

Supplementary Material

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-AAAAAAaccgggGGCGATGAATTACGCTCAC) 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-aaaaaaaGCGGCCGCagagaggcgggctg) and 3-PhoA-SpeI (5-AAactagtTTATTTTCAGCCCCAGAGCGG). For *lacZ* fusions, R6K vectors were constructed similarly, except the flanking primers used were 5-ups-SicP-NotI (5-aaaaaaaGCGGCCGCagagaggcgggctg) and 3-lacZ-SpeI (5-GATCactagtTTATTTTGGACACCAACTGG).

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-aaGGATCCagagaggcgggctg) and 3-dst-SicP-NotI (5-aaaaaaaGCGGCCGCgggctacaccgactg) 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-aaGGATCCagagaggcgggctg) and 3-dst-SicP-NotI (5-aaaaaaaGCGGCCGCgggctacaccgactg) 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-aaaaaaaGCGGCCGCagagagggcggggctg) 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-aaaaaaaGCGGCCGCagagagggcggggctg) and 3-PhoA-SpeI (5-AAactagtTTATTTTCAGCCCCAGAGCGG).

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-aaCTCGAGagagagggcggggctg) 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-aaaaaaaGCGGCCGCagagagggcggggctg) 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-aagaacaGCGGCCGCaattgaagaagcaatcttataaaagatataattc) and 3-PhoA-KpnI (5-AAggtaccTTATTTTCAGCCCCAGAGCGG). 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-aagaacaGCGGCCGCaattgaagaagcaatcttataaaagatataattc) and 3-PhoA-KpnI (5-AAggtaccTTATTTTCAGCCCCAGAGCGG). 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 (<i>rpsL hisG</i>); termed “Wild type” for the purposes of this work	(Hoiseth and Stocker 1981)
SB1404	$\Delta sicP \Delta sptP$ (clean deletion that eliminates <i>sicP</i> and <i>sptP</i> as well as the RBS of <i>sicP</i>)	Sang Ho Lee
SB1653	<i>sptP-phoA</i> , pSB3292	This study; construction of the <i>sptP-phoA</i> fusion described in (Lee and Galan 2004).
SB1654	<i>sicPSTOP sptP-phoA</i> , 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	<i>sicPSTOP</i>	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	<i>sicP^{Δaa 2-118} sptP-phoA</i>	This study
SB2365	<i>sicP^{Δaa 2-110} sptP-phoA</i>	This study
SB2367	<i>sicP^{Δaa 2-102} sptP-phoA</i>	This study
SB2369	<i>sicP^{Δaa 2-93} sptP-phoA</i>	This study
SB2371	<i>sicP^{Δaa 2-64} sptP-phoA</i>	This study
SB2373	<i>sicP^{Δaa 2-31} sptP-phoA</i>	This study
SB2374	<i>sptP-lacZ</i>	This study
SB2375	<i>sicPSTOP sptP-lacZ</i>	This study
SB2376	$\Delta sicP sptP-lacZ$	This study
SB2383	<i>4mut sptP-phoA</i>	This study
SB2388	<i>4mut sptP</i>	This study
SB2392	<i>sicPSTOP A->U sptP-phoA</i> , pSB3292	This study
SB2394	<i>sicPSTOP A->U/U->A sptP-phoA</i> , pSB3292	This study
SB2396	<i>sicP-FS sptP-phoA</i> , pSB3292	This study
SB2398	<i>sicP-FS STOP sptP-phoA</i> , pSB3292	This study
SB2401	<i>sicPSTOP sptP-phoA sicP after</i> , pSB3292	This study
SB2418	<i>weak RBS 3xF-sicP sptP-phoA</i> , pSB3292	This study
SB2420	<i>strong RBS 3xF-sicP sptP-phoA</i> , pSB3292	This study
SB2424	<i>TTG->TTA sptP-phoA</i> , pSB3292	This study

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

Table S2. Plasmids

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

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

	for construction of SB2574	
pSB4153	R6K suicide plasmid carrying <i>sicPSTOP A->U/U->A sptP-phoA</i> for construction of SB2575	This study
pSB4154	pWSK29 expressing <i>sicP extended stem sptP-phoA</i>	This study
pSB4155	pWSK29 expressing <i>sicPSTOP extended stem sptP-phoA</i>	This study
pSB4167	R6K suicide plasmid carrying <i>weak RBS 3xF-sicP sptP-phoA</i> for construction of SB2418	This study
pSB4168	R6K suicide plasmid carrying <i>strong RBS 3xF-sicP sptP-phoA</i> for construction of SB2420	This study
pSB4169	R6K suicide plasmid carrying <i>sicP-FS sptP sicP after</i> 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	aattgaagaagcaatctataa aagatataattc caggaatATGCTAAAG TATGAGGAGAG	Creates a deletion in <i>sicP</i> extending from nt -14 through 372
	3-71-stitch	CTCTCCTCATACTT TAGCATATTCCTGG AATTATATCTTTTA TAAGATTGCTTCTT CAAATT	
SB1658	5-72-stitch	aattgaagaagcaatctataa aagatataattc gcgctcaaaaacatactgcag	Creates a deletion in <i>sicP</i> extending from nt -14 through 354
	3-72-stitch	CTGCAGTATGTTTT TGAGCGCGAATTAT ATCTTTTATAAGAT TGCTTCTTCAAATT	
SB1660	5-73-stitch	aattgaagaagcaatctataa aagatataattc tgagtcatttgaatcagcagg	Creates a deletion in <i>sicP</i> extending from nt -14 through 329
	3-73-stitch	CCTGCTGATTCACA AATGACTCAGAATT ATATCTTTTATAAG ATTGCTTCTTCAA TT	
SB1662	5-74-stitch	aattgaagaagcaatctataa aagatataattc atactaccatattatcgcagc ttgag	Creates a deletion in <i>sicP</i> extending from nt -14 through 304
	3-74-stitch	CTCAAGCTGCGATA TAATATGGTAAGTA TGAATTATATCTTT TATAAGATTGCTTC TTCAAATT	
SB2357	5-75-stitch	aattgaagaagcaatctataa aagatataattc atacatgcaattaccgatctgac aaa	Creates a deletion in <i>sicP</i> extending from nt -14 through 279
	3-75-stitch	TTTGTCAGATCGGT AATTGCATGTATGA ATTATATCTTTTAT AAGATTGCTTCTTC AAATT	

SB2359	5-76-stitch	aatttgaagaagcaatcttataa aagatataattc gcggcagattatggtgattaatg g	Creates a deletion in <i>sicP</i> extending from nt -14 through 191
	3-76-stitch	CCATTAATCACCAT AATCTGCCGCGAAT TATATCTTTTATAA GATTGCTTCTTCAA ATT	
SB2361	5-77-stitch	aatttgaagaagcaatcttataa aagatataattc cgatagcgatattttacgtctatt gaagc	Creates a deletion in <i>sicP</i> 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 <i>sicP</i> that eliminates codons 2 through 118
	3-72-Tlc-stitch	CTGCAGTATGTTTT TGAGCGCCAATTTA CTTTCCTCTTGAAT TATATCTTTTATAA G	
SB2365	5-73-Tlc-stitch	cttataaaagatataattcaaga ggaaagtaaaTTG ct tgagtcatttgaatcagcagg	Creates an in-frame deletion in <i>sicP</i> that eliminates codons 2 through 110
	3-73-Tlc-stitch	CCTGCTGATTCACA AATGACTCAAGCA ATTTACTTTCCTCT TGAATTATATCTTT TATAAG	
SB2367	5-74-Tlc-stitch	cttataaaagatataattcaaga ggaaagtaaaTTG a atacttaccatattatcgcagc ttgag	Creates an in-frame deletion in <i>sicP</i> 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 <i>sicP</i> that eliminates codons 2

		aaa	through 93
	3-75-Tlc-stitch	TTTGTCAGATCGGT AATTGCATGTATCA ATTTACTTTCCTCT TGAATTATATCTTT TATAAG	
SB2371	5-76-Tlc-stitch	cttataaaagatataattcaaga ggaaagtaaaTTG tg gcggcagattatggtgattaatg g	Creates an in-frame deletion in <i>sicP</i> that eliminates codons 2 through 64
	3-76-Tlc-stitch	CCATTAATCACCAT AATCTGCCGCCACA ATTTACTTTCCTCT TGAATTATATCTTT TATAAG	
SB2373	5-77-Tlc-stitch	cttataaaagatataattcaaga ggaaagtaaaTTG ct cgatagcgatattttacgtctatt gaagc	Creates an in-frame deletion in <i>sicP</i> that eliminates codons 2 through 31
	3-77-Tlc-stitch	GCTTCAATAGACGT AAAAATATCGCTAT CGAGCAATTTACTT TCCTCTTGAATTAT ATCTTTTATAAG	
SB2374, 75, 76	5-lacZ-join	CGACTTTATATTGC TAAGGAAAATACT AAGCTT ACCATGATTACGG ATTCACTGGC	Creates a translational fusion of <i>sptP</i> to reporter gene <i>lacZ</i> ; joins the first 27 codons of <i>sptP</i> to codon 2 of <i>lacZ</i> . These primers also insert a <i>HinDIII</i> site (AAGCTT) at the juncture between <i>sptP</i> and <i>lacZ</i>
	3-lacZ-join	GCCAGTGAATCCGT AATCATGGTAAGCT TAGTATTTTCCTTA GCAATATAAAGTC G	
SB2383, 88	5-SicP-4mut-SptP	tcagcaggaagcgcTcaaaaa TatTTtA caggaatATGCTAAAG TATGAGGAGAG	Introduces 4 nt changes into <i>sicP</i> at nt 366: C -> T, at nt 369: A -> T, at nt 370: C -> T, at nt 372: G -> A
	3-SicP-4mut-SptP	CTCTCCTCATACTT TAGCATATTCCTGT AAAATATTTTGGAG CGCTTCTGCTGA	
SB2392	5-1mut2	caggaagcgcTcaaaaacatT CTGCAGGAATATG CTAAAGTATGAGG	Changes nt 369 of <i>sicP</i> from A -> T
	3-1mut2	CCTCATACTTTAGC	

		ATATTCCTGCAGAA TGTTTTTGAGCGCT TCCTG	
SB2394	5-1mut2res	aaacatTctgcaggaatATG CTAAAGaATGAGGA GAGAAAATTGAAT AATTTAACGTTG	Changes nt 369 of <i>sicP</i> from A -> T and nt 389 from T -> A
	3-1mut2res	CAACGTTAAATTAT TCAATTTTCTCTCC TCATTCTTTAGCAT ATTCCTGCAGAATG TTT	
SB2396	5-SicPFS1	ATTGCAAGCACATT CCAGGATATTATC	Inserts “TT” after nt 11 of <i>sicP</i> in order to alter the reading frame of the coding sequence
	3-SicPFS1	GATAATATCCTGGA ATGTGCTTGCAAT	
	5-SicPFS2	GATGATATCTGGTT ATTGtACGGTATcAT TATACCGTTATCGC CTG	Changes nt 154 of <i>sicP</i> from A -> T and nt 153 from G -> C in order to eliminate stop codons from the +2 reading frame
	3-SicPFS2	GCGATAACGGTAT AATGATACCGTAC AATAACCAGATAT C	
	5-SicPFS3	CAGATTATGGTGG ATTAATGGTGAAC	Inserts “G” after nt 206 of <i>sicP</i> in order to alter the reading frame of the coding sequence
	3-SicPFS3	G TTCACCATTAATC CACCATAATCTG	
SB2398	5-FS-stop	ATTGCAAGCACATC CAGGATATTATC	Inserts “T” after nt 11 of <i>sicP</i> in order to alter the reading frame of the coding sequence
	3-FS-stop	GATAATATCCTGGA TGTGCTTGCAAT	
SB2418	5-CheR-SicP	AGCTCttgagaaggcgt A TGGACTACAAAGA CCATGACGG	Replaces nt -15 through +4 of triple-FLAG tagged <i>sicP</i> with RBS ^{CheR} from (Lovdok, Bentele et al. 2009)
	3-CheR-SicP	TagcgccttctcaaGAGCT gaattatatctttataagattgctt ctcaaatt	
SB2420	5-CheRup-SicP	AGCTCgataggaaaggcg ctA TGGACTACAAAGA CCATGACGG	Replaces nt -15 through +4 of triple-FLAG tagged <i>sicP</i> with RBS ^{CheRii} from (Lovdok, Bentele et al. 2009)
	3-CheRup-SicP	TagcgcctttctatcGAGC Tgaattatatctttataagattg cttctcaaatt	

SB2418, 20	5-SptPPhoAmid	CCCGACTTTATATTGCT AAGGAAAATACTAAGC TTCGGACACCAGAAAT G	Creates a translational fusion of <i>sptP</i> to reporter gene <i>phoA</i> ; joins the first 27 codons of <i>sptP</i> to codon 45 of <i>phoA</i> . These primers also insert a <i>HinDIII</i> site (AAGCTT) at the juncture between <i>sptP</i> and <i>phoA</i>
	3-SptPPhoAmid	CATTTCTGGTGTCCGAA GCTTAGTATTTTCCTTA GCAATATAAAGTCGGG	
SB2424	5-SptP-TTG-X	GCTAAAGTATGAG GAGAGAAAAttaAAT AATTTAACGTTGTC TTCG	Changes nt 3 of <i>sptP</i> from G -> A
	3-SptP-TTG-X	CGAAGACAACGTT AAATTATTTAATTT TCTCTCCTCATACT TTAGC	
SB2433	5-2L1mut	GGAGAGAAAATTG AATAATTTAACGTT GTaTTCGTTTTCAA AAGTTGGTG	Changes nt 20 of <i>sptP</i> from C -> A
	3-2L1mut	CACCAACTTTTGAA AACGAATACAACG TTAAATTATTCAAT TTTCTCTCC	
SB2435	5-RBSmut	GCTAAAGTATGAG GtGAGAAAATTGAA TAATTTAACG	Changes nt -8 of <i>sptP</i> from A -> T
	3-RBSmut	CGTTAAATTATTCA ATTTTCTCACCTCA TACTTTAGC	
SB2437	5-2L2mut	GTATGAGGAGAGA cAATTGAATAATTT AACG	Changes nt -3 of <i>sptP</i> from A -> C
	3-2L2mut	CGTTAAATTATTCA ATTGTCTCTCCTCA TAC	
SB2443	5-2SL4mut	GTATGAGGAGAGA AAATTGAATAA cTTgACGcTGTCa TCGTTTTCAAAGT TGGTG	Introduces 4 nt changes into <i>sptP</i> at nt 9: T -> C, at nt 12: A -> G, at nt 16: T -> C, at nt 21: T -> A
	3-2SL4mut	CACCAACTTTTGAA AACGATGACAGCG TCAAGTTATTCAAT TTTCTCTCCTCATA C	

SB2462	5-stem1	caaaaacatactgcaggaatA TcCTAAAGTATGAG GAGAG	Changes nt 382 of <i>sicP</i> from G -> C
	3-stem1	CTCTCCTCATACTT TAGGATATTCCTGC AGTATGTTTTTG	
SB2465	5-stem2	gaagcgctcaaaaacataGtg caggaatATGCTAAAG TATG	Changes nt 370 of <i>sicP</i> from C -> G
	3-stem2	CATACTTTAGCATA TTCCTGCACTATGT TTTTGAGCGCTTC	
SB2571	5-stem2R	cataGtgcaggaatATGCT AAAcTATGAGGAG AGAAAATTG	Changes nt 370 of <i>sicP</i> from C -> G and nt 388 from G -> C
	3-stem2R	CAATTTTCTCTCCT CATAGTTTAGCATA TTCCTGCACTATG	
SB2574	5-stem4	caggaagcgctcaaaaacTta ctgcaggaatATGCTAA AG	Changes nt 367 of <i>sicP</i> from A -> T
	3-stem4	CTTTAGCATATTCC TGCAGTAAGTTTTT GAGCGCTTCCTG	
SB2575	5-stem4R	cTtactgcaggaatATGCT AAAGTAaGAGGAG AGAAAATTG	Changes nt 367 of <i>sicP</i> from A -> T and nt 391 from T -> A
	3-stem4R	CAATTTTCTCTCCT CTTACTTTAGCATA TTCCTGCAGTAAG	
SB2576	5-Pstop5	GTGAATCAGCAGgA AGCGCTcAAAACA TACTGCAG	Changes nt 361 of <i>sicP</i> from A -> T in order to introduce a premature stop codon
	3-Pstop5	CTGCAGTATGTTTT AGAGCGCTTCCTGC TGATTCAC	
SB2577	5-Pstop15	GTcATTTGTGAATC AGCAGtAAGCGCTC AAAACATACTG	Changes nt 352 of <i>sicP</i> from A -> T in order to introduce a premature stop codon
	3-Pstop15	CAGTATGTTTTTGA GCGCTTACTGCTGA TTCACAAATGAC	
SB2578	5-Pstop30	CATATTATATCGCA GCTTGAGTgATTTG TGAATCAGCAGG	Changes nt 335 of <i>sicP</i> from A -> T in order to introduce a premature stop codon
	3-Pstop30	CCTGCTGATTCACA	

		AATCACTCAAGCTG CGATATAATATG	
SB2579	5-Pstop50	CCGATCTGACAAAT ACTTA ^g CATATTAT ATCGCAGCTTGAG	Changes nt 312 of <i>sicP</i> from A -> T in order to introduce a premature stop codon
	3-Pstop50	CTCAAGCTGCGATA TAATATGCTAAGTA TTTGTCAGATCGG	
pSB4154, 55	5-extendSL	gcaggaagcgetcaaCCTc atactgcaggaatATG	Replaces nt 363-365 of <i>sicP</i> with “CCT” in order to extend base pairing in the putative regulatory stem
	3-extendSL	CATATTCCTGCAGT ATGAGGTTGAGCG CTTCCTGC	

Supplementary References

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