

basicsynbio and the BASIC SEVA collection: Software and vectors for an established DNA assembly method: online supplement

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Description of files in the online supplement

The supplementary data contains files which were used to generate and document the BASIC SEVA vector collection. Specifically, a Jupyter Notebook was used to generate and document the collection and is available in both pdf (**addgene_submission_notebook.pdf**) and ipynb (**addgene_submission_notebook.ipynb**) file formats, however rather than execute the code in this notebook, we recommend cloning the GitHub repository and executing code in the equivalent file (available online¹). This avoids exceptions caused by differences in file paths.

Multiple parts and linkers were required to assemble the collection. Sequences of parts used in the assembly of the collection are given in multiple GenBank files: **AbR_markers.gb**, **mScarlet_cs_cassettes.gb**, **oris.gb**, and **TS_ori_gblocks.gb**. The instance of the BasicLinker class representing BSEVA_L1 is pickled in **SEVA-BB1**. These were used along with parts and linkers accessed from within basicsynbio PartLinkerCollection objects.

Several files were exported and include manual instructions to assemble the collection (**BASIC_SEVA_collection_v10_manual.pdf**), instructions to transfer purified clip reactions on a Beckman Echo Acoustic Liquid Handler (**echo_clips_1.csv**), instructions to transfer ddH₂O and 10x assembly buffer using a Beckman Echo Acoustic Liquid Handler (**echo_water_buffer_1.csv**), annotated sequences of the assembled collection (**BASIC_SEVA_collection_v10.gb**). An additional file was exported detailing the mass of T0 + marker part to add during clip reaction setup (**AbR_marker_clip_masses.csv**). This was required as T0 + marker parts were cloned into pJet1.2 which contains an internal Bsal site, meaning a “PartException” exception would be raised when attempting to import the full plasmid sequence as opposed to the corresponding sequence given in **AbR_markers.gb**.

BASIC_SEVA_66-11_&_69-11.gb details the sequences of the corresponding BASIC SEVA vectors that were identified following the attempted assembly of BASIC_SEVA_66.10 & 69.10.

¹ https://github.com/LondonBiofoundry/basicsynbio/blob/main/scripts_nbs/addgene_submission.ipynb

BASIC SEVA modules

As outlined in the main text, we utilised marker 5a sequence over the SEVA tetracycline module and were unable to generate BASIC SEVA plasmids containing a high copy number origin of replication. The following sections provide an overview of these findings.

Marker 5a

After cloning the SEVA tetracycline module into pJET1.2 (Thermo Scientific™ K1231), we identified a 248I>S substitution in the amino acid sequence of the tetracycline efflux transporter, we assigned this variant the identity BS_5axx. We conducted an NCBI BLASTp search against the amino acid sequence but failed to find a variant containing the 248I>S substitution.

We attempted to correct this error via PCR mutagenesis, amplifying the module cloned into pJet1.2 (Thermo Scientific™ K1231) with the BS_5xx_980G>T_F/R primer pair (Table S1) and plating cells transformed with the circularised product on LB-agar supplemented with 100 µg/mL carbenicillin. The equivalent SEVA module sequence (BS_5xx) was confirmed via Sanger Sequencing.

We measured the transformation efficiency (TE) of BS_5xx and compared it to BS_5axx (Figure S4). We found BS_5axx had a larger TE compared to BS_5xx ($P < 0.05$) with mean TEs of 1.8×10^7 and 1.3×10^5 , respectively. The reason for the difference in TE was not investigated further but could indicate BS_5xx does not efficiently confer tetracycline resistance under the conditions tested, requiring additional mutations. We therefore opted to use BS_5axx when generating the BASIC SEVA collection described in this paper.

pUC origin of replication

We attempted to include the SEVA pUC origin of replication module (SEVA #8) in the BASIC SEVA collection. In a similar manner to that conducted for other BASIC SEVA oris (Materials and methods), we assembled the SEVA #8 ori + T1 part with a T0 + ampicillin marker part, a construct we refer to as BS_x8x. Following plasmid DNA preparation and sequencing, we identified a 1089T>A point mutation in the resulting BS_x8x1089T>A plasmid. We attempted to correct this error via PCR mutagenesis using the BS_x8x1089A>T_F/R primer pair (Table S1). We transformed *E. coli* DH5α cells with both the circularised and linear PCR mutagenesis products (Figure S5). Transformation of linear PCR products gives an indication of background, a result of BS_x8x1089A>T PCR template not digested by a subsequent DpnI step. We observed many microcolonies following transformation with the circularised PCR product, an indication of a burdensome construct (Figure S5). We also observed some larger colonies, though not many more than achieved via transformation of linear PCR product. We prepped plasmid DNA from two of the larger colonies, however both contained further mutations to the pUC origin of replication (data not shown). We conclude BS_x8x is too burdensome for DH5α. As discussed in the main text, we recommend the use of BASIC_SEVA_39.10 for applications requiring a high yield of plasmid DNA or where another marker is required, we suggest amplifying a plasmid containing a pBR322 ori with chloramphenicol¹.

Supplementary Figures

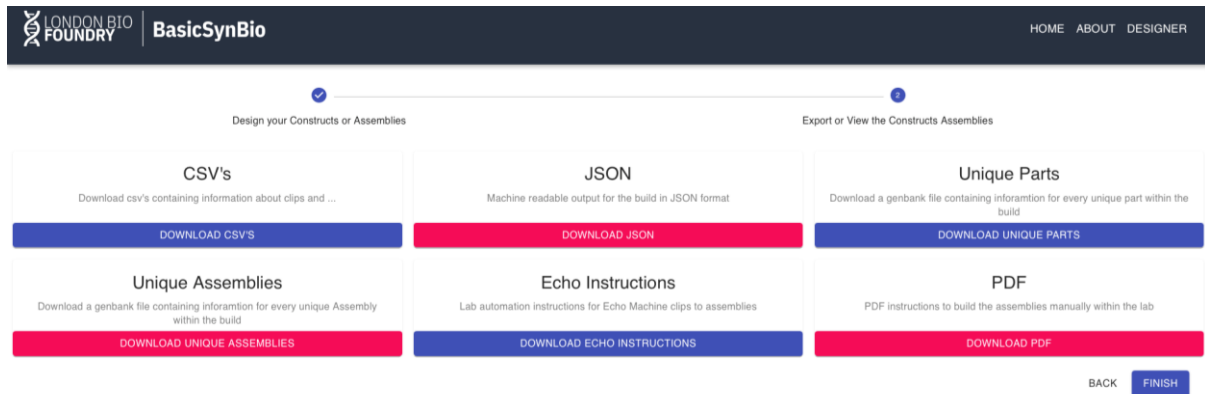


Figure S1. A screenshot displaying the export options available to basicsynbio Web-app users.



Figure S2. A screenshot highlighting the integration of Seqviz within the basicsynbio Web-app which can be used to view the sequence of any BasicPart or BasicAssembly.

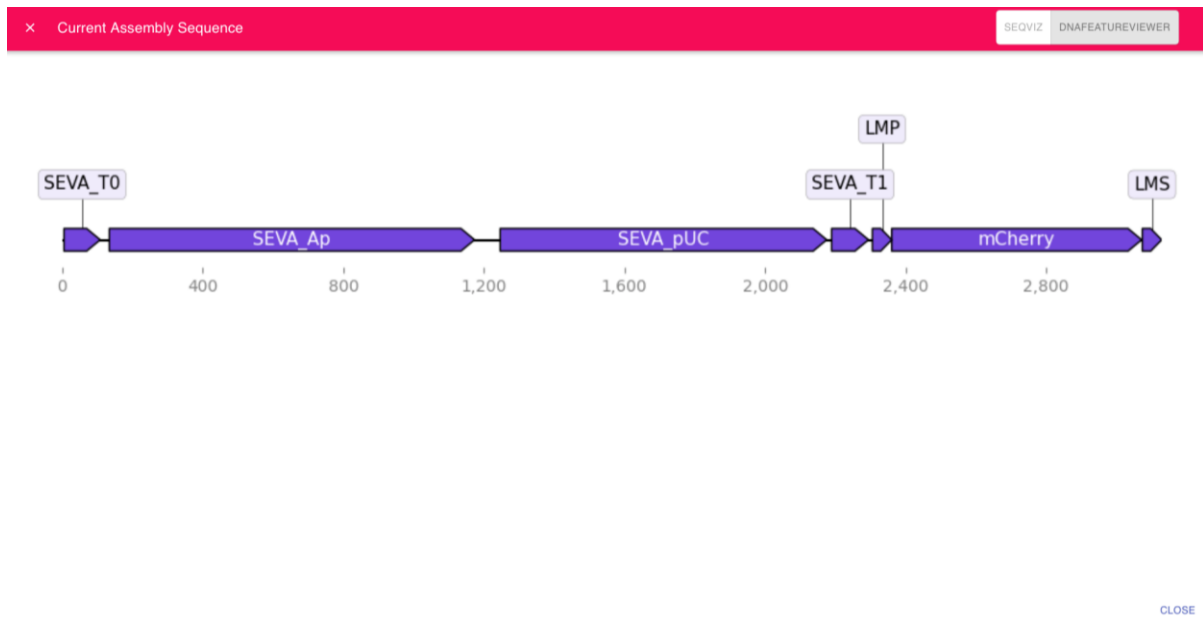


Figure S3. A screenshot highlighting the integration of DNA Features Viewer within the basicsynbio Web-app which can be used to view the sequence of any BasicPart or BasicAssembly.

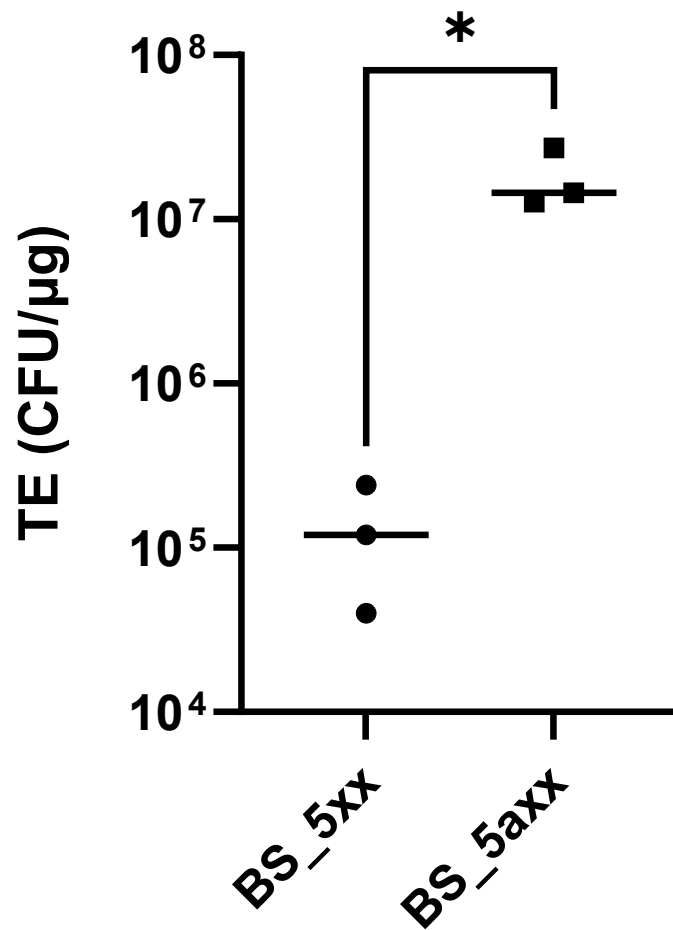


Figure S4. Scatter plot illustrating transformation efficiency (TE) of considered tetracycline marker modules for the BASIC SEVA collection as outlined in section “Marker 5a”. TE was measured by calculating colony forming units (CFU) per μg DNA transformed into chemically competent cells (NEB C2987), as described by the manufacturer, when reactions were plated on LB-agar supplemented with 10 $\mu\text{g}/\text{mL}$ tetracycline. Mean TE is illustrated by a horizontal line. Significance of the difference was measured using Welch’s t-test (One-tail), where * denotes $P < 0.05$.

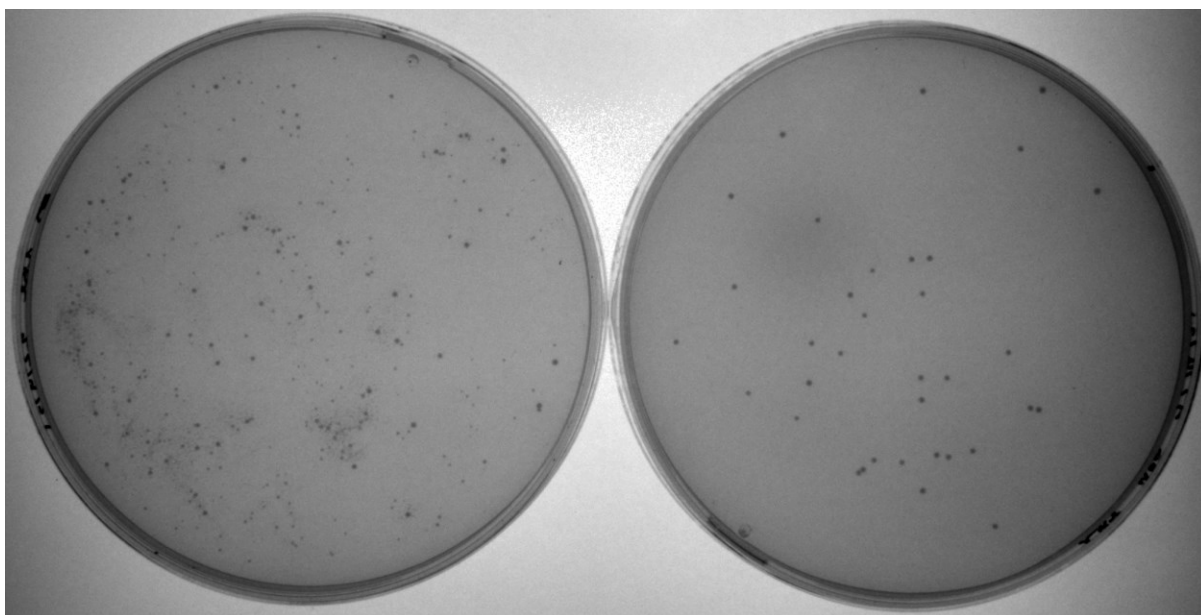


Figure S5. PCR mutagenesis of BS_x8x1089T>A. Left: *E. coli* DH5 α transformed with circularised product of PCR mutagenesis (putative BS_x8x). Right: *E. coli* DH5 α transformed with linear product of PCR mutagenesis.

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SEVA_Gm -----AGCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGC
BASIC_SEVA_66_clone_E11 -----AGCAGCAACGATGTTACGCAGC
BASIC_SEVA_66_clone_F11 -----AGCAGCAACGATGTTACGCAGC
BASIC_SEVA_66_clone_G11 AGCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGC
BASIC_SEVA_66_clone_H11 AGCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGC
BASIC_SEVA_69_clone_E12 -----AGCAGCAACGATGTTACGCAGC
BASIC_SEVA_69_clone_F12 -----AGCAGCAACGATGTTACGCAGC
BASIC_SEVA_69_clone_G12 AGCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGC
BASIC_SEVA_69_clone_H12 AGCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGC

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Figure S6. Multiple sequence alignment of the gentamicin resistance cassette region from BASIC_SEVA_66 & 69 clones. The sequence of the original SEVA_Gm part is given. The predicted ATG start codon of the gentamicin resistance CDS is highlighted by a light blue rectangle. Clones H11 & G12 were designated BASIC_SEVA_66.11 & 69.11, respectively.

Supplementary Tables

Table S1. Oligonucleotide used in this work. “/5Phos/” denotes the presence of a 5' phosphate group.

Primer name	Oligonucleotide sequence	Comment
BS_5xx_980G>T_R	/5Phos/GCTCCAGCGAAAGCGGTC	Mutagenesis primers
BS_5xx_980G>T_F	/5Phos/GCGACGATGAtCGGCCTGTCTG	
BS_x8x1089A>T_R	/5Phos/GTAGGTATCTCAGTTCGG	
BS_x8x1089A>T_F	/5Phos/AGCGTGAGCTtTGAGAAAGCG	
SPAP	CGAGCCGTATTGTGGTGATTTA	Sequencing primers
SP072	GGACCCCTGGATTCTCACC	
pJET1.2 Forward	CGACTCACTATAGGGAGAGCGGC	
pJET1.2 Reverse	AAGAACATCGATTTTCCATGGCAG	
BS_x5x 1	AAAGGCGCTCGATGCAG	Additional sequencing primers to sequence BS_x5ax ori + T1 part
BS_x5x 2	ACACGATAGAGCACCCGG	
BS_x5x 3	AAAGGCTTGCTTCGCGG	
BS_x5x 4	ATTCACGCAGCAGCACC	
BSEVA_L1_prefix_long	/5Phos/GGACACTATCTGTGTGGACCTCAGAATTGTACGAGAC	BSEVA_L1 oligos. All ordered HPLC-purified.
BSEVA_L1_prefix_adapter	/5Phos/CACACAGATAGT	
BSEVA_L1_suffix_long	/5Phos/CTCGGCCCACTTGTGTGTCTCGTACAATTCTGAGGTC	
BSEVA_L1_suffix_adapter	/5Phos/ACACAAGTGGGC	
BSEVA_L1_overhang	GTCTCGTACAATTCTGAGGTC	

Bibliography

1. Sambrook, J. & Russell, D. *Molecular cloning A Laboratory Manual*. (Cold Spring Harbor Laboratory Press, 2001).
2. Geissmann, Q. OpenCFU, a New Free and Open-Source Software to Count Cell Colonies and Other Circular Objects. *PLoS One* **8**, e54072 (2013).