SUPPLEMENTAL MATERIAL

TABLE. S1 Q5 primers. Names, sequences, and melting temperatures of the primers used for Q5 site-directed mutagenesis as manually designed on SnapGene.

Primer Number	**Sequence (5' → 3')**	**Tm (°C)**
Q5_1	TACTAGAGTCACACAGG	48
Q5_2	TAAACTCTAGAAGCGGC	50

TABLE. S2 Primers for amplified regions of pDO6935. Names, sequences, and melting temperatures of the primers used for PCR amplification of regions on pDO6935 as designed on SnapGene (1-6) and control primers for pUC19.

Primer Number	Sequence (5' → 3')	**Tm (°C)**
1	GTGTCTAGAGTGCCACCAAAAGAGAAG	55
2	ACAGAATTCCAACGCGGCCTTTTTACG	57
3	TCAGAATTCCTCATTAGGCACCCCAGGC	59
4	GTCTCTAGACATAGCTGTTTCCTGTGTG	55
5	ACTGAATTCCACACAGGAAACAGCTATGA	53
6	GTGTCTAGAGCGTTGCGCTCACTGCCC	65
puc19cntrlF primer	GTGAAATACCGCACAGATGC	-
puc19cntrlR primer	GGCGTTACCCAACTTAATCG	-

TABLE. S3 pDO6935 Amplified segments and associated primers. The primer pairs used to amplify segments of pDO6935 and their respective amplicon names and sizes as predicted using SnapGene.

Amplicon	**Primers**	**Size**
A	1, 2	1212
B	2, 4	410
C	1, 5	839
D	1, 3	951
E	2, 6	295

TABLE. S4 Nucleotide Regions of Amplified Segments and *lac* Regulatory Elements on pDO6935. Nucleotides start at the 5' end of the origin of replication (ori), denoted as nucleotide 1 and end at the 3' end of *brkA,* denoted as nucleotide 1790. *****During amplification of region D, part of the nucleotide region in the CAP BS was truncated. The top region (858-1791) was the intended amplified region, however sequencing results yielded the bottom region (893-1791) as the amplified region.

Region	**Nucleotide Number**
Origin of Replication	1-597
*lac P*romoter	912-943
lac Operator	950-967
Catabolite Activator Protein Binding Site (CAP BS)	876-898
Amplicon A	597-1791
Amplicon B	597-968
Amplicon C	989-1791
Amplicon D*	858-1791 893-1791
Amplicon E	597-858

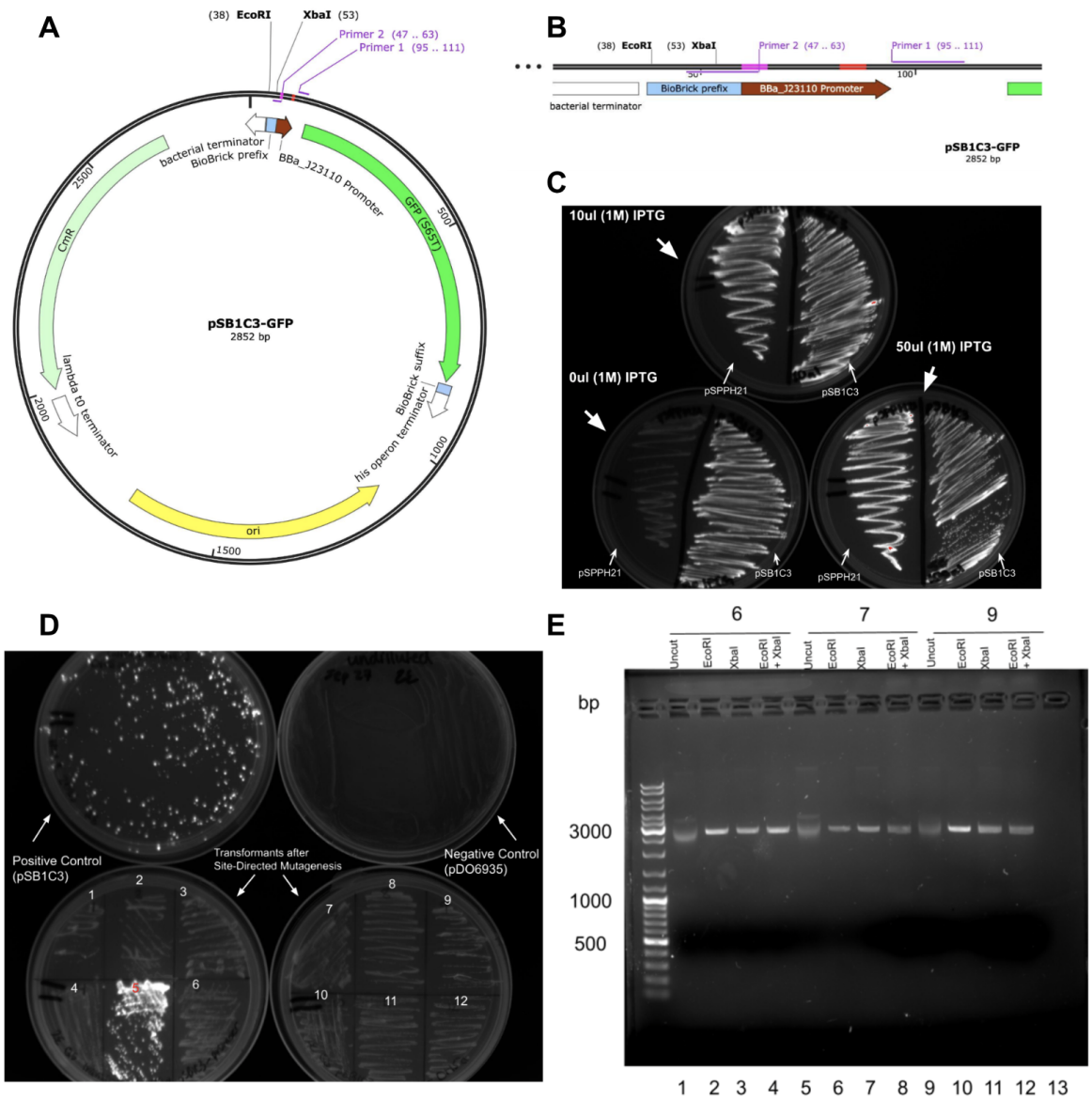

FIG. S1 Removal of BBa_J23110 promoter from pSB1C3 and abolishment of GFP expression. (A) Circular map of pSB1C3, as visualized using SnapGene software. Key genetic elements are annotated and the primers used for the knockout of the BBa_J23110 promoter with site-directed mutagenesis are labelled. (**B)** Linearized close-up of the BBa_J23110 promoter on pSB1C3 and primers for Q5 site-directed mutagenesis, as visualized using SnapGene software. (**C)** GFP expression from *E. coli* DH5α visualized in the ChemiDoc Imaging System (Bio-Rad Laboratories) set to Alexa546 after overnight incubation with 10 µL 1M IPTG (top), 50 µL 1 M IPTG (bottom right), or no IPTG (bottom left). On each plate, pSPPH21 is streaked on the left side and pSB1C3 on the right. (**D)** Evaluation of GFP expression from twelve colonies collected and streaked from the Q5 transformation plate (bottom) as compared to a positive control (pSB1C3; top left) and negative control (pDO6935; top right). Visualization was conducted in the ChemiDoc Imaging System (Bio-Rad Laboratories) set to Alexa546 after overnight incubation of cells on LB-

CHL at 37°C. (**E)** Electrophoresis of products from restriction digests of Q5 Clones 6, 7, and 9 using EcoRI and XbaI run on a 1.5% agarose gel. Visualization was conducted in the ChemiDoc Imaging System (Bio-Rad Laboratories) set to SYBR Safe and samples were stained with GelRed Nucleic Acid Stain (Thermo Scientific). Lanes 1, 5, and 9, contain the uncut Clones 6, 7, and 9, respectively. Lanes 2, 6, and 10 contain single-cut (EcoRI) Clones 6, 7, and 9, respectively. Lanes 3, 7, and 11 contain single-cut (XbaI) Clones 6, 7, and 9 respectively. Lanes 4, 8, and 12 contain double-cut (EcoRI and XbaI) Clones 6, 7, and 9, respectively. The first lane is the O'GeneRuler 1 Kb DNA Ladder (Thermo Scientific).

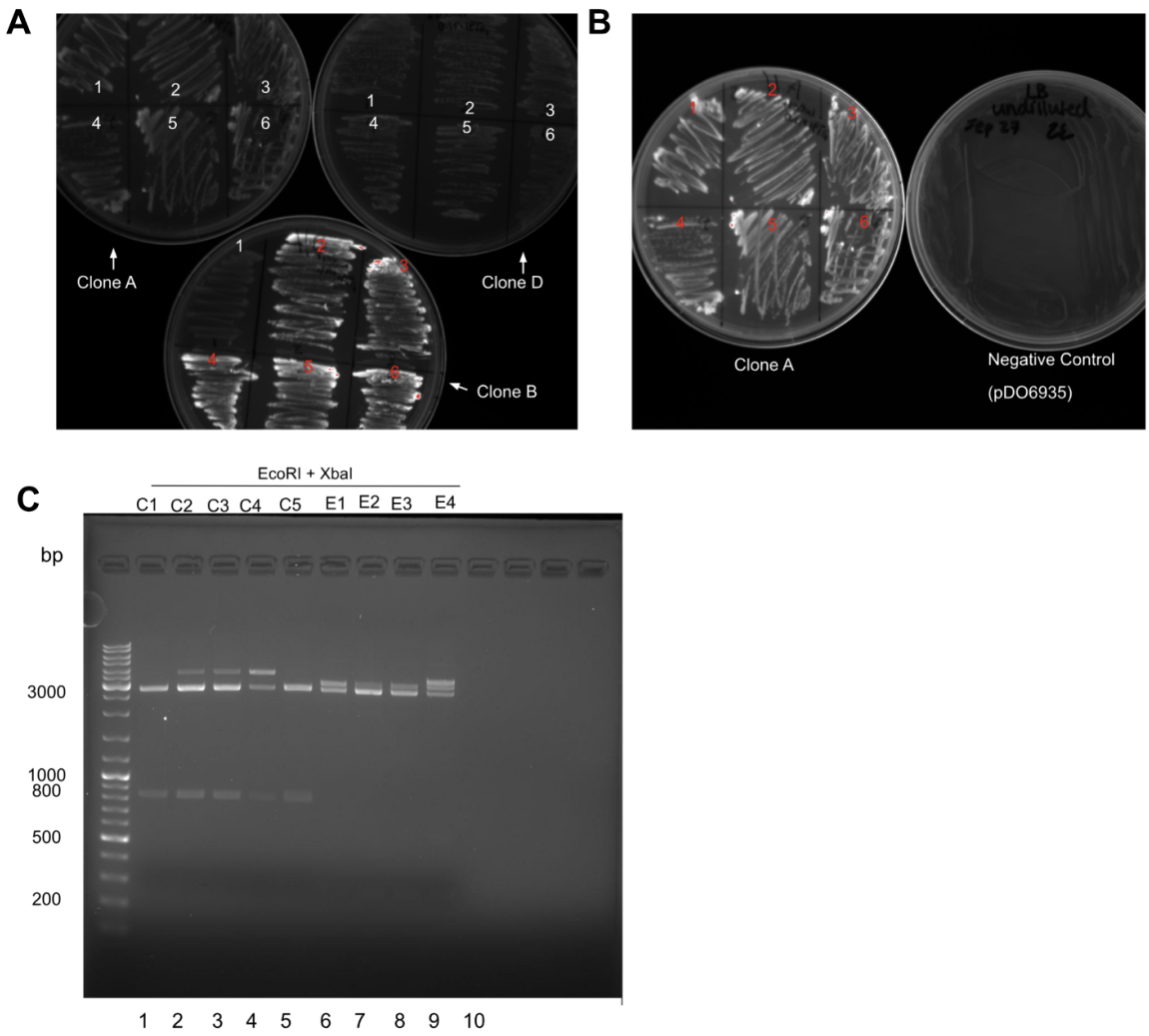

FIG. S2 Confirmation of transformation with ligated clones pEAH23A-E. (A) GFP expression from restreaked colonies of DH5α *E. coli* transformed with pEAH23A, pEAH23B, and pEAH23D. The plates were incubated at 37°C for 16 hours and GFP expression was visualized in the ChemiDoc Imaging System (Bio-Rad Laboratories) set to Alexa546. **(B)** GFP expression from restreaked colonies of DH5α *E. coli* transformed with pEAH23A alongside the negative control (pDO6935) measured in the ChemiDoc Imaging System (Bio-Rad Laboratories) set to Alexa546. **(C)** Electrophoresis of pEAH23C and pEAH23E after digestion with EcoRI and XBaI. Products were run on a 1.5% agarose gel with GelRed Nucleic Acid Stain (Thermo Scientific) and visualized in the ChemiDoc Imaging System (Bio-Rad Laboratories) set to SYBR Safe. Lanes 1-5 contain digested pEAH23C, and lanes 6-9 contain digested pEAH23E. The first lane is the O'GeneRuler 1 Kb DNA Ladder (Thermo Scientific).

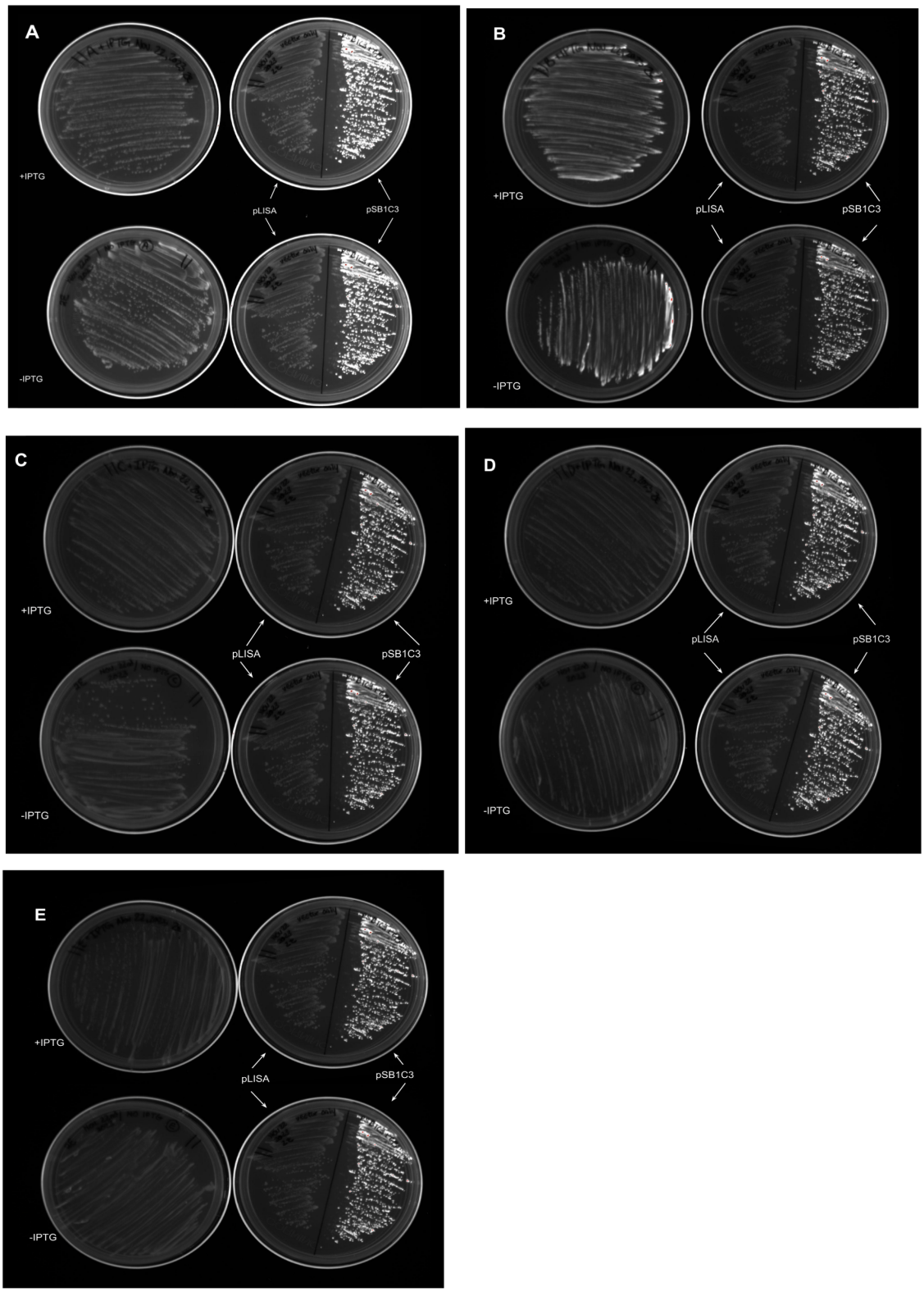

FIG. S3 Clones pEAH23A and pEAH23B express GFP regardless of IPTG induction. (A-E) Visualization of GFP expression from DH5α cells transformed with pEAH23A-pEAH23E. Top left plates were induced with 10μL 1M IPTG via spread-plating on LB-CHL before inoculation.

The bottom left plates are uninduced. GFP-expression controls pLISA (-) and pSB1C3 (+) induced with 10μL 1M IPTG by spread-plating on LB-CHL plates (top and bottom right plates). All plates were incubated at 37°C for 16 hours before GFP expression was visualized in the ChemiDoc Imaging System (Bio-Rad Laboratories) set to Alexa546.

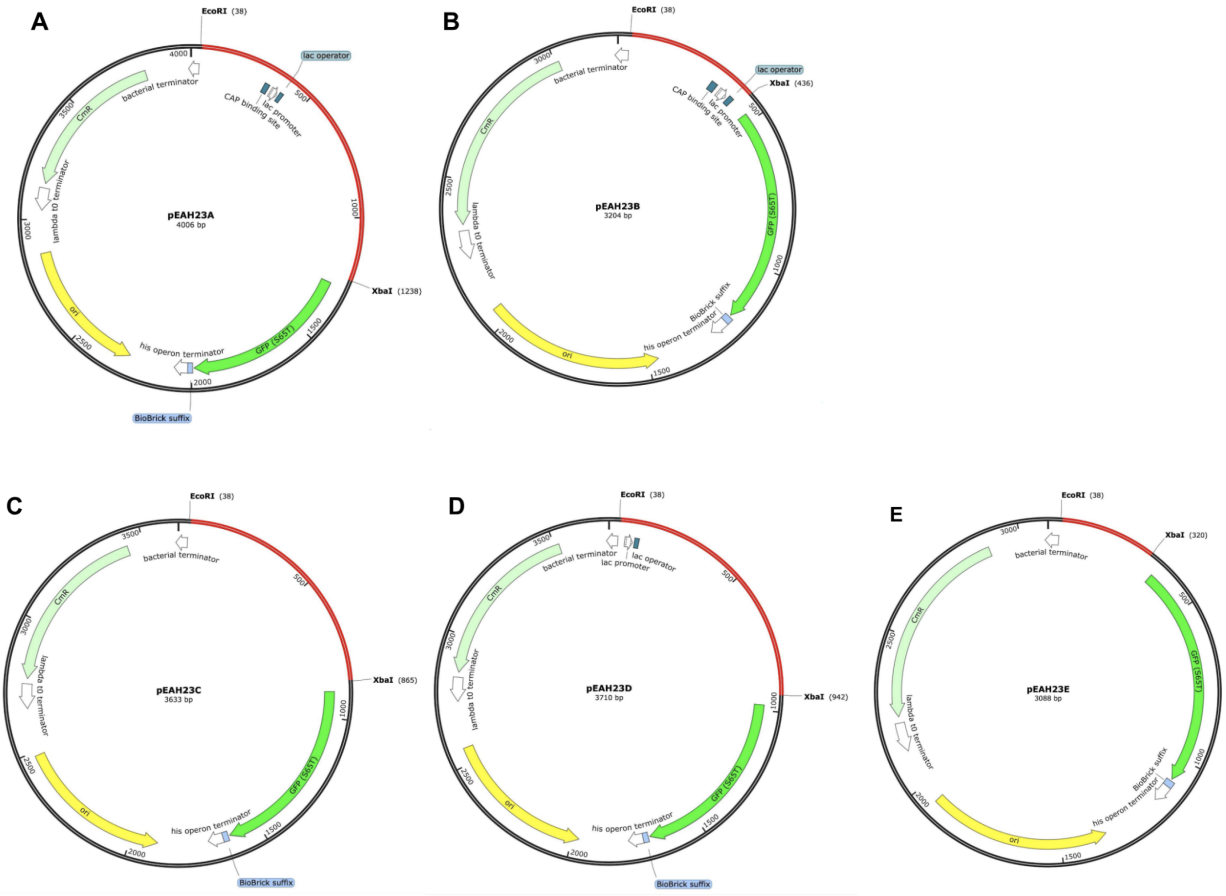

FIG. S4 Confirmation of pEAH23A, pEAH23B, pEAH23C, pEAH23D, and pEAH23E by whole plasmid sequencing. (A-E) These images present the circular maps of pEAH23A-pEAH23E, as visualized using SnapGene software after whole-plasmid nanopore sequencing (Plasmidsaurus). Key features of each plasmid are labelled and the inserted region from pDO6935 is marked in red.