Supporting Information

A Framework for DNA Quantification and Outlier Detection Using Multidimensional Standard Curves

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Supporting information includes synthetic DNA sequences used in the manuscript (Table S1-S4), numerical values of extracted features and sigmoidal fittings for lambda DNA standard (Table S5), breakdown for Figure of Merit of Ct, Cy0, -log10(F0) and M0 (Table S6-S9), numerical values of extracted features and sigmoidal fittings for non-specific outliers (Table S10), numerical values of extracted features and sigmoidal fittings for temperature variation experiment (Table S11), estimated quantification for temperature variation experiment (Table S12), melting curve analysis for lambda DNA standard experiment (Figure S1), melting curve analysis for non-specific outlier detection experiment (Figure S2) and melting curve analysis for temperature variation experiment (Figure S3).

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Table S1. Nucleotide sequence for synthetic double-stranded DNA ordered from Integrated DNA Technologies containing the lambda phage DNA target.

gBlock gene fragment for lambbda	CAGGAACAGGGAATGCCCGTTCTGCGAGGC**GGTGGCAAGGGTAATGAGG**TGCTTTATGACTCTGCCGCCGTCATAA AATGG**TATGCCGAAAGGGATGCTGA**AATTGAGAACGAAAAGCTGCGCCGGGAGGTTGAAGAACTGCGGCAGGCCA GCGAGGCAGATCTCCAGCCAGGAACTATTGAGTACGAACGCCATCGACTTACGCGTGCGCAGGCCGACGCACAGGA ACTGAAGAATGCCAG

Forward lambda PCR primer in bold-red and reverse lambda primer in bold-blue (primers were designed in-house).

Table S2. Nucleotide sequence for synthetic double-stranded DNA ordered from Integrated DNA Technologies containing blaOXA-48 target.

Outlier 1 (5' - 3')	ATGCGTGTATTAGCCTTATCGGCTG**TGTTTTTGGTGGCATCGAT****TATCGGAATGCCTGCGGTAGCAAAGGAATGGCA** **AGAAAACAAAAGTTGGAATGCTCACTTTACTGAACATAAATCACAGGGCGTAGTTGTGCTCTGGAATGAGAATAAG** **CAGCAAGGATTTACCAATAATCTTAAACGG****GCGAACCAAGCATTTTTAC**CCGCATCTACCTTTAAAATTCCCAATAGC TTGATCGCCCTCGATTTGGGCGTGGTTAAGGATGAACACCAAGTCTTTAAGTGGGATGGACAGACGCGC

OXA-48-F (TGTTTTTGGTGGCATCGAT) **in red**;
OXA-48-R (GTAAMRATGCTTGGTTCGC) **in blue**;
amplicon size = 177 bp (**bold and underlined**);
primers from Monteiro et al 2012

Table S3. Nucleotide sequence for synthetic double-stranded DNA ordered from Integrated DNA Technologies containing blaNDM target.

Outlier 2 (5' - 3')	ATGGTTGCGGCGCAACACAGCCTGACTTTCGCCGCCAATGGCTGGGTCGAACCAGCAACCGCGCCCAACTTTGGCCCG CTCAAGGTATTTTACCCCGGCCCCGGCC**ACACCAGTGACAATATCACCG****TTGGGATCGACGGCACCGACATCGCTTTTG** **GTGGCTGCCTGATC****AAGGACAGCAAGGCCAA**GTCGCTCGGCAATCTCGGTGATGCCGACACTGAGCACTACGCCGCG TCAGCGCGCGCGTTTGGTGCGGCGTTCCCCAAGGCCAGCATGATCGTGATGAGCCATTCCGCCCCC

NDM-F (TTGGCCTTGCTGTCCTTG) **in red**;
NDM-R (ACACCAGTGACAATATCACCG) **in blue**;
amplicon size = 82 bp (**bold and underlined**);
primers from Monteiro et al 2012

Table S4. Nucleotide sequence for synthetic double-stranded DNA ordered from Integrated DNA Technologies containing blaKPC target.

Outlier 3 (5' - 3')	AACCAT**TCGCTAAACTCGAACAGG****ACTTTGGCGGCTCCATCGGTGTGTACGCGATGGATACCGGCTCAGGCGCAACT** **GTAAGTTACCGCGCTGAGGAGCGCTTCCCACTGTGCAGCTCATTCAAGGGCTTTCTTGCTGCCGCTGTGCTGGCTCGCA** **GCCAGCAGCAGGCCGGCTTGCTGGACACACCCATCCGTTACGGCAAAAATGCGCTGGTTCCGTGGTCACCCATCTCGG** **AAAAATATCTGACAACAGGCATGACGGTGGCGGAGCTGTCCGCGGCCGCCGTGCAATACAGTGATAACGCCGCCGCC** **AATTTGTTGCTGAAGGAGTTGGGCGGCCCGGCCGGGCTGACGGCCTTCATGCGCTCTATCGGCGATACCACGTTCCGT** **CTGGACCGCTGGGAGCTGGAGCTGAACTCCGCCATCCCAGGCGATGCGCGCGATACCTCATCGCCGCGCGCCGTGACG** **GAAAGCTTACAAAAACTGACACTGGGCTCTGCACTGGCTGCGCCGCAGCGGCAGCAGTTTGTTGATTGGCTAAAGGG** **AAACACGACCGGCAACCACCGCATCCGCGCGGCGGTGCCGGCAGACTGGGCAGTCGGAGACAAAACCGGAACCTGC** **GGAGTGTATGGCACGGCAAATGACTATGCCGTCGTCTGGCCCACTGGGCGCGCACCTATTGTGTTGGCCGTCTACACC** **CGGGCGCCTAACAAGGATGACAAGCACAGCGAGGCCGTCATCGCCGCTGCGGCTAGACTCGCGCTCGAG**GGATTGG GCGTCAACGGGCAGTAAG

KPC-F (TCGCTAAACTCGAACAGG) **in red**;
KPC-R (TTACTGCCCGTTGACGCCCAATCC) **in blue**;
amplicon size = 785 bp (**bold and underlined**);
primers from Monteiro et al 2012

Table S5. Experimental values for lambda DNA standards.

					Sigmoidal Curve-fitting Parameters				
Concentration (copies/reaction)	Ct	Cy0	-log10(F0)	M0	Fb	Fmax	c	b	d
1.00E+02	29.60286	28.14662	9.76812	11.59576	0.00171	0.19864	31.20895	2.00134	1.26013
	30.36106	28.88565	10.01293	12.51620	0.00137	0.19362	31.89412	2.00278	1.28424
	30.49607	29.03997	10.08592	12.72201	0.00148	0.19703	32.06496	1.99031	1.27033
	30.18126	28.59652	9.74092	12.04612	0.00110	0.19011	31.17973	2.16333	1.54270
	30.61087	29.12458	9.93007	12.71221	0.00137	0.18160	32.38258	1.99474	1.19285
	30.52995	28.99942	10.05593	12.66146	0.00109	0.19989	31.77558	2.06643	1.41198
	30.80903	29.22164	10.46644	13.14969	-0.00004	0.19203	31.98968	1.99794	1.39437
	30.48359	28.99977	10.15566	12.72414	0.00105	0.20679	32.00059	1.97305	1.27604
1.00E+03	26.53766	24.98309	8.93031	7.78828	0.00113	0.19464	27.65128	2.11468	1.48081
	26.97111	25.38085	8.88703	8.17403	0.00141	0.19293	28.35725	2.08432	1.33897
	27.04113	25.43462	8.90557	8.24153	0.00131	0.19943	28.15692	2.13202	1.46509
	27.08741	25.45308	8.91666	8.26776	0.00132	0.20711	27.96269	2.20259	1.59319
	27.06787	25.39216	8.83712	8.15487	0.00100	0.18234	27.98259	2.20839	1.56360
	26.95429	25.32104	9.10802	8.25072	0.00095	0.19906	27.91180	2.12276	1.52155
	26.94251	25.33669	9.19890	8.32383	0.00052	0.19659	28.21981	2.02390	1.35499
	27.01954	25.37736	9.00308	8.24342	0.00082	0.20544	27.85523	2.14832	1.58779
1.00E+04	23.06449	21.45487	8.11253	3.61473	0.00082	0.17757	24.12603	2.05981	1.45392
	23.86092	22.23739	8.16096	4.45714	0.00126	0.20219	24.63902	2.19898	1.64095
	23.82437	22.16628	8.08453	4.33559	0.00102	0.20641	24.58259	2.20760	1.64317
	23.87834	22.20694	8.17261	4.43307	0.00094	0.20444	24.41637	2.24269	1.76205
	23.85718	22.20265	8.05278	4.35341	0.00102	0.20003	24.76700	2.20123	1.57268
	23.85051	22.14791	7.99930	4.26329	0.00104	0.20823	24.32139	2.30533	1.80903
	23.77082	22.13842	8.31389	4.45038	0.00017	0.20166	24.60059	2.09622	1.57798
	23.43357	21.87384	8.09965	4.04118	0.00084	0.20425	24.71569	2.05671	1.38458
1.00E+05	19.93326	18.28931	7.31713	-0.16854	-0.00006	0.18707	20.86677	2.03578	1.49672
	20.15876	18.53712	7.24532	0.04347	0.00037	0.18782	21.00119	2.09966	1.57509
	20.19548	18.50961	7.07905	-0.08905	0.00075	0.20186	20.68453	2.24574	1.78447
	20.18913	18.51850	7.06403	-0.08931	0.00091	0.20251	20.76798	2.23613	1.73927
	20.15086	18.48183	7.01717	-0.15675	0.00097	0.20562	20.79512	2.23583	1.70703
	20.27313	18.52587	7.02551	-0.10546	0.00041	0.20629	20.24835	2.36218	2.09772
	20.22384	18.50878	7.00447	-0.13653	0.00068	0.22824	20.47807	2.30008	1.92284
	20.08686	18.45829	7.18246	-0.07771	0.00049	0.19780	21.04207	2.08265	1.51051
1.00E+06	16.17976	14.51421	6.08100	-4.86072	0.00061	0.18155	16.77620	2.18851	1.71329
	17.10805	15.38710	6.23458	-3.85842	0.00036	0.19747	17.37334	2.25699	1.89780
	17.04889	15.30319	6.17615	-3.98202	0.00060	0.20297	17.04251	2.31918	2.06395
	17.06687	15.28346	6.09262	-4.05469	0.00068	0.21577	16.99645	2.36634	2.09972
	17.04314	15.32311	6.17842	-3.96003	0.00043	0.20119	17.40975	2.23856	1.83308
	17.00921	15.31046	6.28314	-3.90760	0.00043	0.19592	17.57628	2.18554	1.71424
	17.03086	15.30684	6.27657	-3.91537	0.00018	0.20555	17.30596	2.23191	1.88232
	16.65264	15.04565	6.21089	-4.22809	0.00047	0.19609	17.49631	2.11557	1.58828
1.00E+07	13.22484	11.56815	5.27775	-8.42135	0.00021	0.17499	13.59601	2.17619	1.83729
	13.35007	11.66265	5.30553	-8.30574	0.00019	0.17182	13.56278	2.23050	1.93622
	13.34625	11.65031	5.26970	-8.34097	0.00036	0.18093	13.33486	2.28242	2.08529
	13.35548	11.61685	5.17543	-8.43461	0.00050	0.19908	13.03536	2.37365	2.29371
	13.33545	11.64715	5.28802	-8.33280	0.00027	0.16484	13.51644	2.25462	1.96335
	13.35185	11.72146	5.31096	-8.24157	0.00017	0.18286	13.89558	2.14029	1.74200
	13.32309	11.68837	5.30690	-8.27843	0.00020	0.18031	13.83290	2.14862	1.76103
	13.28688	11.63747	5.28394	-8.34558	0.00017	0.18015	13.68564	2.17296	1.82563
1.00E+08	10.05275	8.29492	4.33749	-12.40689	-0.00017	0.17118	10.10286	2.24129	2.00471
	10.05378	8.26361	4.32252	-12.44862	-0.00009	0.16975	9.86191	2.30801	2.16317
	10.10387	8.30169	4.27329	-12.43992	0.00010	0.18066	9.33865	2.40134	2.59146
	10.08808	8.28186	4.27340	-12.46041	0.00001	0.18756	9.41329	2.39533	2.52000
	10.05353	8.32742	4.39515	-12.33715	-0.00016	0.16012	10.28119	2.17953	1.88943
	10.04862	8.31133	4.25989	-12.43861	-0.00012	0.20363	10.05777	2.23803	2.03842
	10.09425	8.37908	4.36445	-12.30285	-0.00013	0.18918	10.18256	2.19485	1.98332
	10.08292	8.36027	4.17914	-12.43852	-0.00027	0.22425	10.39602	2.18692	1.84830

Table S6. Breakdown for Figure of Merit of Ct

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
Quantification	1	9.86E+07	1.14E+07	1.54E+06	1.21E+05	1.44E+04	1.36E+03	1.70E+02
	2	9.85E+07	1.05E+07	8.21E+05	1.04E+05	8.39E+03	1.02E+03	1.02E+02
	3	9.52E+07	1.05E+07	8.54E+05	1.01E+05	8.60E+03	9.69E+02	9.29E+01
	4	9.62E+07	1.05E+07	8.44E+05	1.01E+05	8.29E+03	9.39E+02	1.15E+02
	5	9.85E+07	1.06E+07	8.58E+05	1.04E+05	8.41E+03	9.52E+02	8.60E+01
	6	9.88E+07	1.05E+07	8.78E+05	9.58E+04	8.45E+03	1.03E+03	9.08E+01
	7	9.58E+07	1.07E+07	8.65E+05	9.90E+04	8.92E+03	1.04E+03	7.51E+01
	8	9.66E+07	1.10E+07	1.12E+06	1.09E+05	1.12E+04	9.84E+02	9.37E+01

		Concentration						
		1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
CV		1.51	3.12	25.54	7.31	22.67	13.24	28.57

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
RE	1	1.45	14.47	54.09	20.63	44.06	36.41	70.38
	2	1.52	5.15	17.93	3.51	16.09	1.65	1.85
	3	4.81	5.42	14.57	0.96	13.98	3.07	7.07
	4	3.79	4.76	15.61	1.40	17.08	6.07	15.06
	5	1.50	6.20	14.24	4.07	15.88	4.81	14.04
	6	1.17	5.02	12.24	4.22	15.50	2.82	9.18
	7	4.19	7.09	13.52	0.96	10.80	3.64	24.85
	8	3.45	9.75	11.79	8.69	12.14	1.64	6.28

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
LOOCV	1	1.54	15.06	55.68	21.04	45.23	37.94	75.74
	2	1.61	5.34	18.30	3.58	16.43	1.71	1.96
	3	5.10	5.63	14.88	0.98	14.28	3.18	7.49
	4	4.01	4.94	15.93	1.43	17.44	6.28	16.05
	5	1.59	6.43	14.53	4.14	16.21	4.99	14.85
	6	1.24	5.21	12.50	4.29	15.83	2.92	9.73
	7	4.44	7.36	13.80	0.98	11.04	3.78	26.20
	8	3.65	10.14	12.08	8.85	12.43	1.70	6.66

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
Q	1	3.369615427	680.1255825	76914.73423	3172.548417	45181.28958	18284.66405	152285.5769
	2	3.697706432	85.91882195	8380.724989	91.75630779	5994.743495	37.37121997	103.6361771
	3	37.0387709	95.22160658	5536.058531	6.890050818	4528.43351	129.1393161	1513.613468
	4	22.94081953	73.45389592	6350.202408	14.59457257	6751.192477	504.5186376	6904.466029
	5	3.616615164	124.5264551	5285.166586	123.272899	5836.543914	317.5217883	5953.015666
	6	2.205991195	81.67364176	3907.027556	132.3924554	5559.816658	109.0805965	2551.570218
	7	28.06870714	162.9962182	4766.226925	6.868853549	2702.049103	182.0832954	18605.78912
	8	19.0321599	308.559578	3636.013447	562.2663153	3422.092657	36.78207274	1194.270867

Av. Q	4587.427356
Std(Q)	12798.97639

Shaded region is not included in the mean and standard deviation calculation.

Table S7. Breakdown for Figure of Merit of Cy0

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
Quantification	1	9.89E+07	1.10E+07	1.51E+06	1.20E+05	1.43E+04	1.33E+03	1.59E+02
	2	1.01E+08	1.03E+07	8.41E+05	1.01E+05	8.42E+03	1.02E+03	9.66E+01
	3	9.84E+07	1.04E+07	8.90E+05	1.03E+05	8.83E+03	9.82E+02	8.71E+01
	4	9.98E+07	1.06E+07	9.02E+05	1.03E+05	8.60E+03	9.70E+02	1.17E+02
	5	9.67E+07	1.04E+07	8.78E+05	1.05E+05	8.62E+03	1.01E+03	8.22E+01
	6	9.78E+07	9.88E+06	8.86E+05	1.02E+05	8.94E+03	1.06E+03	8.95E+01
	7	9.34E+07	1.01E+07	8.88E+05	1.03E+05	9.00E+03	1.05E+03	7.71E+01
	8	9.46E+07	1.05E+07	1.06E+06	1.07E+05	1.08E+04	1.02E+03	8.94E+01

		Concentration						
		1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
CV		2.61	3.09	22.80	5.67	20.53	10.92	26.76

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
RE	1	1.12	9.57	51.28	19.63	42.51	33.04	58.70
	2	0.99	2.83	15.86	1.28	15.78	1.83	3.42
	3	1.57	3.68	10.98	3.17	11.65	1.78	12.94
	4	0.24	6.04	9.79	2.55	14.03	2.99	17.29
	5	3.25	3.91	12.17	5.11	13.79	1.06	17.75
	6	2.20	1.16	11.42	2.05	10.55	6.01	10.53
	7	6.55	1.07	11.20	3.23	9.98	4.90	22.95
	8	5.37	4.58	5.84	6.79	7.54	2.07	10.55

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
LOOCV	1	1.18	9.95	52.77	20.02	43.63	34.41	63.06
	2	1.05	2.94	16.19	1.30	16.11	1.90	3.63
	3	1.66	3.82	11.22	3.23	11.90	1.85	13.69
	4	0.26	6.28	10.00	2.60	14.33	3.10	18.43
	5	3.45	4.05	12.42	5.21	14.08	1.10	18.76
	6	2.33	1.20	11.66	2.08	10.78	6.24	11.15
	7	6.94	1.11	11.44	3.29	10.20	5.09	24.21
	8	5.68	4.76	5.98	6.92	7.71	2.15	11.17

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
Q	1	3.446499155	293.8727203	61694.83744	2229.153151	38080.94797	12414.46875	99065.56028
	2	2.704335868	25.64423697	5856.179975	9.4490826	5217.224456	38.01580127	332.6606835
	3	6.773396564	43.44835471	2808.754941	58.02041459	2847.704792	35.87320758	4740.053865
	4	0.16475125	117.0191354	2234.028185	37.61831964	4129.709448	101.3143436	8528.344499
	5	29.24540678	48.93045044	3446.670889	150.9393028	3985.124014	12.75766633	8910.135307
	6	13.39485809	4.278144208	3035.066203	24.23629323	2336.676232	409.4133361	3143.759043
	7	118.5810533	3.654988773	2921.458507	60.18102025	2090.351446	272.132678	14865.29181
	8	79.49868599	67.2800651	795.926136	266.35222	1194.209994	48.59985426	3155.958584

Av. Q	3327.111107
Std(Q)	10357.03217

Shaded region is not included in the mean and standard deviation calculation.

Table S8. Breakdown for Figure of Merit of -log10(F0)

		Concentration (copies/reaction)						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
Estimated concentration	1	9.24E+07	9.38E+06	1.33E+06	6.57E+04	9.49E+03	1.30E+03	1.69E+02
	2	9.58E+07	8.77E+06	9.15E+05	7.82E+04	8.44E+03	1.44E+03	9.32E+01
	3	1.08E+08	9.56E+06	1.05E+06	1.17E+05	1.02E+04	1.38E+03	7.81E+01
	4	1.08E+08	1.20E+07	1.29E+06	1.22E+05	8.20E+03	1.34E+03	1.81E+02
	5	8.03E+07	9.15E+06	1.05E+06	1.36E+05	1.10E+04	1.63E+03	1.14E+02
	6	1.12E+08	8.65E+06	8.13E+05	1.34E+05	1.25E+04	8.43E+02	8.40E+01
	7	8.65E+07	8.74E+06	8.26E+05	1.41E+05	5.82E+03	6.75E+02	3.09E+01
	8	1.36E+08	9.24E+06	9.69E+05	9.12E+04	9.79E+03	1.09E+03	6.59E+01

		Concentration						
		1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
CV		17.12	11.62	18.87	25.79	21.25	26.44	49.95

		Concentration (copies/reaction)						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
RE	1	7.65	6.22	32.90	34.29	5.09	29.83	69.15
	2	4.23	12.35	8.53	21.75	15.64	44.24	6.75
	3	7.96	4.37	5.44	17.25	1.60	37.88	21.92
	4	7.93	20.28	29.19	21.62	18.00	34.21	80.72
	5	19.74	8.54	4.86	36.30	9.76	62.86	14.07
	6	11.53	13.50	18.72	33.56	25.01	15.74	16.01
	7	13.51	12.64	17.41	40.58	41.85	32.45	69.06
	8	35.74	7.62	3.11	8.83	2.07	8.77	34.10

		Concentration (copies/reaction)						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
LOOCV	1	8.10	6.44	33.78	34.79	5.20	31.05	74.40
	2	4.48	12.77	8.72	22.10	15.97	46.13	7.16
	3	8.47	4.53	5.56	17.59	1.64	39.47	23.14
	4	8.44	21.12	29.97	22.05	18.37	35.64	87.00
	5	20.79	8.83	4.97	37.07	9.99	65.67	14.99
	6	12.30	13.96	19.10	34.27	25.63	16.28	16.93
	7	14.27	13.07	17.77	41.45	42.59	33.47	71.48
	8	38.41	7.89	3.18	8.98	2.12	9.10	35.85

		Concentration (copies/reaction)						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
Q	1	1060.418654	465.8733045	20973.87734	30776.12302	562.5879396	24487.22015	257007.1228
	2	323.8706979	1832.30566	1403.671426	12400.58407	5306.383246	53951.07103	2413.597403
	3	1154.52433	229.6986509	571.1291957	7827.451923	55.58405516	39524.89899	25335.17332
	4	1145.856701	4977.746725	16505.17504	12296.32648	7024.969033	32226.4529	350820.2001
	5	7024.242078	876.1035038	455.7804334	34709.15559	2071.538524	109116.6425	10533.75
	6	2426.695163	2189.612488	6748.799012	29662.98078	13620.99001	6773.502357	13541.05669
	7	3301.099969	1919.845431	5838.601616	43385.93276	37870.80065	28710.48399	246581.6781
	8	23496.23644	699.0459642	186.2176545	2043.665944	92.9962591	2109.96936	61074.02853

Av. Q	13383.6404
Std(Q)	19965.62983

Shaded region is not included in the mean and standard deviation calculation.

Table S9. Breakdown for Figure of Merit of M0

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
Quantification	1	9.79E+07	1.07E+07	1.49E+06	1.10E+05	1.34E+04	1.33E+03	1.60E+02
	2	1.00E+08	1.01E+07	8.51E+05	9.76E+04	8.42E+03	1.07E+03	9.61E+01
	3	9.97E+07	1.03E+07	9.12E+05	1.05E+05	9.01E+03	1.03E+03	8.57E+01
	4	1.01E+08	1.08E+07	9.49E+05	1.05E+05	8.54E+03	1.02E+03	1.25E+02
	5	9.42E+07	1.02E+07	9.01E+05	1.09E+05	8.92E+03	1.08E+03	8.62E+01
	6	9.97E+07	9.70E+06	8.75E+05	1.06E+05	9.38E+03	1.03E+03	8.87E+01
	7	9.24E+07	9.90E+06	8.79E+05	1.08E+05	8.46E+03	9.85E+02	6.76E+01
	8	9.97E+07	1.03E+07	1.05E+06	1.04E+05	1.06E+04	1.03E+03	8.56E+01

		Concentration						
		1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
CV		3.16	3.66	21.27	3.60	17.82	10.04	29.53

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
RE	1	2.08	7.18	48.51	9.81	34.47	32.59	60.20
	2	0.21	0.51	14.86	2.38	15.76	7.03	3.89
	3	0.27	2.50	8.81	5.07	9.88	3.10	14.26
	4	0.87	7.97	5.06	5.08	14.62	1.61	24.77
	5	5.80	2.04	9.92	9.09	10.76	8.18	13.80
	6	0.34	3.00	12.50	6.03	6.19	2.57	11.33
	7	7.58	1.00	12.13	7.87	15.44	1.51	32.38
	8	0.35	2.76	4.53	4.41	6.12	2.99	14.37

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
LOOCV	1	2.21	7.45	49.90	9.99	35.35	33.94	64.68
	2	0.23	0.53	15.17	2.43	16.09	7.30	4.12
	3	0.28	2.59	9.00	5.16	10.09	3.21	15.09
	4	0.93	8.28	5.17	5.18	14.94	1.67	26.44
	5	6.14	2.11	10.13	9.26	11.00	8.49	14.59
	6	0.36	3.11	12.77	6.14	6.32	2.67	12.00
	7	8.02	1.03	12.38	8.02	15.77	1.56	34.06
	8	0.37	2.87	4.64	4.49	6.27	3.10	15.19

		Concentration						
	Replicate	1.00E+08	1.00E+07	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02
Q	1	14.48355173	196.0455799	51492.29758	352.4987999	21721.63372	11102.42442	114977.4461
	2	0.151085871	0.996125511	4794.369153	20.80382594	4518.99283	515.2650383	473.4225522
	3	0.240514715	23.74490618	1688.21065	94.08412553	1776.523547	100.031615	6354.210365
	4	2.54494666	241.6626081	556.8389089	94.55462541	3892.730628	26.9552615	19337.51929
	5	112.3744416	15.77897784	2138.129748	302.7398044	2109.697609	697.2277884	5945.29875
	6	0.38928774	34.24071887	3395.910064	133.1499656	697.2143991	68.87569779	4015.152291
	7	191.6758201	3.782885288	3193.689604	226.9843175	4338.91238	23.57942485	32567.2971
	8	0.399221687	28.97160266	446.8837465	71.19091061	683.6123534	93.11477616	6444.590448

Av. Q	2546.596033
Std(Q)	8058.439539

Shaded region is not included in the mean and standard deviation calculation.

Table S10. Experimental values for non-specific outlier detection experiment.

					Sigmoidal Curve-fitting Parameters				
Outlier ID	Ct	Cy0	-log10(F0)	M0	Fb	Fmax	c	b	d
Outlier 1	22.18460	20.16701	6.24107	N/A	0.00108	0.16458	22.37300	2.98314	2.06180
	21.63717	19.66722	6.00429	N/A	0.00165	0.20330	21.78228	2.98460	2.09782
	21.49195	19.51880	6.04543	N/A	0.00127	0.17532	21.76057	2.94959	2.03027
	21.61322	19.64198	6.04332	N/A	0.00142	0.18405	21.85918	2.96545	2.04505
	21.55848	19.57242	6.02639	N/A	0.00113	0.19108	21.75289	2.94269	2.06273
	21.43270	19.45167	5.98519	N/A	0.00141	0.19163	21.45900	2.98925	2.15545
	21.44939	19.45573	5.98497	N/A	0.00132	0.18354	21.65420	2.97424	2.05931
	21.73830	19.77457	6.02388	N/A	0.00159	0.18908	22.14562	2.96286	1.97109
Outlier 2	18.44049	16.09981	5.61749	N/A	0.00098	0.19616	12.36939	3.70596	8.27322
	18.37323	16.03334	5.62648	N/A	0.00103	0.21221	12.06129	3.66681	8.86532
	18.38343	16.04607	5.64900	N/A	0.00101	0.20760	12.16554	3.66055	8.68182
	18.37301	16.01949	5.61605	N/A	0.00102	0.21130	12.00102	3.68541	8.92312
	18.43692	16.05071	5.62262	N/A	0.00082	0.20214	11.98671	3.73028	8.93332
	18.36191	16.05032	5.64676	N/A	0.00115	0.21558	12.02351	3.62638	9.07374
	18.34952	16.04050	5.68474	N/A	0.00099	0.21375	12.08867	3.59851	8.99036
	18.38125	16.04822	5.66437	N/A	0.00099	0.20719	12.08793	3.64746	8.88694
Outlier 3	19.93116	17.55704	5.13044	N/A	0.00124	0.20157	18.06961	3.73834	3.18304
	18.84150	16.52545	5.05112	N/A	0.00127	0.21137	16.20053	3.68401	3.79378
	18.89363	16.52140	5.05550	N/A	0.00116	0.20746	16.12094	3.72917	3.85576
	18.97990	16.62387	5.05235	N/A	0.00129	0.21675	16.25445	3.71713	3.82810
	19.15945	16.79429	5.13383	N/A	0.00101	0.19113	16.76119	3.71036	3.57039
	18.63558	16.31977	5.04156	N/A	0.00117	0.20856	15.72623	3.68477	4.02451
	18.53768	16.24235	5.07549	N/A	0.00099	0.20603	15.96533	3.58936	3.77196
	19.01092	16.68804	5.05829	N/A	0.00175	0.21230	16.77984	3.68891	3.45259

Table S11. Experimental values for temperature variation experiment.

					Sigmoidal Curve-fitting Parameters				
Temperature (˚C)	Ct	Cy0	-log10(F0)	M0	Fb	Fmax	c	b	d
54.0	20.36472	18.71671	7.52739	0.40680	0.00019	0.16920	20.82675	2.14960	1.77959
	20.34576	18.70276	7.35635	0.28505	0.00062	0.20736	20.81465	2.18309	1.78861
	20.37727	18.70277	7.42406	0.32766	0.00029	0.18938	20.61377	2.21799	1.92214
	20.37547	18.71779	7.39350	0.32401	0.00044	0.18941	20.72816	2.20445	1.85649
	20.34697	18.73254	7.37018	0.32450	0.00078	0.19933	20.98318	2.14943	1.69672
55.0	20.35076	18.70896	7.41330	0.32720	0.00066	0.19168	20.74375	2.19071	1.83181
	20.34589	18.70457	7.38593	0.30547	0.00051	0.20034	20.88222	2.16314	1.74592
	20.39589	18.72499	7.38224	0.32448	0.00049	0.20133	20.56239	2.24494	1.97382
	20.39395	18.72994	7.33733	0.30143	0.00069	0.20441	20.67652	2.23723	1.90351
	20.42425	18.77627	7.46880	0.43186	0.00008	0.20008	20.88380	2.15034	1.78424
56.9	20.37027	18.75150	7.36937	0.34368	0.00045	0.19184	21.08252	2.12646	1.65080
	20.37564	18.74796	7.35993	0.33413	0.00040	0.19838	21.04501	2.13617	1.67370
	20.39991	18.77594	7.33519	0.34764	0.00067	0.20141	20.94867	2.17618	1.75209
	20.42563	18.77232	7.24463	0.28723	0.00078	0.21952	20.80642	2.23909	1.85573
	20.41883	18.78839	7.42493	0.41685	0.00030	0.18988	21.04506	2.13321	1.69389
59.3	20.37572	18.75679	7.19605	0.24051	0.00091	0.21538	21.08690	2.17507	1.66996
	20.38818	18.78148	7.31135	0.33836	0.00036	0.21527	21.24931	2.09322	1.57093
	20.39076	18.78063	7.25616	0.30289	0.00082	0.21567	21.08949	2.15789	1.67288
	20.40582	18.78285	7.17554	0.25470	0.00099	0.23383	21.07078	2.19077	1.69844
	20.40556	18.81165	7.34236	0.38905	0.00060	0.19931	21.21351	2.11276	1.60749
61.9	20.38439	18.74872	7.40130	0.36089	-0.00020	0.21077	20.97150	2.10500	1.70538
	20.33584	18.74770	7.31287	0.30417	0.00048	0.21610	21.27799	2.06409	1.52489
	20.37290	18.72687	7.14594	0.17816	0.00077	0.21721	20.89255	2.22958	1.78190
	20.43562	18.72142	7.03779	0.10500	0.00088	0.21644	20.39958	2.38595	2.12478
	20.36003	18.72843	7.28283	0.26554	0.00038	0.20818	20.99268	2.15006	1.69807
62.0	20.41051	18.76012	7.48086	0.42268	0.00014	0.14534	20.90459	2.17275	1.77418
	20.39197	18.76013	7.33928	0.33383	0.00056	0.17423	21.02490	2.16699	1.70140
	20.42831	18.78184	7.44075	0.42005	0.00032	0.15973	20.89417	2.18726	1.79439
	20.52006	18.79240	7.51415	0.47739	-0.00040	0.16194	20.55288	2.22705	2.03337
	20.38991	18.77583	7.35947	0.36270	0.00053	0.16913	21.01541	2.16170	1.71138
62.6	20.38280	18.73967	7.36062	0.32604	0.00078	0.16352	20.83232	2.23319	1.81676
	20.39852	18.77213	7.38646	0.37584	0.00047	0.16690	21.07822	2.14955	1.67338
	20.43431	18.78175	7.34838	0.36206	0.00042	0.17135	20.95350	2.19847	1.76730
	20.49831	18.82070	7.54599	0.52649	-0.00039	0.16673	20.85542	2.14898	1.83399
	20.46540	18.82146	7.40142	0.43649	0.00013	0.18356	21.01592	2.14776	1.73624
63.7	20.41626	18.77712	7.41314	0.39781	0.00047	0.16384	20.93468	2.19208	1.76969
	20.43215	18.79243	7.44092	0.43111	0.00035	0.16397	20.97034	2.17289	1.75241
	20.44733	18.78596	7.36589	0.37745	0.00046	0.16898	20.84960	2.22591	1.83667
	20.43170	18.79295	7.40845	0.41129	0.00045	0.16867	20.93550	2.18689	1.77562
	20.46906	18.81612	7.49067	0.48695	0.00020	0.16283	20.87581	2.18652	1.82512
64.7	20.36327	18.65122	7.34938	0.22752	0.00036	0.20699	20.18481	2.30557	2.19262
	20.31742	18.64796	7.22387	0.14525	0.00107	0.22005	20.55469	2.27274	1.93658
	20.37853	18.65491	7.25185	0.17024	0.00080	0.21916	20.15218	2.35872	2.22943
	20.37585	18.64610	7.16426	0.10621	0.00107	0.21438	20.11381	2.40256	2.25970
	20.34921	18.61443	7.25960	0.13315	0.00068	0.20605	20.05181	2.36940	2.27506
65.2	20.43822	18.71953	7.24305	0.23176	0.00105	0.18152	20.25138	2.40375	2.21454
	20.42829	18.71954	7.37928	0.31714	0.00048	0.17588	20.49530	2.29242	2.03097
	20.46003	18.73462	7.33735	0.30657	0.00071	0.17844	20.31844	2.35687	2.17127
	20.50101	18.76079	7.40170	0.37414	0.00035	0.17237	20.13990	2.36654	2.31995
	20.45599	18.70472	7.41535	0.32457	0.00052	0.16909	20.26672	2.34996	2.18669
67.4	20.45377	18.48566	6.92595	-0.20862	0.00127	0.20727	17.52534	2.90144	4.61491
	20.37989	18.45652	7.01548	-0.18293	0.00125	0.20657	18.26642	2.76729	3.73532
	20.39153	18.44212	6.95625	-0.23488	0.00119	0.21009	17.69905	2.84843	4.36246
	20.38347	18.42486	6.92194	-0.27427	0.00136	0.21405	17.28423	2.90740	4.85197
	20.39764	18.44359	7.02391	-0.19091	0.00094	0.20133	17.67030	2.83803	4.41447
68.4	20.53432	18.42980	6.89546	-0.28509	0.00043	0.16600	16.14991	3.10894	6.54409
	20.49266	18.44686	6.83167	-0.30764	0.00119	0.17262	16.70523	3.09419	5.59168
	20.53705	18.41536	6.81213	-0.35224	0.00072	0.17192	16.17232	3.16518	6.37963
	20.53589	18.43272	6.93089	-0.25985	0.00021	0.16609	16.23338	3.07248	6.46712
	20.51385	18.39090	6.79396	-0.38901	0.00063	0.17053	15.93598	3.17754	6.75246
69.8	20.41249	18.23849	6.40807	-0.78888	0.00109	0.20294	15.33253	3.36855	7.27589
	20.41747	18.26823	6.45102	-0.73121	0.00098	0.20575	15.22802	3.31863	7.63894
	20.41780	18.24772	6.44430	-0.75660	0.00104	0.20434	15.17342	3.35466	7.63455
	20.61922	18.32978	6.23095	-0.80469	0.00065	0.20668	14.60788	3.60700	8.50319
	20.42243	18.22635	6.41143	-0.79927	0.00084	0.19605	15.24092	3.38203	7.42636

					Sigmoidal Curve-fitting Parameters				
Temperature (˚C)	Ct	Cy0	-log10(F0)	M0	Fb	Fmax	c	b	d
70.0	20.74806	18.44930	6.03475	-0.80365	0.00082	0.16904	15.16445	3.68778	7.46727
	20.60356	18.35253	6.26511	-0.75984	0.00088	0.16672	15.02772	3.60165	7.67607
	20.62427	18.34432	6.25985	-0.77153	0.00078	0.16474	15.05547	3.62830	7.57422
	20.64095	18.35823	6.40274	-0.66749	0.00008	0.15721	15.38570	3.48703	7.30104
	20.76420	18.43413	6.06676	-0.79918	0.00049	0.16405	15.06840	3.68114	7.66155
71.5	22.00085	19.55338	5.72672	0.14952	0.00110	0.15902	18.25436	3.96320	4.59345
	22.06136	19.64796	5.83418	0.31489	0.00066	0.15096	18.91699	3.87376	4.12782
	21.89426	19.47358	5.83790	0.13631	0.00063	0.15639	17.47154	3.94200	5.37595
	21.95624	19.47948	6.01417	0.25321	-0.00028	0.14694	16.78302	3.85915	6.42304
	22.26285	19.82939	5.77029	0.46318	0.00048	0.15164	18.16812	3.95244	5.00574
71.9	21.82854	19.45337	5.68766	0.02093	0.00126	0.19959	16.57795	4.00858	6.39325
	21.70507	19.32861	5.71016	-0.09441	0.00081	0.20207	17.06401	3.89975	5.71849
	21.68929	19.30635	5.75117	-0.09177	0.00076	0.18518	16.97161	3.91519	5.79432
	21.68989	19.27806	5.72871	-0.13507	0.00061	0.19773	16.54539	3.95119	6.30403
	22.14798	19.75238	5.84279	0.42856	0.00075	0.16901	17.73599	3.95168	5.38159
72.7	29.27482	27.23517	6.07609	8.33772	0.00139	0.13414	32.16381	3.44074	1.32696
	27.30637	24.96968	5.89784	5.87651	0.00101	0.13731	27.47575	3.94222	2.20929
	26.29511	23.91018	5.88241	4.76767	0.00072	0.13070	25.93275	3.95333	2.41984
	27.14658	24.77912	5.92470	5.69576	0.00093	0.13004	26.75693	4.00640	2.44168
	37.14676	33.62933	6.07270	14.97709	0.00036	0.19567	36.62494	6.13472	2.48132
73.3	29.76530	27.62594	6.18037	8.80903	0.00138	0.15107	31.95165	3.60649	1.52664
	27.75944	25.50957	5.77067	6.35660	0.00145	0.16679	28.94448	3.76794	1.83080
	28.11433	25.71665	5.81668	6.60099	0.00131	0.16450	28.41290	3.99881	2.14824
	26.05641	23.67020	5.73693	4.42744	0.00113	0.17675	25.33975	3.94051	2.57152
	32.59289	29.76913	6.51494	11.24574	0.00029	0.16556	32.74108	4.55253	2.21121
73.6	33.94676	30.78618	5.96904	11.96032	0.00155	0.16958	33.74045	6.02777	2.43656
	32.61359	29.77271	5.99564	10.92393	0.00128	0.14406	33.53012	5.11220	2.05708
	35.49216	32.25844	5.97058	13.48930	0.00112	0.18466	36.00561	5.95166	2.22506
	34.29546	31.07854	6.25448	12.44291	0.00102	0.15645	33.57587	5.94813	2.57798
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table S12. Estimated quantification (copies/reaction) for temperature variation experiments

Temperature (˚C)	Ct_quant	Cy_quant	-log10(F0)_quant	M0_quant	MahalDistSq	Outlier (p<0.01)	Outlier (p<0.001)
54.0	9.00E+04	8.98E+04	3.94E+04	7.98E+04	15.80888	1	1
	9.12E+04	9.06E+04	5.97E+04	8.54E+04	3.72041	0	0
	8.92E+04	9.06E+04	5.07E+04	8.34E+04	9.81176	1	0
	8.94E+04	8.97E+04	5.46E+04	8.35E+04	6.17608	0	0
	9.11E+04	8.88E+04	5.78E+04	8.35E+04	3.43553	0	0
55.0	9.09E+04	9.02E+04	5.20E+04	8.34E+04	6.61385	0	0
	9.12E+04	9.05E+04	5.56E+04	8.44E+04	5.09417	0	0
	8.81E+04	8.93E+04	5.61E+04	8.35E+04	6.29589	0	0
	8.82E+04	8.90E+04	6.26E+04	8.46E+04	3.44637	0	0
	8.64E+04	8.62E+04	4.54E+04	7.87E+04	9.51227	1	0
56.9	8.97E+04	8.77E+04	5.79E+04	8.26E+04	3.22295	0	0
	8.93E+04	8.79E+04	5.92E+04	8.31E+04	2.98724	0	0
	8.79E+04	8.63E+04	6.29E+04	8.25E+04	1.89064	0	0
	8.64E+04	8.65E+04	7.84E+04	8.53E+04	0.23503	0	0
	8.68E+04	8.55E+04	5.05E+04	7.93E+04	5.56744	0	0
59.3	8.93E+04	8.74E+04	8.82E+04	8.75E+04	0.97742	0	0
	8.86E+04	8.59E+04	6.66E+04	8.29E+04	1.59929	0	0
	8.84E+04	8.60E+04	7.62E+04	8.45E+04	1.04814	0	0
	8.75E+04	8.59E+04	9.27E+04	8.68E+04	1.19408	0	0
	8.75E+04	8.42E+04	6.18E+04	8.06E+04	2.38773	0	0
61.9	8.88E+04	8.79E+04	5.35E+04	8.18E+04	5.05873	0	0
	9.18E+04	8.79E+04	6.64E+04	8.45E+04	2.48206	0	0
	8.95E+04	8.92E+04	9.96E+04	9.06E+04	0.52527	0	0
	8.58E+04	8.95E+04	1.30E+05	9.43E+04	2.40121	0	0
	9.03E+04	8.91E+04	7.14E+04	8.63E+04	0.95897	0	0
62.0	8.73E+04	8.72E+04	4.41E+04	7.91E+04	10.94358	1	0
	8.84E+04	8.72E+04	6.23E+04	8.31E+04	2.18943	0	0
	8.62E+04	8.59E+04	4.86E+04	7.92E+04	7.36837	0	0
	8.10E+04	8.53E+04	4.07E+04	7.67E+04	23.23125	1	1
	8.85E+04	8.63E+04	5.93E+04	8.18E+04	2.64154	0	0
62.6	8.89E+04	8.84E+04	5.91E+04	8.34E+04	3.47163	0	0
	8.80E+04	8.65E+04	5.55E+04	8.12E+04	3.78358	0	0
	8.59E+04	8.59E+04	6.09E+04	8.18E+04	2.81780	0	0
	8.22E+04	8.37E+04	3.77E+04	7.47E+04	18.17028	1	1
	8.41E+04	8.37E+04	5.35E+04	7.85E+04	4.38302	0	0
63.7	8.69E+04	8.62E+04	5.20E+04	8.02E+04	5.41980	0	0
	8.60E+04	8.53E+04	4.86E+04	7.87E+04	6.82898	0	0
	8.51E+04	8.57E+04	5.84E+04	8.11E+04	3.93775	0	0
	8.60E+04	8.53E+04	5.26E+04	7.96E+04	4.92475	0	0
	8.39E+04	8.40E+04	4.31E+04	7.63E+04	10.74028	1	0
64.7	9.01E+04	9.38E+04	6.07E+04	8.81E+04	9.41522	1	0
	9.29E+04	9.40E+04	8.24E+04	9.23E+04	0.71597	0	0
	8.92E+04	9.36E+04	7.70E+04	9.10E+04	5.12620	0	0
	8.93E+04	9.41E+04	9.53E+04	9.43E+04	2.88542	0	0
	9.10E+04	9.62E+04	7.56E+04	9.29E+04	7.25503	0	0
65.2	8.56E+04	8.96E+04	7.87E+04	8.79E+04	3.65354	0	0
	8.62E+04	8.96E+04	5.65E+04	8.39E+04	9.87237	1	0
	8.44E+04	8.87E+04	6.26E+04	8.44E+04	8.83050	0	0
	8.21E+04	8.71E+04	5.35E+04	8.12E+04	15.21127	1	1
	8.46E+04	9.05E+04	5.17E+04	8.35E+04	19.65390	1	1
67.4	8.47E+04	1.05E+05	1.70E+05	1.12E+05	43.32151	1	1
	8.91E+04	1.07E+05	1.37E+05	1.11E+05	34.53592	1	1
	8.84E+04	1.08E+05	1.58E+05	1.14E+05	39.33326	1	1
	8.89E+04	1.09E+05	1.72E+05	1.16E+05	40.76519	1	1
	8.80E+04	1.08E+05	1.34E+05	1.11E+05	44.56879	1	1
68.4	8.02E+04	1.09E+05	1.83E+05	1.17E+05	95.13760	1	1
	8.25E+04	1.08E+05	2.14E+05	1.19E+05	66.43159	1	1
	8.01E+04	1.10E+05	2.24E+05	1.22E+05	97.58370	1	1
	8.01E+04	1.09E+05	1.68E+05	1.16E+05	97.36687	1	1
	8.13E+04	1.12E+05	2.35E+05	1.24E+05	97.38880	1	1
69.8	8.71E+04	1.24E+05	6.00E+05	1.55E+05	113.71959	1	1
	8.68E+04	1.21E+05	5.40E+05	1.50E+05	102.96621	1	1
	8.68E+04	1.23E+05	5.49E+05	1.52E+05	111.38571	1	1
	7.57E+04	1.16E+05	9.23E+05	1.56E+05	173.79202	1	1
	8.66E+04	1.25E+05	5.95E+05	1.56E+05	122.96730	1	1

Temperature (˚C)	Ct_quant	Cy_quant	-log10(F0)_quant	M0_quant	MahalDistSq	Outlier (p<0.01)	Outlier (p<0.001)
70.0	6.94E+04	1.07E+05	1.49E+06	1.56E+05	196.83714	1	1
	7.65E+04	1.15E+05	8.49E+05	1.52E+05	154.38650	1	1
	7.55E+04	1.15E+05	8.60E+05	1.53E+05	168.08292	1	1
	7.46E+04	1.14E+05	6.08E+05	1.45E+05	166.31021	1	1
	6.86E+04	1.09E+05	1.38E+06	1.56E+05	207.39946	1	1
71.5	2.97E+04	5.12E+04	3.15E+06	9.20E+04	372.42053	1	1
	2.85E+04	4.80E+04	2.42E+06	8.40E+04	339.53794	1	1
	3.19E+04	5.40E+04	2.40E+06	9.27E+04	330.72542	1	1
	3.06E+04	5.38E+04	1.56E+06	8.69E+04	328.50617	1	1
	2.48E+04	4.25E+04	2.83E+06	7.73E+04	375.62239	1	1
71.9	3.33E+04	5.47E+04	3.46E+06	9.88E+04	346.99193	1	1
	3.62E+04	5.95E+04	3.28E+06	1.05E+05	332.46719	1	1
	3.66E+04	6.04E+04	2.96E+06	1.05E+05	323.48567	1	1
	3.66E+04	6.16E+04	3.13E+06	1.08E+05	338.15955	1	1
	2.68E+04	4.48E+04	2.37E+06	7.88E+04	337.40204	1	1
72.7	2.13E+02	2.93E+02	1.34E+06	9.77E+02	1353.71622	1	1
	8.10E+02	1.34E+03	2.07E+06	3.83E+03	940.99661	1	1
	1.61E+03	2.74E+03	2.15E+06	7.09E+03	777.50706	1	1
	9.02E+02	1.53E+03	1.94E+06	4.24E+03	890.94052	1	1
	1.02E+00	3.98E+00	1.36E+06	2.45E+01	3083.72685	1	1
73.3	1.53E+02	2.25E+02	1.04E+06	7.52E+02	1308.98610	1	1
	5.95E+02	9.34E+02	2.83E+06	2.94E+03	1143.44541	1	1
	4.68E+02	8.13E+02	2.53E+06	2.56E+03	1129.26146	1	1
	1.89E+03	3.22E+03	3.07E+06	8.56E+03	821.55818	1	1
	2.24E+01	5.33E+01	4.62E+05	1.95E+02	1490.49511	1	1
73.6	8.93E+00	2.69E+01	1.74E+06	1.31E+02	2257.84980	1	1
	2.21E+01	5.32E+01	1.64E+06	2.33E+02	1884.37851	1	1
	3.13E+00	1.00E+01	1.74E+06	5.60E+01	2647.27747	1	1
	7.05E+00	2.21E+01	8.71E+05	1.00E+02	2136.30201	1	1
	N/A	N/A	N/A	N/A	N/A	N/A	N/A

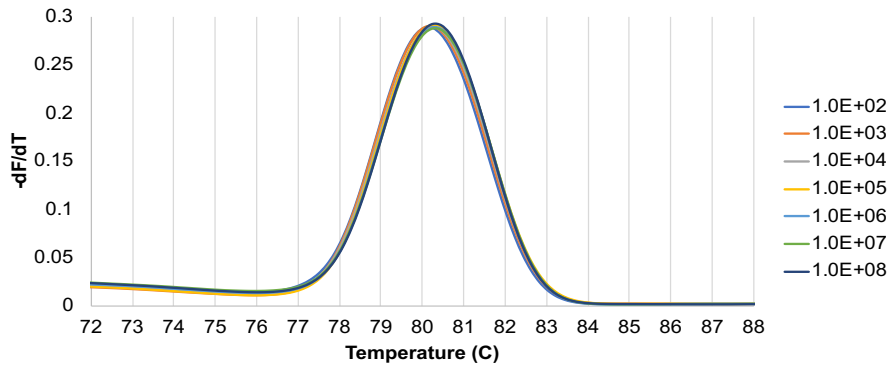

Figure S1. Melting curve analysis for lambda DNA standard experiment.

This figure shows average melting curves peaks for synthetic lambda DNA standard experiments using the 242bp double-stranded DNA molecule (gBlock gene fragment ordered from IDT) using in-house lambda primers. Ten-fold dilution from 10^8 to 10^2 copies/reaction were used in this experiment, 8-reactions per tested concentration. Average melting curve peak was 80.26°C (SD = 0.1°C) for all positive reactions and no secondary melting event was observed at other annealing temperatures.

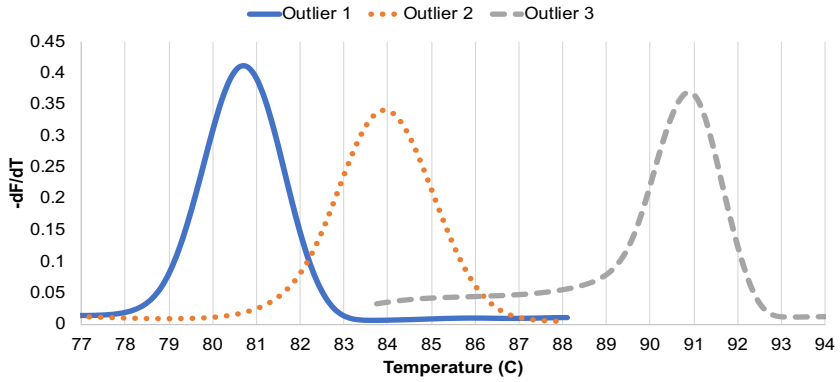

Figure S2. Melting curve analysis for non-specific outlier detection experiment.

This figure shows average melting curves peaks of 80.66°C (SD = 0.07°C) for Outlier 1, 83.97°C (SD = 0.10°C) for Outlier 2 and 90.76°C (SD = 0.10°C) for Outlier 3. Octuplicate reactions were performed per reaction condition. No secondary melting event was observed at other annealing temperatures. Specific primers sets were selected from Monteiro et al 2012.

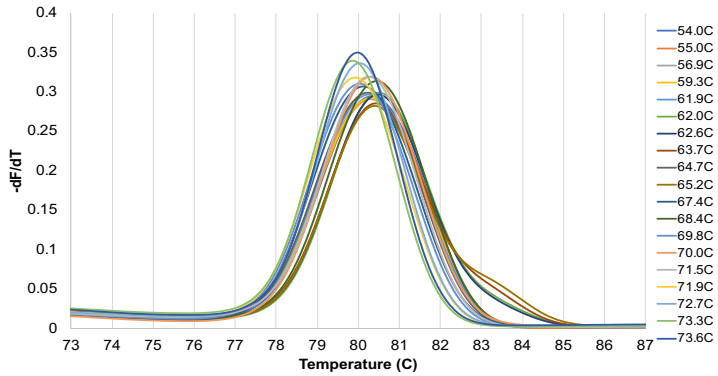

Figure S3. Melting curve analysis for temperature variation experiment.

This figure shows average melting curves peaks for temperature variation experiments ranging from 54°C to 73.6°C using phage lambda DNA and in-house primers. Observed average melting curve peaks for tested temperatures is 80.07°C (SD = 1.12 °C). Quintuplicate reactions were performed per tested temperature.