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Building an Orthogonal Replication Systems for Performing Directed Evolution in *Escherichia coli*: A Strategic Review and a Summary of the Initial Steps in Cloning Bacteriophage T7 gp4 Primase/Helicase

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SUPPLEMENTAL MATERIAL

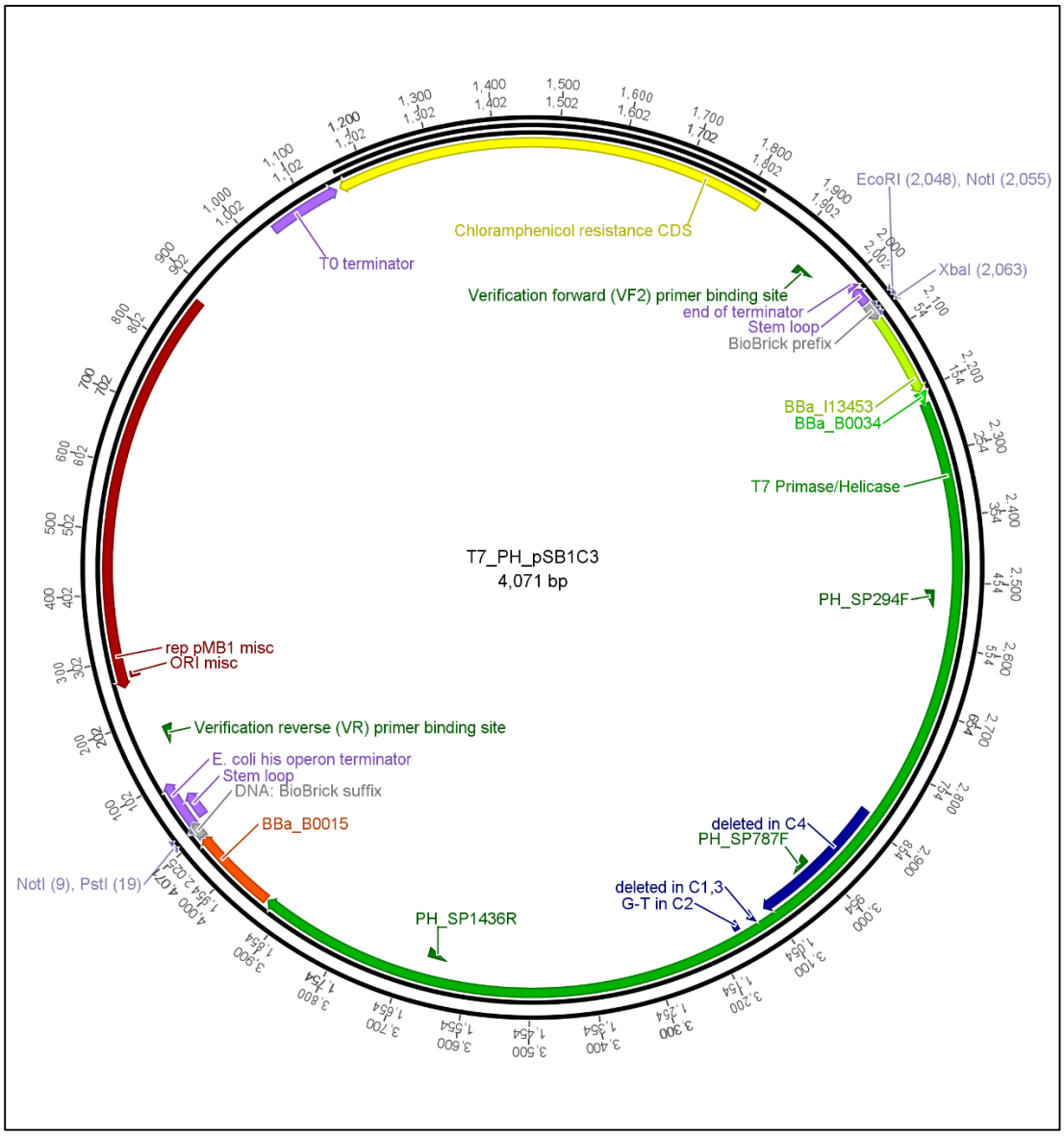

FIG S1 The pSB1C3-B Plasmid Containing pBAD promoter. This is the recommended plasmid to perform future studies on because it contains the pBAD promoter. In this map, all restriction sites as well as the sequencing primers used for the studies highlighted in this paper, are shown. The four clones containing the mutations obtained from iGEM are shown in dark blue in the bottom right section of the plasmid, and they are all in the same region of gp4 primase/helicase.

TABLE S1 Characteristics of isolated plasmids from the transformation of *E. coli* DH5α with the pBS13-A plasmids that have undergone site directed mutagenesis (SDM) to correct the T^{938} into the G^{938}. The colonies were spread plated on Luria agar with 25 µg/mL chloramphenicol and either 0.2% L-Arabinose or D-Glucose at 37°C and incubated overnight twice. Colony counts were for colonies that appeared homogenous. Agarose gel electrophoresis of the restriction digested samples was performed and characteristics of the bands were recorded.

Plasmid Sample	Colony Count	Colony Colour from X-Gal + IPTG	Nanodrop Spectrometer Reading (ng/uL)	Band Characteristics
SDM Point Mutation – L-Arabinose	1	N/A	N/A	N/A
SDM Point Mutation – D-Glucose	14	N/A	27.2* 33.7*	No bands* No bands*
Negative Control for SDM – No DNA template	0	N/A	N/A	N/A
Negative Control for SDM – No DNA polymerase	0	N/A	N/A	N/A
Positive Control for SDM – *lacZ* gene knockout	TNTC	Blue colonies	77.6	N/A
Positive Control for SDM – no *lacZ* gene knockout	TNTC	White colonies	121.3	N/A
Positive Control for Transformation – pBSC13-A plasmid	TNTC	N/A	317	Approximately 2 kb** Approximately 2 kb**
Positive Control for Restriction Enzyme Digest – Original and Uncut pBSC13-A plasmid	N/A	N/A	205.4	Approximately 4 kb

*Plasmid isolation was done in replicates and those replicates were also loaded onto the gel
**There were two bands similar in size resulting from the use of EcoRI and PstI restriction enzyme digest