0.747	4.43E-03		
NMB0647		hypothetical protein							0.732	2.37E-05
NMB0654		hypothetical protein							0.896	2.61E-05
NMB0655		hypothetical protein							1.485	2.35E-13
NMB0656		hypothetical protein	-0.730	6.07E-04						
NMB0695		hypothetical protein							1.545	8.51E-05
NMB0701		hypothetical protein							1.009	2.14E-05
NMB0715		hypothetical protein							1.828	1.08E-09

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Table C.27 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0716		hypothetical protein							0.839	1.96E-04
NMB0730		hypothetical protein							1.341	1.25E-08
NMB0731		hypothetical protein							1.303	1.06E-11
NMB0734		hypothetical protein							0.724	5.53E-06
NMB0744		hypothetical protein	0.886	5.79E-04					-0.961	2.94E-05
NMB0761		hypothetical protein							-0.886	2.10E-03
NMB0762		hypothetical protein							-0.837	3.02E-05
NMB0821		hypothetical protein							0.832	2.02E-09
NMB0841		hypothetical protein							-1.463	7.77E-15
NMB0847		hypothetical protein							-0.729	5.97E-08
NMB0850		hypothetical protein	0.931	9.95E-03						
NMB0856		hypothetical protein							1.688	1.43E-11
NMB0860		hypothetical protein							0.972	1.02E-08
NMB0863		hypothetical protein							1.168	3.17E-11
NMB0865		hypothetical protein							0.825	5.89E-04
NMB0898		hypothetical protein							-0.695	2.05E-03
NMB0899		hypothetical protein							-0.725	5.26E-04
NMB0907		hypothetical protein	0.829	2.17E-04					0.821	4.47E-05
NMB0928		hypothetical protein							-0.850	7.14E-09
NMB0973		hypothetical protein							0.907	2.65E-03
NMB1006		hypothetical protein							1.897	5.72E-08
NMB1008		hypothetical protein							1.166	8.15E-06
NMB1010		hypothetical protein							1.140	1.46E-05
NMB1020		hypothetical protein					-0.669	2.44E-03		
NMB1089		hypothetical protein							-1.066	1.18E-03
NMB1100		hypothetical protein							0.751	6.05E-04
NMB1103		hypothetical protein							-0.987	6.36E-05
NMB1107		hypothetical protein							-1.445	2.92E-08
NMB1117		hypothetical protein							-0.744	5.13E-03
NMB1124		hypothetical protein							-0.900	1.23E-04
NMB1144		hypothetical protein							-1.288	2.67E-07
NMB1149		hypothetical protein							-0.760	9.87E-03
NMB1157		hypothetical protein							-1.152	3.69E-03
NMB1162		hypothetical protein							-0.807	1.22E-03
NMB1182		hypothetical protein							-1.413	7.77E-15
NMB1187		hypothetical protein							-0.912	1.21E-03
NMB1202		hypothetical protein							1.163	1.22E-08
NMB1211		hypothetical protein							1.492	2.51E-05
NMB1212		hypothetical protein							1.045	1.15E-03
NMB1296		hypothetical protein							0.614	1.67E-03
NMB1311		hypothetical protein							0.780	5.36E-06

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Table C.27 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1317		hypothetical protein					0.746	5.31E-03		
NMB1334		hypothetical protein	1.165	1.29E-08			-0.598	4.60E-03		
NMB1340		hypothetical protein							-2.010	2.01E-13
NMB1343		hypothetical protein							-0.990	2.37E-05
NMB1402		hypothetical protein	-0.649	6.29E-04					0.863	7.36E-07
NMB1404		hypothetical protein	-0.732	2.59E-03						
NMB1408		hypothetical protein	-1.165	1.08E-05					-0.835	3.92E-04
NMB1410		hypothetical protein	-0.874	3.66E-05					0.493	8.94E-03
NMB1468		hypothetical protein							-2.497	3.83E-14
NMB1469		hypothetical protein							-1.749	3.42E-03
NMB1486		hypothetical protein							-0.792	1.20E-05
NMB1495		hypothetical protein							0.971	1.47E-04
NMB1503		hypothetical protein							-0.773	8.76E-07
NMB1517		hypothetical protein							1.013	4.45E-08
NMB1523		hypothetical protein							-1.551	7.12E-11
NMB1534		hypothetical protein							-1.098	7.24E-07
NMB1544		hypothetical protein							1.187	2.04E-08
NMB1580		hypothetical protein							1.221	2.64E-08
NMB1596		hypothetical protein							0.824	4.90E-03
NMB1631		hypothetical protein							1.123	8.15E-04
NMB1633		hypothetical protein							1.164	5.72E-08
NMB1666		hypothetical protein							-1.664	1.71E-04
NMB1667		hypothetical protein							-0.675	4.65E-03
NMB1675		hypothetical protein							0.909	8.56E-05
NMB1681		hypothetical protein							-0.877	6.24E-06
NMB1699		hypothetical protein							0.599	2.18E-04
NMB1700		hypothetical protein							0.653	1.15E-03
NMB1742		hypothetical protein							1.598	3.27E-05
NMB1743		hypothetical protein							1.492	1.59E-04
NMB1744		hypothetical protein							0.678	6.71E-03
NMB1757		hypothetical protein							1.437	2.62E-06
NMB1758		hypothetical protein							0.714	1.35E-03
NMB1772		hypothetical protein					0.960	7.70E-03		
NMB1774		hypothetical protein							-0.886	6.79E-05
NMB1775		hypothetical protein					1.034	4.29E-03		
NMB1776		hypothetical protein					0.871	2.11E-03	-0.621	6.03E-03
NMB1777		hypothetical protein					0.593	5.31E-03	-1.570	3.77E-12
NMB1784		hypothetical protein							0.847	8.93E-05
NMB1786		hypothetical protein					0.780	2.53E-03		
NMB1800		hypothetical protein							0.652	4.78E-04
NMB1865		hypothetical protein							-0.767	1.04E-03

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Table C.27 – continued from previous page

ID	Name	Annotation	20→40 log$_2$ ratio	20→40 P-value*	40→60 log$_2$ ratio	40→60 P-value*	60→90 log$_2$ ratio	60→90 P-value*	90→240 log$_2$ ratio	90→240 P-value*
NMB1870		hypothetical protein							-0.706	9.86E-05
NMB1931		hypothetical protein							1.110	1.08E-07
NMB1977		hypothetical protein							1.036	2.24E-05
NMB2013		hypothetical protein							-1.697	1.00E-10
NMB2015		hypothetical protein							-1.339	2.45E-10
NMB2074		hypothetical protein							0.793	1.02E-05
NMB2094		hypothetical protein							1.153	4.03E-06
NMB2125		hypothetical protein							-0.586	7.76E-03
NMB2130		hypothetical protein							-0.912	9.40E-04
NMB2137		hypothetical protein							-4.052	7.21E-18
NMB2141		hypothetical protein							-2.130	9.64E-14
NMB2146		hypothetical protein							-0.992	8.35E-04
NMB2147		hypothetical protein							0.693	2.34E-06
NMB2149		hypothetical protein							0.898	2.02E-07
NMB2152		hypothetical protein							1.048	3.28E-08

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.28: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *mobile and extra chromosomal element functions*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40 log$_2$ ratio	20→40 P-value*	40→60 log$_2$ ratio	40→60 P-value*	60→90 log$_2$ ratio	60→90 P-value*	90→240 log$_2$ ratio	90→240 P-value*
Prophage functions										
NMB0748	*hfq*	host factor-I							-1.030	8.51E-04
NMB1104		phage sheath protein					1.223	2.11E-03		
Transposon functions										
NMB0225		transposase, IS30 family							0.861	8.76E-07
NMB0583		IS1016C2 transposase							1.255	2.89E-09
NMB0805		transposase, IS30 family							1.152	4.77E-09
NMB0991		IS1106 transposase							0.983	1.37E-07
NMB1022		transposase, IS30 family							0.592	2.03E-03
NMB1081		bacteriophage transposase							0.853	6.49E-03
NMB1099		transposase, IS30 family							0.699	7.61E-05
NMB1401		IS1016C2 transposase							1.289	1.04E-11
NMB1411		IS1016C2 transposase							1.436	1.04E-11
NMB1602		putative transposase							1.454	1.36E-17
NMB1713		transposase, IS30 family							1.111	2.27E-08

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Table C.28 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1770		transposase, IS30 family							0.748	4.37E-05
NMB2148		transposase, IS30 family							0.996	2.06E-07

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.29: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *protein fate*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Degradation of proteins, peptides and glycopeptides										
NMB0214	*prlC*	oligopeptidase A	2.227	2.07E-15						
NMB1231	*lon*	ATP-dependent protease La	1.681	4.27E-08					-1.754	1.71E-10
NMB1312	*clpP*	ATP-dependent Clp protease, proteolytic subunit							1.307	4.46E-09
NMB1332	*prc*	carboxy-terminal peptidase							1.367	5.02E-10
NMB1472	*clpB*	clpB protein	2.058	4.63E-16						
NMB1587		putative protease	-0.686	9.26E-04						
NMB1802	*gcp*	O-sialoglycoprotein endopeptidase					0.605	4.29E-03	-0.637	3.32E-04
NMB1969	*aspA/nalP*	putative serotype-1-specific antigen							-0.643	2.10E-04
Protein and peptide secretion and trafficking										
NMB0608	*secF*	protein-export membrane protein							-0.759	3.33E-05
NMB1789	*secB*	protein-export protein	1.223	1.46E-06					-1.255	1.34E-07
Protein folding and stabilization										
NMB0059	*dnaJ*	dnaJ protein	1.319	3.86E-14					-0.699	1.21E-06
NMB0278	*dsbA-1*	thiol:disulfide interchange protein	0.607	6.47E-05					-0.736	2.09E-07
NMB0294	*dsbA-2*	thiol:disulfide interchange protein	0.639	1.17E-03					-0.869	1.38E-06
NMB0554	*dnaK*	dnaK protein	1.187	9.51E-05					-0.821	2.35E-03
NMB0561	*grpE*	grpE protein	1.534	2.11E-08						
NMB0791	*ppiB*	peptidyl-prolyl cis-trans isomerase	1.481	1.63E-10					-1.612	1.55E-12
NMB1262	*ppiA*	peptidyl-prolyl cis-trans isomerase							0.668	7.45E-05
NMB1313	*tig*	trigger factor							-0.862	3.51E-03
NMB1522	*slyD*	FKBP-type peptidyl-prolyl cis-trans isomerase							-0.689	5.41E-04
NMB1972	*groEL*	chaperonin, 60 kDa	1.694	1.08E-05						
NMB1973	*groES*	chaperonin, 10 kDa	1.739	3.66E-05						
Protein modification and repair										
NMB0044	*pilB*	trifunctional thioredoxin/methionine sulfoxide reductase A/B protein	1.642	1.45E-08						

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.30: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *protein synthesis*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Ribosomal proteins: synthesis and modification										
NMB0127	*rplK*	50S ribosomal protein L11					-0.766	6.17E-03		
NMB0128	*rplA*	50S ribosomal protein L1					-0.690	9.05E-03		
NMB0130	*rplJ*	50S ribosomal protein L10							-1.216	4.53E-04
NMB0131	*rplL*	50S ribosomal protein L7 L12							-2.177	2.27E-15
NMB0136	*rpsL*	30S ribosomal protein S12							-0.851	5.65E-04
NMB0137	*rpsG*	30S ribosomal protein S7							-0.840	7.88E-04
NMB0140	*rpsJ*	30S ribosomal protein S10					-0.809	4.20E-03	-0.716	2.03E-03
NMB0144	*rplW*	50S ribosomal protein L23					-0.914	6.97E-03		
NMB0152	*rplN*	50S ribosomal protein L14							-0.877	4.14E-05
NMB0153	*rplX*	50S ribosomal protein L24							-0.761	4.69E-04
NMB0160	*rpmD*	50S ribosomal protein L30					-1.114	4.24E-03		
NMB0161	*rplO*	50S ribosomal protein L15					-0.911	2.81E-03		
NMB0164	*rpmJ*	50S ribosomal protein L36							1.090	4.03E-03
NMB0165	*rpsM*	30S ribosomal protein S13					-0.919	3.04E-03		
NMB0167	*rpsD*	30S ribosomal protein S4					-1.030	4.54E-03		
NMB0169	*rplQ*	50S ribosomal protein L17					-0.909	9.46E-03	-1.413	4.24E-06
NMB0321	*rpmB*	50S ribosomal protein L28							-1.988	4.03E-11
NMB0322	*rpmG*	50S ribosomal protein L33							-0.985	2.43E-04
NMB0324	*rpmA*	50S ribosomal protein L27							-1.983	3.61E-10
NMB0325	*rplU*	50S ribosomal protein L21							-1.614	1.06E-08
NMB0589	*rplS*	50s ribosomal protein L19					-0.767	3.21E-03	-0.902	2.72E-05
NMB0592	*rpsP*	30S ribosomal protein S16							-1.175	7.48E-06
NMB0609	*rpsO*	30s ribosomal protein S15							-1.442	5.85E-10
NMB0722	*rpmI*	50S ribosomal protein L35							-2.100	7.88E-13
NMB0723	*rplT*	50S ribosomal protein L20							-1.785	2.95E-11
NMB0876		50S ribosomal protein L25							-0.977	3.44E-09
NMB0941	*rpmJ2*	50S ribosomal protein L36	1.571	2.33E-07			-1.589	1.51E-04		
NMB0942	*rpmE2*	putative 50S ribosomal protein L31	2.361	2.55E-11			-1.241	1.21E-03	-1.556	3.78E-07
NMB1320	*rplI*	50S ribosomal protein L9							-1.719	3.85E-10
NMB1321	*rpsR*	30S ribosomal protein S18					-0.746	4.14E-03	-2.518	2.19E-18
NMB1323	*rpsF*	30S ribosomal protein S6							-2.945	1.01E-15
NMB1911	*rpmF*	50S ribosomal protein L32							-0.825	4.01E-03
NMB1950	*rpsU*	30S ribosomal protein S21							-1.946	1.10E-10
NMB1956	*rpmE*	50S ribosomal protein L31							-2.061	3.81E-09
NMB2056	*rpsI*	30S ribosomal protein S9					-0.643	6.82E-03	-1.370	6.48E-10
NMB2057	*rplM*	50S ribosomal protein L13							-1.377	3.81E-08

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Table C.30 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB2101	*rpsB*	30S ribosomal protein S2					-0.819	6.06E-03	-1.912	2.67E-11
Translation factors										
NMB0163	*infA*	translation initiation factor IF-1							1.393	2.08E-04
NMB0187	*frr*	ribosome recycling factor							-0.848	7.45E-05
NMB0626	*prfC*	peptide chain release factor 3							0.635	1.29E-05
NMB1643	*infB*	translation initiation factor IF-2	-0.674	8.94E-04					-1.351	3.69E-12
tRNA aminoacylation										
NMB0003	*gltX*	glutamyl-tRNA synthetase							0.701	6.40E-05
NMB0347		D-tyrosyl-tRNA deacylase							0.790	1.24E-04
NMB0466	*aspS*	aspartyl-tRNA synthetase					-0.619	6.22E-03	0.876	8.26E-06
NMB0720	*thrS*	threonyl-tRNA synthetase					-0.691	3.21E-03	0.930	2.18E-06
NMB1356	*gatA*	Glu-tRNA(Gln) amidotransferase, subunit A							-1.272	1.83E-12
tRNA and rRNA base modification										
NMB0590	*trmD*	tRNA (guanine-N1)-methyltransferase					-0.725	4.54E-03		
NMB0697	*ksgA*	dimethyladenosine transferase					-0.614	2.81E-03	1.051	4.15E-09

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.31: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *purines, pyrimidines, nucleosides and nucleotides biosynthesis*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
2'-Deoxyribonucleotide metabolism										
NMB0639		putative deoxyribonucleotide triphosphate pyrophosphatase							0.655	6.05E-04
Nucleotide and nucleoside interconversions										
NMB0849		putative deoxycytidine triphosphate deaminase	0.890	4.78E-04						
NMB1307	*ndk*	nucleoside diphosphate kinase					-0.628	5.31E-03		
NMB2103	*pyrH*	uridylate kinase							0.586	2.52E-04
Purine ribonucleotide biosynthesis										
NMB0757	*purC*	phosphoribosylaminoimidazole-succinocarboxamide synthase							1.159	3.08E-06
NMB1019	*purK*	phosphoribosylaminoimidazole carboxylase, ATPase subunit							0.595	1.91E-03
NMB1201	*guaB*	IMP dehydrogenase							-0.949	3.55E-07
NMB1439	*purE*	phosphoribosylaminoimidazole carboxylase, catalytic subunit							-0.666	9.69E-04
NMB1920	*guaA*	GMP synthase							1.624	4.91E-11
NMB1996	*purL*	phosphoribosylformylglycinamidine synthase							1.011	5.49E-07
Pyrimidine ribonucleotide biosynthesis										
NMB0106	*pyrB*	aspartate carbamoyltransferase, catalytic subunit							-0.607	5.43E-07

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Table C.31 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB0682	*pyrC*	dihydroorotase							-0.819	2.02E-07
NMB0824	*pyrF*	orotidine 5'-phosphate decarboxylase							1.044	8.20E-09
NMB1554	*pyrG*	CTP synthase							-0.767	1.90E-05
Salvage of nucleosides and nucleotides										
NMB0774	*upp*	uracil phosphoribosyltransferase							-0.762	8.46E-05

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.32: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *regulatory functions*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Other										
NMB0044	*pilB*	trifunctional thioredoxin/methionine sulfoxide reductase A/B protein	1.642	1.45E-08						
NMB0115	*ntrX*	nitrogen assimilation regulatory protein NtrX					0.736	1.51E-04		
NMB0173	*oxyR*	transcriptional regulator, LysR family							-0.848	3.58E-08
NMB0205	*fur*	ferric uptake regulation protein							-1.307	1.44E-11
NMB0573		transcriptional regulator, AsnC family							0.808	3.60E-06
NMB0595		DNA-binding response regulator	0.598	6.75E-03					-0.811	2.37E-05
NMB0729	*himA*	integration host factor, α subunit							-1.643	4.94E-13
NMB0748	*hfq*	host factor-I							-1.030	8.51E-04
NMB0810		transcriptional regulator, TetR family							1.045	3.86E-05
NMB1007		transcriptional regulator							1.969	1.51E-15
NMB1204		transcriptional regulator	-0.664	9.15E-04						
NMB1249		putative nitrate nitrite sensory protein NarX							0.730	1.07E-07
NMB1302	*hip*	integration host factor, β subunit							-1.296	8.49E-08
NMB1563		transcriptional regulator, GntR family	0.749	1.66E-03						
NMB1792		sensor histidine kinase					0.652	9.46E-03		
NMB1967		transcriptional regulator, AraC family							1.085	1.10E-04
NMB2012		transcriptional regulator, HTH 3 family							-1.066	1.15E-10
NMB2075	*birA*	BirA protein Bvg accessory factor							-0.647	1.52E-04

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.33: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into functional group *transcription*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Degradation of RNA										
NMB0662		putative ribonuclease							-0.718	7.49E-05
DNA-dependent RNA polymerase										
NMB0132	*rpoB*	DNA-directed RNA polymerase, β subunit					-0.869	5.31E-03		
NMB0133	*rpoC*	DNA-directed RNA polymerase, β' subunit					-0.755	7.00E-03		
NMB0168	*rpoA*	DNA-directed RNA polymerase, α subunit							-1.130	1.40E-06
NMB1660	*rpoZ*	DNA-directed RNA polymerase, ω subunit							1.621	3.82E-03
RNA processing										
NMB0196	*rne*	ribonuclease E							-0.771	2.86E-06
NMB1905	*rnpA*	ribonuclease P, protein component							-0.864	1.21E-05
Transcription factors										
NMB0075		putative transcriptional accessory protein Tex							1.416	4.30E-11
NMB0689	*greB*	transcription elongation factor							0.595	9.49E-03
NMB1368		putative ATP-dependent RNA helicase	-0.991	8.46E-05						
NMB1430	*greA*	transcription elongation factor	-0.673	7.69E-03						
NMB1538	*rpoD*	RNA polymerase sigma factor	1.024	2.10E-05					0.676	7.89E-04
NMB1642	*nusA*	N utilization substance protein A							-0.743	8.82E-04

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.34: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) categorised into TIGR role category transport and binding proteins. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Amino acids, peptides and amines										
NMB0623	*potD-2*	spermidine/putrescine ABC transporter, periplasmic substrate-binding protein							-0.787	8.30E-05
NMB0787		amino acid ABC transporter, periplasmic substrate-binding protein					-0.615	7.00E-03	-0.735	1.64E-04
NMB0789		amino acid ABC transporter, ATP-binding protein							0.694	3.27E-07
NMB1594	*potD-3*	spermidine/putrescine ABC transporter, periplasmic substrate-binding protein					-0.626	8.96E-03	-0.853	3.78E-05
NMB1612	*hisJ*	amino acid ABC transporter, periplasmic substrate-binding protein							-0.654	1.66E-03
NMB1965		L-glutamate ABC transporter, putative periplasmic transport protein							1.168	1.94E-06

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Table C.34 – continued from previous page

ID	Name	Annotation	20→40		40→60		60→90		90→240	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Anions										
NMB0880	*cysW*	sulfate ABC transporter, permease protein							1.570	1.88E-06
NMB0881	*cysU*	sulfate ABC transporter, permease protein							0.770	2.04E-03
Carbohydrates, organic alcohols, and acids										
NMB0073	*ctrC*	capsule polysaccharide export, inner-membrane protein					-0.609	2.11E-03		
Cations and iron carrying compounds										
NMB0175		putative zinc transporter ZupT					0.939	3.04E-03		
NMB0293		putative TonB-dependent receptor					0.694	4.20E-03		
NMB0460	*tbpB*	transferrin-binding protein 2	0.975	3.25E-05						
NMB0578	*nosD*	copper ABC transporter, periplasmic substrate-binding protein							0.667	4.28E-03
NMB0586		putative zinc/manganese transporter	1.141	7.65E-09						
NMB0634	*fbpA*	iron(III) ABC transporter, periplasmic substrate-binding protein	1.141	6.13E-06					-1.225	1.90E-07
NMB0752		putative bacterioferritin-associated ferredoxin	0.998	6.71E-05						
NMB0964		TonB-dependent receptor	1.005	2.95E-03						
NMB1206	*bfrB*	bacterioferritin B	-1.289	8.01E-08					0.950	1.04E-05
NMB1207	*bfrA*	bacterioferritin A	-1.217	2.58E-07					1.285	1.07E-08
NMB1271		putative mercury transport periplasmic protein							0.690	3.83E-04
NMB1497		TonB-dependent receptor	0.634	1.11E-03						
NMB1541	*lbpB*	lactoferrin-binding protein B	0.615	6.11E-04					0.623	7.74E-04
NMB1730	*tonB*	TonB protein	0.585	5.19E-03					-0.824	7.85E-06
NMB1882		TonB-dependent receptor							-0.677	2.82E-03
NMB1989	*fetB*	iron(III) ABC transporter, periplasmic substrate-binding protein							0.827	3.04E-05
Other										
NMB0318	*farA*	fatty acid efflux system protein							0.796	1.47E-04
NMB0319	*farB*	fatty acid efflux system protein					0.588	3.26E-03		
NMB0812	*norM*	multidrug efflux protein					0.870	3.21E-03		
NMB1277		transporter, BCCT family							0.720	8.57E-05
NMB1716	*mtrC*	membrane fusion protein							0.802	1.96E-04
NMB1728	*exbD*	biopolymer transport protein							-1.566	4.44E-13
NMB1729	*exbB*	biopolymer transport protein							-1.141	2.95E-04
NMB1738	*hlyD*	putative secretion protein					0.823	4.20E-03		
NMB1429	*porA*	outer membrane protein PorA							-1.690	5.01E-08
NMB2039	*porB*	major outer membrane protein PIB							-2.222	2.02E-11
Unknown substrate										
NMB0387		ABC transporter, ATP-binding protein							-0.596	4.54E-06
NMB0549		ABC transporter, ATP-binding protein							0.723	2.09E-06
NMB1051	*abcZ*	ABC transporter, ATP-binding protein							0.720	1.65E-05
NMB1400		ABC transporter family protein					0.887	2.11E-03		
NMB1707		sodium- and chloride-dependent transporter							0.845	2.02E-05
NMB2026		ABC transporter, permease protein							-0.689	4.41E-03

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.35: Differentially expressed genes between time-points in blood (20→40, 40→60, 60→90 and 90→240) with unknown function. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

			20→40		40→60		60→90		90→240	
ID	**Name**	**Annotation**	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***
NMB0009		BolA/YrbA family protein							-0.742	7.16E-04
NMB0255		cell filamentation protein Fic-related protein							1.081	2.22E-09
NMB0360		AmpG-related protein					0.691	8.07E-03		
NMB0374	*mafB2*	MafB-related protein							0.916	6.32E-09
NMB0490		PspA-related protein					0.631	4.28E-03	0.834	8.88E-06
NMB0496		hemolysin activator-related protein							0.798	4.00E-04
NMB0548		AcrA/AcrE family protein							-0.661	2.82E-03
NMB0577		NosR-related protein							0.679	2.83E-03
NMB0653		MafB-related protein							0.994	8.40E-05
NMB0963		phosphatidylserine decarboxylase precursor-related protein							0.592	9.49E-03
NMB0985		E16-related protein							1.547	7.21E-18
NMB1335	*creA*	creA protein	1.441	6.52E-15			-0.618	1.16E-03		
NMB1337		putative Holliday junction resolvase-like protein	0.678	2.72E-06						
NMB1347	*suhB*	extragenic suppressor protein SuhB					-0.586	3.21E-03		
NMB1380		nifU protein	0.727	1.48E-03						
NMB1381		HesB/YadR/YfhF family protein	0.588	2.83E-03					-0.954	1.26E-07
NMB1409		FrpA/C-related protein	-0.976	5.01E-07						
NMB1516		fixS protein							0.699	2.41E-05
NMB1657		comE operon protein 1-related protein							-0.620	3.91E-03
NMB1689		putative dedA protein							-0.644	5.27E-05
NMB1692		chorismate mutase-related protein					0.749	5.31E-03	-0.876	1.24E-04
NMB1747		putative tspB protein					0.745	9.46E-03		
NMB2008		ABC transporter, ATP-binding protein-related protein							-0.659	8.13E-05
NMB2105	*mafB*	mafB protein							0.641	7.69E-06

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.36: Genes differentially expressed between time-points in blood divided into cellular locations based on predictions and/or published experimental data. Only those genes which are up-regulated between any of the five time-points in blood (indicated by 'Up') and which encode proteins that have a known or putative location in the periplasm or the outer membrane are listed in the table.

ID	Name	20→40	40→60	60→90	90→240	Experimental	PSORTb Location	PSLpred Location	CBS Prediction	SignalP
NMB0003	*gltX*				Up	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0035		Up			Down	OMP (Arenas *et al.*, 2006); OM and OMVs (Ferrari *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	LipoP	√
NMB0044	*pilB*	Up				One type is associated with OM (Skaar *et al.*, 2002);OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	LipoP	√
NMB0046					Up		Unknown	Periplasmic	Cytoplasmic	
NMB0078	*galE2*			Up			Unknown	Periplasmic	SignalP	√
NMB0123				Down	Up		Cytoplasmic	Extracellular	Cytoplasmic	
NMB0176	*dadA*				Up		Unknown	Cytoplasmic	SignalP	√
NMB0214	*prlC*	Up				Surface located (Grifantini *et al.*, 2002a); OM and OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0216	*katA*	Up					Periplasmic	Periplasmic	Cytoplasmic	
NMB0225					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0278	*dsbA-1*	Up			Down	OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	LipoP	√
NMB0293				Up			Outer membrane	Outer membrane	SignalP	√
NMB0294	*dsbA-2*	Up			Down	OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Periplasmic	Periplasmic	LipoP	√
NMB0318	*farA*				Up		Unknown	Outer membrane	SignalP	√
NMB0334	*pgi-2*			Up			Cytoplasmic	Outer membrane	Cytoplasmic	
NMB0337	*ilvE*	Up			Down	OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0345		Up		Down		OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	√
NMB0346		Up				OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Multiple locations	Cytoplasmic	SignalP	√
NMB0359	*glnA*	Up		Down		OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0369					Up		Unknown	Cytoplasmic	SecretomeP	
NMB0372					Up		Unknown	Periplasmic	SecretomeP	
NMB0374	*mafB2*				Up		Unknown	Outer membrane	SignalP	√
NMB0392	*nadB*			Up	Down		Periplasmic	Periplasmic	Cytoplasmic	
NMB0460	*tbpB*	Up				OMVs (Ferrari *et al.*, 2006)	Outer membrane	Outer membrane	SecretomeP	
NMB0476					Up		Unknown	Periplasmic	Cytoplasmic	
NMB0490				Up	Up		Unknown	Outer membrane	SecretomeP	
NMB0493					Up		Outer membrane	Outer membrane	SecretomeP	
NMB0496					Up		Outer membrane	Outer membrane	Cytoplasmic	
NMB0497				Up	Up		Outer membrane	Extracellular	SecretomeP	
NMB0499				Up			Unknown	Periplasmic	TMHMM	

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Table C.36 – continued from previous page

ID	Name	20→40	40→60	60→90	90→240	Experimental	PSORTb Location	PSLpred Location	CBS Prediction	SignalP
NMB0509				Up			Unknown	Periplasmic	SecretomeP	
NMB0540	*aspC*				Up	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0549	*yjbZ*				Up	OM (Williams *et al.*, 2007)	Inner membrane	Inner membrane	TMHMM	
NMB0554	*dnaK*	Up			Down	Plasminogen binding (Knaust *et al.*, 2007); OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	Cytoplasmic	
NMB0561	*grpE*	Up				OM (Williams *et al.*, 2007)	Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0578	*nosD*				Up		Periplasmic	Periplasmic	Cytoplasmic	
NMB0586		Up				OMVs (Vipond *et al.*, 2006)	Inner membrane	Periplasmic	SignalP	√
NMB0595		Up			Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0618	*ppsA*	Up				OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0631		Up			Down	OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0634	*fbpA*	Up			Down	OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Periplasmic	Periplasmic	SignalP	√
NMB0639					Up		Unknown	Periplasmic	SecretomeP	
NMB0647					Up		Unknown	Cytoplasmic	SecretomeP	
NMB0653					Up		Unknown	Extracellular	SignalP	√
NMB0655					Up	Surface located (Grifantini *et al.*, 2002a)	Unknown	Periplasmic	SecretomeP	
NMB0681					Up		Unknown	Periplasmic	Cytoplasmic	
NMB0701					Up		Unknown	Extracellular	Cytoplasmic	
NMB0717					Up		Unknown	Periplasmic	SignalP	√
NMB0720	*thrS*			Down	Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0752		Up					Unknown	Extracellular	Cytoplasmic	
NMB0791	*ppiB*	Up			Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0798	*ftsH*	Up				OMVs (Williams *et al.*, 2007)	Inner membrane	Inner membrane	TMHMM	√
NMB0805					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0821					Up		Unknown	Outer membrane	TMHMM	
NMB0850		Up					Unknown	Periplasmic	SignalP	√
NMB0855					Up		Unknown	Cytoplasmic	SignalP	√
NMB0884	*sodB*				Up	OM (Williams *et al.*, 2007)	Unknown	Extracellular	SecretomeP	
NMB0907		Up			Up		Unknown	Extracellular	SecretomeP	
NMB0942	*rpmE2*	Up		Down	Down		Unknown	Periplasmic	SecretomeP	
NMB0946		Up			Down	Plasminogen binding (Knaust *et al.*, 2007); OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	SecretomeP	
NMB0947	*lpdA2*	Up			Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0957	*lpdA3*				Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0964		Up				OMVs (Vipond *et al.*, 2006)	Unknown	Outer membrane	SignalP	√
NMB1006					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1009					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1010					Up		Unknown	Periplasmic	LipoP	√
NMB1022					Up		Cytoplasmic	Periplasmic	Cytoplasmic	

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Table C.36 – continued from previous page

ID	Name	20→40	40→60	60→90	90→240	Experimental	PSORTb Location	PSLpred Location	CBS Prediction	SignalP
NMB1081					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1099					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1100					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1206	*bfrB*	Down			Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1207	*bfrA*	Down			Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1211					Up		Unknown	Periplasmic	LipoP	√
NMB1212					Up		Unknown	Periplasmic	LipoP	√
NMB1213					Up		Unknown	Cytoplasmic	LipoP	√
NMB1214					Up		Outer membrane	Outer membrane	TMHMM	
NMB1262	*ppiA*				Up		Periplasmic	Periplasmic	SignalP	√
NMB1294	*plsC*			Up			Unknown	Cytoplasmic	SignalP	√
NMB1311					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1312	*clpP*				Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1317				Up			Unknown	Extracellular	TatP	√
NMB1332	*prc*				Up	OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	SignalP	√
NMB1334		Up		Down			Unknown	Periplasmic	Cytoplasmic	
NMB1366	*trxA*	Up			Down	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1377	*lldA*	Up				OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1379	*nifS*	Up				OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1380		Up				OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1412					Up		Unknown	Periplasmic	SignalP	√
NMB1472	*clpB*	Up				OM and OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1475		Up					Unknown	Periplasmic	SignalP	√
NMB1497		Up				OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	√
NMB1538	*rpoD*	Up			Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1541	*lbpB*	Up			Up		Outer membrane	Cytoplasmic	LipoP	√
NMB1544					Up		Unknown	Periplasmic	SecretomeP	
NMB1578					Up	OMVs (Ferrari *et al.*, 2006)	Unknown	Periplasmic	LipoP	√
NMB1579	*hisG*				Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1596					Up		Unknown	Outer membrane	Cytoplasmic	
NMB1602					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1609	*metZ*				Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Inner membrane	Cytoplasmic	
NMB1633					Up		Unknown	Periplasmic	SecretomeP	
NMB1654					Up		Unknown	Periplasmic	SignalP	√
NMB1660	*rpoZ*				Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1683					Up		Cytoplasmic	Outer membrane	Cytoplasmic	
NMB1700					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1716	*mtrC*				Up	OMVs (Williams *et al.*, 2007)	Inner membrane	Cytoplasmic	LipoP	√
NMB1738	*hlyD*			Up			Inner membrane	Periplasmic	Cytoplasmic	

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Table C.36 – continued from previous page

ID	Name	20→40	40→60	60→90	90→240	Experimental	PSORTb Location	PSLpred Location	CBS Prediction	CBS SignalP
NMB1747				Up			Outer membrane	Periplasmic	TMHMM	
NMB1749					Up		Unknown	Periplasmic	TMHMM	
NMB1762					Up		Outer membrane	Outer membrane	SignalP	√
NMB1772				Up			Unknown	Periplasmic	TMHMM	
NMB1775				Up			Outer membrane	Periplasmic	TMHMM	√
NMB1776				Up	Down		Unknown	Extracellular	SignalP	√
NMB1779				Up	Up		Outer membrane	Extracellular	SecretomeP	
NMB1784					Up		Unknown	Periplasmic	SecretomeP	
NMB1789	*secB*	Up			Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1800					Up		Unknown	Extracellular	SecretomeP	
NMB1802	*gcp*			Up	Down		Extracellular	Extracellular	Cytoplasmic	
NMB1972	*groEL*	Up				OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1973	*groES*	Up				OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1977					Up	OM (Williams *et al.*, 2007)	Unknown	Outer membrane	LipoP	√
NMB1989	*fetB*				Up		Periplasmic	Periplasmic	LipoP	√
NMB1996	*purL*				Up	OMVs (Vaughan *et al.*, 2006)	Outer membrane	Cytoplasmic	Cytoplasmic	
NMB2000	*hslO*	Up			Down	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB2074					Up		Unknown	Periplasmic	Cytoplasmic	
NMB2105	*mafB*				Up		Unknown	Periplasmic	SignalP	√
NMB2147					Up		Unknown	Extracellular	LipoP	√
NMB2148					Up		Cytoplasmic	Periplasmic	Cytoplasmic	

OM or OMVs in the table indicate that the protein has been detected in outer membrane or outer membrane vesicles preparations, respectively. For predictions the algorithms PSORTb, PSLpred, and tools at CBS were used. Denominations from CBS: SignalP, the protein encodes a signal peptide; TMHMM, the protein encodes transmembrane helices; LipoP, the proteins is a lipoprotein; SecretomeP, the protein is secreted through a non-classical i.e. not signal peptide triggered protein secretion and TatP, the protein has a twin-arginine signal peptide cleavage sites.

Table C.37: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *amino acid biosynthesis*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Aromatic amino acid family										
NMB0307	*aroG*	phospho-2-dehydro-3-deoxyheptonate aldolase, phe-sensitive							1.288	3.32E-06
NMB0440		putative prephenate dehydrogenase					0.617	7.12E-06		
NMB0678	*trpA*	tryptophan synthase, α subunit							-0.599	8.77E-05
NMB1432	*aroA*	3-phosphoshikimate 1-carboxyvinyltransferase							-0.711	5.34E-03
Aspartate family										
NMB0203	*dapB*	dihydrodipicolinate reductase							-1.005	3.86E-11
NMB0943	*metF*	5,10-methylenetetrahydrofolate reductase			-0.728	3.99E-03	-1.503	1.15E-10	-1.290	1.96E-08
NMB0944	*metH*	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase					-1.212	8.61E-06	-2.135	1.85E-12
NMB1046	*thrC*	threonine synthase							-0.594	1.76E-03
NMB1498	*lysC*	aspartokinase, α and β subunits							-1.346	1.46E-07
NMB2079	*asd*	aspartate-semialdehyde dehydrogenase							-0.656	3.99E-03
Glutamate family										
NMB0055	*proC*	pyrroline-5-carboxylate reductase							0.923	1.49E-05
NMB0224	*glnE*	glutamate-ammonia-ligase adenylyltransferase					0.768	4.07E-05	0.937	6.91E-07
NMB0359	*glnA*	glutamate ammonia ligase	0.591	1.75E-03						
NMB1476	*gluD*	glutamate dehydrogenase, NAD-specific							1.089	3.12E-04
NMB1573	*argF*	ornithine carbamoyltransferase, catabolic							-0.671	2.93E-05
NMB1710	*gdhA*	glutamate dehydrogenase, NADP-specific					-0.990	1.65E-03	-1.987	4.41E-09
NMB1787	*argC*	N-acetyl-gamma-glutamyl-phosphate reductase					0.649	7.94E-03		
NMB1995	*glnB*	nitrogen regulatory protein P-II							-0.973	2.30E-05
Histidine family										
NMB0603	*hisE*	phosphoribosyl-ATP pyrophosphatase					-0.623	1.34E-04		
NMB0627	*hisI*	phosphoribosyl-AMP cyclohydrolase							-0.954	9.43E-03
NMB1579	*hisG*	ATP phosphoribosyltransferase			0.621	1.29E-03			1.362	2.01E-12
NMB1581	*hisD*	histidinol dehydrogenase					0.648	8.86E-03		
Pyruvate family										
NMB0337	*ilvE*	putative branched-chain amino acid aminotransferase	0.748	1.11E-03	0.657	7.14E-03				
NMB1031	*leuB*	3-isopropylmalate dehydrogenase					-0.600	2.08E-04		
NMB1034	*leuD*	3-isopropylmalate dehydratase, small subunit					-0.595	1.15E-03	-0.679	1.77E-04
NMB1150	*ilvD-1*	dihydroxy-acid dehydratase							-1.108	1.31E-05
NMB1188	*ilvD-2*	dihydroxy-acid dehydratase							-1.073	5.34E-06
NMB1574	*ilvC*	ketol-acid reductoisomerase							-1.446	8.80E-11
Serine family										
NMB0381	*cysB*	cys regulon transcriptional activator							0.750	1.22E-07
NMB0763	*cysK*	cysteine synthase							-1.965	2.05E-17
NMB1055	*glyA*	serine hydroxymethyltransferase							-0.761	3.84E-05

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.38: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *biosynthesis of co-factors, prosthetic groups and carriers*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Biotin										
NMB0474	*bioC*	putative biotin synthesis protein							0.840	2.43E-06
NMB1146	*bioB-1*	biotin synthetase							-1.073	3.57E-08
NMB1184	*bioB-2*	biotin synthetase							-1.096	2.38E-08
Folic acid										
NMB0966	*trpG*	para-aminobenzoate synthase glutamine amidotransferase, component II					0.658	6.67E-04		
NMB1055	*glyA*	serine hydroxymethyltransferase							-0.761	3.84E-05
NMB1063	*folB*	dihydroneopterin aldolase							1.176	2.83E-10
Heme, porphyrin, and cobalamin										
NMB0801	*hemB*	delta-aminolevulinic acid dehydratase							-0.648	6.46E-04
Menaquinone and ubiquinone										
NMB0743	*ubiE*	ubiquinone menaquinone biosynthesis methlytransferase							-0.737	5.36E-06
Other										
NMB0186	*uppS*	undecaprenyl pyrophosphate synthetase							-0.632	3.39E-05
NMB0527		putative 6-pyruvoyl tetrahydrobiopterin synthase			-0.702	1.62E-03				
NMB1130		putative phytoene synthase							-0.681	3.14E-05
NMB1168		putative phytoene synthase							-0.727	1.37E-05
NMB1379	*nifS*	nifS protein	0.943	4.14E-04	0.956	1.29E-03	0.899	5.36E-04	1.387	2.41E-07
Pantothenate and coenzyme A										
NMB0870	*panB*	3-methyl-2-oxobutanoate hydroxymethyltransferase							-0.778	1.15E-06
NMB1282	*panD*	aspartate 1-decarboxylase							-0.686	2.26E-07
Pyridine nucleotides										
NMB0392	*nadB*	l-aspartate oxidase					1.031	1.04E-06		
NMB0394	*nadA*	quinolinate synthetase A			0.776	1.43E-05	1.482	1.56E-14	1.523	4.01E-15
NMB0396	*nadC*	nicotinate-nucleotide pyrophosphorylase	0.742	2.73E-04	0.807	1.55E-04	1.258	1.63E-09	1.288	3.22E-10
NMB1364	*nadE*	putative NH(3)-dependent NAD+ synthetase					0.676	7.16E-04		
NMB2157	*pncA*	putative pyrazinamidase/ nicotinamidase					-0.805	2.11E-04		
Pyridoxine										
NMB1360	*pdxH*	pyridoxamine 5-phosphate oxidase					0.719	5.23E-03	1.046	5.11E-05
Riboflavin, FMN, and FAD										
NMB1817	*ribD*	riboflavin biosynthesis protein					0.615	6.15E-03		
Thiamine										
NMB2040	*thiC*	thiamine biosynthesis protein							1.184	9.36E-07
NMB2067	*cytX*	probable hydroxymethylpyrimidine transporter CytX					0.735	3.17E-03	0.908	2.34E-04
NMB2069	*thiE*	thiamin-phosphate pyrophosphorylase							1.419	1.69E-07
NMB2071	*thiG*	thiG protein					0.728	7.61E-04	0.972	2.27E-05

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.39: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into TIGR designation *cell envelope*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Biosynthesis and degradation of murein sacculus and peptidoglycan										
NMB0414	*murE*	UDP-N-acetylmuramoylalanyl-D-glutamate 2,6-diaminopimelate ligase							-0.603	8.61E-06
NMB0422	*murG*	UDP-N-acetylglucosamine N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase							0.936	1.24E-06
NMB0423	*murC*	UDP-N-acetylmuramate alanine ligase							-0.630	4.90E-06
NMB0749		penicillin-binding protein 4							0.620	5.35E-05
NMB0877		penicillin-binding protein							-3.722	4.39E-09
NMB1085		putative N-acetylmuramoyl-L-alanine amidase							1.414	9.61E-08
NMB1145	*mpl-1*	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase							-0.780	1.68E-03
NMB1183	*mpl-2*	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase							-0.648	3.69E-04
NMB1797		penicillin-binding protein 3							-0.831	3.35E-07
Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides										
NMB0014	*kdtA*	3-deoxy-D-manno-octulosonic-acid transferase							1.048	1.10E-05
NMB0062	*rfbA-1*	glucose-1-phosphate thymidylyltransferase							1.169	1.19E-10
NMB0063	*rfbB-1*	dTDP-D-glucose 4,6-dehydratase							1.423	2.88E-16
NMB0064	*galE-1*	UDP-glucose 4-epimerase					0.642	1.91E-03	0.801	1.02E-04
NMB0068	*siaC*	polysialic acid capsule biosynthesis protein					-0.862	1.35E-03	-0.757	4.05E-03
NMB0069	*siaB*	polysialic acid capsule biosynthesis protein					-1.007	2.79E-05	-1.185	8.65E-07
NMB0070	*siaA*	polysialic acid capsule biosynthesis protein					-1.012	1.08E-05	-0.877	7.55E-05
NMB0078	*galE-2*	UDP-glucose 4-epimerase (truncation)					0.697	1.33E-04		
NMB0079	*rfbB-2*	dTDP-D-glucose 4,6-dehydratase							1.503	1.15E-13
NMB0080	*rfbA-2*	glucose-1-phosphate thymidylyltransferase							1.013	8.04E-09
NMB0082	*lipA*	capsule polysaccharide modification protein					0.592	6.49E-03		
NMB0083	*lipB*	capsule polysaccharide modification protein					0.587	3.40E-03	0.775	1.01E-04
NMB0199	*lpxB*	lipid-A-disaccharide synthase					0.837	2.79E-05	0.803	3.67E-05
NMB0544		UDP-2,3-diacylglucosamine hydrolase					0.635	9.64E-05	0.588	2.09E-04
NMB0790	*pgm*	phosphoglucomutase	0.688	1.40E-05						
NMB0825	*rfaE*	putative ADP-heptose synthase					-0.879	3.15E-06	-1.412	5.76E-12
NMB0828	*rfaD*	ADP-L-glycero-D-mannoheptose-6-epimerase					-0.940	2.36E-05		
NMB1279	*mltB*	putative membrane-bound lytic murein transglycosylase B							-0.890	1.54E-05
NMB1283	*kdsA*	2-dehydro-3-deoxyphosphooctonate aldolase							-0.603	6.82E-07
NMB1297	*mltD*	membrane-bound lytic murein transglycosylase D							-0.901	6.44E-10
NMB2156	*rfaC*	heptosyltransferase I					-0.736	2.16E-04		
Other										
NMB0345		putative cell-binding factor	0.709	9.37E-03	0.920	2.63E-03				

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Table C.39 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0382	*rmpM*	outer membrane protein class 4							-1.698	1.01E-11
NMB0663	*nspA*	outer membrane protein	-1.122	1.30E-07	-1.662	1.23E-11	-2.138	2.09E-17	-3.197	2.41E-25
NMB0703	*comL*	competence lipoprotein							-0.699	2.98E-07
NMB0946		peroxiredoxin 2 family protein/glutaredoxin	1.062	4.02E-04					-3.224	1.62E-18
NMB1053	*opcA*	class 5 outer membrane protein					-0.985	2.60E-03	-1.746	2.98E-07
NMB1213		putative lipoprotein					-0.915	6.90E-03		
NMB1533		H.8 outer membrane protein							-2.072	9.49E-14
NMB1592		putative lipoprotein					-0.771	4.33E-05	-0.709	1.12E-04
NMB1623	*aniA*	major anaerobically induced outer membrane protein					-1.167	1.04E-03		
NMB1716	*mtrC*	membrane fusion protein							1.301	3.08E-07
NMB1907	*yidC*	putative inner membrane protein, translocase component							-0.676	5.76E-05
NMB1946		outer membrane lipoprotein							-0.618	5.31E-03
Surface structures										
NMB0018	*pilE*	pilin							-1.394	2.69E-08
NMB0333	*pilG*	pilus assembly protein							-0.855	2.02E-07
NMB0770		putative type IV pilus assembly protein PilZ							-0.882	5.33E-04
NMB0888	*pilW*	type IV pilus assembly protein PilW			-0.669	4.82E-03	-1.007	2.57E-06	-0.894	1.33E-05
NMB1808	*pilM*	pilM protein					-0.770	3.37E-03		
NMB1810	*pilO*	pilO protein					-0.702	6.90E-03	-0.725	4.58E-03
NMB1811	*pilP*	pilP protein					-0.652	5.81E-03	-0.698	2.73E-03
NMB1812	*pilQ*	pilQ protein							-1.108	3.00E-04
NMB1847	*pilC-1*	pilC1 protein (authentic frameshift)							0.781	1.98E-05

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.40: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *cellular processes*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
Cell division										
NMB0171	*minD*	septum site-determining protein							-1.031	5.18E-05
NMB0172	*minE*	cell division topological specificity factor					-0.618	4.76E-04	-1.448	1.19E-12
NMB0426	*ftsA*	cell division protein							-0.710	1.08E-06
NMB0427	*ftsZ*	cell division protein							-0.708	3.61E-04
NMB0798	*ftsH*	cell division protein	0.672	1.88E-03						
NMB0799	*ftsJ*	cell division protein							0.786	5.27E-09

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Table C.40 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Chemotaxis and motility										
NMB0051	*pilU*	twitching motility protein							-1.221	6.13E-17
NMB0052	*pilT-1*	twitching motility protein							-0.894	4.16E-07
NMB0768	*pilT-2*	twitching motility protein							-0.764	1.57E-06
Detoxification										
NMB0005	*arsC*	arsenate reductase							0.862	4.64E-05
NMB0216	*katA*	catalase	1.831	5.31E-05	1.925	3.26E-05	2.476	4.27E-08	1.658	4.92E-05
NMB0525		putative aluminum resistance protein	-0.682	1.53E-05	-0.851	3.10E-07	-0.608	3.96E-05		
NMB1398	*sodC*	Cu-Zn-superoxide dismutase					-0.918	2.60E-04	-1.186	2.97E-06
NMB1621	*gpxA*	glutathione peroxidase							-1.135	4.35E-09
NMB1714	*mtrE*	multidrug efflux pump channel protein					0.611	1.10E-03	0.698	1.59E-04
DNA transformation										
NMB0702	*comA*	competence protein							0.923	4.11E-04
NMB0703	*comL*	competence lipoprotein							-0.699	2.98E-07
Other										
NMB1981	*luxS*	S-ribosylhomocysteinase	0.756	6.54E-04						
Pathogenesis										
NMB0018	*pilE*	pilin PilE							-1.394	2.69E-08
NMB0277	*mviN*	virulence factor MviN							0.594	9.77E-05
NMB0333	*pilG*	pilus assembly protein							-0.855	2.02E-07
NMB0585		putative iron-regulated protein FrpA					-0.614	3.56E-03		
NMB0770		putative type IV pilus assembly protein PilZ							-0.882	5.33E-04
NMB0926		opacity protein (authentic frameshift)							-1.383	5.83E-09
NMB1552	*pivNM-1A*	pilin gene inverting protein							0.792	1.77E-05
NMB1567		macrophage infectivity potentiator							-1.108	1.16E-04
NMB1625	*pivNM-1B*	pilin gene inverting protein							0.870	1.31E-04
NMB1808	*pilM*	pilM protein					-0.770	3.37E-03		
NMB1810	*pilO*	pilO protein					-0.702	6.90E-03	-0.725	4.58E-03
NMB1811	*pilP*	pilP protein					-0.652	5.81E-03	-0.698	2.73E-03
NMB1812	*pilQ*	pilQ protein							-1.108	3.00E-04
NMB1847	*pilC-1*	pilC1 protein (authentic frameshift)							0.781	1.98E-05
NMB1985	*iga2*	adhesion and penetration protein					-0.817	3.96E-05	-1.214	5.50E-09
NMB2095		putative adhesin complex protein							-0.957	1.24E-04
Toxin production and resistance										
NMB0103		putative bacteriocin resistance protein					-0.778	2.49E-05	-0.807	8.69E-06
NMB0408	*bacA*	bacitracin resistance protein							0.645	7.88E-06
NMB0493		hemagglutinin-related protein							1.086	2.45E-09
NMB0497		hemagglutinin-related protein					1.139	8.04E-07	1.910	1.27E-13
NMB0582		putative bacteriocin resistance protein							1.457	3.33E-03
NMB0697	*ksgA*	dimethyladenosine transferase							0.741	4.19E-05
NMB0839	*pmbA*	pmbA protein					-0.650	3.28E-04	-3.571	1.71E-29

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Table C.40 – continued from previous page

ID	Name	Annotation	40 min log$_2$ ratio	40 min P-value*	60 min log$_2$ ratio	60 min P-value*	90 min log$_2$ ratio	90 min P-value*	240 min log$_2$ ratio	240 min P-value*
NMB1210		putative toxin-activating protein							0.736	2.62E-04
NMB1214		hemagglutinin-related protein							0.943	1.34E-05
NMB1603		putative tellurite resistance protein							1.199	3.67E-03
NMB1762		putative hemolysin activation protein HecB							1.136	3.60E-07
NMB1779		hemagglutinin-related protein					0.990	2.88E-05	1.575	4.70E-10
NMB2091		putative hemolysin							-0.812	1.36E-04

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.41: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *central intermediary metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min log$_2$ ratio	40 min P-value*	60 min log$_2$ ratio	60 min P-value*	90 min log$_2$ ratio	90 min P-value*	240 min log$_2$ ratio	240 min P-value*
Other										
NMB1391	*pgl*	oxidoreductase, Sol/DevB family							-0.620	1.88E-04
NMB1473		aminotransferase, class I							-0.811	1.55E-04
NMB1584		3-hydroxyacid dehydrogenase	0.589	4.49E-03						
NMB1799	*metK*	S-adenosylmethionine synthetase							-0.777	9.56E-07
Phosphorus compounds										
NMB0641	*ppa*	inorganic pyrophosphatase							-1.078	1.65E-08
Sulfur metabolism										
NMB1155	*cysH-1*	phosphoadenosine phosphosulfate reductase					0.780	9.95E-03		
NMB1192	*cysD-2*	sulfate adenylyltransferase, subunit 2							0.835	9.72E-03
NMB1193	*cysH-2*	phosphoadenosine phosphosulfate reductase					0.855	6.99E-03		

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.42: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *DNA metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min log$_2$ ratio	40 min P-value*	60 min log$_2$ ratio	60 min P-value*	90 min log$_2$ ratio	90 min P-value*	240 min log$_2$ ratio	240 min P-value*
Chromosome-associated proteins										
NMB1230	*hupB*	DNA-binding protein HU-β							-0.972	3.21E-06

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Table C.42 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
DNA replication, recombination, and repair										
NMB0056	*dksA*	DnaK suppressor protein					-0.669	2.08E-04	-1.290	7.97E-11
NMB0212	*gyrB*	DNA gyrase, subunit B							-0.728	5.71E-07
NMB0287	*dinG*	probable ATP-dependent helicase							0.879	3.05E-07
NMB0453	*mutT*	mutT protein					0.683	2.81E-05	0.828	4.67E-07
NMB0533	*nth*	endonuclease III							0.635	7.34E-05
NMB0729	*himA*	integration host factor, α subunit					-0.972	3.15E-06	-2.615	1.18E-20
NMB0740	*recN*	DNA repair protein							0.712	9.03E-07
NMB0751	*xerD*	integrase recombinase					0.652	2.23E-03	1.503	1.96E-10
NMB0785	*recB*	exodeoxyribonuclease V 135 KD polypeptide							0.618	5.38E-04
NMB0826	*nmgII*	C-5 cytosine-specific DNA methylase					-0.917	3.76E-05	-1.450	9.18E-10
NMB1222	*ung*	uracil-DNA glycosylase					0.992	9.77E-04		
NMB1233	*recD*	exodeoxyribonuclease V, α subunit							-1.016	1.46E-03
NMB1237	*recR*	recombination protein							-0.716	4.60E-07
NMB1295	*fpg*	formamidopyrimidine-DNA glycosylase					0.675	4.09E-03	1.183	1.07E-06
NMB1302	*hip*	integration host factor, β subunit							-1.825	9.63E-12
NMB1322	*priB*	putative primosomal replication protein n					-1.077	1.07E-05	-3.017	4.41E-20
NMB1396	*mutY*	A/G-specific adenine glycosylase							1.315	3.02E-06
NMB1445	*recA*	recA protein							-1.593	1.68E-10
NMB1460	*ssb*	single-strand binding protein							-1.129	7.83E-03
NMB1720	*recC*	exodeoxyribonuclease V 125 kD polypeptide							0.586	1.63E-03
Restriction modification										
NMB0725		modification methylase HgaI-1	-0.778	1.44E-04	-1.098	3.62E-07	-1.523	4.05E-12	-0.639	5.49E-04
NMB0726		type II restriction enzyme HgaI	-0.936	6.71E-05	-1.067	1.38E-05	-1.301	3.17E-08	-1.794	9.05E-13
NMB0727		N-6 adenine-specific DNA methylase	-0.708	3.85E-03	-0.924	2.24E-04	-1.210	2.47E-07	-1.585	8.04E-11
NMB0829	*hsdM*	type I restriction enzyme EcoR124II M protein					-0.798	5.79E-05	-0.686	3.69E-04
NMB0831		type I restriction enzyme S protein (degenerate)							-0.925	5.78E-06
NMB0835		putative type I restriction enzyme EcoR124II R protein							0.601	6.50E-03
NMB1033		modification methylase NlaIV					-0.946	3.21E-05	-0.894	5.39E-05
NMB1260		type III restriction-modification system EcoPI enzyme, subunit res							1.557	1.58E-05
NMB1289		putative type II restriction enzyme							-1.849	1.74E-14
NMB1290		C-5 cytosine-specific DNA-methylase							-0.995	2.39E-03

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.43: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *energy metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Aerobic										
NMB0434		putative AcnD-accessory protein PrpF							1.095	8.30E-07
Amino acids and amines										
NMB0176	*dadA*	D-amino acid dehydrogenase, small subunit							1.250	2.60E-09
NMB0401	*putA*	proline dehydrogenase							-0.595	5.83E-04
NMB0574	*gcvT*	glycine cleavage system T protein							0.719	2.53E-06
NMB1573	*argF*	ornithine carbamoyltransferase, catabolic							-0.671	2.93E-05
NMB1609	*metZ*	trans-sulfuration enzyme family protein							1.288	4.26E-07
ATP-proton motive force interconversion										
NMB1933	*atpC*	ATP synthase F1, ϵ subunit					-0.914	6.73E-06	-1.800	2.16E-14
NMB1934	*atpD*	ATP synthase F1, β subunit					-1.290	3.60E-06	-1.438	2.07E-07
NMB1935	*atpG*	ATP synthase F1, γ subunit					-1.293	1.95E-05	-2.278	8.88E-12
NMB1936	*atpA*	ATP synthase F1, α subunit					-1.181	2.04E-03	-1.959	2.51E-06
NMB1938	*atpF*	ATP synthase F0, B subunit					-1.262	9.57E-04		
NMB1939	*atpE*	ATP synthase F0, C subunit					-1.177	1.18E-05	-1.841	1.63E-08
NMB1940	*atpB*	ATP synthase F0, A subunit					-0.769	4.67E-03		
Electron transport										
NMB0123		ferredoxin, 4Fe-4S bacterial type					-1.245	4.68E-07		
NMB0241	*nuoA*	NADH dehydrogenase I, A subunit			-0.649	4.98E-04	-0.607	2.16E-04		
NMB0243	*nuoC*	NADH dehydrogenase I, C subunit			-0.695	5.34E-03	-0.833	1.28E-04		
NMB0244	*nuoD*	NADH dehydrogenase I, D subunit			-0.625	6.11E-04				
NMB0245	*nuoE*	NADH dehydrogenase I, E subunit			-0.617	7.37E-03	-0.658	8.45E-04	0.859	1.40E-05
NMB0251	*nuoI*	NADH dehydrogenase I, I subunit			-0.827	3.68E-03	-1.262	1.08E-06		
NMB0253	*nuoJ*	NADH dehydrogenase I, J subunit			-0.682	2.14E-04				
NMB0254	*nuoK*	NADH dehydrogenase I, K subunit					-1.258	6.75E-06		
NMB0258	*nuoM*	NADH dehydrogenase I, M subunit							0.948	9.77E-05
NMB0564	*nqrF*	Na(+)-translocating NADH-quinone reductase, subunit F					-0.762	2.60E-04	-1.069	3.87E-07
NMB0565	*nqrE*	Na(+)-translocating NADH-quinone reductase, subunit E					-0.855	1.74E-04	-0.881	8.37E-05
NMB0566	*nqrD*	Na(+)-translocating NADH-quinone reductase, subunit D					-0.897	9.29E-05	-0.973	1.77E-05
NMB0567	*nqrC*	Na(+)-translocating NADH-quinone reductase, subunit C					-1.149	3.91E-06	-1.385	3.51E-08
NMB0568	*nqrB*	Na(+)-translocating NADH-quinone reductase, subunit B					-0.856	1.49E-03	-0.959	3.19E-04
NMB0569	*nqrA*	Na(+)-translocating NADH-quinone reductase, subunit A					-1.088	1.59E-06	-1.207	8.89E-08
NMB0717		putative cytochrome							1.099	3.33E-08
NMB0923		cytochrome c							-1.093	1.48E-09
NMB1044	*fpr-1*	ferredoxin NADP reductase							-1.334	1.40E-12
NMB1366	*trxA*	thioredoxin	1.305	5.23E-09	0.944	2.74E-05	0.755	1.73E-04		
NMB1677		cytochrome c5					-0.885	3.34E-03	-0.935	1.60E-03

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Table C.43 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1723	*fixP*	cytochrome c oxidase, subunit III			-0.801	4.06E-03	-1.397	7.17E-08	-2.732	5.36E-18
NMB1724	*fixO*	cytochrome c oxidase, subunit II			-0.890	8.45E-03	-1.617	1.68E-07	-1.951	5.84E-10
NMB1734	*grxB*	glutaredoxin							0.604	2.20E-03
NMB1790	*grxC*	glutaredoxin 3	1.088	1.38E-03	1.098	2.05E-03				
NMB1805	*cyc*	cytochrome c4					-0.633	9.29E-05	-0.870	1.69E-07
NMB2051	*petC*	ubiquinol cytochrome c reductase, cytochrome c1					-1.078	5.49E-07	-1.845	2.97E-14
NMB2052	*petB*	ubiquinol cytochrome c reductase, cytochrome b					-1.273	4.00E-06	-1.388	3.98E-07
NMB2053	*petA*	ubiquinol cytochrome c reductase, iron-sulfur subunit					-0.960	1.79E-04	-1.161	6.01E-06
NMB2154	*etfA*	electron transfer flavoprotein, α subunit					-0.696	1.05E-03	-0.644	1.91E-03
NMB2155	*etfB*	electron transfer flavoprotein, β subunit					-0.758	3.15E-03	-1.013	7.54E-05
Fermentation										
NMB0546	*adhA*	alcohol dehydrogenase, propanol-preferring							-1.039	1.19E-04
NMB0604		alcohol dehydrogenase, zinc-containing							-0.669	1.82E-03
NMB0631		putative phosphate acetyltransferase Pta	0.597	1.48E-03	0.632	1.29E-03			-1.268	3.69E-11
NMB1395		alcohol dehydrogenase, zinc-containing	0.650	1.17E-03			0.700	1.51E-04		
NMB1518	*ackA-2*	acetate kinase							-2.415	5.14E-21
NMB0431	*prpC*	methylcitrate synthase citrate synthase 2							1.691	4.64E-11
Glycolysis/gluconeogenesis										
NMB0089	*pykA*	pyruvate kinase II							-0.595	1.54E-03
NMB0207	*gapA-1*	glyceraldehyde 3-phosphate dehydrogenase							0.677	5.34E-06
NMB0334	*pgi-1*	glucose-6-phosphate isomerase					0.701	9.70E-04		
NMB0618	*ppsA*	phosphoenolpyruvate synthase	0.668	8.84E-03	0.740	4.48E-03				
NMB1285	*eno*	enolase					-0.707	5.71E-03	-1.788	2.71E-10
NMB1377	*lldA*	L-lactate dehydrogenase	1.201	6.45E-07	1.556	3.11E-09	1.907	3.82E-13	1.854	5.92E-13
NMB1388	*pgi*	glucose-6-phosphate isomerase							-0.990	3.60E-11
NMB1390	*glk*	glucokinase							-0.849	5.10E-09
NMB1604	*gpm*	phosphoglycerate mutase							-1.201	1.10E-09
NMB2159	*gapA-2*	glyceraldehyde 3-phosphate dehydrogenase							-1.757	1.25E-14
Other										
NMB0340	*gloA*	lactoylglutathione lyase							-1.648	2.33E-12
NMB1493	*cstA*	carbon starvation protein A	-1.393	1.30E-07	-1.660	4.35E-09	-1.635	1.27E-09	-1.179	1.58E-06
Pentose phosphate pathway										
NMB0351	*tal*	transaldolase	0.638	2.38E-05						
NMB1244	*rpe*	ribulose-phosphate 3-epimerase	-0.871	7.46E-07	-0.878	2.04E-06	-0.857	6.13E-07	-0.746	5.34E-06
NMB1392	*zwf*	glucose-6-phosphate 1-dehydrogenase							-0.985	3.22E-10
NMB1457	*tktA*	transketolase							-1.047	7.80E-07
Pyruvate dehydrogenase										
NMB1341	*aceE*	pyruvate dehydrogenase, E1 component							-1.517	1.19E-10
NMB1342	*aceF*	dihydrolipoamide acetyltransferase (pyruvate dehydrogenase, E2 component)							-1.570	5.65E-12
NMB1344	*lpdA*	lipoamide dehydrogenase (pyruvate dehydrogenase, E3 component)							-1.098	2.30E-06

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Table C.43 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Sugars										
NMB0064	*galE-1*	UDP-glucose 4-epimerase					0.642	1.91E-03	0.801	1.02E-04
NMB0078	*galE-2*	UDP-glucose 4-epimerase (truncation)					0.697	1.33E-04		
NMB0390	*mapA*	maltose phosphorylase							-1.009	8.16E-10
NMB0391	*pgmB*	β-phosphoglucomutase							-1.152	4.86E-12
NMB0620	*pgp*	phosphoglycolate phosphatase					0.679	2.30E-03		
NMB0790	*pgm*	phosphoglucomutase	0.688	1.40E-05						
TCA cycle										
NMB0920	*icd*	isocitrate dehydrogenase, NADP-dependent, monomeric type							-0.868	3.55E-04
NMB0947	*lpdA2*	lipoamide dehydrogenase (putative 2-oxoglutarate dehydrogenase, E3 component)	0.959	2.17E-04					-1.113	3.90E-06
NMB0948	*sdhC*	succinate dehydrogenase, cytochrome b556 subunit					-0.731	2.81E-03	-1.318	2.35E-07
NMB0949	*sdhD*	succinate dehydrogenase, hydrophobic membrane anchor protein					-0.805	8.67E-04	-1.117	4.98E-06
NMB0951	*sdhB*	succinate dehydrogenase, iron-sulfur protein					-0.843	3.14E-04	-0.899	9.77E-05
NMB0954	*gltA*	citrate synthase							-1.086	2.68E-05
NMB0957	*lpdA3*	lipoamide dehydrogenase (2-oxoglutarate dehydrogenase, E3 component)					-0.593	1.72E-03		
NMB1572	*acnB*	aconitate hydratase 2							-1.368	3.07E-09
NMB2096	*mqo*	malate:quinone oxidoreductase					0.633	3.55E-04		

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.44: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *fatty acid and phospholipid metabolism*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Biosynthesis										
NMB0185	*cdsA*	phosphatidate cytidylyltransferase							0.617	3.41E-05
NMB0219	*fabF-1*	3-oxoacyl-(acyl-carrier-protein) synthase II							-1.331	1.10E-09
NMB0220	*acpP-1*	acyl carrier protein							-2.482	6.11E-15
NMB0336	*fabI*	enoyl-(acyl-carrier-protein) reductase							-1.618	1.18E-20
NMB0672	*lpxK*	tetraacyldisaccharide 4'-kinase							0.598	2.89E-03
NMB0679	*accD*	acetyl-CoA carboxylase carboxyl transferase, β subunit							-1.097	6.37E-11
NMB1139	*accA-1*	acetyl-CoA carboxylase carboxyl transferase, α subunit							-1.037	4.01E-05
NMB1177	*accA-2*	acetyl-CoA carboxylase carboxyl transferase α subunit					-0.668	8.00E-03	-1.130	9.67E-06
NMB1294	*plsC*	1-acyl-sn-glycerol-3-phosphate acyltransferase					0.687	1.76E-03	0.914	3.31E-05
NMB1588	*pgsA*	CDP-diacylglycerol glycerol-3-phosphate 3-phosphatidyltransferase			-0.642	2.05E-04				
NMB1697		putative acyl carrier protein							0.705	2.37E-03

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Table C.44 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB1860	*accB*	acetyl-CoA carboxylase, biotin carboxyl carrier protein							-1.144	1.26E-04
NMB1861	*accC*	acetyl-CoA carboxylase, biotin carboxylase							-0.702	7.40E-03
NMB1913	*plsX*	fatty acid phospholipid synthesis protein							-0.592	1.06E-03
Degradation										
NMB0464		putative phospholipase A1					-0.672	3.21E-05	-0.966	9.35E-09

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.45: Differentially expressed genes in blood at 40, 60, 90 and 240 min in comparison to the 20 min time-point categorised into TIGR role category *hypothetical proteins* or unassigned. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
Conserved										
NMB0035		conserved hypothetical protein	0.696	6.01E-03	1.169	4.28E-06	1.187	6.27E-07		
NMB0036		conserved hypothetical protein					0.982	6.41E-04		
NMB0050		conserved hypothetical protein							0.683	2.45E-03
NMB0053		conserved hypothetical protein							0.623	3.90E-03
NMB0109		conserved hypothetical protein							-1.007	1.39E-06
NMB0121		conserved hypothetical protein							1.096	4.03E-07
NMB0227		conserved hypothetical protein					0.756	5.79E-05	0.953	5.80E-07
NMB0228		conserved hypothetical protein					1.074	3.69E-06	1.192	2.38E-07
NMB0230		conserved hypothetical protein					0.982	6.04E-07	1.612	1.68E-13
NMB0248		conserved hypothetical protein			-0.729	5.84E-03	-1.027	1.18E-05		
NMB0263		conserved hypothetical protein							0.657	4.56E-03
NMB0276		conserved hypothetical protein					0.667	2.04E-03		
NMB0310		conserved hypothetical protein							0.786	5.49E-04
NMB0313		conserved hypothetical protein					1.018	4.50E-05		
NMB0315		conserved hypothetical protein					0.806	1.15E-04	0.713	4.42E-04
NMB0316		conserved hypothetical protein	-0.858	6.56E-04	-1.178	5.77E-06				
NMB0317		conserved hypothetical protein	-0.718	8.13E-04	-1.421	1.12E-09	-1.306	1.67E-09	-1.163	1.83E-08
NMB0395		conserved hypothetical protein	0.689	1.32E-04	1.106	5.50E-08	1.614	3.39E-14	0.676	1.07E-04
NMB0404		conserved hypothetical protein							-0.652	1.84E-04
NMB0409		conserved hypothetical protein							0.756	1.03E-03
NMB0444		conserved hypothetical protein							0.857	6.67E-05
NMB0455		conserved hypothetical protein					0.600	2.05E-04	1.204	2.17E-11
NMB0459		conserved hypothetical protein					0.618	8.67E-04	0.610	7.93E-04
NMB0477		conserved hypothetical protein					-0.619	2.94E-03		

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Table C.45 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0479		conserved hypothetical protein							-1.107	1.82E-05
NMB0534		conserved hypothetical protein							1.256	3.13E-08
NMB0545		conserved hypothetical protein							0.725	9.35E-06
NMB0557		conserved hypothetical protein	0.715	5.81E-04	0.585	8.53E-03			-0.858	7.23E-06
NMB0571		conserved hypothetical protein					0.670	1.66E-03	1.209	6.92E-08
NMB0606		conserved hypothetical protein					-0.697	2.81E-04	-2.086	3.24E-17
NMB0619		conserved hypothetical protein					0.601	2.10E-03		
NMB0621		conserved hypothetical protein							1.288	1.25E-07
NMB0625		conserved hypothetical protein							-0.596	1.78E-04
NMB0742		conserved hypothetical protein					-0.866	2.43E-05	-0.742	1.71E-04
NMB0771		conserved hypothetical protein							-0.878	8.07E-09
NMB0773		conserved hypothetical protein							-1.114	2.00E-08
NMB0800		conserved hypothetical protein					-0.882	1.34E-06	-0.690	6.40E-05
NMB0840		conserved hypothetical protein					-0.993	1.86E-07	-1.514	3.51E-13
NMB0874		conserved hypothetical protein							-0.651	4.05E-06
NMB0883		conserved hypothetical protein					-0.767	4.65E-05		
NMB0952		conserved hypothetical protein					-0.798	3.92E-03		
NMB1014		conserved hypothetical protein							2.309	4.42E-04
NMB1026		conserved hypothetical protein							-0.726	4.98E-06
NMB1039		conserved hypothetical protein							-1.265	3.78E-10
NMB1095		conserved hypothetical protein							1.485	4.51E-04
NMB1096		conserved hypothetical protein					0.932	2.90E-03	1.080	5.25E-04
NMB1268		conserved hypothetical protein							-0.831	1.55E-06
NMB1286		conserved hypothetical protein							-0.707	2.47E-05
NMB1308		conserved hypothetical protein					-0.608	3.76E-05	-0.592	3.92E-05
NMB1319		conserved hypothetical protein							-0.789	3.96E-04
NMB1327		conserved hypothetical protein					0.647	7.08E-03		
NMB1336		conserved hypothetical protein	1.357	3.14E-11	0.920	4.23E-06				
NMB1365		conserved hypothetical protein			-0.727	4.30E-04				
NMB1378		conserved hypothetical protein	0.742	6.47E-05	0.917	2.51E-06	1.036	2.53E-08	1.358	3.74E-12
NMB1382		conserved hypothetical protein							-0.599	3.27E-04
NMB1417		conserved hypothetical protein							0.690	2.02E-06
NMB1423		conserved hypothetical protein							0.599	9.67E-06
NMB1436		conserved hypothetical protein							-1.011	1.01E-05
NMB1438		conserved hypothetical protein							-1.170	1.75E-07
NMB1475		conserved hypothetical protein	2.505	1.85E-10	2.408	3.23E-09	1.587	8.61E-06	1.387	5.22E-05
NMB1494		conserved hypothetical protein			-1.433	8.56E-05	-1.170	7.74E-04	-0.949	5.14E-03
NMB1543		conserved hypothetical protein							0.961	5.34E-03
NMB1557		conserved hypothetical protein							-0.701	1.26E-05
NMB1564		conserved hypothetical protein	1.192	9.59E-10						
NMB1570		conserved hypothetical protein							0.665	1.81E-08

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Table C.45 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
NMB1575		conserved hypothetical protein							-0.826	1.18E-04
NMB1578		conserved hypothetical protein			0.637	5.34E-03			1.081	1.39E-07
NMB1590		conserved hypothetical protein							-1.006	2.02E-06
NMB1634		conserved hypothetical protein							1.282	6.01E-05
NMB1641		conserved hypothetical protein	-0.730	1.11E-03			-0.723	4.22E-04	-1.221	1.70E-08
NMB1653		conserved hypothetical protein							-1.580	7.94E-10
NMB1654		conserved hypothetical protein							1.102	1.35E-08
NMB1663		conserved hypothetical protein							-0.746	1.58E-05
NMB1665		conserved hypothetical protein							-2.767	4.26E-16
NMB1748		conserved hypothetical protein							1.468	8.42E-04
NMB1749		conserved hypothetical protein							2.706	1.20E-14
NMB1759		conserved hypothetical protein					-0.680	1.10E-03	-0.793	1.19E-04
NMB1796		conserved hypothetical protein	0.879	3.66E-05	0.897	5.47E-05			-1.340	4.45E-10
NMB1806	*engB*	conserved hypothetical protein					-0.585	3.55E-04		
NMB1866		conserved hypothetical protein							-0.639	7.92E-03
NMB1886		conserved hypothetical protein							0.899	1.79E-04
NMB1906		conserved hypothetical protein							-1.484	1.57E-07
NMB1908		conserved hypothetical protein							0.718	5.35E-05
NMB1910		conserved hypothetical protein					-0.868	3.88E-04	-1.943	3.29E-12
NMB1912		conserved hypothetical protein					-0.607	9.12E-05	-0.949	6.12E-09
NMB1979		conserved hypothetical protein							-0.591	1.24E-04
NMB2000	*hslO*	conserved hypothetical protein	0.833	7.20E-07			0.937	2.53E-08		
NMB2003		conserved hypothetical protein					0.858	4.09E-03	1.013	6.04E-04
NMB2020		conserved hypothetical protein	0.655	1.41E-03					-1.042	9.75E-08
NMB2078		conserved hypothetical protein							-0.585	5.39E-05
NMB2098		conserved hypothetical protein					0.788	6.96E-04		
NMB2139		conserved hypothetical protein							0.795	8.39E-03
NMB2140		conserved hypothetical protein					0.712	4.67E-05		
NMB2143		conserved hypothetical protein							0.770	4.24E-08
NMB2153		conserved hypothetical protein					-0.706	5.34E-04		
Unassigned										
NMB0016		hypothetical protein							0.912	2.13E-03
NMB0046		hypothetical protein							0.759	4.29E-08
NMB0054		hypothetical protein							0.654	6.28E-04
NMB0086		hypothetical protein					0.635	1.08E-06		
NMB0091		hypothetical protein							-0.955	1.21E-05
NMB0092		hypothetical protein							-1.284	1.00E-04
NMB0095		hypothetical protein							-0.833	2.06E-05
NMB0096		hypothetical protein					-0.733	2.74E-04	-0.945	1.92E-06
NMB0104		hypothetical protein			-0.752	5.36E-06	-0.787	4.38E-07	-1.340	2.50E-14
NMB0129		hypothetical protein					-0.930	3.42E-03		

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Table C.45 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0197		hypothetical protein							-1.061	1.22E-08
NMB0213		hypothetical protein					0.753	2.36E-05	0.657	1.29E-04
NMB0223		hypothetical protein							0.994	2.04E-03
NMB0239		hypothetical protein					-0.966	1.07E-05		
NMB0240		hypothetical protein							-0.757	5.20E-06
NMB0247		hypothetical protein			-0.894	5.34E-03	-1.209	2.13E-05	-1.036	1.49E-04
NMB0252		hypothetical protein			-0.965	2.28E-04	-1.540	1.63E-09		
NMB0271		hypothetical protein					0.804	9.89E-05		
NMB0298		hypothetical protein							-1.148	1.72E-05
NMB0305		hypothetical protein					-0.781	5.26E-03		
NMB0314		hypothetical protein					0.602	1.62E-03	0.644	6.11E-04
NMB0320		hypothetical protein					-0.735	6.33E-03	-1.882	2.76E-10
NMB0346		hypothetical protein	0.753	4.02E-04	0.700	1.68E-03				
NMB0350		hypothetical protein	0.679	1.11E-03	0.585	8.53E-03			1.086	5.03E-08
NMB0367		hypothetical protein							0.585	5.20E-04
NMB0368		hypothetical protein					-0.673	1.12E-03	0.622	2.07E-03
NMB0369		hypothetical protein					-0.714	2.11E-04		
NMB0370		hypothetical protein							0.629	1.08E-04
NMB0371		hypothetical protein							0.627	3.45E-04
NMB0372		hypothetical protein			-0.693	1.21E-03	-0.683	2.60E-04	0.588	1.21E-03
NMB0373		hypothetical protein			-0.905	1.95E-03	-0.857	3.55E-04		
NMB0376		hypothetical protein							0.799	4.24E-03
NMB0397		hypothetical protein					0.668	2.29E-03		
NMB0429		hypothetical protein							1.054	7.55E-04
NMB0449		hypothetical protein							-1.442	1.61E-10
NMB0450		hypothetical protein							-1.119	2.24E-07
NMB0451		hypothetical protein							-0.712	8.39E-03
NMB0476		hypothetical protein					-0.665	8.88E-03	0.675	6.85E-03
NMB0478		hypothetical protein							-1.220	2.27E-08
NMB0485		hypothetical protein							1.718	8.22E-07
NMB0499		hypothetical protein					1.118	3.81E-04	1.444	5.33E-06
NMB0500		hypothetical protein							-1.023	1.98E-06
NMB0502		hypothetical protein							0.749	7.16E-04
NMB0505		hypothetical protein							1.085	1.94E-03
NMB0507		hypothetical protein							-1.996	7.31E-16
NMB0509		hypothetical protein					0.857	1.47E-04	0.872	8.37E-05
NMB0511		hypothetical protein					0.820	4.95E-03	1.357	4.99E-06
NMB0519		hypothetical protein							-0.671	6.46E-04
NMB0541		hypothetical protein							0.994	9.22E-12
NMB0552		hypothetical protein					0.706	1.81E-03	0.878	9.91E-05
NMB0572		hypothetical protein					0.924	1.75E-06	0.607	7.61E-04

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Table C.45 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0613		hypothetical protein							-1.087	8.17E-04
NMB0644		hypothetical protein					-0.708	4.33E-05	-0.610	2.80E-04
NMB0647		hypothetical protein							0.721	4.01E-05
NMB0648		hypothetical protein							-0.927	3.22E-06
NMB0651		hypothetical protein					-0.677	5.75E-03		
NMB0654		hypothetical protein			-0.709	4.83E-03	-0.906	4.07E-05		
NMB0655		hypothetical protein			-0.712	4.07E-04	-0.810	8.61E-06	0.675	1.07E-04
NMB0656		hypothetical protein	-0.730	6.07E-04	-0.630	9.16E-03				
NMB0660		hypothetical protein							-0.785	1.66E-05
NMB0695		hypothetical protein							1.402	7.38E-05
NMB0701		hypothetical protein							0.915	1.26E-04
NMB0709		hypothetical protein							-0.722	6.73E-04
NMB0715		hypothetical protein							2.131	2.29E-11
NMB0716		hypothetical protein							1.012	1.40E-05
NMB0730		hypothetical protein							1.194	3.73E-07
NMB0731		hypothetical protein							0.865	1.11E-06
NMB0734		hypothetical protein							0.791	1.47E-06
NMB0744		hypothetical protein	0.886	5.79E-04	0.964	3.26E-04	1.113	4.82E-06		
NMB0761		hypothetical protein							-1.270	1.56E-04
NMB0762		hypothetical protein							-1.135	1.09E-07
NMB0813		hypothetical protein					1.086	3.90E-03	0.964	9.05E-03
NMB0816		hypothetical protein					-0.748	9.24E-05		
NMB0818		hypothetical protein					-0.960	1.33E-03	-1.585	5.59E-09
NMB0841		hypothetical protein							-1.459	1.95E-14
NMB0850		hypothetical protein	0.931	9.95E-03						
NMB0856		hypothetical protein							1.136	6.90E-06
NMB0860		hypothetical protein							0.919	7.53E-08
NMB0863		hypothetical protein					-0.676	4.00E-05		
NMB0864		hypothetical protein							0.837	6.08E-03
NMB0882		hypothetical protein							-0.593	8.55E-03
NMB0897		hypothetical protein					-1.012	1.33E-04	-1.119	1.93E-05
NMB0898		hypothetical protein					-0.934	8.23E-05	-1.628	2.40E-10
NMB0899		hypothetical protein							-1.106	6.63E-07
NMB0900		hypothetical protein							-0.707	4.88E-05
NMB0907		hypothetical protein	0.829	2.17E-04	0.663	5.38E-03			1.303	3.61E-09
NMB0908		hypothetical protein							1.176	9.08E-05
NMB0909		hypothetical protein							0.679	7.56E-04
NMB0928		hypothetical protein							-0.889	1.91E-08
NMB0945		hypothetical protein							-0.931	7.45E-04
NMB0965		hypothetical protein					0.740	4.58E-03	1.180	8.31E-06
NMB0971		hypothetical protein							1.350	5.53E-03

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Table C.45 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0979		hypothetical protein			1.115	1.01E-03			1.090	4.98E-04
NMB0986		hypothetical protein			-0.738	1.21E-03	-0.802	6.86E-05	-0.632	1.14E-03
NMB0989		hypothetical protein							1.432	1.26E-04
NMB1006		hypothetical protein							1.179	2.62E-04
NMB1008		hypothetical protein			-0.825	7.48E-03	-1.217	8.61E-06		
NMB1010		hypothetical protein			-0.766	5.44E-03	-0.802	2.53E-03		
NMB1011		hypothetical protein					-0.858	6.57E-03	-1.127	3.25E-04
NMB1013		hypothetical protein							1.457	3.83E-04
NMB1020		hypothetical protein					-0.786	2.50E-05	-0.774	2.08E-05
NMB1035		hypothetical protein							-0.741	3.54E-04
NMB1056		hypothetical protein					-0.680	4.74E-03	-0.597	4.59E-03
NMB1082		hypothetical protein							0.948	3.77E-03
NMB1086		hypothetical protein							1.315	1.29E-04
NMB1094		hypothetical protein							1.114	3.07E-03
NMB1100		hypothetical protein							0.780	4.65E-04
NMB1106		hypothetical protein							0.720	5.75E-03
NMB1107		hypothetical protein							-1.564	6.93E-09
NMB1108		hypothetical protein					0.898	9.26E-03		
NMB1120		hypothetical protein							1.171	4.62E-03
NMB1124		hypothetical protein					-0.816	7.30E-04	-1.716	1.26E-10
NMB1125		hypothetical protein					-0.978	5.64E-04	-1.255	1.04E-05
NMB1132		hypothetical protein							0.613	1.59E-03
NMB1143		hypothetical protein					-0.701	1.71E-03	-1.044	4.51E-06
NMB1144		hypothetical protein							-1.173	3.15E-06
NMB1149		hypothetical protein							-0.859	3.82E-03
NMB1162		hypothetical protein					-0.843	1.12E-03	-1.650	3.12E-09
NMB1163		hypothetical protein					-0.986	4.62E-04	-1.250	9.65E-06
NMB1170		hypothetical protein							0.722	1.07E-04
NMB1173		hypothetical protein							0.601	9.06E-04
NMB1181		hypothetical protein					-0.675	2.53E-03	-1.118	1.19E-06
NMB1182		hypothetical protein							-1.241	1.74E-12
NMB1187		hypothetical protein							-1.152	7.12E-05
NMB1202		hypothetical protein					-0.757	1.42E-04		
NMB1205		hypothetical protein			-0.892	2.54E-03	-1.080	1.15E-04	-1.537	1.03E-07
NMB1208		hypothetical protein					-2.279	4.91E-04		
NMB1211		hypothetical protein					-0.991	5.66E-03		
NMB1229		hypothetical protein							-0.863	3.80E-06
NMB1284		hypothetical protein					-0.617	2.17E-04	-0.733	1.09E-05
NMB1292		hypothetical protein							1.213	5.13E-03
NMB1293		hypothetical protein					0.665	4.99E-03		
NMB1296		hypothetical protein							1.003	1.27E-06

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Table C.45 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB1311		hypothetical protein					-0.693	7.78E-05		
NMB1317		hypothetical protein							0.632	5.67E-03
NMB1334		hypothetical protein	1.165	1.29E-08	0.746	4.07E-04				
NMB1340		hypothetical protein							-2.374	8.48E-16
NMB1343		hypothetical protein							-1.188	1.15E-06
NMB1350		hypothetical protein							-0.669	7.32E-04
NMB1387		hypothetical protein							-0.852	4.05E-09
NMB1402		hypothetical protein	-0.649	6.29E-04	-0.652	1.08E-03	-0.782	1.18E-05		
NMB1404		hypothetical protein	-0.732	2.59E-03	-0.750	3.16E-03	-0.837	6.46E-05	-0.999	6.41E-06
NMB1408		hypothetical protein	-1.165	1.08E-05	-1.366	8.92E-07	-1.605	2.46E-09	-2.440	8.48E-16
NMB1410		hypothetical protein	-0.874	3.66E-05	-1.077	1.27E-06	-1.193	1.99E-08	-0.700	2.69E-04
NMB1468		hypothetical protein							-2.525	5.25E-14
NMB1469		hypothetical protein							-1.782	2.52E-03
NMB1486		hypothetical protein							-0.877	2.60E-06
NMB1503		hypothetical protein							-0.818	4.08E-07
NMB1517		hypothetical protein							0.639	3.22E-04
NMB1523		hypothetical protein							-1.722	5.65E-12
NMB1534		hypothetical protein							-1.331	1.27E-08
NMB1544		hypothetical protein							1.313	2.61E-09
NMB1546		hypothetical protein							0.852	5.27E-03
NMB1547		hypothetical protein							0.889	6.38E-05
NMB1549		hypothetical protein					1.165	1.41E-03		
NMB1580		hypothetical protein							1.430	3.83E-09
NMB1599		hypothetical protein							-0.608	8.52E-03
NMB1633		hypothetical protein							1.370	1.48E-09
NMB1639		hypothetical protein							0.596	1.38E-03
NMB1644		hypothetical protein					0.807	4.98E-03		
NMB1666		hypothetical protein							-1.810	8.14E-05
NMB1667		hypothetical protein					-0.830	8.12E-04	-1.505	1.08E-08
NMB1675		hypothetical protein							1.140	7.67E-06
NMB1681		hypothetical protein							-1.437	4.78E-11
NMB1695		hypothetical protein							0.626	6.32E-03
NMB1699		hypothetical protein							0.825	1.39E-06
NMB1700		hypothetical protein							0.632	8.12E-04
NMB1733		hypothetical protein							1.958	6.36E-03
NMB1739		hypothetical protein							1.407	1.90E-06
NMB1740		hypothetical protein							0.856	3.54E-03
NMB1742		hypothetical protein							2.124	1.95E-07
NMB1743		hypothetical protein							1.594	8.10E-05
NMB1757		hypothetical protein							0.861	3.73E-03
NMB1772		hypothetical protein					1.016	1.33E-03	1.706	2.23E-07

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Table C.45 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***	**log_2 ratio**	**P-value***
NMB1773		hypothetical protein							-0.780	2.32E-03
NMB1774		hypothetical protein							-1.092	2.51E-06
NMB1775		hypothetical protein					1.021	1.10E-03	1.741	1.03E-07
NMB1776		hypothetical protein					1.064	1.07E-05		
NMB1777		hypothetical protein					0.808	8.23E-05	-0.762	1.36E-04
NMB1784		hypothetical protein							1.106	1.16E-06
NMB1800		hypothetical protein					-0.637	9.77E-04		
NMB1865		hypothetical protein					0.685	9.02E-03		
NMB1870		hypothetical protein							-0.677	2.25E-04
NMB1931		hypothetical protein							0.964	3.56E-06
NMB1941	*atpI*	hypothetical protein					-0.855	4.86E-05		
NMB1977		hypothetical protein							1.416	5.61E-08
NMB2013		hypothetical protein							-1.882	5.93E-11
NMB2015		hypothetical protein							-1.656	4.49E-12
NMB2074		hypothetical protein							0.708	8.77E-05
NMB2081		hypothetical protein							0.684	6.88E-03
NMB2085		hypothetical protein					-0.762	1.62E-04	-0.644	1.01E-03
NMB2094		hypothetical protein							1.030	4.02E-05
NMB2116		hypothetical protein							-0.776	1.36E-03
NMB2118		hypothetical protein							-0.648	3.58E-03
NMB2120		hypothetical protein							-0.641	5.33E-04
NMB2121		hypothetical protein							-0.851	5.18E-05
NMB2123		hypothetical protein							-1.099	3.14E-09
NMB2125		hypothetical protein							-0.830	2.18E-04
NMB2130		hypothetical protein							-0.991	4.28E-04
NMB2137		hypothetical protein							-3.680	8.48E-16
NMB2141		hypothetical protein							-1.913	7.75E-12
NMB2149		hypothetical protein							0.656	2.37E-04
NMB2152		hypothetical protein					-0.601	1.10E-03		

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.46: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *mobile and extra chromosomal element functions*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Prophage functions										
NMB0748	*hfq*	host factor-I					-1.013	1.49E-03	-2.043	3.01E-09
NMB0896		integrase (authentic frameshift)					-0.816	1.30E-05	-0.772	2.19E-05
NMB0961	*funZ*	funZ protein					-0.662	1.66E-04	-1.226	1.02E-09
NMB1110		tail protein, 43 kDa					0.855	5.75E-03		
Transposon functions										
NMB0225		transposase, IS30 family							1.075	7.52E-09
NMB0583		IS1016C2 transposase							0.860	1.94E-05
NMB0635		transposase, IS30 family							0.751	9.05E-05
NMB0805		transposase, IS30 family							1.388	4.12E-11
NMB0911		transposase, IS30 family							0.746	1.56E-03
NMB0919		putative IS1106 transposase							0.718	2.26E-03
NMB0991		IS1106 transposase							1.191	1.94E-09
NMB1022		transposase, IS30 family							0.772	9.01E-05
NMB1050		transposase, IS30 family							0.769	3.57E-05
NMB1081		bacteriophage transposase							1.604	1.34E-06
NMB1083		putative bacteriophage, DNA transposition protein B					0.756	4.13E-03	1.255	3.29E-06
NMB1099		transposase, IS30 family							0.790	1.40E-05
NMB1251		transposase, IS30 family							0.745	9.98E-04
NMB1259		transposase, IS30 family							0.868	1.77E-04
NMB1401		IS1016C2 transposase							1.038	1.15E-08
NMB1411		IS1016C2 transposase							1.028	2.09E-07
NMB1539		IS1106 transposase							0.763	7.27E-04
NMB1601		IS1106 transposase							0.685	2.40E-03
NMB1602		putative transposase							1.345	8.48E-16
NMB1713		transposase, IS30 family							1.154	1.57E-08
NMB1770		transposase, IS30 family							0.941	9.41E-07
NMB2148		transposase, IS30 family							1.258	9.61E-10

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.47: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *protein fate*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Degradation of proteins, peptides, and glycopeptides										
NMB0214	*prlC*	oligopeptidase A	2.227	2.07E-15	1.816	2.96E-11	1.296	7.37E-08	0.957	1.82E-05
NMB0700	*iga*	IgA-specific serine endopeptidase					-0.730	9.67E-03	-1.311	5.06E-06
NMB0927	*pip*	proline iminopeptidase							0.625	8.16E-03
NMB1231	*lon*	ATP-dependent protease La	1.681	4.27E-08	1.342	1.86E-05	0.720	9.16E-03	-1.034	5.36E-05
NMB1312	*clpP*	ATP-dependent Clp protease, proteolytic subunit					-0.796	2.11E-04		
NMB1332	*prc*	carboxy-terminal peptidase							1.540	2.94E-11
NMB1372	*clpX*	ATP-dependent Clp protease, ATP-binding subunit							-0.783	1.40E-05
NMB1472	*clpB*	ClpB protein	2.058	4.63E-16	1.556	6.53E-11	1.257	5.31E-09	0.756	1.03E-04
NMB1587		putative protease	-0.686	9.26E-04	-0.704	1.21E-03	-0.969	1.12E-06		
NMB1664		putative protease							-0.739	1.33E-08
NMB1802	*gcp*	O-sialoglycoprotein endopeptidase					0.615	8.12E-04		
NMB2127		putative protease							0.732	5.29E-06
Protein and peptide secretion and trafficking										
NMB0125	*secE*	preprotein translocase, SecE subunit					-1.066	7.77E-06	-1.304	2.64E-07
NMB0162	*secY*	preprotein translocase, SecY subunit					-1.038	2.50E-05	-0.883	1.95E-04
NMB0607	*secD*	protein-export membrane protein					-0.680	1.58E-03	-0.963	9.55E-06
NMB0608	*secF*	protein-export membrane protein					-0.635	6.89E-04	-1.395	2.83E-11
NMB1313	*tig*	trigger factor							-1.362	9.90E-06
NMB1789	*secB*	protein-export protein SecB	1.223	1.46E-06	1.218	4.24E-06	0.759	1.15E-03		
Protein folding and stabilization										
NMB0059	*dnaJ*	dnaJ protein	1.319	3.86E-14	0.901	5.50E-08	0.782	3.45E-07		
NMB0278	*dsbA-1*	thiol:disulfide interchange protein	0.607	6.47E-05						
NMB0294	*dsbA-2*	thiol:disulfide interchange protein	0.639	1.17E-03					-0.795	1.09E-05
NMB0550	*dsbC*	thiol:disulfide interchange protein							-0.719	5.33E-07
NMB0554	*dnaK*	dnaK protein	1.187	9.51E-05	0.909	5.25E-03				
NMB0561	*grpE*	grpE protein	1.534	2.11E-08	0.964	6.49E-04			0.701	3.72E-03
NMB0791	*ppiB*	peptidyl-prolyl cis-trans isomerase	1.481	1.63E-10	0.976	1.86E-05			-1.223	1.24E-08
NMB1262	*ppiA*	peptidyl-prolyl cis-trans isomerase							0.741	1.82E-05
NMB1313	*tig*	trigger factor							-1.362	9.90E-06
NMB1522	*slyD*	FKBP-type peptidyl-prolyl cis-trans isomerase							-1.012	1.55E-06
NMB1972	*groEL*	chaperonin, 60 kDa	1.694	1.08E-05	1.492	2.21E-04				
NMB1973	*groES*	chaperonin, 10 kDa	1.739	3.66E-05	1.448	1.21E-03				
Protein modification and repair										
NMB0044	*pilB*	trifunctional thioredoxin/methionine sulfoxide reductase A/B protein	1.642	1.45E-08	1.278	1.47E-05	1.226	2.00E-06	1.488	5.54E-08

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.48: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *protein synthesis*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Ribosomal proteins: synthesis and modification										
NMB0127	*rplK*	50S ribosomal protein L11					-1.175	4.30E-06	-0.906	1.84E-04
NMB0128	*rplA*	50S ribosomal protein L1					-1.059	1.07E-05	-1.066	6.06E-06
NMB0130	*rplJ*	50S ribosomal protein L10					-0.998	5.00E-03	-2.214	7.08E-09
NMB0131	*rplL*	50S ribosomal protein L7 L12					-0.774	7.05E-04	-2.951	2.08E-20
NMB0136	*rpsL*	30S ribosomal protein S12					-0.931	2.87E-04	-1.783	2.31E-10
NMB0137	*rpsG*	30S ribosomal protein S7					-0.787	2.26E-03	-1.627	4.52E-09
NMB0140	*rpsJ*	30S ribosomal protein S10					-1.167	3.39E-06	-1.883	5.76E-12
NMB0142	*rplC*	50S ribosomal protein L3					-0.883	8.67E-04	-1.352	7.05E-07
NMB0143	*rplD*	50S ribosomal protein L4							-1.340	1.78E-03
NMB0144	*rplW*	50S ribosomal protein L23					-1.331	1.33E-05	-1.904	2.83E-09
NMB0145	*rplB*	50S ribosomal protein L2							-1.246	2.19E-05
NMB0146	*rpsS*	30S ribosomal protein S19					-1.353	2.14E-05	-1.152	1.67E-04
NMB0147	*rplV*	50S ribosomal protein L22					-0.891	2.89E-04	-0.806	7.76E-04
NMB0148	*rpsC*	30S ribosomal protein S3					-0.855	9.57E-04	-1.095	2.29E-05
NMB0149	*rplP*	50S ribosomal protein L16							-0.941	7.18E-05
NMB0150	*rpmC*	50S ribosomal protein L29					-1.348	1.49E-04	-0.940	1.00E-02
NMB0151	*rpsQ*	30S ribosomal protein S17					-1.146	1.19E-03	-1.760	1.21E-06
NMB0152	*rplN*	50S ribosomal protein L14					-0.908	4.33E-05	-1.785	1.40E-12
NMB0153	*rplX*	50S ribosomal protein L24					-0.791	4.60E-04	-1.552	3.21E-10
NMB0154	*rplE*	50S ribosomal protein L5					-0.860	4.06E-03		
NMB0155	*rpsN*	30S ribosomal protein S14					-0.885	3.46E-03	-0.796	7.47E-03
NMB0156	*rpsH*	30S ribosomal protein S8					-0.952	7.24E-05	-1.344	5.90E-08
NMB0157	*rplF*	50S ribosomal protein L6					-0.846	2.17E-04	-1.185	4.25E-07
NMB0158	*rplR*	50S ribosomal protein L18					-0.848	4.64E-04	-1.176	1.79E-06
NMB0159	*rpsE*	30s ribosomal protein S5					-1.048	1.17E-03	-1.035	1.08E-03
NMB0160	*rpmD*	50S ribosomal protein L30					-1.604	3.72E-06		
NMB0161	*rplO*	50S ribosomal protein L15					-1.297	1.12E-06	-1.192	3.47E-06
NMB0164	*rpmJ*	50S ribosomal protein L36					-1.531	1.19E-04		
NMB0165	*rpsM*	30S ribosomal protein S13					-1.224	5.00E-06	-1.551	1.48E-08
NMB0167	*rpsD*	30S ribosomal protein S4					-1.228	1.19E-04	-1.723	1.57E-07
NMB0169	*rplQ*	50S ribosomal protein L17					-1.416	9.42E-06	-2.828	2.59E-14
NMB0321	*rpmB*	50S ribosomal protein L28							-2.520	2.84E-14
NMB0322	*rpmG*	50S ribosomal protein L33							-1.603	4.41E-09
NMB0324	*rpmA*	50S ribosomal protein L27							-2.668	4.28E-14
NMB0325	*rplU*	50S ribosomal protein L21							-2.097	9.22E-12
NMB0589	*rplS*	50s ribosomal protein L19					-1.272	7.37E-08	-2.174	1.22E-15

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Table C.48 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0592	*rpsP*	30S ribosomal protein S16					-1.089	5.09E-05	-2.264	2.89E-13
NMB0609	*rpsO*	30s ribosomal protein S15					-0.913	4.23E-05	-2.356	6.13E-17
NMB0722	*rpmI*	50S ribosomal protein L35					-0.859	8.87E-04	-2.960	4.50E-18
NMB0723	*rplT*	50S ribosomal protein L20					-0.850	5.62E-04	-2.634	6.38E-17
NMB0941	*rpmJ2*	50S ribosomal protein L36	1.571	2.33E-07	1.612	2.51E-06				
NMB0942	*rpmE2*	putative 50S ribosomal protein L31	2.361	2.55E-11	1.959	3.40E-08			-0.839	4.62E-03
NMB1301	*rpsA*	30S ribosomal protein S1							-1.067	1.46E-05
NMB1320	*rplI*	50S ribosomal protein L9					-0.666	9.16E-03	-2.384	1.73E-14
NMB1321	*rpsR*	30S ribosomal protein S18					-1.030	6.36E-06	-3.548	4.83E-25
NMB1323	*rpsF*	30S ribosomal protein S6					-0.781	8.86E-03	-3.726	2.16E-18
NMB1911	*rpmF*	50S ribosomal protein L32					-0.820	5.49E-03	-1.645	1.16E-07
NMB1950	*rpsU*	30S ribosomal protein S21					-0.800	3.99E-03	-2.746	1.84E-15
NMB1956	*rpmE*	50S ribosomal protein L31							-2.806	8.62E-10
NMB2056	*rpsI*	30S ribosomal protein S9					-0.814	1.16E-04	-2.184	1.72E-16
NMB2057	*rplM*	50S ribosomal protein L13					-0.746	2.09E-03	-2.124	1.52E-13
NMB2101	*rpsB*	30S ribosomal protein S2					-1.118	2.98E-05	-3.030	2.95E-18
Translation factors										
NMB0124	*tufA2*	translation elongation factor Tu							-0.782	2.04E-03
NMB0138	*fusA*	elongation factor G (EF-G)							-1.515	5.66E-04
NMB0139	*tufA1*	translation elongation factor Tu							-0.963	2.50E-04
NMB0163	*infA*	translation initiation factor IF-1					-1.523	1.04E-04		
NMB0187	*frr*	ribosome recycling factor							-0.823	3.32E-04
NMB0721	*infC*	translation initiation factor 3					-0.934	6.21E-04	-0.909	6.73E-04
NMB0937	*efp*	elongation factor P (EF-P)					-0.901	5.37E-04		
NMB1643	*infB*	translation initiation factor IF-2	-0.674	8.94E-04					-1.903	3.06E-16
NMB2102	*tsf*	elongation factor TS (EF-TS)							-2.157	9.86E-06
tRNA aminoacylation										
NMB0003	*gltX*	glutamyl-tRNA synthetase							0.592	7.66E-04
NMB0347		D-tyrosyl-tRNA deacylase							1.092	5.54E-07
NMB0466	*aspS*	aspartyl-tRNA synthetase					-0.704	4.16E-04		
NMB0719	*tgt*	queuine tRNA-ribosyltransferase					-0.585	1.54E-05		
NMB0720	*thrS*	threonyl-tRNA synthetase					-0.940	4.30E-06		
NMB0724	*pheS*	phenylalanyl-tRNA synthetase, α chain			-0.805	1.08E-03	-1.144	4.81E-07	-0.929	1.48E-05
NMB0728	*pheT*	phenylalanyl-tRNA synthetase, β chain					-0.613	5.36E-04		
NMB1356	*gatA*	Glu-tRNA(Gln) amidotransferase, subunit A							-1.376	3.42E-13
NMB1425	*lysS*	lysyl-tRNA synthetase, heat inducible							-0.637	9.02E-04
NMB1684	*serS*	seryl-tRNA synthetase							-0.726	4.07E-05
NMB1835	*tyrS*	tyrosyl-tRNA synthetase							-0.862	6.63E-04
tRNA and rRNA base modification										
NMB0348		tRNA-dihydrouridine synthase A							0.819	4.64E-06
NMB0590	*trmD*	tRNA (guanine-N1)-methyltransferase					-1.010	1.07E-05	-1.161	4.32E-07

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Table C.48 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB0697	*ksgA*	dimethyladenosine transferase							0.741	4.19E-05
NMB1298	*rsuA*	ribosomal small subunit pseudouridine synthase A					-1.007	3.50E-05	-0.640	5.14E-03
NMB1556	*trmU*	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase							-0.620	4.98E-06
NMB2036	*truA*	tRNA pseudouridine synthase A					0.636	6.36E-03		

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.49: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *purines, pyrimidines, nucleosides and nucleotides biosynthesis*. genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
2'-Deoxyribonucleotide metabolism										
NMB0639		putative deoxyribonucleotide triphosphate pyrophosphatase							0.624	1.27E-03
Nucleotide and nucleoside interconversions										
NMB0849	*dcd*	putative deoxycytidine triphosphate deaminase	0.890	4.78E-04	0.889	8.76E-04	0.896	1.38E-04	1.350	3.42E-08
NMB1307	*ndk*	nucleoside diphosphate kinase					-0.687	5.19E-04	-0.649	1.53E-03
Purine ribonucleotide biosynthesis										
NMB0690	*purF*	amidophosphoribosyltransferase							-0.621	7.16E-04
NMB0757	*purC*	phosphoribosylaminoimidazole-succinocarboxamide synthase							0.741	4.21E-03
NMB0815	*purA*	adenylosuccinate synthetase					-0.609	2.29E-03		
NMB1201	*guaB*	IMP dehydrogenase							-1.446	5.76E-12
NMB1252	*purM*	phosphoribosylformylglycinamidine cyclo-ligase					-0.956	8.43E-07	-1.519	9.05E-13
NMB1920	*guaA*	GMP synthase							1.459	2.61E-09
Pyrimidine ribonucleotide biosynthesis										
NMB0682	*pyrC*	dihydroorotase							-0.691	1.01E-05
NMB0824	*pyrF*	orotidine 5'-phosphate decarboxylase							0.928	2.60E-07
NMB1554	*pyrG*	CTP synthase							-1.309	9.25E-11
Salvage of nucleosides and nucleotides										
NMB0774	*upp*	uracil phosphoribosyltransferase							-1.185	1.78E-08

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.50: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *regulatory functions*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Other										
NMB0044	*pilB*	trifunctional thioredoxin/methionine sulfoxide reductase A/B protein	1.642	1.45E-08	1.278	1.47E-05	1.226	2.00E-06	1.488	5.54E-08
NMB0115	*ntrX*	nitrogen assimilation regulatory protein NtrX					0.638	1.60E-04	0.723	1.61E-05
NMB0173	*oxyR*	transcriptional regulator, LysR family							-1.365	2.84E-14
NMB0205	*fur*	ferric uptake regulation protein							-1.064	1.17E-08
NMB0290		putative transcriptional regulator					0.869	2.64E-04	0.674	3.54E-03
NMB0381	*cysB*	cys regulon transcriptional activator							0.750	1.22E-07
NMB0573		transcriptional regulator, AsnC family							1.176	7.27E-10
NMB0595		DNA-binding response regulator	0.598	6.75E-03	0.662	3.41E-03			-0.633	9.32E-04
NMB0646		ribonuclease inhibitor barstar							-1.144	1.84E-03
NMB0729	*himA*	integration host factor, α subunit					-0.972	3.15E-06	-2.615	1.18E-20
NMB0748	*hfq*	host factor-I					-1.013	1.49E-03	-2.043	3.01E-09
NMB0810		transcriptional regulator, TetR family							1.599	3.51E-08
NMB1007		transcriptional regulator			-0.983	1.86E-05	-1.364	1.63E-09	0.605	2.30E-03
NMB1204		transcriptional regulator	-0.664	9.15E-04						
NMB1249		putative nitrate nitrite sensory protein NarX							0.848	4.35E-09
NMB1302	*hip*	integration host factor, β subunit							-1.825	9.63E-12
NMB1303		transcriptional regulator, MerR family					-0.940	2.08E-06	-0.726	1.05E-04
NMB1563		transcriptional regulator, GntR family	0.749	1.66E-03						
NMB1585		transcriptional regulator, MarR family							0.693	3.73E-03
NMB1650	*lrp*	leucine-responsive regulatory protein					-1.085	1.01E-05		
NMB1659	*spoT*	guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase					-0.771	1.20E-06		
NMB1735	*relA*	GTP pyrophosphokinase					0.638	1.06E-03	1.162	1.79E-08
NMB1792		sensor histidine kinase					0.716	1.25E-03		
NMB1856		transcriptional regulator, LysR family					0.785	2.20E-04	0.743	1.14E-03
NMB1967		transcriptional regulator, AraC family							1.247	5.35E-05
NMB1995	*glnB*	nitrogen regulatory protein P-II							-0.973	2.30E-05
NMB2012		transcriptional regulator, HTH 3 family					-0.731	4.57E-06	-1.796	1.04E-16

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.51: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into functional group *transcription*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Degradation of RNA										
NMB0646		ribonuclease inhibitor barstar							-1.144	1.84E-03
NMB0662		putative ribonuclease							-0.816	1.27E-05
NMB0758	*pnp*	polyribonucleotide nucleotidyltransferase					-0.726	1.44E-03	-1.175	6.05E-07
DNA-dependent RNA polymerase										
NMB0132	*rpoB*	DNA-directed RNA polymerase, β subunit					-1.139	4.07E-05		
NMB0133	*rpoC*	DNA-directed RNA polymerase, β' subunit					-0.858	5.34E-04	-0.782	1.21E-03
NMB0168	*rpoA*	DNA-directed RNA polymerase, α subunit					-0.897	1.46E-04	-2.027	1.71E-13
RNA processing										
NMB0196	*rne*	ribonuclease E							-0.812	1.56E-06
NMB0591	*rimM*	16S rRNA processing protein					-0.806	2.35E-03	-1.219	5.92E-06
NMB0832	*prrC*	anticodon nuclease					-0.670	2.79E-04	-1.110	1.22E-08
NMB1241	*cca*	tRNA nucleotidyltransferase							-0.747	3.29E-06
NMB1373	*rbfA*	ribosome-binding factor A							-0.746	2.02E-06
NMB1905	*rnpA*	ribonuclease P, protein component					-0.596	2.79E-03	-1.459	5.83E-11
Transcription factors										
NMB0075		putative transcriptional accessory protein Tex							1.691	2.68E-13
NMB0126	*nusG*	transcription antitermination protein					-0.811	1.12E-03	-1.181	9.42E-06
NMB0217		putative RNA polymerase sigma-54 factor RpoN					0.716	8.28E-05	0.946	3.38E-07
NMB1368		putative ATP-dependent RNA helicase	-0.991	8.46E-05						
NMB1430	*greA*	transcription elongation factor	-0.673	7.69E-03			-0.969	2.26E-05	-1.434	2.45E-09
NMB1538	*rpoD*	RNA polymerase sigma factor RpoD	1.024	2.10E-05	0.793	1.85E-03			0.880	6.47E-05
NMB1642	*nusA*	N utilization substance protein A							-1.035	9.21E-06
NMB2144		putative RNA polymerase sigma-70 factor							0.753	1.92E-06

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.52: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point categorised into TIGR designation *transport and binding proteins*. Genes are further subdivided into subroles according to TIGR designations. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
Amino acids, peptides and amines										
NMB0085	*gltS*	sodium/glutamate symporter					-0.812	1.23E-03	-0.641	9.15E-03
NMB0611	*potB*	spermidine/putrescine ABC transporter, permease protein					-0.915	8.47E-07		
NMB0612	*potC*	spermidine/putrescine ABC transporter, permease protein					-0.833	7.57E-08	-0.878	1.18E-08
NMB0623	*potD-2*	spermidine/putrescine ABC transporter, periplasmic substrate-binding protein							-1.318	2.57E-09
NMB0787		amino acid ABC transporter, periplasmic substrate-binding protein					-1.077	4.74E-07	-1.812	4.28E-14
NMB0788		amino acid ABC transporter, permease protein			-0.723	5.40E-05	-1.116	3.86E-10	-1.336	4.34E-13
NMB0789		amino acid ABC transporter, ATP-binding protein					-0.871	4.00E-09		
NMB1594	*potD-3*	spermidine/putrescine ABC transporter, periplasmic substrate-binding protein					-0.732	5.53E-04	-1.584	2.48E-11
NMB1612	*hisJ*	amino acid ABC transporter, periplasmic substrate-binding protein							-1.189	4.72E-07
NMB1963		L-glutamate ABC transporter, putative periplasmic transport protein							-0.791	7.59E-06
NMB1965		L-glutamate ABC transporter, putative inner membrane permease							1.001	1.13E-05
Anions										
NMB0378		putative phosphate permease					-1.085	1.85E-03	-0.994	3.67E-03
NMB0879	*cysA*	sulfate ABC transporter, ATP-binding protein							0.746	2.65E-06
NMB0880	*cysW*	sulfate ABC transporter, permease protein							1.779	2.00E-07
NMB0881	*cysU*	sulfate ABC transporter, permease protein							0.821	2.49E-03
Carbohydrates, organic alcohols, and acids										
NMB0072	*ctrB*	capsule polysaccharide export, inner-membrane protein							-0.801	1.56E-05
NMB0073	*ctrC*	capsule polysaccharide export, inner-membrane protein					-0.722	1.30E-05		
NMB0470		C4-dicarboxylate transporter							0.691	9.90E-06
NMB1362		putative oxalate/formate antiporter					-1.171	6.48E-04		
NMB1794		citrate transporter							0.795	7.76E-04
Cations and iron carrying compounds										
NMB0034		putative high-affinity iron transporter					1.172	9.96E-06	0.899	3.76E-04
NMB0175		putative zinc transporter ZupT					0.764	3.64E-03		
NMB0293		putative TonB-dependent receptor					0.987	4.23E-06	1.503	5.83E-11
NMB0460	*tbpB*	transferrin-binding protein 2	0.975	3.25E-05	1.351	5.63E-08	1.236	9.97E-08	1.740	2.25E-12
NMB0461	*tbpA*	transferrin-binding protein 1			0.818	3.47E-06	1.099	3.84E-10	0.813	4.05E-07
NMB0578	*nosD*	copper ABC transporter, periplasmic substrate-binding protein							0.927	1.10E-04
NMB0579	*nosF*	copper ABC transporter, ATP-binding protein					0.776	3.99E-03	1.046	9.85E-05
NMB0586		putative zinc/manganese transporter	1.141	7.65E-09	1.013	5.38E-07				
NMB0634	*fbpA*	iron(III) ABC transporter, periplasmic substrate-binding protein	1.141	6.13E-06	1.463	5.47E-08	1.122	3.60E-06		
NMB0752		putative bacterioferritin-associated ferredoxin	0.998	6.71E-05						
NMB0964		TonB-dependent receptor	1.005	2.95E-03	1.198	5.85E-04	1.094	3.51E-04	0.970	1.14E-03

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Table C.52 – continued from previous page

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*	log_2 ratio	P-value*
NMB1206	*bfrB*	bacterioferritin B	-1.289	8.01E-08	-1.849	1.23E-11	-2.431	2.09E-17	-1.481	5.22E-10
NMB1207	*bfrA*	bacterioferritin A	-1.217	2.58E-07	-1.789	2.11E-11	-2.307	6.77E-17	-1.022	3.32E-06
NMB1497		TonB-dependent receptor	0.634	1.11E-03						
NMB1540	*lbpA*	lactoferrin-binding protein A					0.976	1.76E-03		
NMB1541	*lbpB*	lactoferrin-binding protein B	0.615	6.11E-04			0.866	2.64E-06	1.489	5.57E-13
NMB1668	*hmbR*	hemoglobin receptor					0.695	4.38E-07		
NMB1730	*tonB*	TonB protein	0.585	5.19E-03	0.987	3.15E-06	1.054	1.35E-07		
NMB1829		TonB-dependent receptor							2.232	1.62E-03
NMB1880	*fetB2*	putative ABC transporter, periplasmic solute-binding protein							1.058	1.21E-03
NMB1882		TonB-dependent receptor					0.699	2.82E-03		
NMB1988	*fetA*	iron-regulated outer membrane protein FrpB					0.652	5.76E-03	1.170	1.55E-06
NMB1989	*fetB*	iron(III) ABC transporter, periplasmic substrate-binding protein							1.066	1.86E-06
NMB1990		iron(III) ABC transporter, permease protein							0.862	4.60E-03
Nucleosides, purines and pyrimidines										
NMB2067	*cytX*	probable hydroxymethylpyrimidine transporter CytX					0.735	3.17E-03	0.908	2.34E-04
Other										
NMB0097		putative secretion protein, degenerate			-0.606	2.46E-03	-0.622	3.54E-04	-0.878	8.12E-07
NMB0318	*farA*	fatty acid efflux system protein					0.743	5.96E-04	1.539	1.21E-10
NMB0319	*farB*	fatty acid efflux system protein					0.668	9.29E-05	0.730	1.59E-05
NMB0393		multidrug resistance protein					1.142	2.67E-09	0.780	8.69E-06
NMB0812		multidrug efflux protein					0.771	1.84E-03	0.918	1.81E-04
NMB1277		transporter, BCCT family							1.116	2.07E-08
NMB1714	*mtrE*	multidrug efflux pump channel protein					0.611	1.10E-03	0.698	1.59E-04
NMB1716	*mtrC*	membrane fusion protein							1.301	3.08E-07
NMB1719	*mtrF*	efflux pump component MtrF			-0.769	4.07E-04	-0.789	4.32E-05	-1.304	3.52E-10
NMB1728	*exbD*	biopolymer transport protein ExbD			0.860	5.92E-05	0.639	7.45E-04	-0.927	1.59E-06
NMB1738	*hlyD*	putative secretion protein							0.791	9.69E-04
NMB1429	*porA*	outer membrane protein PorA					-0.860	4.16E-03	-2.550	4.55E-13
NMB2039	*porB*	major outer membrane protein PIB							-2.823	1.24E-14
Unknown substrate										
NMB0098		ABC transporter, ATP-binding protein (authentic frameshift)					-0.719	2.71E-04	-0.820	2.83E-05
NMB0387		ABC transporter, ATP-binding protein							-0.615	3.67E-06
NMB0549	*yjbZ*	ABC transporter, ATP-binding protein							0.947	6.12E-09
NMB1240		ABC transporter, ATP-binding protein					-0.645	8.13E-04	-0.899	4.09E-06
NMB1707		sodium- and chloride-dependent transporter							0.734	2.20E-04

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.53: Differentially expressed genes in blood at 40, 60, 90 and/or 240 min in comparison to the 20 min time-point with unknown function. Only genes with a fold change of ≥ 1.5 and P-value* ≤ 0.01 are listed in the table.

ID	Name	Annotation	40 min		60 min		90 min		240 min	
			$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*	$\log_2$ ratio	P-value*
General										
NMB0004		EpiH/GdmH-related protein					0.711	1.11E-03	1.043	2.61E-06
NMB0009		BolA/YrbA family protein							-1.344	2.14E-07
NMB0193	*gidA*	glucose inhibited division protein A							0.616	3.08E-05
NMB0255		cell filamentation protein Fic-related protein			-0.678	5.53E-04	-0.983	9.97E-08		
NMB0349		glutamyl-tRNA synthetase-related protein					0.683	2.74E-04		
NMB0360		AmpG-related protein					0.753	1.07E-03	0.843	2.05E-04
NMB0374	*mafB2*	MafB-related protein							0.790	4.05E-07
NMB0490		PspA-related protein					0.794	4.07E-05	1.628	2.68E-13
NMB0496		hemolysin activator-related protein							1.067	5.78E-06
NMB0577		NosR-related protein							0.757	1.06E-03
NMB0624		galactosyltransferase-related protein (authentic frameshift)					-0.621	1.87E-03		
NMB0653		MafB-related protein							0.971	1.50E-04
NMB0827	*nmgI*	type II restriction enzyme-related protein (authentic frameshift)					-0.989	2.00E-05	-0.641	6.21E-03
NMB0901		D-lactate dehydrogenase-related protein							-0.967	9.20E-06
NMB0985		E16-related protein							1.218	1.71E-13
NMB1238		peptidyl-prolyl cis-trans isomerase-related protein							-0.736	5.78E-06
NMB1335	*creA*	creA protein	1.441	6.52E-15	0.945	5.47E-08				
NMB1337		putative Holliday junction resolvase-like protein	0.678	2.72E-06						
NMB1347	*suhB*	extragenic suppressor protein SuhB					-1.080	4.05E-09	-1.546	1.81E-14
NMB1380		nifU protein	0.727	1.48E-03	0.798	8.76E-04	0.604	3.68E-03	0.595	3.52E-03
NMB1381		HesB/YadR/YfhF family protein	0.588	2.83E-03	0.591	4.16E-03				
NMB1409		FrpA/C-related protein	-0.976	5.01E-07	-1.093	9.18E-08	-1.394	1.19E-11	-1.688	7.65E-15
NMB1482		acyl CoA thioester hydrolase family protein							0.959	1.23E-03
NMB1526	*smpB*	small major protein B							-0.630	6.37E-03
NMB1669	*hemO*	iron-starvation protein PigA							-0.966	1.38E-03
NMB1689		putative dedA protein							-0.886	1.77E-07
NMB1692		chorismate mutase-related protein					0.756	1.27E-03		
NMB1747		putative tspB protein					0.798	1.58E-03	0.967	1.16E-04
NMB1753		VapD-related protein							-0.720	3.04E-03
NMB2008		ABC transporter, ATP-binding protein-related protein							-1.099	2.69E-09
NMB2105	*mafB*	mafB protein							0.689	2.89E-06
NMB2128		CinA-related protein					0.590	3.04E-03		
NMB2132		transferrin-binding protein-related protein			-0.616	5.53E-04				

* P-value adjusted for multiple testing using Benjamini and Hochberg step down correction

Table C.54: Genes up-regulated at 40, 60, 90 and/or 240 min in blood compared to the 20 min time-point divided into cellular locations based on predictions and/or published experimental data. Only those genes which are up-regulated at any of the four time-points in blood (indicated by 'Up') and which encode proteins that have a known or putative location in the periplasm or the outer membrane are listed in the table.

		Time-point (min)					PSORTb	PSLpred	CBS	
ID	**Name**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB0003	*gltX*				Up	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0035		Up	Up	Up		OMP (Arenas *et al.*, 2006); OM and OMVs (Ferrari *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	LipoP	✓
NMB0036				Up			Unknown	Cytoplasmic	SignalP	✓
NMB0044	*pilB*	Up	Up	Up	Up	Associated with OM (Skaar *et al.*, 2002); OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	LipoP	✓
NMB0046					Up		Unknown	Periplasmic	Cytoplasmic	
NMB0054					Up		Unknown	Cytoplasmic	SignalP	✓
NMB0078	*galE-2*			Up			Unknown	Periplasmic	SignalP	✓
NMB0086				Up		OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Multiple locations	Cytoplasmic	Cytoplasmic	
NMB0121					Up	Surface located (Kolberg *et al.*, 2008)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0176	*dadA*				Up		Unknown	Cytoplasmic	SignalP	✓
NMB0207	*gapA-1*				Up	Surface located (Grifantini *et al.*, 2002a); OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0214	*prlC*	Up	Up	Up	Up	Surface located (Grifantini *et al.*, 2002a); OM and OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0216	*katA*	Up	Up	Up	Up		Periplasmic	Periplasmic	Cytoplasmic	
NMB0225					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0278	*dsbA-1*	Up				OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	LipoP	✓
NMB0293				Up	Up		Outer membrane	Outer membrane	SignalP	✓
NMB0294	*dsbA-2*	Up			Down	OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Periplasmic	Periplasmic	LipoP	✓
NMB0313				Up		OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Outer membrane	SignalP	✓
NMB0315				Up	Up	Surface located (Grifantini *et al.*, 2002a)	Unknown	Cytoplasmic	SignalP	✓
NMB0318	*farA*			Up	Up		Unknown	Outer membrane	SignalP	✓
NMB0334	*pgi-2*			Up			Cytoplasmic	Outer membrane	Cytoplasmic	
NMB0337	*ilvE*	Up	Up			OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0345		Up	Up			OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	✓
NMB0346		Up	Up			OM and OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Multiple locations	Cytoplasmic	SignalP	✓
NMB0359	*glnA*	Up				OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0372			Down	Down	Up		Unknown	Periplasmic	SecretomeP	
NMB0374	*mafB2*				Up		Unknown	Outer membrane	SignalP	✓
NMB0376					Up		Unknown	Periplasmic	Cytoplasmic	
NMB0392	*nadB*			Up			Periplasmic	Periplasmic	Cytoplasmic	
NMB0397				Up			Unknown	Extracellular	SecretomeP	
NMB0460	*tbpB*	Up	Up	Up	Up	OM (Williams *et al.*, 2007); OMVs (Ferrari *et al.*, 2006)	Outer membrane	Outer membrane	SecretomeP	

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Table C.54 – continued from previous page

		Time-point (min)					PSORTb	PSLpred	CBS	
ID	**Name**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB0461	*tbpA*		Up	Up	Up	OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	√
NMB0476				Down	Up		Unknown	Periplasmic	Cytoplasmic	
NMB0490				Up	Up		Unknown	Outer membrane	SecretomeP	
NMB0493					Up		Outer membrane	Outer membrane	SecretomeP	
NMB0496					Up		Outer membrane	Outer membrane	Cytoplasmic	
NMB0497				Up	Up		Outer membrane	Extracellular	SecretomeP	
NMB0499				Up	Up		Unknown	Periplasmic	TMHMM	
NMB0502					Up		Unknown	Periplasmic	TMHMM	
NMB0509				Up	Up		Unknown	Periplasmic	SecretomeP	
NMB0511				Up	Up		Unknown	Cytoplasmic	SecretomeP	
NMB0545					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0549	*yjbZ*				Up	OM (Williams *et al.*, 2007)	Inner membrane	Inner membrane	TMHMM	
NMB0554	*dnaK*	Up	Up			Plasminogen binding (Knaust *et al.*, 2007); OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	Cytoplasmic	
NMB0561	*grpE*	Up	Up		Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0578	*nosD*				Up		Periplasmic	Periplasmic	Cytoplasmic	
NMB0586		Up	Up			OMVs (Vipond *et al.*, 2006)	Inner membrane	Periplasmic	SignalP	√
NMB0595		Up	Up		Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0618	*ppsA*	Up	Up			OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB0631		Up	Up		Down	OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB0634	*fbpA*	Up	Up	Up		OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Periplasmic	Periplasmic	SignalP	√
NMB0635					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0639					Up		Unknown	Periplasmic	SecretomeP	
NMB0647					Up		Unknown	Cytoplasmic	SecretomeP	
NMB0653					Up		Unknown	Extracellular	SignalP	√
NMB0655			Down	Down	Up	Surface located (Grifantini *et al.*, 2002a)	Unknown	Periplasmic	SecretomeP	
NMB0701					Up		Unknown	Extracellular	Cytoplasmic	
NMB0717					Up		Unknown	Periplasmic	SignalP	√
NMB0749					Up		Periplasmic	Periplasmic	SignalP	√
NMB0752		Up					Unknown	Extracellular	Cytoplasmic	
NMB0791	*ppiB*	Up	Up		Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0805					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB0850		Up					Unknown	Periplasmic	SignalP	√
NMB0907		Up	Up		Up		Unknown	Extracellular	SecretomeP	
NMB0942	*rpmE2*	Up	Up		Down		Unknown	Periplasmic	SecretomeP	
NMB0946		Up			Down	Plasminogen binding (Knaust *et al.*, 2007); OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	SecretomeP	
NMB0947	*lpdA2*	Up			Down	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	

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Table C.54 – continued from previous page

		Time-point (min)					PSORTb	PSLpred	CBS	
ID	**Name**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB0964		Up	Up	Up	Up	OMVs (Vipond *et al.*, 2006)	Unknown	Outer membrane	SignalP	✓
NMB0965				Up	Up		Unknown	Periplasmic	Cytoplasmic	
NMB1006					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1013					Up		Unknown	Periplasmic	SecretomeP	
NMB1014					Up		Unknown	Periplasmic	TMHMM	
NMB1022					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1050					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1081					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1082					Up		Unknown	Periplasmic	SecretomeP	
NMB1096				Up	Up		Unknown	Periplasmic	Cytoplasmic	
NMB1099					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1100					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1106					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1108				Up			Unknown	Periplasmic	Cytoplasmic	
NMB1120					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1173					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1214					Up		Outer membrane	Outer membrane	TMHMM	
NMB1251					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1259					Up		Cytoplasmic	Periplasmic	Cytoplasmic	
NMB1262	*ppiA*				Up		Periplasmic	Periplasmic	SignalP	✓
NMB1292					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1293				Up			Unknown	Periplasmic	SignalP	✓
NMB1294	*plsC*			Up	Up		Unknown	Cytoplasmic	SignalP	✓
NMB1317					Up		Unknown	Extracellular	TatP	✓
NMB1327				Up		OMVs (Vipond *et al.*, 2006)	Extracellular	Periplasmic	Cytoplasmic	
NMB1332	*prc*				Up	OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Periplasmic	SignalP	✓
NMB1334		Up	Up				Unknown	Periplasmic	Cytoplasmic	
NMB1364	*nadE*			Up		OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1366	*trxA*	Up	Up	Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1377	*lldA*	Up	Up	Up	Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1379	*nifS*	Up	Up	Up	Up	OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1380		Up	Up	Up	Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1472	*clpB*	Up	Up	Up	Up	OM and OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1475		Up	Up	Up	Up		Unknown	Periplasmic	SignalP	✓
NMB1497		Up				OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	✓
NMB1538	*rpoD*	Up	Up		Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1540	*lbpA*			Up		OMVs (Vaughan *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	SignalP	✓
NMB1541	*lbpB*	Up		Up	Up		Outer membrane	Cytoplasmic	LipoP	✓
NMB1544					Up		Unknown	Periplasmic	SecretomeP	
NMB1578			Up		Up	OMVs (Ferrari *et al.*, 2006)	Unknown	Periplasmic	LipoP	✓

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Table C.54 – continued from previous page

		Time-point (min)					PSORTb	PSLpred	CBS	
ID	**Name**	**40**	**60**	**90**	**240**	**Experimental**	**Location**	**Location**	**Prediction**	**SignalP**
NMB1579	*hisG*		Up		Up	OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1602					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1609	*metZ*				Up	OM (Williams *et al.*, 2007)	Cytoplasmic	Inner membrane	Cytoplasmic	
NMB1633					Up		Unknown	Periplasmic	SecretomeP	
NMB1654					Up		Unknown	Periplasmic	SignalP	✓
NMB1668	*hmbR*			Up			Outer membrane	Outer membrane	SignalP	✓
NMB1700					Up		Unknown	Periplasmic	Cytoplasmic	
NMB1714	*mtrE*			Up	Up	OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Outer membrane	Outer membrane	Cytoplasmic	
NMB1716	*mtrC*				Up	OMVs (Williams *et al.*, 2007)	Inner membrane	Cytoplasmic	LipoP	✓
NMB1738	*hlyD*				Up		Inner membrane	Periplasmic	Cytoplasmic	
NMB1747				Up	Up		Outer membrane	Periplasmic	TMHMM	
NMB1749					Up		Unknown	Periplasmic	TMHMM	
NMB1762					Up		Outer membrane	Outer membrane	SignalP	✓
NMB1772				Up	Up		Unknown	Periplasmic	TMHMM	
NMB1775				Up	Up		Outer membrane	Periplasmic	TMHMM	✓
NMB1776				Up			Unknown	Extracellular	SignalP	✓
NMB1779				Up	Up		Outer membrane	Extracellular	SecretomeP	
NMB1784					Up		Unknown	Periplasmic	SecretomeP	
NMB1789	*secB*	Up	Up	Up		OM and OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1802	*gcp*			Up			Extracellular	Extracellular	Cytoplasmic	
NMB1829					Up		Outer membrane	Outer membrane	SignalP	✓
NMB1880	*fetB2*				Up		Unknown	Periplasmic	LipoP	✓
NMB1882				Up			Outer membrane	Outer membrane	SignalP	✓
NMB1972	*groEL*	Up	Up			OM and OMVs (Ferrari *et al.*, 2006; Vaughan *et al.*, 2006; Vipond *et al.*, 2006; Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1973	*groES*	Up	Up			OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB1977					Up	OM (Williams *et al.*, 2007)	Unknown	Outer membrane	LipoP	✓
NMB1988	*fetA*			Up	Up	OMVs (Ferrari *et al.*, 2006)	Outer membrane	Outer membrane	SignalP	✓
NMB1989	*fetB*				Up		Periplasmic	Periplasmic	LipoP	✓
NMB2000	*hslO*	Up		Up		OM (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB2036	*truA*			Up		OM (Williams *et al.*, 2007)	Unknown	Cytoplasmic	Cytoplasmic	
NMB2074					Up		Unknown	Periplasmic	Cytoplasmic	
NMB2096	*mqo*			Up		OMVs (Williams *et al.*, 2007)	Cytoplasmic	Cytoplasmic	Cytoplasmic	
NMB2105	*mafB*				Up		Unknown	Periplasmic	SignalP	✓
NMB2139					Up	OMVs (Ferrari *et al.*, 2006; Williams *et al.*, 2007)	Unknown	Cytoplasmic	LipoP	✓
NMB2148					Up		Cytoplasmic	Periplasmic	Cytoplasmic	

OM or OMVs in the table indicate that the protein has been detected in outer membrane or outer membrane vesicles preparations, respectively. For predictions the algorithms PSORTb, PSLpred, and tools at CBS were used. Denominations from CBS: SignalP, the protein encodes a signal peptide; TMHMM, the protein encodes transmembrane helices; LipoP, the proteins is a lipoprotein; SecretomeP, the protein is secreted through a non-classical i.e. not signal peptide triggered protein secretion and TatP, the protein has a twin-arginine signal peptide cleavage sites.

Table C.55: Genes up-regulated during incubation in blood, but down-regulated upon meningococcal attachment to epithelial cells (Grifantini *et al.*, 2002a), which encodes potential surface exposed proteins. Predictions of cellular locations were performed using PSORTB v.2 (**P1**), PLSpred (**P2**), or tools at CBS (**P3**). Abbreviations: OM, outer membrane; CP, cytoplasmic; P, periplasmic; L, Lipoprotein; U, no clear location; ML, multiple locations.

		versus T = 0 min					Between T				versus T = 20 min			16HBE14 cells[A]				Cellular location				
ID	**Name**	**20 min**	**40 min**	**60 min**	**90 min**	**240 min**	**40 min**	**60 min**	**90 min**	**240 min**	**60 min**	**90 min**	**240 min**	**30 min**	**60 min**	**120 min**	**180 min**	**Experimental**	**P1**	**P2**	**P3**	**SignalP**
NMB0130	*rplJ*	Up				Down									Down			OM and OMVs [B]	U	CP	CP	
NMB0139	*tufA-1*	Up	Up	Up										Down				OM [B] and OMVs [B,D,E]	CP	CP	CP	
NMB0140	*rpsJ*	Up	Up	Up		Down								Down				OM [B]	CP	CP	CP	
NMB0153	*rplX*	Up				Down								Down	Down			OM and OMVs [B]	U	CP	CP	
NMB0158	*rplR*	Up												Down					CP	OM	CP	
NMB0160	*rpmD*	Up	Up											Down	Down			OM and OMVs [B]	CP	CP	CP	
NMB0161	*rplO*	Up												Down	Down			OM and OMVs [B]	U	CP	CP	
NMB0165	*rpsM*	Up				Down								Down				OM and OMVs [B]	CP	CP	CP	
NMB0168	*rpoA*	Up	Up			Down								Down	Down			OM [B] and OMVs [B,C]	CP	CP	CP	
NMB0390	*mapA*	Up	Up														Down	OMVs [B,E]	OM	CP	CP	
NMB0550	*dsbC*		Up	Up										Down				OM and OMVs [B]	P	P	L	Y
NMB0554	*dnaK*		Up	Up	Up		Up			Down	Up			Down				Plasminogen binding [F]. OM [B] and OMVs [C,E,F,H]	U	P	CP	
NMB0609	*rpsO*	Up				Down								Down			Down	OM and OMVs [B]	U	CP	CP	
NMB0723	*rplT*	Up				Down								Down	Down		Down	OM and OMVs [B]	U	OM	CP	
NMB0791	*ppiB*		Up	Up		Down	Up			Down	Up		Down	Down				OM and OMVs [B]	CP	P	CP	
NMB0947	*lpdA2*		Up			Down	Up			Down			Down	Down				OM and OMVs [B]	CP	CP	CP	
NMB1206	*bfrB*	Up	Up	Up		Up	Down			Up				Down	Up	Down	Down	OM [B]	CP	CP	CP	
NMB1207	*bfrA*	Up	Up			Up	Down			Up				Down		Down	Down	OM and OMVs [B]	CP	CP	CP	
NMB1312	*clpP*	Up	Up	Up		Up				Up				Down				OM [B]	CP	CP	CP	
NMB1366	*trxA*		Up	Up	Up		Up			Down	Up	Up		Down	Down			OM [B]	CP	CP	CP	
NMB1523		Up	Up	Up		Down								Down	Down				ML	EC	L	Y
NMB1533		Up	Up	Up		Down								Down	Down			OM [B] and OMVs [B,C]	P	P	L	Y
NMB1802	*gcp*								Up	Down		Up		Down	Down				EC	EC	CP	
NMB1811	*pilP*		Up											Down			Down		U	EC	CP	
NMB1934	*atpD*	Up	Up	Up										Down	Down		Down	OM [B] and OMVs [B,C,E]	CP	CP	CP	
NMB1935	*atpG*	Up				Down									Down		Down	OM and OMVs [B]	U	CP	CP	
NMB1972	*groEL*		Up	Up	Up	Up	Up				Up			Down				OM [B] and OMVs [B,C,D,E]	CP	CP	CP	
NMB1973	*groES*		Up	Up	Up		Up				Up			Down				OM [B]	CP	CP	CP	

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Table C.55 – continued from previous page

ID	Name	versus T = 0 min				Between T				versus T = 20 min			16HBE14 cells[A]				Cellular location				
		20 min	40 min	60 min	90 min	240 min	40 min	60 min	90 min	240 min	60 min	90 min	240 min	30 min	60 min	120 min	180 min	Experimental	P1	P2	P3	SignalP
NMB2056	*rpsI*	Up	Up	Up		Down								Down	Down			OM and OMVs [B]	U	CP	CP	
NMB2101	*rpsB*	Up	Up	Up	Up	Down								Down		Down		OMVs [C,E]	U	CP	CP	

A (Grifantini *et al.*, 2002a), B (Williams *et al.*, 2007), C (Ferrari *et al.*, 2006), D (Vipond *et al.*, 2006), E (Vaughan *et al.*, 2006), F (Knaust *et al.*, 2007)

Table C.56: Genes encoding potential surface exposed proteins that were up-regulated during incubation in blood, which were not reported to be differentially expressed upon attachment to epithelial cells (Grifantini *et al.*, 2002a). Predictions of cellular locations were performed using PSORTB v.2 (**P1**), PLSpred (**P2**), or tools at CBS (**P3**). Abbreviations used in the table: OM, outer membrane; IM, inner membrane; CP, cytoplasmic; P, periplasmic; L, Lipoprotein; U, no clear location; ML, multiple locations; TM, Trans-membrane helices.

ID	Name	versus T = 0 min				Between T				versus T = 20 min			Cellular location				
		20 min	40 min	60 min	90 min	240 min	40 min	60 min	90 min	240 min	60 min	90 min	240 min	Experimental	P3	P2	P1	SignalP
NMB0003	*gltX*					Up				Up			Up	OMVs [B]	CP	CP	CP	
NMB0035							Up			Down	Up	Up		OMP [C]; OM and OMVs [B,D,E]	U	CP	L	Y
NMB0044	*pilB*		Up		Up	Up	Up				Up	Up	Up	Surface located [F]. OMVs [B]	U	P	L	Y
NMB0086					Up							Up		OMVs [B]	ML	CP	CP	
NMB0109		Up	Up											OM [B] and OMVs [B,D,G]	U	IM	CP	
NMB0121													Up	Surface located [H]	CP	CP	CP	
NMB0128	*rplA*	Up												OM [B] and OMVs [B,G]	U	CP	S	
NMB0137	*rpsG*	Up		Up		Down								OM and OMVs [B]	U	CP	CP	
NMB0142	*rplC*	Up												OM and OMVs [B]	CP	P	S	
NMB0144	*rplW*	Up	Up	Up										OM and OMVs [B]	U	P	CP	
NMB0146	*rpsS*	Up	Up	Up										OM [B]	U	P	CP	
NMB0147	*rplV*	Up	Up	Up										OM [B]	U	OM	CP	
NMB0150	*rpmC*	Up	Up	Up										OM [B]	U	CP	CP	
NMB0151	*rpsQ*	Up	Up	Up										OMVs [B]	U	P	CP	
NMB0152	*rplN*	Up				Down								OMVs [B]	U	CP	CP	
NMB0174	*valS*	Up	Up	Up										OM and OMVs [B]	CP	CP	CP	
NMB0179	*fabZ*			Up		Up								OM [B]	CP	CP	CP	
NMB0219	*fabF-1*	Up	Up	Up	Up									OM [B] and OMVs [B,D,E]	IM	C	CP	
NMB0265	*ruvA*	Up	Up	Up	Up	Up								OM [B]	U	CP	CP	
NMB0284	*purB*		Up	Up										OMVs [B]	U	CP	CP	
NMB0293									Up			Up	Up		OM	OM		Y
NMB0294	*dsbA-2*						Up			Down			Down	Surface located [D]. OM and OMVs [B]	P	P	L	Y
NMB0313												Up		Surface located [D]. OMVs [B,G]	U	OM		Y
NMB0317		Up	Up		Up	Up								OM [B]	CP	CP	CP	
NMB0318	*farA*				Up	Up				Up		Up	Up		U	OM		Y
NMB0324	*rpmA*	Up	Up	Up		Down								OM and OMVs [B]	U	CP	CP	

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Table C.56 – continued from previous page

ID	Name	versus T = 0 min					Between T				versus T = 20 min			Cellular location Experimental	P3	P2	P1	SignalP
		20 min	40 min	60 min	90 min	240 min	40 min	60 min	90 min	240 min	60 min	90 min	240 min					
NMB0334	*pgi-2*								Up			Up			CP	OM	CP	
NMB0337	*ilvE*						Up			Down	Up			OM [B]	U	CP	CP	
NMB0345							Up		Down		Up			Surface located [D]. OM [B] and OMVs [B,G]	OM	OM		Y
NMB0346							Up				Up			Surface located [D]. OM [B]	ML	CP		Y
NMB0359	*glnA*		Up	Up			Up		Down					OM and OMVs [B,D]	CP	CP	CP	
NMB0374	*mafB2*			Down		Up				Up			Up		U	OM		Y
NMB0397												Up			U	EC	S	
NMB0464		Up	Up	Up										OMVs [B,E]	OM	OM	S	
NMB0469	*speB*		Up	Up										OM [B]	CP	CP	CP	
NMB0496										Up			Up		OM	OM	CP	
NMB0497		Down		Down		Up			Up	Up		Up	Up		OM	EC	S	
NMB0540	*aspC*		Up	Up		Up				Up				OMVs [B]	CP	CP	CP	
NMB0549	*ybjZ*					Up				Up			Up	OM [B]	IM	IM	TM	
NMB0561	*grpE*	Up	Up	Up	Up	Up	Up				Up		Up	OM [B]	CP	P	CP	
NMB0563	*apbE*	Up	Up	Up		Up									U	EC	L	Y
NMB0569	*nqrA*	Up												OMVs [B]	U	CP	CP	
NMB0586			Up				Up				Up			OMVs [D,E]	IM	P		Y
NMB0589	*rplS*	Up	Up	Up										OM [B] and OMVs [B,D,G]	CP	CP	CP	
NMB0592	*rpsP*	Up	Up	Up		Down								OM [B] and OMVs [B,D]	U	OM	CP	
NMB0595							Up			Down	Up		Down	OM [B] and OMVs [B,D]	CP	CP	CP	
NMB0618	*ppsA*						Up				Up			OM [B] and OMVs [B,D]	CP	CP	CP	
NMB0623	*potD-2*	Up	Up	Up										OM [B] and OMVs [B,D]	P	P	L	Y
NMB0631							Up			Down	Up		Down	OMVs [B,D,E,G]	U	CP	CP	
NMB0653										Up			Up		U	EC		Y
NMB0663	*nspA*	Up	Up	Up	Up									OM [B] and OMVs [B,D]	OM	OM		Y
NMB0701						Up				Up			Up		U	EC	CP	
NMB0720	*thrS*	Up	Up	Up		Up			Down	Up				OM and OMVs [B]	CP	CP	CP	
NMB0727		Up													U	EC	CP	
NMB0752							Up								U	EC	CP	
NMB0758	*pnp*	Up												OM [B] and OMVs [B,D,E]	CP	CP	CP	
NMB0798	*ftsH*						Up							OMVs [B]	IM	IM	TM	Y
NMB0821		Up	Up	Up	Up	Up				Up					U	OM	TM	
NMB0866		Up													OM	EC		Y
NMB0884	*sodB*									Up				OM [B]	U	EC	S	
NMB0907						Up	Up			Up	Up		Up		U	EC	S	
NMB0922	*lst*	Up	Up	Up		Up								Surface located [J]. OMVs [E]	U	CP	CP	
NMB0946			Up	Up		Down	Up			Down			Down	Plasminogen binding [I]. OMVs [B,D,G]	U	CP	S	
NMB0957	*lpdA3*									Up				OM [B] and OMVs [B,D]	CP	CP	CP	
NMB0964				Up	Up		Up				Up	Up	Up	OMVs [E]	U	OM		Y
NMB0983	*purH*	Up	Up	Up		Up								OMVs [B,D]	CP	CP	CP	

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Table C.56 – continued from previous page

ID	Name	versus T = 0 min					Between T				versus T = 20 min			Cellular location Experimental	P3	P2	P1	SignalP
		20 min	40 min	60 min	90 min	240 min	40 min	60 min	90 min	240 min	60 min	90 min	240 min					
NMB1020		Up	Up	Up											CP	EC	CP	
NMB1035			Up	Up										OM [B]	U	P	L	Y
NMB1037	*gshA*	Up	Up	Up	Up	Up								OMVs [B]	CP	CP	CP	
NMB1038	*radC*					Up								OM [B]	U	CP	CP	
NMB1040				Up	Up										CP	OM	SignalP	Y
NMB1044	*fpr-1*	Up	Up	Up										OMVs [B]	U	CP	CP	
NMB1053	*opcA*	Up	Up											OM and OMVs [B,D,E]	OM	OM		Y
NMB1070	*leuA*	Up	Up	Up										OMVs [B]	CP	CP	CP	
NMB1073		Up	Up	Up	Up									OM and OMVs [B]	U	OM	CP	
NMB1164		Up	Up	Up		Up								OM [B] and OMVs [B,D,E]	U	OM	L	Y
NMB1195					Up										U	OM	CP	
NMB1214										Up			Up		OM	OM	TM	
NMB1228	*metM*	Up	Up	Up	Up	Up								OM[B] and OMVs [B,D]	CP	CP	CP	
NMB1285	*eno*	Up	Up	Up										Plasminogen binding [I]. OM and OMVs [B,D,G]	CP	CP	CP	
NMB1301	*rpsA*	Up	Up	Up										OM [B] and OMVs [B,D,E]	CP	CP	CP	
NMB1317									Up				Up		U	EC	TATP	Y
NMB1327					Up							Up		OMVs [E]	EC	P	CP	
NMB1332	*prc*					Up				Up			Up	OM [B] and OMVs [B,D,G]	U	P		Y
NMB1339	*proS*		Up											OMVs [B]	CP	CP	CP	
NMB1347	*suhB*	Up	Up	Up	Up									OM [B]	CP	CP	CP	
NMB1437		Up	Up	Up	Up	Up								OMVs [B,D]	U	CP	CP	
NMB1457	*tktA*		Up	Up										OMVs [G]	U	CP	CP	
NMB1472	*clpB*	Down	Up	Up			Up				Up	Up	Up	OM [B] and OMVs [B,G]	CP	CP	CP	
NMB1497							Up							OMVs [B,D,G]	OM	OM		Y
NMB1538	*rpoD*		Up	Up		Up	Up			Up	Up		Up	OM [B]	CP	CP	CP	
NMB1540	*lbpA*											Up		OMVs [B,G]	OM	OM		Y
NMB1554	*pyrG*	Up	Up	Up										OMVs [B,D]	CP	CP	TM	
NMB1594	*potD-3*	Up	Up	Up		Down									P	P	L	Y
NMB1596										Up					U	OM	CP	
NMB1609	*metZ*					Up				Up			Up	OM [B]	CP	IM	CP	
NMB1612	*hisJ*		Up			Down								OM and OMVs [B,D]	P	P	L	Y
NMB1643	*infB*	Up		Up		Down								OMVs [B]	CP	CP	S	
NMB1648		Up	Up	Up	Up										CP	OM	CP	
NMB1652		Up	Up											OM and OMVs [B]	U	CP	CP	
NMB1660	*rpoZ*									Up				OM [B]	CP	CP	CP	
NMB1668	*hmbR*											Up			OM	OM		Y
NMB1680	*aroC*	Up	Up	Up	Up									OMVs [B]	CP	CP	CP	
NMB1681		Up	Up	Up		Down								OM [B]	U	P	CP	
NMB1683						Up				Up					CP	OM	CP	
NMB1714	*mtrE*											Up	Up	OM [B] and OMVs [B,D,E,G]	OM	OM	CP	

Continued on next page ...

Table C.56 – continued from previous page

ID	Name	versus T = 0 min					Between T				versus T = 20 min			Cellular location Experimental	P3	P2	P1	SignalP
		20 min	40 min	60 min	90 min	240 min	40 min	60 min	90 min	240 min	60 min	90 min	240 min					
NMB1716	*mtrC*									Up			Up	OMVs [B]	IM	CP	L	Y
NMB1747						Up			Up			Up	Up		OM	P	TM	
NMB1762										Up			Up		OM	OM		Y
NMB1775		Down		Down		Up			Up			Up	Up		OM	P	TM	Y
NMB1776					Up				Up	Down		Up			U	EC		Y
NMB1779						Up			Up	Up		Up	Up		OM	EC	S	
NMB1789	*secB*						Up			Down	Up	Up		OM and OMVs [B]	CP	CP	CP	
NMB1800		Up	Up	Up		Up				Up					U	EC	S	
NMB1805	*cyc*	Up	Up	Up										OM [B]	P	P		Y
NMB1812	*pilQ*		Up			Down								OM [B] and OMVs [B,D]	OM	OM		Y
NMB1829													Up		OM	OM		Y
NMB1849	*carA*		Up	Up	Up	Up								OM [B] and OMVs [B,D]	U	CP	CP	
NMB1870			Up											Surface located [K]. OM [B]	U	CP	CP	
NMB1882					Up							Up			OM	OM		Y
NMB1939	*atpE*		Up			Down								OM [B]	IM	IM	TM	
NMB1950	*rpsU*	Up				Down								OM [B]	CP	CP	CP	
NMB1961		Up	Up	Up	Up									OM [B]	OM	OM		Y
NMB1963		Up	Up	Up										OM [B]	U	P		Y
NMB1977				Up	Up	Up				Up			Up	OM [B]	U	OM	L	Y
NMB1988	*fetA*											Up	Up	OMVs [D]	OM	OM		Y
NMB1996	*purL*					Up				Up				OMVs [G]	OM	CP	CP	
NMB2000	*hslO*		Up	Up	Up		Up			Down		Up		OM [B]	CP	CP	CP	
NMB2036	*truA*											Up		OM [B]	U	CP	CP	
NMB2053	*petA*	Up	Up	Up	Up									OM [B]	IM	P	S	
NMB2096	*mqo*											Up		OMVs [B,D]	CP	CP	CP	
NMB2132		Up												Surface located [L]	U	OM	L	Y
NMB2139						Up							Up	OMVs [B,D]	U	CP	L	Y
NMB2147										Up					U	EC	L	Y

A (Grifantini *et al.*, 2002a), B (Williams *et al.*, 2007), C (Arenas *et al.*, 2006), D (Ferrari *et al.*, 2006), E (Vipond *et al.*, 2006), F (Skaar *et al.*, 2002), G (Vaughan *et al.*, 2006), H (Kolberg *et al.*, 2008), I (Knaust *et al.*, 2007), J (Shell *et al.*, 2002), K (Masignani *et al.*, 2003), L (Pizza *et al.*, 2000)

Appendix D

Supplementary data to Chapter 7

```
MC58        MKAKILTSVA LLACSGSLFA QTLATVNGQK IDSSVIDAQV AAFRAENSRA EDTPQLRQSL LENEVVNTVV AQEVKRLKLD RSAEFKNALA KLRAEAKKSG  100
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
1000        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
528         .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
FAM18       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGP165      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
90/18311    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
Z2491       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
F6124       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ133       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ232       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGH36       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
2996        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ198       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
297-0       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100

MC58        DDKKPSFKTV WQAVKYGLNG EAYALHIAKT QPVSEQEVKA AYDNISGFYK GTQEVQLGEI LTDKEENAKK AVADLKAKKG FDAVLKQYSL NDRTKQTGAP  200
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
1000        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
528         .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
FAM18       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGP165      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
90/18311    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
Z2491       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
F6124       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ133       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ232       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGH36       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
2996        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ198       .......... .......... .......... .......I.. .......... .......... .......... .......... .......... ..........  200
297-0       .......... .......... .......... .......I.. .......... .......... .......... .......... .......... ..........  200
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .....R.... .......... ..........  200
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200

MC58        VGYVPLKDLE QGVPPLYQAI KDLKKGEFTA TPLKNGDFYG VYYVNDSREV KVPSFDEMKG QIAGNLQAER IDRAVGALLG KANIKPAK 288
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
1000        .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
528         .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
FAM18       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
NGP165      .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
90/18311    .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
Z2491       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
F6124       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
BZ133       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
BZ232       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
NGH36       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
2996        .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
BZ198       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
297-0       .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .......... ........ 288
NGH15       .......... .......R.. .......... .......... .......... .......... .......... .......... ........ 288
```

Figure D.1: Multiple sequence alignment of NMB0345 amino acid sequences from 24 meningococcal strains, including MC58 (labelled in blue), using ClustalW with default settings. The amino acid sequences were compared to the sequence from strain MC58. All strains used were MenB strains, except F6124 (MenA), 90/18311 (MenC) and NG P165 (NT). The MenA (strain Z2491) and MenC (strain FAM18) genome sequence strains were also included for comparison (labelled in blue).

```
MC58        -------MPS ETRLPNFIRV LIFALGFIFL NACSEQTAQT VTLQGETMGT TYTVKYLSNN RDKLPSPAEI QKRIDDALKE VNRQMSTYQP DSEISRFNQH  93
BZ169       -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
NGH38       -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
BZ83        -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
H44/76      -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
BZ198       -------... .........A .......... .......... .......... ..........G......... .......... .......... ..........  93
297-0       -------... .........A .......... .......... .......... ..........G......... .......... .......... ..........  93
BZ133       -------... .........T .....S.... .......... .......... .......... .......... .......... .......... N........T  93
NGE28       -------... .........T .....S.... .......... .......... .......... .......... .......... .......... ..........  93
2996        -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
NGH15       -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
F6124       -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
MO1-240185  -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
NGP165      -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
FAM18       LPKPSNT... .......... .......... .......... .......... .......... .......... .......... .......... .......... 100
Z2491       -------... .........T .....S.... .......... .......... .......... .......... .......... .......... ..........  93
90/18311    -------... .........T .....S.... .......... .......... .......... .......... .......... .......... ..........  93
BZ147       -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
BZ232       -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
1000        -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93
528         -------... .......... .......... .......... .......... .......... .......... .......... .......... ..........  93

MC58        TAGKPLRISS DFAHVTAEAV RLNRLTHGAL DVTVGPLVNL WGFGPDKSVT REPSPEQIKQ AASYTGIDKI ILKQGKDYAS LSKTHPKAYL DLSSIAKGFG  193
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
BZ198       .......... ...Y...... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
297-0       .......... ...Y...... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
BZ133       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
NGE28       .......... ...Y...... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
2996        .......... ...Y...... .......... .......... .....G.... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
F6124       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
NGP165      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
FAM18       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
Z2491       .......... .......... H......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
90/18311    .......... .......... H......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
BZ232       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
1000        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193
528         .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  193

MC58        VDKVAGELEK YGIQNYLVEI GGELHGKGKN ARGEPWRIGI EQPNIVQGGN TQIIVPLNNR SLATSGDYRI FHVDKNGKRL SHIINPNNKR PISHNLASIS  293
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  293
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  293
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  293
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  293
BZ198       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  293
297-0       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  293
BZ133       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  293
NGE28       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  293
2996        .......... .......... .......... .......... .....I.... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293
NGH15       .......... .......... .......... .....V.... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293
F6124       .......... .......... .......... .......... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293
NGP165      .......... .......... .......... .......... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293
FAM18       .......... .......... .......... .......... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  300
Z2491       .......... .......... .......... .......... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293
90/18311    .......... .......... .......... .......... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293
BZ147       .......... .......... .......... .......... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293
BZ232       .......... .......... .......... .......... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293
1000        .......... .......... .......... .......... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293
528         .......... .......... .......... .......... .......... .......... .......... .....S.... .......... .......... .......... .......... .......... ..........  293

MC58        VVADSAMTAD GLSTGLFVLG ETEALKLAER EKLAVFLIVR DKGGYRTAMS SEFEKLLR 351
BZ169       .......... .......... .......... .......... .......... ........ 351
NGH38       .......... .......... .......... .......... .......... ........ 351
BZ83        .......... .......... .......... .......... .......... ........ 351
H44/76      .......... .......... .......... .......... .......... ........ 351
BZ198       .......... .......... .......... .......... .......... ........ 351
297-0       .......... .......... .......... .......... .......... ........ 351
BZ133       .......... .......... .......... .......... .....HA... ........ 351
NGE28       .......... .......... ..K....... .......... .......... ........ 351
2996        .......... .......... .......... .......... .......... ........ 351
NGH15       .......... .......... .......... .......... .......... ........ 351
F6124       .......... .......... .......... .......... .T........ ........ 351
MO1-240185  .......... .......... .......... .......... .T........ ........ 351
NGP165      .......... .......... .......... .......... .T........ ........ 351
FAM18       .......... .......... .......... .......... .T........ ........ 358
Z2491       .......... .......... .......... .......... .......... ........ 351
90/18311    .......... .......... .......... .......... .......... ........ 351
BZ147       .......... .......... .......... .......... .......... ........ 351
BZ232       .......... .......... .......... .......... .......... ........ 351
1000        .......... .......... .........Q ...S...... .T........ ...A.... 351
528         .......... .......... .........Q ...S...... .T........ ...A.... 351
```

Figure D.2: Multiple sequence alignment of *apbE* amino acid sequences from 21 meningococcal strains, including MC58 (labelled in blue), using ClustalW with default settings. The amino acid sequences were compared to the sequence from strain MC58. All strains used were MenB strains, except F6124 (MenA), 90/18311 (MenC) and NG P165 (NT). The MenA (strain Z2491) and MenC (strain FAM18) genome sequence strains were also included for comparison (labelled in blue).

```
MC58            781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
BZ169           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
BZ83            781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
H44/76          781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
BZ198           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
1000            781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
BZ147           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
528             781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
NGH15           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
90/18311        781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
MO1-240185      781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
2996            781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
SWZ107          781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
NGH38           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
NGF26           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
NGP165          781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
NG6/88          781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
FAM18           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
NGH36           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
Z2491           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTGGAACACAACCTTAA 840
297-0           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
BZ232           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTTAA---------- 828
F6124           781 GCAGAAGCAGCAGGGGAAGCCGAAACGCAACCTGGAACACAACCTGGAACACAACCTTAA 840
                    ******************************************  *
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Figure D.3: Multiple alignment, using ClustalW, of the nucleotide sequences of the gene NMB1961 in 23 meningococcal strains. All strains used were MenB strains, except F6124 (MenA), 90/18311 (MenC), NG P165 (NT). The amino acid sequences were compared to the sequence in strain MC58. Sequences of orthologues genes in serogroup A (Z2491) and serogroup C (FAM18) sequence strains were also included in the alignment. Only the last 60 nt of the alignment is shown, to highlight the repetitive element in the end of the gene in strains F6124 and Z2491; both serogroup A strains.

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MC58        MKKTAYAFLL LIGFASAPAF AETRPADPYE GYNRAVFKFN DQADRYIFAP AARGYRKVAP KPVRAGVSNF FNNLCDVVSF GSNILRLDIK RASEDLVRVG  100
FAM18       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
90/18311    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
2996        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGH36       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NGP165      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
528         .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
1000        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ198       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
Z2491       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
297-0       .......A.. .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
BZ232       .......A.. .......... .......... .......... .......... .......... .......... .......... .......... ..........  100
F6124       .......A.. .......... .......... .......... .......... .......... .......... .......... .......... ..........  100

MC58        INTTFGLGGL IDIAGAGGIP DNKNTLGDTF ASWGWKNSNY FVLPVLGPST VRDALGTGIT SVYSPKNIVF RTPVGRWGTT AVSAVSTREG LLDLTDSLDE  200
FAM18       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
90/18311    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
2996        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGH36       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NGP165      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
528         .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
1000        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ198       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
Z2491       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
297-0       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
BZ232       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200
F6124       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  200

MC58        AAIDKYSYTR DLYMKVRARQ TGATPAEGTE DNIDIDELVE SAETGAAETA VQEDSVSETQ AEAAGEAETQ PGTQP---- 275
FAM18       .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
90/18311    .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
MO1-240185  .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
2996        .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
H44/76      .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
BZ83        .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
NGH36       .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
NGH15       .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
SWZ107      .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
NGH38       .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
NGF26       .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
NGP165      .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
528         .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
BZ169       .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
BZ147       .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
1000        .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
BZ198       .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
NG6/88      .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
Z2491       .......... .......... .......... .......... .......... .......... .......... .......... .....GTQP 279
297-0       .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
BZ232       .......... .......... .......... .......... .......... .......... .......... .......... .....---- 275
F6124       .......... .......... .......... .......... .......... .......... .......... .......... .....GTQP 279
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Figure D.4: Multiple sequence alignment, using ClustalW, of the amino acid sequences of the gene NMB1961 in 23 meningococcal strains. All strains used were MenB strains, except F6124 (MenA), 90/18311 (MenC), NG P165 (NT). The amino acid sequences were compared to the sequence in strain MC58. Sequences of the homologues genes in serogroup A (Z2491) and serogroup C (FAM18) sequence strains were also included in the alignment (Blue text).

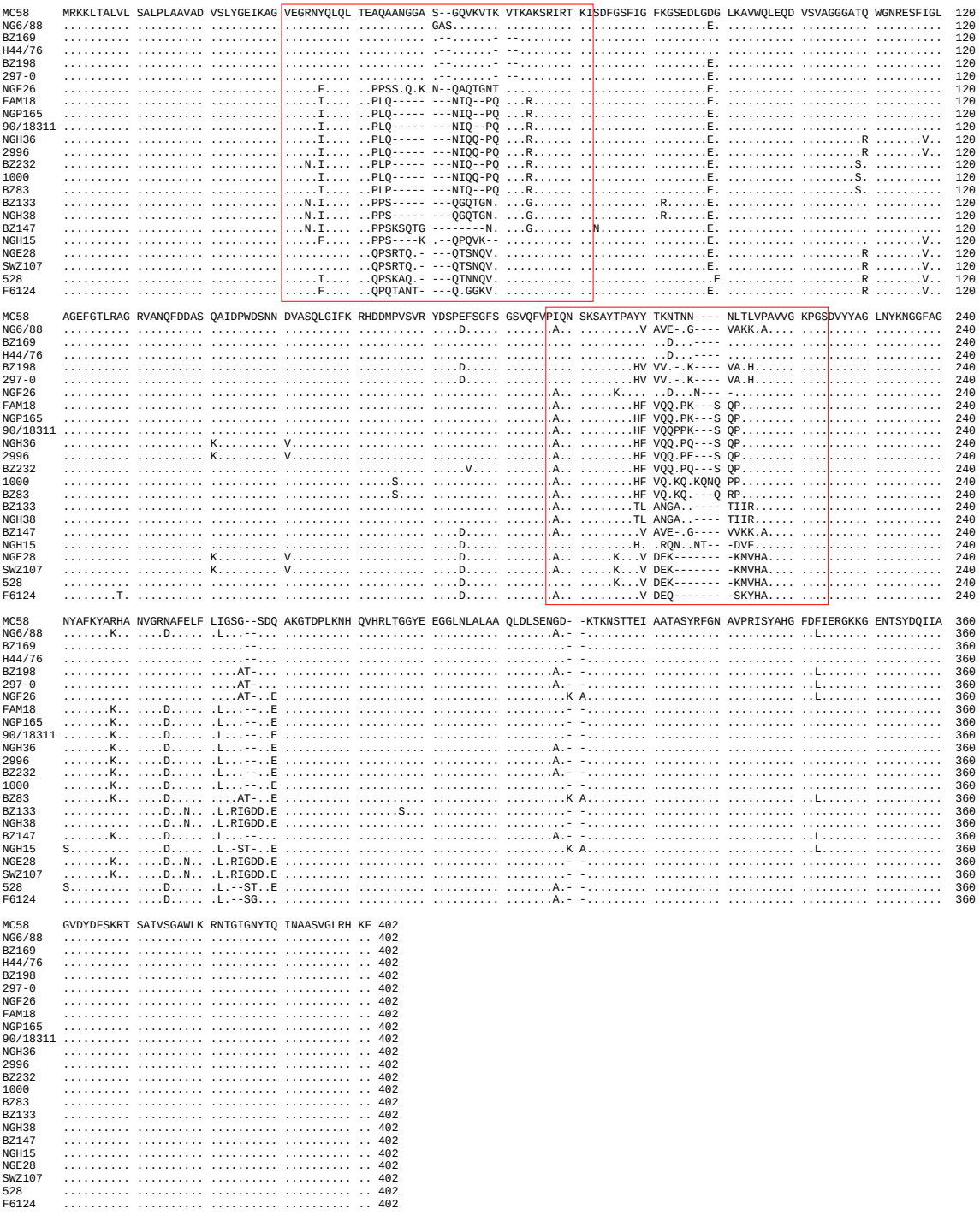


Figure D.5: Multiple sequence alignmentof PorA amino acid sequences available in the NCBI database for 24 of the meningococcal strains used in sequencing (using ClustalW with default settings). Regions corresponding to the variable surface exposed loops (VR1 and VR2) are indicated in the alignment