# Supplementary Material

## Supplementary Figure:

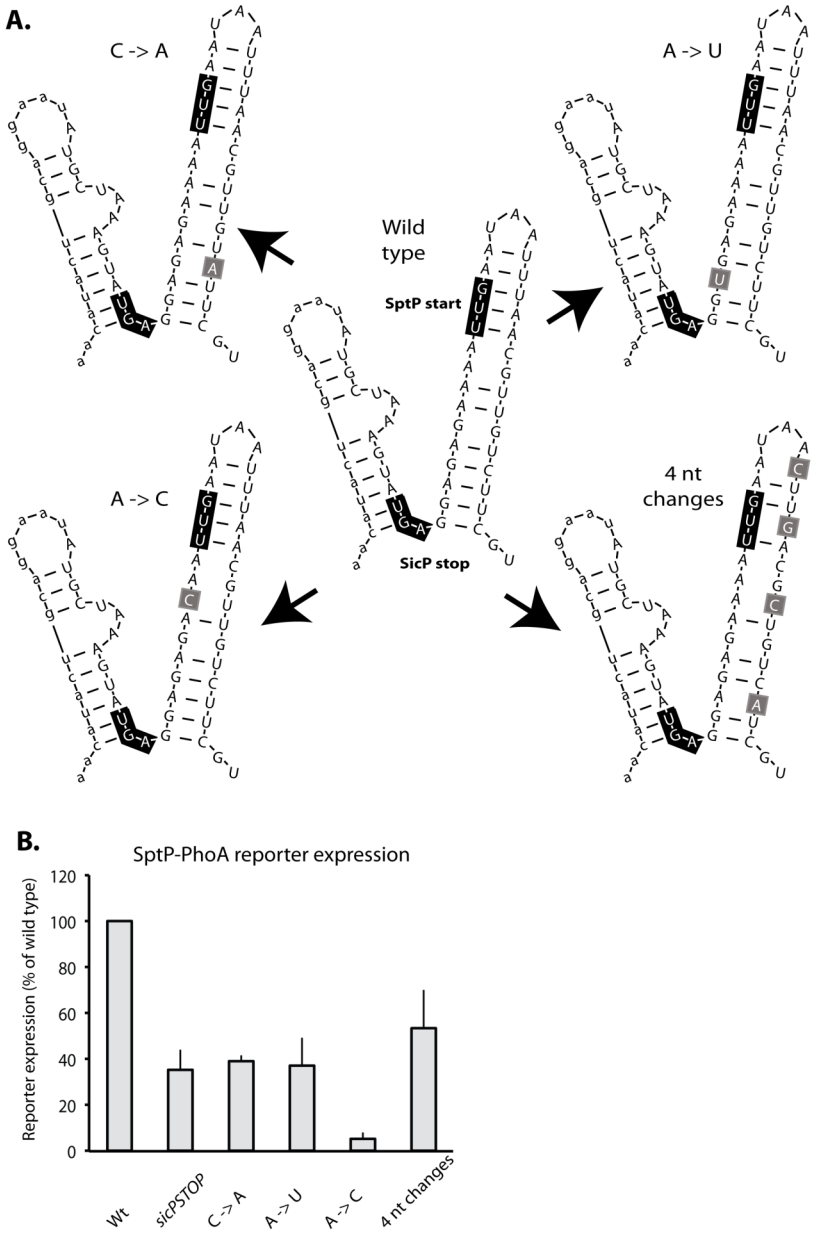

**Figure S1. Altering base-pairing in one of two predicted stem-loop structures does not result in an increase in *sptP-phoA* expression. (A)** A schematic of the predicted stem-loop structures (designated "Wild type") and schematics indicating the mutations that were introduced into the region (highlighted in gray). **(B)** Whole cell lysates of the various mutants (C->A, A->U, A->C, and 4 nt changes) were analyzed by western immunoblot with an anti-PhoA antibody. SptP-PhoA reporter signal was quantified and normalized to wild type. Results represent the average of at least three independent experiments. Student's *t* test was used to determine *p* values. For all mutants, $p<0.05$ with respect to Wt.

## Supplementary Methods:

### Plasmid construction

All plasmids were constructed using standard recombinant DNA techniques and are listed in Table S2. Restriction enzymes and T4 DNA ligase were from New England BioLabs (Ipswich, MA, USA). DNA digestion and ligation were performed according to the recommendations of the manufacturer. PCR, unless otherwise noted, was performed according to standard protocols using Taq DNA Polymerase (New England BioLabs, Ipswich, MA, USA) or KOD Hot Start DNA Polymerase (Novagen, Madison, WI, USA). Mutagenic primers used in strain construction are listed in Table S3.

To incorporate mutant *sicP* and *sptP* alleles into the bacterial chromosome, R6K replication origin-based suicide vectors were designed to include approximately 1kb of homologous sequence both upstream and downstream of the mutated operon. For mutations upstream of *sptP-phoA*, Primers 5-dst-SptP-SpeI (5-aaACTAGTTTATGACGACGGCAAGCTG) and 3-dst-SptP-SmaI (5-AAAAAAcccgggGGCGATGAATTACGCTCAC) were used to amplify ~1kb downstream of *sptP* for cloning into the SpeI and SmaI restriction sites of pSB0890. A subsequent cloning step introduced the mutant allele plus ~1kb upstream of *sicP* into the SpeI and NotI sites of pSB890. Mutagenic primers (Table S3) were designed to introduce the desired nucleotide change(s) through a process called overlap extension PCR (Ho, Hunt et al. 1989). The flanking primers used were 5-ups-SicP-NotI (5-aaaaaaaGCGGCCGCagagaggcggggctg) and 3-PhoA-SpeI (5-AAactagtTTATTTCAGCCCCAGAGCGG). For *lacZ* fusions, R6K vectors were construcuted similarly, except the flanking primers used were 5-ups-SicP-NotI (5-aaaaaaaGCGGCCGCagagaggcggggctg) and 3-lacZ-SpeI (5-GATCactagtTTATTTTTGACACCAGACCAACTGG).

To incorporate mutations upstream of full-length *sptP*, appropriate mutagenic primers (Table S3) were used to introduce the desired mutations, and flanking primers 5-ups-SicP-BamHI (5-aaGGATCCagagaggcggggctg) and 3-dst-SicP-NotI (5-aaaaaaaGCGGCCGCgggctacaccgacctg) were used for amplification. The resulting PCR product was digested and cloned into the BamHI and NotI sites of pSB0890.

To construct a triple-FLAG tagged allele of *sicP*, mutagenic primers 5-SicP-3xFLAG (5-GACGGTGATTATAAAGATCATGACATTGATTAC AAGGATGACGATGACAAG CAAGCACACCAGGATATTATCGC) and 3-SicP-3xFLAG (5-GTAATCAATGTCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCCAATTTACTT TCCTCTTGAATTATATCTTTTATAAG) were used to introduce the tag at codon 2 of *sicP*. Flanking primers 5-ups-SicP-BamHI (5-aaGGATCCagagaggcggggctg) and 3-dst-SicP-NotI (5-

aaaaaaaGCGGCCGCgggctacaccgacctg) were used for amplification. The resulting PCR product was digested and cloned into the BamHI and NotI sites of pSB0890.

To construct the R6K plasmid to introduce *sicPSTOP sptP-phoA sicP after* into the bacterial chromosome, triple-FLAG tagged *sicP* flanked by SpeI sites was amplified using primers 5-3xFSicP-SpeI (5-aaACTAGTgatataattcaagaggaaagtaaaTTGGAC) and 3-SicP-SpeI (5-AAactagtGCCTCATACTTTAGCATATTCCTGC).

To construct pSB4167 and pSB4168 (R6K suicide plasmids carrying *weak* or *strong RBS 3xF-sicP sptP-phoA,* respectively) two rounds of overlap extension PCR were required. The first replaced the native *sicP* RBS with either the "weak" or "strong" RBS using mutagenic primers described in Table S3 and flanking primers 5-ups-SicP-NotI (5-aaaaaaaGCGGCCGCagagaggcggggctg) and 3-SptP-HindIII (5-agtacAAGCTTAGTATTTTCCTTAGCAATATAAAGTCGGG). The second round of overlap extension PCR used mutagenic primers described in Table S3 to fuse triple-FLAG tagged *sicP* preceded by mutant RBSs to *sptP-phoA*. The flanking primers used were 5-ups-SicP-NotI (5-aaaaaaaGCGGCCGCagagaggcggggctg) and 3-PhoA-SpeI (5-AAactagtTTATTTCAGCCCCAGAGCGG).

To construct pSB4169 (an R6K suicide plasmid carrying *sicP-FS sptP sicP after*), first, three DNA fragments were created for sequential cloning into pSKII (Stratagene, Heidelberg, Germany). First, ~1kb of sequence immediately downstream of *sptP* was amplified using primers 5-SptP-insEcoRI (5-TTATGACGACGGCAAGCTGAtaaaGAATTCaacatagcttacttttagaactatc) and 3-dst-SptP-NotI (5-aaaaaaaGCGGCCGCTAGGCGATGAATTACGCTCAC) and cloned into the vector. Then, to create frameshifted *sicP* upstream of full-length *sptP*, mutagenic primers (Table S3) were used with the flanking primers 5-ups-SicP-XhoI (5-aaCTCGAGagagaggcggggctg) and 3-SptP-insEcoRI (5-GATAGTTCTAAAAGTAAGCTATGTTGAATTCTTTATCAGCTTGCCGTCGTCATAA). The resulting fragment was digested and cloned into the pSKII vector. Finally, wild type *sicP* was amplified using primers 5-SicP-EcoRI (5-aaGAATTCgcaatcttataaaagatataattcaagaggaaag) and 3-SicP-EcoRI (5- AAgaattcGCCTCATACTTTAGCATATTCCTG), digested, and cloned into the vector. Correct orientation of the open reading frame was verified by PCR, and the entire insert was amplified using primers 5-ups-SicP-NotI (5-aaaaaaaGCGGCCGCagagaggcggggctg) and 3-dst-SptP-SmaI (5-AAAAAAcccgggGGCGATGAATTACGCTCAC) and cloned into pSB0890.

Plasmid pSB2978, which expresses epitope-tagged SicP was constructed by excising a ~600 bp fragment encoding *SicP-m45* from plasmid pSB0680 (Fu and Galan) using restriction enzymes NheI and AccI. The fragment was cloned into pWSK129 (Wang and Kushner) digested with NheI and AccI.

Plasmids pSB4065, pSB4066, and pSB4067 were constructed by amplifying the desired allele using primers 5-SicP-NotI (5-aagaacaGCGGCCGCaatttgaagaagcaatcttataaaagatataattc) and 3-PhoA-KpnI (5-AAggtaccTTATTTCAGCCCCAGAGCGG). The resulting fragments were digested and cloned into pWSK29 (Wang and Kushner).

Inserts for plasmids pSB4154, pSB4155, and pSB4156 were constructed by overlap extension PCR using mutagenic primers to introduce the desired mutations upstream of *sptP-phoA* and flanking primers 5-SicP-NotI (5-aagaacaGCGGCCGCaatttgaagaagcaatcttataaaagatataattc) and 3-PhoA-KpnI (5-

AAggtaccTTATTTCAGCCCCAGAGCGG). The resulting fragments were digested and cloned into pWSK29 (Wang and Kushner).

**Table S1. Bacterial Strains**

| Bacterial strain | Relevant Genotype or Description | Reference or Source |
|---|---|---|
| SB300 | Mouse passaged SL1344 (*rpsL hisG*); termed "Wild type" for the purposes of this work | (Hoiseth and Stocker 1981) |
| SB1404 | Δ*sicP*Δ*sptP* (clean deletion that eliminates *sicP* and *sptP* as well as the RBS of *sicP*) | Sang Ho Lee |
| SB1653 | *sptP-phoA,* pSB3292 | This study; construction of the *sptP-phoA* fusion described in (Lee and Galan 2004). |
| SB1654 | *sicPSTOP sptP-phoA*, pSB3292 | This study |
| SB1656 | $\Delta sicP^{nt\ -14-372}$ *sptP-phoA,* pSB3292 | This study |
| SB1658 | $\Delta sicP^{nt\ -14-354}$ *sptP-phoA,* pSB3292 | This study |
| SB1660 | $\Delta sicP^{nt\ -14-329}$ *sptP-phoA,* pSB3292 | This study |
| SB1662 | $\Delta sicP^{nt\ -14-304}$ *sptP-phoA,* pSB3292 | This study |
| SB1664 | *sicPSTOP* | This study |
| SB2357 | $\Delta sicP^{nt\ -14-279}$ *sptP-phoA,* pSB3292 | This study |
| SB2359 | $\Delta sicP^{nt\ -14-191}$ *sptP-phoA,* pSB3292 | This study |
| SB2361 | $\Delta sicP^{nt\ -14-92}$ *sptP-phoA,* pSB3292 | This study |
| SB2363 | $sicP^{\Delta aa\ 2-118}$ *sptP-phoA* | This study |
| SB2365 | $sicP^{\Delta aa\ 2-110}$ *sptP-phoA* | This study |
| SB2367 | $sicP^{\Delta aa\ 2-102}$ *sptP-phoA* | This study |
| SB2369 | $sicP^{\Delta aa\ 2-93}$ *sptP-phoA* | This study |
| SB2371 | $sicP^{\Delta aa\ 2-64}$ *sptP-phoA* | This study |
| SB2373 | $sicP^{\Delta aa\ 2-31}$ *sptP-phoA* | This study |
| SB2374 | *sptP-lacZ* | This study |
| SB2375 | *sicPSTOP sptP-lacZ* | This study |
| SB2376 | Δ*sicP sptP-lacZ* | This study |
| SB2383 | *4mut sptP-phoA* | This study |
| SB2388 | *4mut sptP* | This study |
| SB2392 | *sicPSTOP A->U sptP-phoA,* pSB3292 | This study |
| SB2394 | *sicPSTOP A->U/U->A sptP-phoA,* pSB3292 | This study |
| SB2396 | *sicP-FS sptP-phoA,* pSB3292 | This study |
| SB2398 | *sicP-FS STOP sptP-phoA,* pSB3292 | This study |
| SB2401 | *sicPSTOP sptP-phoA sicP after*, pSB3292 | This study |
| SB2418 | *weak RBS 3xF-sicP sptP-phoA*, pSB3292 | This study |
| SB2420 | *strong RBS 3xF-sicP sptP-phoA*, pSB3292 | This study |
| SB2424 | *TTG->TTA sptP-phoA*, pSB3292 | This study |

| | | |
|---|---|---|
| SB2433 | *sicPSTOP C->A sptP-phoA,* pSB3292 | This study |
| SB2435 | *sicPSTOP A->U sptP-phoA,* pSB3292 | This study |
| SB2437 | *sicPSTOP A->C sptP-phoA,* pSB3292 | This study |
| SB2443 | *sicPSTOP 4 nt changes sptP-phoA,* pSB3292 | This study |
| SB2462 | *ATG->ATC sptP-phoA,* pSB3292 | This study |
| SB2465 | *sicPSTOP C->G sptP-phoA,* pSB3292 | This study |
| SB2571 | *sicPSTOP C->G/G->C sptP-phoA,* pSB3292 | This study |
| SB2574 | *sicPSTOP A->U sptP-phoA,* pSB3292 | This study |
| SB2575 | *sicPSTOP A->U/U->A sptP-phoA,* pSB3292 | This study |
| SB2576 | *sicPK121* sptP-phoA*, pSB3292 | This study |
| SB2577 | *sicPE118* sptP-phoA*, pSB3292 | This study |
| SB2578 | *sicPS112* sptP-phoA*, pSB3292 | This study |
| SB2579 | *sicPY104* sptP-phoA*, pSB3292 | This study |
| SB2580 | *sicP-FS sptP sicP after* | This study |

**Table S2. Plasmids**

| Plasmid | Relevant Genotype or Description | Reference or Source |
|---|---|---|
| pSB0890 | *oriR6K* from pGP704 (Miller and Mekalanos), *oriT* from plasmid RK2 (Schmidhauser and Helinski), tet resistance cassette from Tn10 (Jorgensen and Reznikoff), multiple cloning site from pSKII (Stratagene, Heidelberg, Germany), *sacB* gene from pKNG101 (Kaniga, Delor et al.) | Constructed by K. Kaniga |
| pSB2971 | R6K suicide plasmid carrying *sptP-phoA* for construction of SB1653 | This study |
| pSB2973 | R6K suicide plasmid carrying *sicPSTOP sptP-phoA* for construction of SB1654 | This study |
| pSB2974 | R6K suicide plasmid carrying Δ$sicP^{nt\ -14-372}$ *sptP-phoA* for construction of SB1656 | This study |
| pSB2975 | R6K suicide plasmid carrying Δ$sicP^{nt\ -14-354}$ *sptP-phoA* for construction of SB1658 | This study |
| pSB2976 | R6K suicide plasmid carrying Δ$sicP^{nt\ -14-329}$ *sptP-phoA* for construction of SB1660 | This study |
| pSB2977 | R6K suicide plasmid carrying Δ$sicP^{nt\ -14-304}$ *sptP-phoA* for construction of SB1662 | This study |
| pSB2978 | pWSK29 expressing SicP-m45 | This study |
| pSB2979 | R6K suicide plasmid carrying Δ$sicP^{nt\ -14-279}$ *sptP-phoA* for construction of SB2357 | This study |
| pSB2980 | R6K suicide plasmid carrying *sicPSTOP* for construction of SB1664 | This study |
| pSB2981 | R6K suicide plasmid carrying Δ$sicP^{nt\ -14-191}$ *sptP-phoA* for construction of SB2359 | This study |
| pSB2982 | R6K suicide plasmid carrying Δ$sicP^{nt\ -14-92}$ *sptP-phoA* for construction of SB2361 | This study |
| pSB2983 | R6K suicide plasmid carrying $sicP^{\Delta aa\ 2-118}$ *sptP-phoA* for construction of SB2363 | This study |
| pSB2984 | R6K suicide plasmid carrying $sicP^{\Delta aa\ 2-110}$ *sptP-phoA* for construction of SB2365 | This study |
| pSB2985 | R6K suicide plasmid carrying $sicP^{\Delta aa\ 2-102}$ *sptP-phoA* for construction of SB2367 | This study |
| pSB2986 | R6K suicide plasmid carrying $sicP^{\Delta aa\ 2-93}$ *sptP-phoA* for construction of SB2369 | This study |
| pSB2988 | R6K suicide plasmid carrying $sicP^{\Delta aa\ 2-64}$ *sptP-phoA* for construction of SB2371 | This study |
| pSB2989 | R6K suicide plasmid carrying $sicP^{\Delta aa\ 2-31}$ *sptP-phoA* for construction of SB2373 | This study |
| pSB2990 | R6K suicide plasmid carrying *sptP-lacZ* for construction of SB2374 | This study |
| pSB3061 | R6K suicide plasmid carrying Δ*sicP sptP-lacZ* for construction of SB2376 | This study |

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| pSB3062 | R6K suicide plasmid carrying *sicPSTOP sptP-lacZ* for construction of SB2375 | This study |
| pSB3064 | R6K suicide plasmid carrying *4mut sptP-phoA* for construction of SB2383 | This study |
| pSB3068 | R6K suicide plasmid carrying *4mut sptP* for construction of SB2388 | This study |
| pSB3076 | R6K suicide plasmid carrying *sicPSTOP A->U sptP-phoA* for construction of SB2392 | This study |
| pSB3077 | R6K suicide plasmid carrying *sicPSTOP A->U/U->A sptP-phoA* for construction of SB2394 | This study |
| pSB3292 | *hilA* in pBAD24 | (Lara-Tejero, Kato et al. 2011) |
| pSB4051 | R6K suicide plasmid carrying *sicP-FS sptP-phoA* for construction of SB2396 | This study |
| pSB4052 | R6K suicide plasmid carrying *sicP-FS STOP sptP-phoA* for construction of SB2398 | This study |
| pSB4054 | R6K suicide plasmid carrying *TTG->TTA sptP-phoA* for construction of SB2424 | This study |
| pSB4057 | R6K suicide plasmid carrying *sptP-phoA sicP after* for construction of SB2401 | This study |
| pSB4058 | R6K suicide plasmid carrying *sicPSTOP C->A sptP-phoA* for construction of SB2433 | This study |
| pSB4059 | R6K suicide plasmid carrying *sicPSTOP A->U sptP-phoA* for construction of SB2435 | This study |
| pSB4060 | R6K suicide plasmid carrying *sicPSTOP A->C sptP-phoA* for construction of SB2437 | This study |
| pSB4065 | pWSK29 expressing *sicPsptP-phoA* | This study |
| pSB4066 | pWSK29 expressing *sicPSTOPsptP-phoA* | This study |
| pSB4067 | pWSK29 expressing Δ*sicPsptP-phoA* | This study |
| pSB4069 | R6K suicide plasmid carrying *sicPSTOP 4 nt changes sptP-phoA* for construction of SB2443 | This study |
| pSB4071 | R6K suicide plasmid carrying *sicPK121* sptP-phoA* for construction of SB2576 | This study |
| pSB4072 | R6K suicide plasmid carrying *sicPE118* sptP-phoA* for construction of SB2577 | This study |
| pSB4073 | R6K suicide plasmid carrying *sicPS112* sptP-phoA* for construction of SB2578 | This study |
| pSB4074 | R6K suicide plasmid carrying *sicPY104** for construction of SB2579 | This study |
| pSB4075 | R6K suicide plasmid carrying *ATG->ATC sptP-phoA* for construction of SB2462 | This study |
| pSB4078 | R6K suicide plasmid carrying *sicPSTOP C->G sptP-phoA* for construction of SB2465 | This study |
| pSB4079 | R6K suicide plasmid carrying *sicPSTOP C->G/G->C sptP-phoA* for construction of SB2571 | This study |
| pSB4152 | R6K suicide plasmid carrying *sicPSTOP A->U sptP-phoA* | This study |

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|---|---|---|
| | for construction of SB2574 | |
| pSB4153 | R6K suicide plasmid carrying *sicPSTOP A->U/U->A sptP-phoA* for construction of SB2575 | This study |
| pSB4154 | pWSK29 expressing *sicP extended stem sptP-phoA* | This study |
| pSB4155 | pWSK29 expressing *sicPSTOP extended stem sptP-phoA* | This study |
| pSB4167 | R6K suicide plasmid carrying *weak RBS 3xF-sicP sptP-phoA* for construction of SB2418 | This study |
| pSB4168 | R6K suicide plasmid carrying *strong RBS 3xF-sicP sptP-phoA* for construction of SB2420 | This study |
| pSB4169 | R6K suicide plasmid carrying *sicP-FS sptP sicP after* for construction of SB2580 | This study |

**Table S3. Mutagenic Primers**

| Used in the construction of strain: | Primer name | Primer sequence | Description |
|---|---|---|---|
| SB1656 | 5-71-stitch | aatttgaagaagcaatcttataa aagatataattc caggaatATGCTAAAG TATGAGGAGAG | Creates a deletion in *sicP* extending from nt -14 through 372 |
| | 3-71-stitch | CTCTCCTCATACTT TAGCATATTCCTGG AATTATATCTTTTA TAAGATTGCTTCTT CAAATT | |
| SB1658 | 5-72-stitch | aatttgaagaagcaatcttataa aagatataattc gcgctcaaaaacatactgcag | Creates a deletion in *sicP* extending from nt -14 through 354 |
| | 3-72-stitch | CTGCAGTATGTTTT TGAGCGCGAATTAT ATCTTTTATAAGAT TGCTTCTTCAAATT | |
| SB1660 | 5-73-stitch | aatttgaagaagcaatcttataa aagatataattc tgagtcatttgtgaatcagcagg | Creates a deletion in *sicP* extending from nt -14 through 329 |
| | 3-73-stitch | CCTGCTGATTCACA AATGACTCAGAATT ATATCTTTTATAAG ATTGCTTCTTCAAA TT | |
| SB1662 | 5-74-stitch | aatttgaagaagcaatcttataa aagatataattc atacttaccatattatatcgcagc ttgag | Creates a deletion in *sicP* extending from nt -14 through 304 |
| | 3-74-stitch | CTCAAGCTGCGATA TAATATGGTAAGTA TGAATTATATCTTT TATAAGATTGCTTC TTCAAATT | |
| SB2357 | 5-75-stitch | aatttgaagaagcaatcttataa aagatataattc atacatgcaattaccgatctgac aaa | Creates a deletion in *sicP* extending from nt -14 through 279 |
| | 3-75-stitch | TTTGTCAGATCGGT AATTGCATGTATGA ATTATATCTTTTAT AAGATTGCTTCTTC AAATT | |

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|---|---|---|---|
| SB2359 | 5-76-stitch | aatttgaagaagcaatcttataa aagatataattc gcggcagattatggtgattaatg g | Creates a deletion in *sicP* extending from nt -14 through 191 |
| | 3-76-stitch | CCATTAATCACCAT AATCTGCCGCGAAT TATATCTTTTATAA GATTGCTTCTTCAA ATT | |
| SB2361 | 5-77-stitch | aatttgaagaagcaatcttataa aagatataattc cgatagcgatatttttacgtctatt gaagc | Creates a deletion in *sicP* extending from nt -14 through 92 |
| | 3-77-stitch | GCTTCAATAGACGT AAAAATATCGCTAT CGGAATTATATCTT TTATAAGATTGCTT CTTCAAATT | |
| SB2363 | 5-72-Tlc-stitch | cttataaaagatataattcaaga ggaaagtaaaTTG gcgctcaaaaacatactgcag | Creates an in-frame deletion in *sicP* that eliminates codons 2 through 118 |
| | 3-72-Tlc-stitch | CTGCAGTATGTTTT TGAGCGCCAATTTA CTTTCCTCTTGAAT TATATCTTTTATAA G | |
| SB2365 | 5-73-Tlc-stitch | cttataaaagatataattcaaga ggaaagtaaaTTG ct tgagtcatttgtgaatcagcagg | Creates an in-frame deletion in *sicP* that eliminates codons 2 through 110 |
| | 3-73-Tlc-stitch | CCTGCTGATTCACA AATGACTCAAGCA ATTTACTTTCCTCT TGAATTATATCTTT TATAAG | |
| SB2367 | 5-74-Tlc-stitch | cttataaaagatataattcaaga ggaaagtaaaTTG a atacttaccatattatatcgcagc ttgag | Creates an in-frame deletion in *sicP* that eliminates codons 2 through 102 |
| | 3-74-Tlc-stitch | CTCAAGCTGCGATA TAATATGGTAAGTA TTCAATTTACTTTC CTCTTGAATTATAT CTTTTATAAG | |
| SB2369 | 5-75-Tlc-stitch | cttataaaagatataattcaaga ggaaagtaaaTTG atacatgcaattaccgatctgac | Creates an in-frame deletion in *sicP* that eliminates codons 2 |

| | | | |
|---|---|---|---|
| | | aaa | through 93 |
| | 3-75-Tlc-stitch | TTTGTCAGATCGGTAATTGCATGTATCAATTTACTTTCCTCTTGAATTATATCTTTTATAAG | |
| SB2371 | 5-76-Tlc-stitch | cttataaaagatataattcaagaggaaagtaaaTTG tggcggcagattatggtgattaatgg | Creates an in-frame deletion in *sicP* that eliminates codons 2 through 64 |
| | 3-76-Tlc-stitch | CCATTAATCACCATAATCTGCCGCCACAATTTACTTTCCTCTTGAATTATATCTTTTATAAG | |
| SB2373 | 5-77-Tlc-stitch | cttataaaagatataattcaagaggaaagtaaaTTG ctcgatagcgatatttttacgtctattgaagc | Creates an in-frame deletion in *sicP* that eliminates codons 2 through 31 |
| | 3-77-Tlc-stitch | GCTTCAATAGACGTAAAAATATCGCTATCGAGCAATTTACTTTCCTCTTGAATTATATCTTTTATAAG | |
| SB2374, 75, 76 | 5-lacZ-join | CGACTTTATATTGCTAAGGAAAATACTAAGCTT ACCATGATTACGGATTCACTGGC | Creates a translational fusion of *sptP* to reporter gene *lacZ;* joins the first 27 codons of *sptP* to codon 2 of *lacZ.* These primers also insert a HinDIII site (AAGCTT) at the juncture between *sptP* and *lacZ* |
| | 3-lacZ-join | GCCAGTGAATCCGTAATCATGGTAAGCTTAGTATTTTCCTTAGCAATATAAAGTCG | |
| SB2383, 88 | 5-SicP-4mut-SptP | tcagcaggaagcgctcaaaaaTatTTtAcaggaatATGCTAAAGTATGAGGAGAG | Introduces 4 nt changes into *sicP* at nt 366: C -> T, at nt 369: A -> T, at nt 370: C -> T, at nt 372: G -> A |
| | 3-SicP-4mut-SptP | CTCTCCTCATACTTTAGCATATTCCTGTAAAATATTTTTGAGCGCTTCCTGCTGA | |
| SB2392 | 5-1mut2 | caggaagcgctcaaaaacatTCTGCAGGAATATGCTAAAGTATGAGG | Changes nt 369 of *sicP* from A -> T |
| | 3-1mut2 | CCTCATACTTTAGC | |

| | | | |
|---|---|---|---|
| | | ATATTCCTGCAGAATGTTTTTGAGCGCTTCCTG | |
| SB2394 | 5-1mut2res | aaacatTctgcaggaatATGCTAAAGaATGAGGAGAGAAAATTGAATAATTTAACGTTG | Changes nt 369 of *sicP* from A -> T and nt 389 from T -> A |
| | 3-1mut2res | CAACGTTAAATTATTCAATTTTCTCTCCTCATTCTTTAGCATATTCCTGCAGAATGTTT | |
| SB2396 | 5-SicPFS1 | ATTGCAAGCACATTCCAGGATATTATC | Inserts "TT" after nt 11 of *sicP* in order to alter the reading frame of the coding sequence |
| | 3-SicPFS1 | GATAATATCCTGGAATGTGCTTGCAAT | |
| | 5-SicPFS2 | GATGATATCTGGTTATTGtACGGTATcATTATACCGTTATCGCCTG | Changes nt 154 of *sicP* from A -> T and nt 153 from G -> C in order to eliminate stop codons from the +2 reading frame |
| | 3-SicPFS2 | GCGATAACGGTATAATGATACCGTACAATAACCAGATATC | |
| | 5-SicPFS3 | CAGATTATGGTGGATTAATGGTGAAC | Inserts "G" after nt 206 of *sicP* in order to alter the reading frame of the coding sequence |
| | 3-SicPFS3 | GTTCACCATTAATCCACCATAATCTG | |
| SB2398 | 5-FS-stop | ATTGCAAGCACATCCAGGATATTATC | Inserts "T" after nt 11 of *sicP* in order to alter the reading frame of the coding sequence |
| | 3-FS-stop | GATAATATCCTGGATGTGCTTGCAAT | |
| SB2418 | 5-CheR-SicP | AGCTCttgagaaggcgctA TGGACTACAAAGACCATGACGG | Replaces nt -15 through +4 of triple-FLAG tagged *sicP* with $RBS^{CheR}$ from (Lovdok, Bentele et al. 2009) |
| | 3-CheR-SicP | TagcgccttctcaaGAGCTgaattatatcttttataagattgcttcttcaaatt | |
| SB2420 | 5-CheRup-SicP | AGCTCgataggaaaggcgctA TGGACTACAAAGACCATGACGG | Replaces nt -15 through +4 of triple-FLAG tagged *sicP* with $RBS^{CheRîî}$ from (Lovdok, Bentele et al. 2009) |
| | 3-CheRup-SicP | TagcgcctttcctatcGAGCTgaattatatcttttataagattgcttcttcaaatt | |

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| SB2418, 20 | 5-SptPPhoAmid | CCCGACTTTATATTGCTAAGGAAAATACTAAGCTTCGGACACCAGAAATG | Creates a translational fusion of *sptP* to reporter gene *phoA;* joins the first 27 codons of *sptP* to codon 45 of *phoA*. These primers also insert a HinDIII site (AAGCTT) at the juncture between *sptP* and *phoA* |
| | 3-SptPPhoAmid | CATTTCTGGTGTCCGAAGCTTAGTATTTTCCTTAGCAATATAAAGTCGGG | |
| SB2424 | 5-SptP-TTG-X | GCTAAAGTATGAGGAGAGAAAAttaAATAATTTAACGTTGTCTTCG | Changes nt 3 of *sptP* from G -> A |
| | 3-SptP-TTG-X | CGAAGACAACGTTAAATTATTTAATTTTCTCTCCTCATACTTTAGC | |
| SB2433 | 5-2L1mut | GGAGAGAAAATTGAATAATTTAACGTTGTaTTCGTTTTCAAAAGTTGGTG | Changes nt 20 of *sptP* from C -> A |
| | 3-2L1mut | CACCAACTTTTGAAAACGAATACAACGTTAAATTATTCAATTTTCTCTCC | |
| SB2435 | 5-RBSmut | GCTAAAGTATGAGGtGAGAAAATTGAATAATTTAACG | Changes nt -8 of *sptP* from A -> T |
| | 3-RBSmut | CGTTAAATTATTCAATTTTCTCACCTCATACTTTAGC | |
| SB2437 | 5-2L2mut | GTATGAGGAGAGAcAATTGAATAATTTAACG | Changes nt -3 of *sptP* from A -> C |
| | 3-2L2mut | CGTTAAATTATTCAATTGTCTCTCCTCATAC | |
| SB2443 | 5-2SL4mut | GTATGAGGAGAGAAAATTGAATAAcTTgACGcTGTCaTCGTTTTCAAAAGTTGGTG | Introduces 4 nt changes into *sptP* at nt 9: T -> C, at nt 12: A -> G, at nt 16: T -> C, at nt 21: T -> A |
| | 3-2SL4mut | CACCAACTTTTGAAAACGATGACAGCGTCAAGTTATTCAATTTTCTCTCCTCATAC | |

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| SB2462 | 5-stem1 | caaaaacatactgcaggaatATcCTAAAGTATGAGGAGAG | Changes nt 382 of *sicP* from G -> C |
| | 3-stem1 | CTCTCCTCATACTTTAGGATATTCCTGCAGTATGTTTTTG | |
| SB2465 | 5-stem2 | gaagcgctcaaaaacataGtgcaggaatATGCTAAAGTATG | Changes nt 370 of *sicP* from C -> G |
| | 3-stem2 | CATACTTTAGCATATTCCTGCACTATGTTTTTGAGCGCTTC | |
| SB2571 | 5-stem2R | cataGtgcaggaatATGCTAAAcTATGAGGAGAGAAAATTG | Changes nt 370 of *sicP* from C -> G and nt 388 from G -> C |
| | 3-stem2R | CAATTTTCTCTCCTCATAGTTTAGCATATTCCTGCACTATG | |
| SB2574 | 5-stem4 | caggaagcgctcaaaaacTtactgcaggaatATGCTAAAG | Changes nt 367 of *sicP* from A -> T |
| | 3-stem4 | CTTTAGCATATTCCTGCAGTAAGTTTTTGAGCGCTTCCTG | |
| SB2575 | 5-stem4R | cTtactgcaggaatATGCTAAAGTAaGAGGAGAGAAAATTG | Changes nt 367 of *sicP* from A -> T and nt 391 from T -> A |
| | 3-stem4R | CAATTTTCTCTCCTCTTACTTTAGCATATTCCTGCAGTAAG | |
| SB2576 | 5-Pstop5 | GTGAATCAGCAGgAAGCGCTCtAAAACATACTGCAG | Changes nt 361 of *sicP* from A -> T in order to introduce a premature stop codon |
| | 3-Pstop5 | CTGCAGTATGTTTTAGAGCGCTTCCTGCTGATTCAC | |
| SB2577 | 5-Pstop15 | GTcATTTGTGAATCAGCAGtAAGCGCTCAAAAACATACTG | Changes nt 352 of *sicP* from A -> T in order to introduce a premature stop codon |
| | 3-Pstop15 | CAGTATGTTTTTGAGCGCTTACTGCTGATTCACAAATGAC | |
| SB2578 | 5-Pstop30 | CATATTATATCGCAGCTTGAGTgATTTGTGAATCAGCAGG | Changes nt 335 of *sicP* from A -> T in order to introduce a premature stop codon |
| | 3-Pstop30 | CCTGCTGATTCACA | |

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| | | AATCACTCAAGCTG CGATATAATATG | |
| SB2579 | 5-Pstop50 | CCGATCTGACAAAT ACTTAgCATATTAT ATCGCAGCTTGAG | Changes nt 312 of *sicP* from A -> T in order to introduce a premature stop codon |
| | 3-Pstop50 | CTCAAGCTGCGATA TAATATGCTAAGTA TTTGTCAGATCGG | |
| pSB4154, 55 | 5-extendSL | gcaggaagcgctcaaCCTc atactgcaggaatATG | Replaces nt 363-365 of *sicP* with "CCT" in order to extend base pairing in the putative regulatory stem |
| | 3-extendSL | CATATTCCTGCAGT ATGAGGTTGAGCG CTTCCTGC | |

**Supplementary References**


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