# **Supporting Information**

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#### **SI Materials and Methods**

**Bacterial Strains and Plasmids.** As mentioned in the main text, the plasmids, strains, and oligonucleotide sequences are listed in Tables S1–S3 and the plasmid structures are shown in Fig. S1. The sRNAs, target mRNAs, and *hfq* gene were PCR-amplified from *E. coli* MG1655 genomic DNA. The target mRNA sequences for *ompC* (-81 to +36 bp) and *sodB* (-56 to +141 bp) that were fused to *gfp*, *mCherry*, or T7RNAP sequences were based on the constructs used by Urban and Vogel (1). For T7RNAP, only the first 300 bp was fused to mCherry in the RyhB-*sodB::gfp* competition studies and the first 900 bp was fused to the target mRNA sequences in the DsrA-*rpoS::gfp* competition studies. The *fhlA* target mRNA sequence (-309 to +96 bp) is the same as previously reported (2). We fused the *rpoS* target mRNA sequence between -150 and +30 bp to *gfp*. Nucleotide numbering is relative to the start codon.

The *gfp* gene and the T1T2 terminator sequence were obtained from pTAK102 (3). The mCherry gene was amplified from a plasmid provided by R. Tsien (University of California, San Diego, CA) (4). The T7RNAP gene was amplified from the strain BL21DE3 (Stratagene). The Asp terminator sequence was PCR-amplified from pLex (Invitrogen). The pLlacO-1 and pLtetO-1 promoters were obtained from pZE21 (5). Mutations in the -10 and -35 sites of pLtetO-1 were introduced by PCR to generate pLtetO-1m9. The pcon promoter, which was generated by PCR synthesis, is a constitutive promoter (5' tcgagcaccgtcgttgttgacatttttaAgcttggcggttataatggattccacaca 3') modified from the sequences described by Lanzer and Bujard (6) (in some promoters, the capitalized A was replaced with T to abolish the *Hind*III site). The st7 and st3 RBS sequences were synthesized (7). Genes were deleted by the  $\lambda$ -Red method (8).

**Data Collection and Analysis.** Single-cell measurements of GFP expression were collected with a Beckman–Coulter EPICS XL-MCL, and the data were analyzed using Flow explorer 4.1 (R. Hoebe, University of Amsterdam, Amsterdam, The Netherlands) and custom programs written with Matlab software (Math-Works). All cultures were grown at 37 °C in LB with 100 µg/mL ampicillin (Sigma). Bacterial cultures were generated by diluting overgrown cultures 1:10,000 in LB media, growing them for 3 h, and diluting them again (1:1,000) with the appropriate concentration of IPTG (Fisher Scientific) and were then grown for another 150 min. The dilutions were adjusted for slower growing cultures, such that the cell density at fluorescence measurement was approximately the same for all strains. Slower growing cultures include strains with *hfq* deleted, highly transcribed *hfq*, pcon-OxyS, or pLlacO-1-*sodB::gfp* when RyhB was absent.

**Western Blotting.** Four milliliters of exponentially growing cells ( $\approx 0.3 \text{ OD}_{600}$ ) was centrifuged, and the cell pellet was dissolved in 5% (wt/vol) SDS buffer. The samples were sonicated, heated at 70 °C for 10 min in LDS sample buffer (Invitrogen), and separated on 10% (wt/vol) Bis-Tris gel (Invitrogen) with MES buffer (50 mM MES, 50 mM Tris base, 0.1% SDS, and 1 mM EDTA)

at 200 V for 30 min. Equal amounts of protein were loaded onto the gel by normalizing the OD. The gels were blotted onto PVDF membranes (GE Healthcare Life Sciences) in transfer buffer (Invitrogen) at 15 V for 35 min in transfer buffer, blocked in 10% wt/vol dry milk and TTBS20 [50 mM Tris, 150 mM NaCL, and 0.1% (vol/vol) Tween 20], and then incubated overnight in 5% wt/ vol dry milk at 4 °C with a 1:5,000 dilution of rabbit anti-Hfq (80 mg/mL; kindly provided by Udo Blasi and Branislav Vecerek, Max F. Perutz Laboratories, University of Vienna, Vienna) or a 1:5,000 dilution of goat anti-L9 antibody (143 mg/mL; kindly provided by Isabella Moll, Max F. Perutz Laboratories, University of Vienna, Vienna). The secondary antibodies were donkey antirabbit IgG HRP (GE Healthcare Life Sciences) and donkey antigoat IgG-HRP (Santa Cruz Biotechnology) used at a 1:10,000 dilution. The secondary antibodies were visualized with ECL Plus Western Blotting Detection Reagents and radiographic film (both from GE Healthcare Life Sciences). Digital images were captured by transillumination of the film using the Gel Doc XR imaging system (Bio-Rad). Band intensity was quantified on nonsaturated exposures with an algorithm based on the area and intensity of the bands using Quantity One Analysis software (Bio-Rad). A spectra multicolor low-range ladder (Fermentas) was used to size the bands. Western blots were performed in triplicate.

Quantitative RT-PCR. The sRNA and target mRNA concentrations were measured by quantitative RT-PCR. The RNA measurements were made as follows. Total RNA was extracted from five exponentially growing cell cultures using TRIzol (Invitrogen) and then treated with DNase I (New England Biolabs). The cDNA was synthesized from the DNase I-treated RNA using the iScript select cDNA synthesis kit and random primers (Bio-Rad). Quantitative PCR was then performed to determine the concentration of cDNA using iQ SYBR Green Supermix (Bio-Rad) with the iQ5 Real-Time PCR detection system (Bio-Rad). An identical amount of DNase I-treated RNA was prepared exactly as for the cDNA sample except that water was added instead of reverse transcriptase (-RT). The -RT sample was amplified in exactly the same manner as the cDNA sample to determine the concentration of contaminating DNA. Differences in RNA extraction, loading, and the efficiency of cDNA synthesis were normalized by measuring the stable 5S ribosomal RNA (rrfB) in each sample. The sRNA and target mRNA ("sample") and *rrfB* were amplified with the oligonucleotides shown in Table S3. The amount of mRNA was calculated by the following equation:

$$\frac{E_{sample}^{CT(cDNA)} - E_{sample}^{CT(-RT)}}{E_{rfB}^{CT(cDNA)} - E_{rfB}^{CT(-RT)}}.$$

The cycle threshold (CT) values were determined automatically by the iQ5 software. The PCR efficiency was determined for every oligonucleotide pair ( $E_{sample}$ ) and the control pair ( $E_{rr/B}$ ) from five samples at five different dilutions. Measurements were performed in at least triplicate.

<sup>1.</sup> Urban JH, Vogel J (2007) Translational control and target recognition by Escherichia coli small RNAs in vivo. *Nucleic Acids Res* 35:1018–1037.

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Fig. S1. Plasmid construction. The general construction of the plasmids used for the experiments is shown. (A) Plasmids shown in Figs. 1 A–C, 2E, and 3. (B) Plasmids shown in Figs. 1D and 2 B–D. (C) Plasmids shown in Fig. 4. (D) Plasmids shown in Fig. 5.

Table S1.	Strains	
Strain		Description
MG1655		Yale E. coli genetic stock
		center (CGSC#7740)
HL 1		MG1655 + pkd46
HL 716		HL 1 + <i>laclq</i> inserted into the
		chromosome at the Ints site
		HL 716 + 2009
		HI 716 + pHI 100
HL 839		HL 716 + pHL 108
HL 862		HL 716 + $\Delta micC$
HL 865		HL 716 + $\Delta dsrA$
HL 1128		HL 862 + ∆ <i>hfq</i>
HL 1178		HL 862 + pHL 282
HL 1188		HL 865 + ∆ <i>hfq</i>
HL 1329		HL 865 + pHL 344
HL 1474		HL 862A + pHL 404
HL 1543		HL 1188 + pHL 344
HI 2304		HI 716 $\pm$ pHI 269
HL 2305		HL 716 + pHL 765
HL 2307		HL 716 + pHL 777
HL 2308		HL 716 + pHL 814
HL 2311		HL 716 + pHL 762
HL 2312		HL 716 + pHL 787
HL 2314		HL 716 + pHL 818
HL 2478		HL 770 + pHL 98
HL 2479		HL 770 + pHL 108
HL 2625		
HL 2027		HI 716 + $\Lambda r b v B$
HL 2817		HL 716 + pHL 720
HL 2818		HL 865A + pHL 489
HL 2839		HL 2752 + pHL 720
HL 2869		HL 2752 + pHL 991
HL 3075		HL 716 + pHL 1019
HL 3076		HL 716 + pHL 1069
HL 3077		HL 716 + pHL 1020
HL 3078		HL /16 + pHL 10/0
HL 3089		HL 716 + PHL 1074
HL 3090		HL 716 + pHL 1035 HL 716 + pHL 1075
HL 3098		HI 716 + pHI 1095
HL 3099		HL 716 + pHL 1096
HL 3105		HL 716 + pHL 1032
HL 3106		HL 716 + pHL 1073
HL 3107		HL 716 + pHL 1033
HL 3151		HL 716 + pHL 1037
HL 3152		HL 716 + pHL 1056
HL 3153		HL /16 + pHL 105/
HL 31/6		HL 716 + PHL 1039
HL 3100		HL 716 + pHL 262 HL 716 + pHL 990
HL 3223		HL 1128 + pHL 282
HL 3233		HL 716 + pHL 1147
HL 3234		HL 716 + pHL 1148
HL 3236		HL 716 + pHL 1022
HL 3262		HL 716 + $\Delta oxyS$
HL 3270		HL 716 + pHL 1158
HL 3326		HL 716 + pHL 1008
HL 3327		HL 716 + pHL 1009
HL 3328		HL /16 + pHL 1011
HL 3329		HL //U + PHL 1008

Strain	Description
HL 3330	HL 770 + pHL 1009
HL 3331	HL 770 + pHL 1011
HL 3338	HL 770 + $\Delta rhyB$
HL 3339	HL 770 + pHL 100
HL 3347	HL 3338 + pHL 720
HL 3357	HL 716 + pHL 1191
HL 3358	HL 770 + pHL 1191
HL 3373	HL 2752 + pHL 1011
HL 3374	HL 3338 + pHL 1011
HL 3375	HL 865 + pHL 1008
HL 3376	HL 1188 + pHL 1008
HL 3395	HL 862 + pHL 1009
HL 3396	HL 1128 + pHL 1009
HL 3425	HL 770 + ∆ <i>oxy</i> S
HL 3447	HL 3425 + pHL 1179
HL 3448	HL 3425 + pHL 1191
HL 3449	HL 3262 + pHL 1179
HL 3450	HL 3262 + pHL 1191
HL 3545	HL 770 + pHL 1213
HL 3612	HL 716 + pHL 841
HL 3619	HL 716 + pHL 1228
HL 3722	HL 716 + pHL 404
HL 3723	HL 716 + pHL 489
HL 3724	HL 716 + pHL 991
HL 3857	HL 716 + pHL 1303
HL 3858	HL 716 + pHL 1304
HL 3859	HL 716 + pHL 1305
HL 3860	HL 716 + pHL 802
HL 3902	HL 770 + pHL 404
HL 3903	HL 770 + pHL 489
HL 3904	HL 770 + pHL 991
HL 4120	HL 716 + pHL 1370
HL 4168	HL 716 + pHL 1394
HL 4175	HL 716 + pHL 1391
HL 4178	HL 862 + pHL 1360
HL 4179	HL 865 + pHL 1381
HL 4180	HL 2752+ pHL 1358
HL 4202	HL 716 + pHL 1389
HL 4203	HL 716 + pHL 1405
HL 4209	HL 716 + pHL 1410
HL 4211	HL 716 + pHL 1413
HL 4212	HL 716 + pHL 1414
HL 4218	HL 716 + pHL 1411
HL 4219	HL 716 + pHL 1416

Plasmid	Description
 pHL 67	lack from pTrc99a + CoIE1 from pZE21 + KanR
p.12 07	cassette from pKD13 (including the P1 and P4
	oligonucleotide sites). Template for <i>laclq</i> insertion
	into the genome.
pHL 98	pLlacO-1:DsrA-mCherry, pLtetO-1: <i>rpoS::gfp</i>
pHL 100	pLlacO-1:OxyS-mCherry, pLtetO-1: <i>fhlA::gfp</i>
pHL 108	pLlacO-1:MicC-mCherry, pLtetO-1: <i>ompC::gfp</i>
pHL 269	pLtetO-1:MicC-mCherry, pLlacO-1:ompC::gfp
pHL 282	pLtetO-1:mCherry, pLlacO-1:ompC::gfp
pHL 344	pLtetO-1:mCherry, pLlacO-1: <i>rpoS::gfp</i>
pHL 404	pLtetO-1:MicC, pLlacO-1:hfq, pLtetO-1:ompC::gfp
pHL 489	pLtetO-1:DsrA, pLlacO-1:hfq, pLtetO-1:rpoS::gfp
pHL 720	pLtetO-1:mCherry, pLlacO-1:sodB::gfp
pHL 745	pLtetO-1:RhyB-mCherry, pLlacO-1:sodB::gfp
pHL 762	pLtetO-1:MicC, pLtetO-1:sodB::mCherry, pLlacO-1:ompC::gfp
PHL 765	pLtetO-1:MICC, pLtetO-1:DsrA, pLlacO-1:ompC::gtp
PHL 772	pLtetO-1:KNyB, pLtetO-1: <i>htq</i> , pLlacO-1: <i>sodB::gtp</i>
PHL 777	pLtetO-TIMICC, pLtetO-TIRNYB, pLlacO-TIOMPC.:grp
рпь /o/ БЦ 902	pliero-rivino, pliero-ripos::monerry, pliaco-riompl::gfp
	pLtetO-1:OxyS-mCherry, pLlacO-1:ompC::grp
	pleteto 1:Micc, pleteto 1:0xy3, placo 1:0mpcgip
	platero-1.micc, platero-1.miA.mcherry, placo-1.ompcgrp
	plieco-1.RhyB-incherry, pliaco-1.onpcgrp
pHL 990	nl tetO-1:DsrA_pcon:RhyB_nl lacO-1:rpoS::afp
nHI 991	place $1.237$ , place $1.257$ , place $1.253.37$
pHL 1008	place-1:DsrA-mCherry, platete-1:RBS (st7) gfp
pHI 1009	pl lacO-1:MicC-mCherry, pl tetO-1:RBS (st7) gfp
pHL 1011	pLlacO-1:RhyB-mCherry, pLtetO-1:RBS (st7) gfp
pHL 1019	pLtetO-1:RhvB, pcon:MicC, pLlacO-1:sodB;;gfp
pHL 1020	pLtetO-1:RhyB, pcon:DsrA, pLlacO-1:sodB::gfp
pHL 1022	pLtetO-1:RhyB, pcon:OxyS, pLlacO-1:sodB::gfp
pHL 1032	pLtetO-1:RhyB, pcon:ompC::mCherry, pLlacO-1:sodB::gfp
pHL 1033	pLtetO-1:RhyB, pcon:rpoS::mCherry, pLlacO-1:sodB::gfp
pHL 1035	pLtetO-1:RhyB, pcon:fhlA::mCherry, pLlacO-1:sodB::gfp
pHL 1037	pLtetO-1:DsrA, pcon:MicC, pLlacO-1:rpoS::gfp
pHL 1039	pLtetO-1:DsrA, pcon:OxyS, pLlacO-1:rpoS::gfp
pHL 1056	pcon:MicC, pLlacO-1: <i>rpoS::gfp</i>
pHL 1057	pcon:RhyB, pLlacO-1: <i>rpoS::gfp</i>
pHL 1069	pcon:MicC, pLlacO-1:sodB::gfp
pHL 1070	pcon:DsrA, pLlacO-1: <i>sodB::gfp</i>
pHL 1073	pcon:ompC::mCherry, pLlacO-1:sodB::gfp
pHL 1074	pcon:rpoS::mCherry, pLlacO-1:sodB::gfp
pHL 1075	pcon: <i>fhlA</i> ::mCherry, pLlacO-1:sodB::gfp
pHL 1095	pLtetO-1:RhyB, pcon:MicC, pcon: <i>omp</i> C::mCherry, pLlacO-1:sodB::gfp
pHL 1096	pLtetO-1:RhyB, pcon:DsrA, pcon: <i>rpoS</i> ::mCherry, pLlacO-1:sodB::gfp
pHL 1147	pcon:OxyS, pLlacO-1: <i>rpoS::gfp</i>
pHL 1148	pcon:OxyS, pLlacO-1:sodB::gfp
pHL 1158	pLtetO-1:RhyB, pcon:DsrA, pcon: <i>ompC</i> ::mCherry, pLlacO-1:sodB::gfp
pHL 1179	pLtetO-1:mCherry, pLlacO-1: <i>fhlA::gfp</i>
pHL 1191	pLlacO-1:OxyS-mCherry, pLtetO-1:RBS (st/) gfp
pHL 1213	pLlacO-1:hfq, pLtetO-1:RBS (st7) gfp
PHL 1228	pLtetO-1:DsrA-mCherry, pLlacO-1: <i>ompC::gfp</i>
PHL 1303	pLtetO-1:sodB::mCherry, pLlacO-1:ompC::gfp
рпс 1304 «Ш. 1305	pLtetO-1:rpos::mCnerry, pLlacO-1:ompC::grp
рпь 1305 «Ш. 1359	pLtetO-1:m/A::mCnerry, pLlacO-1:ompC::gfp
рпь 1358 БШ 1360	pLiacO-1:nrg, pLtetO-1m9:sodb::gtp
рпь 1300 nul 1270	pLiaco-i:nrg, pLieto-i:ompC::gtp
рпь 1370 БЦГ 1291	pLtetO-1:DsrA, pCon:17(st3) (900pps), pLlacO-1:rpoS::gtp
ערר וססו העו 1200	pliaco-i.my, pliaco-i.mpos::grp
рпь 1389 nu 1381	pLtetO-1:DsrA, pLtacO-1: <i>rpoS::gtp</i>
ארר וסטו דיר וחטי	$\mu_{\text{LIdU}} = 1.7\mu_{\text{U}}g_{\mu}$
UIL 1374	$p_{Let}(0, 1, n) \neq 0$ , $p_{L$

Plasmid	Description	
pHL 1405		
pHL 1410	pLtetO-1:DsrA, pcon: <i>ompC::</i> T7noRBS(900bps), pLlacO-1: <i>rpoS::gfp</i>	
pHL 1411	pLtetO-1:DsrA, pcon: <i>fhlA:</i> T7noRBS(900bps), pLlacO-1: <i>rpoS::gfp</i>	
pHL 1413	pcon: <i>ompC::</i> T7noRBS(900bps), pLlacO-1: <i>rpoS::gfp</i>	
pHL 1414	pcon: <i>fhlA::</i> T7noRBS(900bps), pLlacO-1: <i>rpoS::gfp</i>	
pHL 1416	pcon:T7 (st3) (900bps), pLlacO-1: <i>rpoS::gfp</i>	

### Table S3. Oligonucleotides

Name	Function	Sequence
AatpLacSalF	Fuses pLac directly to gfp, with Aatll and Sall sites	ctagacgtcataaatgtgagcggataacattgacattgtgagcgga taacaagatactgt
AspTermBamF	Clones Asp terminator from pLex	ggccggatcctttaatcgtacagggtagtacaaata
AspTermHindIIIF	Clones Asp terminator from pLex	ggccaagctttaatcgtacaggtagtagtagaata
AspTermEcoBIB	Clones Asp terminator from plex	ccanaatteactactacaanaaaaaaaaaaaaaaa
ColEApolE	Adds Appl restriction site to ColE 1 origin	
ColEDom	Adds Apai restriction site to cole 1 origin	
COIEBamHIF	Amplifies Cole origin with terminators from pZE21	cgcggatcccatggtacgcgtgctaga
ColeAatIIR	Amplifies ColE origin with terminators from pZE21	ctagacgtcgttcgagagctcgcttggactcct
DsrA1XmalF	PCR amplifies DsrA with native terminator	tcctcccgggaacacatcagatttcctggtgtaa
DsrA2ApaR	PCR amplifies DsrA with native terminator	catgggcccagcgtctctgaagtgaatcgttga
DsrA3NoTermHindIIIR	RT-PCR amplifies DsrA without native terminator	ggccaagcttcccgaccctgaggggggggggat
DsrAKO1pkD1F	Deletes chromosomal DsrA using pKD13 and the λ-Red method	atatggcgaatattttcttgtcagcgaaaaaaattgcggataaggtgatg
DsrAKO2nkD4R	Deletes chromosomal DsrA using nKD13 and the	tattcatgacttcaggetgette
	$\lambda$ -Red method	aaattccggggatccgtcgacc
DsrA2HIndilik	PCR amplifies DSrA with native terminator	ggccaagcttagcgtctctgaagtgaatcgttga
DsrA2NotIR	PCR amplifies DsrA with native terminator	tcctgcggccgcagcgtctctgaagtgaatcgttga
FhIA1SallF	PCR amplifies <i>fhlA</i> sequence to fuse to <i>gfp</i>	tactgtcgacgcgaattgctgggactggacgccc
FhIA3 fusion SphIR	PCR amplifies <i>fhlA</i> sequence to fuse to <i>gfp</i>	tacgcatgctctcacacagcgaggccagatcggg
GfpRBSSalF	PCR amplifies <i>qfp</i> with Sall site and RBS (st7)	cctgtcgactaaggaggaaaaaaaatgcgtaaaggagaagaacttttc
GFPseaR	RT-PCR amplifies mRNA sequences fused to afp	gtatgttgcatcaccttcaccctctccactgacag
HfaNoStonSnhB	PCR amplifies <i>bfa</i> sequence to fuse to <i>afp</i>	ttacqcatqctttcqqtttcttcqctqtcctqttq
HfaPBSYmalE	PCP amplifies htq with PPS (st7)	cctcccagataagagagaaaaaatagctaagagagaatctttacaag
HfqPromAatF	PCR amplifies <i>hfq</i> , native <i>hfq</i> promoter, and	cgcgacgtcgcgtgacgaagtattacaggttgtt
	DCD amplifies hfromith DDC (st7)	
нтадрав	PCR amplifies <i>htq</i> with RBS (st7)	gacgggcccttattcggtttcttcgctgtcctg
HtqHindR	PCR amplifies htq with RBS (st/)	ggccaagcttattcggtttcttcgctgtcctg
HfqpkD1F	Deletes chromosomal <i>hfq</i> using pKD13 and the λ-Red method	tcagaatcgaaaggttcaaagtacaaataagcatataaggaaaagagag agtgtaggctggagctgcttc
HfqpkD4R	Deletes chromosomal hfq using pKD13 and the	ggaacgcaggatcgctggctccccgtgtaaaaaaacagcccgaaacctta
	λ-Red method	attccggggatccgtcgacc
IntSpKD1F	PCR amplifies <i>laclq</i> and the KanR cassette	ccgtagatttacagttcgtcatggttcgcttcagatcgttgacagccgcag
l aclOintS	PCB amplifies lock and KanB consette	
LaciQints	for a shift GT to incort at int Gits	
	trom pHL67 to insert at ints site	agctaactcacattaattgcgttgc
mCherryRBSBsiWIHindIIIF	PCR amplifies mCherry with RBS (st7)	tcttaaaagcttattaaagaggagaaacgtacgatggtgagcaagggc gaggagg
mCherryCYFPRBSSphXmalF	Clones mCherry in the absence of an sRNA with <i>Xma</i> l and <i>Apa</i> l sites	tacgcatgccctcccgggtaaggaggaaaaaaatggtgagcaaggg cgaggag
mCherryCYFPRBSXmaSphIF	Clones mCherry fused to target mRNA with Sph and Apal sites	cctcccgggtacgcatgctaaggaggaaaaaaaatggtgagcaagg
mCherryAnalR	PCR amplifies mCherry with BRS (st7)	catgoggegg
MicClYmalE	PCP amplifies MicC with native terminator	
MicClanap	PCR amplifies MicC with native terminator	
MICCZAPAR	PCR amplifies MICC with native terminator	taagggccctctggataaggattatccaattcta
MicC2HindIIIR	PCR amplifies MicC with native terminator	ggccaagcttctggataaggattatccaattcta
MicC2NotIR	PCR amplifies MicC with native terminator	tcctgcggccgcctggataaggattatccaattcta
MicC3NoTermHindIIIR	RT-PCR amplifies MicC without native terminator	ggccaagcttgttcgggcttgtctttttatatgt
MicCKO1pkD1F	Deletes chromosomal MicC using pKD13 and the λ-Red method	atacaaaataaaaattatacttttaatttgctatacgttattctgcgcgggtgtagg ctggagctgcttc
MicCKO2pkD4R	Deletes chromosomal MicC using pKD13 and the λ-Red method	aaaaagcaacaccgattaaatgctctggataaggattatccaattctaaaattccg
OxySXmalF	PCR amplifies OxyS with native terminator	
OxySAman	PCR amplifies Oxy5 with native terminator	toogaassetttaoosstaasttotsossaaas
Охузарак	PCR amplifies Oxys with halive terminator	
OxySHindliR	PCR amplifies OxyS with native terminator	ggccaagctttgagcctggcttatcgccgggc
OxySNotIR	PCR amplities OxyS with native terminator	tcctgcggccgctgagcctggcttatcgccgggc
OxySNoTermHindIIIR	RT-PCR amplifies OxyS without native terminator	ggccaagcttgcggatcctggagatccgcaaaag
OxyS1pkD1F	Deletes chromosomal OxyS using pKD13 and the $\lambda$ -Red method	agcaatgaacgattatccctatcaagcattctgactgataattgctcacagtgta ggctggagctgcttc
OxyS2pkD4R	Deletes chromosomal OxyS using pKD13 and the λ-Red method	atttatatgtataaatttgagcctggcttatcgccgggcttttttatggc attccggggatccgtcgacc
partpTetOMicCXmaF	First round PCR cloning of pLtetO-1 upstream of MicC	gattgacatccctatcagtgatagagatactcccgggttatatgcctttat tgtcacaga
PconBamF	PCR synthesis of pCon promoter	cgcggatcctcgagcaccgtcgttgttgacatttttaagcttggcggttataat

## Table S3. Cont.

PconEcoRFPCR synthesis of pCon promoterccggaattctgagcaccgtcgttgttgacatttttagcttgggggttatatPconNoHindBamHFPCR synthesis of pCon promoter with no HindIII siteccggaattctgagcaccgtcgttgttgacattttagcttgggggttatatPconNoHindXmaRPCR synthesis of pCon promoter with no HindIII sitecctcggggttgtgggaatccattaatcgccagcagcagggttataatPconNoHindXmaRPCR synthesis of pCon promoter with no HindIII sitecctccgggtgttgtggaatccattaatcgccagcatggtgttgacatttagctgcaggttataatPconNoHindXmaRPCR synthesis of pCon promoter with no HindIII sitecctccgggtgttgtgggaatccattaatcggcgggtataatPconNoHindXmaRPCR synthesis of pLaCo-1 promoter with FacoIIcctgggattctagtctagttgtaggggggatacattgcagttaggttgagatccattaatgggggggatacattgagtgggttaatPactoTsBalRPCR synthesis of pLeto-1 promoter with FacoIIcctgggattcttggttggaggggattcasttggtgggattcasttggtgggggttcasttgptetOBamHIFPCR synthesis of pLeto-1 promoter with KanIcgggattctccatagtggaggggtacattggtggattcattc	Name	Function	Sequence
PconNoHindBamHFPCR synthesis of pCon promoter with no Hindlil sitecgggattctgggacccgttgttgtgacatttttagcttggcgttataatPconNoHindXmaRPCR synthesis of pCon promoter with no Hindlil siterctccggggtgtgggaatcattalaccgccaagctaaaaatgtcaacaacPconNoHindXmaRPCR synthesis of pCon promoter with no Hindlil siterctccgggtgtgggaatcattalaccgccaagctaaaaatgtcaacaacPconXmaRPCR synthesis of pLaCO-1 promoter with coRlirctcccgggtgtgtggaatcattalaccgrcaagctaaaatgtcaacaacpLacCamBamHTITZRPCR synthesis of pLaCO-1 promoter with CoRlircggattcttagtgcggtatcattalaccgrcaagtcaaaatgtcaacacpLetCOIMSARPCR synthesis of pLaCO-1 promoter with SalitcctcccgggggttgtgaatcattalaccgrcaagtcaaaatgtcaacacpTetO-1SallRPCR synthesis of pLetO-1 promoter with SalitcctcccggggattccttatcatgtgataggagtgtacttpTetOXmaRPCR synthesis of pLetO-1 promoter with SaliccccgggggtattctatcatcatgtgataggagtgtacttpTetOXmaRPCR synthesis of pLetO-1 promoter with SalicccccgggggtattctatcatcatgtgataggagtgtacttctacctgtgataggagtgtacttctacctgtgtggaggtgtattctpTetOXmaRPCR synthesis of pLetO-1 promoter with Salicccccgggggtattctatcatcatgatggaggtgtacttctacctgtgtggaggtgtattcttacctgtgagggtgtattcttacctgtgaggggtatccttatactgtgagggtgtattcttacctgtgaggggtatcttatactgtgagggttgtaggtgtgtgt	PconEcoRF	PCR synthesis of pCon promoter	ccggaattctcgagcaccgtcgttgttgacatttttaagcttggcggttataat
PconNoHindEcoRFPCR synthesis of pCon promoter with no Hindlil siteccggaattcttagagcaccgtcgttgttgaattttagcttgcggttataatPconNoHindNotHFPCR synthesis of pCon promoter with no Hindlil sitecctccgggtgtgggaatcattaacgccaagcttaaaatgtcaacaacPconNoHindNotHFPCR synthesis of pLacO-1 promoter with AmalicctcggggtgtggaatcattaacgccaagttaaaatgtcaacaacPLacEcoRFPCR synthesis of pLaCO-1 promoter with EcoRIccggaattcattaacgccaagttaaatgtcacacacpLacEcoRFPCR synthesis of pLaCO-1 promoter with SalltcctcaggggtgtggaatcattaagtgaatcattgacttgpTetO-1SallRPCR synthesis of pLteO-1 promoter with SalltcctcatggaagtacttagtaagtgaatcattgacttpTetOAmalFFPCR synthesis of pLteO-1 promoter with SamHtcctcatggaagtacttcatacgtgaaggagtactpDmpC3allFPCR synthesis of pLteO-1 promoter with SamHcccggaattcttatactgatgaaggatgaattcatcatagtaatgpOmpC1SallFPCR amplifies ompC sequence to fuse to gfptactgtcagctggatgactcctatacgtgatggpDmpC2SphIRPCR amplifies rpoS sequence to fuse to gfptactgtcagccggtggaacgtaaattgaactcctatcatggaggaggaagtactptsGfpSphSallFPCR amplifies rpoS sequence to fuse to gfptactgcagctgtcaactcataccttggaggaggagaagtrhyBAnaRPCR amplifies rpoS sequence to fuse to gfptactgcagctgtaatattggaacgaagatrhyBAnaRPCR amplifies RybB with native terminatortcctcggggtgtgaaattggaacgaagatrhyBAnARPCR amplifies RybB with native terminatortcctcggggttgggaatgaattggaacgaagatrhyBAnARPCR amplifies RybB without arite terminatortcagcagctgtggaaattggaacgaagatrhyBAnARPCR amplifies RybB without arite terminatortcagcagtgtggaagatgga	PconNoHindBamHF	PCR synthesis of pCon promoter with no HindIII site	cgcggatcctcgagcaccgtcgttgttgacatttttatgcttggcggttataat
PconNoHindXmaRPCR synthesis of pCon promoter with no Hindill sitecctccggggtgtggggatcattataacgccaagctaaaatgtcaacaaPconXmaRPCR synthesis of pCon promotercctccggggtgtgggaatcattataacgccaagctaaaatgtcaacaapLacCcoRFPCR synthesis of pLaC-1 promoter with coRccggatgtgggaatcattataacgccaagttaaaatgtcaacagpLacXmaBamHT12RPCR synthesis of pLaC-1 promoter with CoRccggatgtgggaatcattataacgccaagttaaaatgtcaacagtpLacXmaBamHT12RPCR synthesis of pLeC-1 promoter with Solltcctccgggatgtggaatgaagtggatggagagatgtgggggggg	PconNoHindEcoRF	PCR synthesis of pCon promoter with no HindIII site	ccggaattctcgagcaccgtcgttgttgacatttttatgcttggcggttataat
PconNoHindNotiHFPCR synthesis of pCon promoter with no HindIII siteCcccccgggtgtgtgggatcattttagcttggcggttataaPconXmaRPCR synthesis of pLaCo-1 promoter with EcoRIcccccgggtgtgtggatacatttagcgcaacttaaaatgtcaacagctagatgtaatttagcgtcaacatpLacEcoRFPCR synthesis of pLaCo-1 promoter with EcoRItcccccgggtgtatggaggtagggtagggggtaggggggtgggggg	PconNoHindXmaR	PCR synthesis of pCon promoter with no HindIII site	cctcccgggtgtgtggaatccattataaccgccaagcataaaaatgtcaacaac
PconXmaRPCR synthesis of pLacO-1 promoterccccggagtgtgtggaatccattaacacgcagctaacattgaacggataacttgpLacEcoRFPCR synthesis of pLlacO-1 promoter withccggaattggatcgactacaattgaactggagtaacttggaccaattgaactgaattgagcggcggattgpLacXmaBamHT1T2RPCR synthesis of pLtetO-1 promoter withtcctccgggagtgtggagtcccctaatggtaaggggggagtactagtggaggagtacattggagggggatactcctataagtgaagggagtactcctataagtgaggggagtactacttgagggagg	PconNoHindNotIHF	PCR synthesis of pCon promoter with no HindIII site	tcctgcggccgctcgagcaccgtcgttgttgacatttttatgcttggcggttataat
pLackCoRFPCR synthesis of pLlacO-1 promoter with EcoRIccggaattcgatccataaatgtgacggatacattgacttgactpLacXmaBamHTIT2RPCR synthesis of pLlacO-1 promoter withtcctccggaattcgatcttgatacgtcataatgtaagtgatacattgactactpTetO-1SalIRPCR synthesis of pLtetO-1 promoter with SalltcctcccggagattcgatcttatacgtaagagatgatacatgtcaattpLtetOImSSalRPCR synthesis of pLtetO-1 promoter with SalltcctcccggagattcgatcttatacgtaagagatgatacttgaagpLtetOImSSalRPCR synthesis of pLtetO-1 promoter with ManlcgcggattctgatcgagagttctatctatacgtaaggagtgtcaattpOmpCISalIFPCR synthesis of pLtetO-1 promoter with XmalcccccggagattgtctatatagtaggggtacatcttatactgpOmpCISalIFPCR amplifies ompC sequence to fuse to gfptactgtcgacctgataggagagtgcaacattcaggagactpNeSOSphIRPCR amplifies fpo S sequence to fuse to gfptactgtcgaccaggtaggacttcatcatcaggagagtpNeSOSphIRPCR amplifies fpo S sequence to fuse to gfptactgtcgaccaggtaggaacttcatcatrpwBXmAFPCR amplifies fpNB with native terminatorcagcggccgtggagaacttcaggagagattrhyBAnARPCR amplifies RyhB with native terminatortacggaccgtggaaaattgagaagaagaattrhyBNDIFDeletes chromosomal RyhB using pKD13 and the λ-Red methodtacgcataggagacattgacaagagattgagagagaagaagaagaagaagaagagagag	PconXmaR	PCR synthesis of pCon promoter	cctcccgggtgtgtggaatccattataaccgccaagcttaaaaatgtcaacaac
pLacXmaBamHTIT2RPCR synthesis of pLlacO-1 promoter with BamHI and Xmal sitestcctcccgggagtacttgttaggcgggggttagttgtaggagtagggggggg	pLacEcoRF	PCR synthesis of pLlacO-1 promoter with EcoRI	ccggaattcgatccataaatgtgagcggataacattgacattg
BamHI and Xmal sitestggatccctaggtctaggcggggtttgpTetO-TSallRPCR synthesis of pLtetO-1 promoter with SalltccctatcagtgataggagttgcactcctatcagtgataggagtgcagttgptetOBamHIFPCR synthesis of pLtetO-1 promoter with BamHIccgcggatctcctatcagtgataggagtgcaatccttatcagtgatagpTetOXmalRPCR synthesis of pLtetO-1 promoter with BamHIccgcggatcttcctatcagtgataggagtgcaatccttatcagtgatagpTetOXmalRPCR synthesis of pLtetO-1 promoter with XmalcctcccgggagtattctatcatgataggagtgcaatccttatcagtgatagpOmpC1SallFPCR amplifies ompC sequence to fuse to gfptacgttgcgactgggaacgaacggaacgaaggaggaagga	pLacXmaBamHT1T2R	PCR synthesis of pLlacO-1 promoter with	tcctcccgggagtatcttgttatccgctcacaatgtcaatgttatccgctcacattta
pTetO-ISallRPCR synthesis of pLtetO-1 promoter with SalltccctatcagtgatagagattgacatcctatcagtgatagagatatgtcatcpLtetOIm9SalRMutates pLtetO-1 promoter with BamHIcgatggacagttcttatcatgatagagattgcatcctatcagtgatagpTetOBamHIFPCR synthesis of pLtetO-1 promoter with BamHIcgrggatcttctatcatggatagaggtgcaatcctagtagpOmpCISalIFPCR amplifies ompC sequence to fuse to gfptacgtgacaggaggacaggaggaggtagattctatcatcgggagggtapOmpCISalIFPCR amplifies ompC sequence to fuse to gfptacgtgacagtgaggagaggtagattctatcatcgggaggacgtaggggacgtaggaggtcaattctatcatgggagggtaPDMpCISblIRPCR amplifies rpoS sequence to fuse to gfptacgtgacagtggagaggtagaggttagattctatcatPtSGgfpSphISalIFPCR amplifies rpoS sequence to fuse to gfptacgtgacgtgataggagagttgacattctatcatggaggttagatttgattggaggttagagggtgaggtcagggggaggtgaggtgagggggggg		BamHI and XmaI sites	tggatcccctaggtctagggcggcggatttg
pLtetO1m9SalRMutates pLtetO-1 promoter by PCRcaagtogaagtctcttatcattgatagggagtcaattptetOBamHIFPCR synthesis of pLtetO-1 promoter with BamHIcgcggatctcctatcatgtatagggattgcaattpTetOXmalRPCR synthesis of pLtetO-1 promoter with XmalccccgggagtattctatcatcgtaggggattgcaattpOmpC1SallFPCR amplifies ompC sequence to fuse to gfptacgtagcatgcgggaggagagagagagacagtacPtsGgfpSphlSallFPCR amplifies ompC sequence to fuse to gfptacgtagcacggtggaagagagagagagacagtacPtsGgfpSphlSallFPCR amplifies rpoS sequence to fuse to gfptacgtagcacggtgaagagaagaactttcatcRpo5-150SallFPCR amplifies rpoS sequence to fuse to gfptacgtagcacaggtagagagaagaactttcatcremoved with Sall and SphI)caagcatggtaaaggagaagaactttcatPhyBNaFPCR amplifies rpoS sequence to fuse to gfptacgtagtcatcggaaggtaaggagagagaactttcatcrhyBApaRPCR amplifies RyhB with native terminatortacgcatgttaaattgagaaggaagaatrhyBApaRPCR amplifies RyhB with native terminatortaaggacgttgtgataattgagaacgaagatrhyBhotIRPCR amplifies RyhB with native terminatortacgatatcggaagcaagagatryhBhotIRPCR amplifies RyhB without native terminatortacgatactacggaagacgaagatryhBhotIFDeletes chromosomal RyhB using pKD13 andtacgcaacacagtccctgggataattgagacgaagatgatgatattattgtcrmB1361FRT-PCR amplifies rodB sequencescaagtggacgaagatggtataggtgtaatcaggtggatgaatga	pTetO-1SallR	PCR synthesis of pLtetO-1 promoter with Sall	tccctatcagtgatagagattgacatccctatcagtgatagagatactgtcgac
ptetDBamHIFPCR synthesis of pLtetO-1 promoter with BamHIcgcggatcttcctatagtgatagagttgacattcctatagtgatagpTetOXmaIRPCR synthesis of pLtetO-1 promoter with XmalcctccgggagtatcttatataggagttgacattctatactgpOmpC1SallFPCR amplifies ompC sequence to fuse to gfptactgttgacttgcattgatagaggttapOmpC2SphIRPCR amplifies gfp fytsg sequence to fuse to gfptactgtcgactagtatagaggttaaaaaagaacccatatcaggaggagacagtaPtSGgfpSphISallFPCR amplifies gfp fytsg sequence to fuse to gfptactgtcgaccacggaggaacagaacgaaccattcaggaggagacagtaRpoS-150SallFPCR amplifies rpoS sequence to fuse to gfptactgtcgacaccggagaaccagttcaacacgttgcatttgRpoS-150SallFPCR amplifies rpoS sequence to fuse to gfptactgtcgacaccggaaccagttagaagaatttcactrmoved with Shl with native terminatorcctccgggggatcatggaaagtagaagaagatrhyBApaRPCR amplifies RyhB with native terminatortacggatgctggataattgagaaggaagatrhyBNotlRPCR amplifies RyhB with native terminatortcctgggcgctggataattgagaacgaaagatryhBokD1FDeletes chromosomal RyhB using pKD13 andtacgatagtggactggtggatagtggaatgatggaatgaat	pLtetO1m9SalR	Mutates pLtetO-1 promoter by PCR	caagtcgacagtctctctatcactgatagggatgtcaatct
pTetOXmalRPCR synthesis of pLtetO-1 promoter with XmalcctcccgggagtatctcatcactgataggggtgtaatctatcactgpOmpC1SallFPCR amplifies ompC sequence to fuse to gfptactgtcgactggggacaggagggacagtacPtsGgfpSphlSallFPCR amplifies ompC sequence to fuse to gfptactgtcgactcggggacagggacagtacPtsGgfpSphlSallFPCR amplifies rpoS sequence to fuse to gfptactgtcgaccaggtgaacagtacacagttgaatagggacaggacagtacRpoS-150SallFPCR amplifies rpoS sequence to fuse to gfptactgtcgaccaggtgaacagtacacagttgaataggggacaggacgtacRpoS30SphIRPCR amplifies RpS sequence to fuse to gfptacgatgtagtcgggcacaggagacgtacaggaggrhyBAmaFPCR amplifies RyhB with native terminatorcctcccggggcgataggaagactttcggggarhyBNotIRPCR amplifies RyhB with native terminatortacgcagttggataaattggaacgaaagatrhyBNotIRPCR amplifies RyhB with native terminatortacgacacacgtggataaattggaacgaaagatryhBpkD1FDeletes chromosomal RyhB using pKD13 andtaagagaccagtcggggacagtggacagtacagaagatcaaaarmB1361FRT-PCR amplifies the control, rrfB sequencescaaagtgtggaaggccgtggtgataattgagaaggaagattttattgtcrrnB1361FPCR amplifies T7 RNA polymerase with an RBS (st3)cctcccgggagagagaatgatagttatattgtcgtgagaggagagatttacataggagagaga	ptetOBamHIF	PCR synthesis of pLtetO-1 promoter with BamHI	cgcggatcctccctatcagtgatagagattgacatccctatcagtgatag
pOmpC1SallFPCR amplifies ompC sequence to fuse to gfptactgtcgacttgccgactgattaatgagggttapOmpC2SphIRPCR amplifies ompC sequence to fuse to gfptacgcatgctagctgcgacaggaggacagtacPtsGgfpSphISallFPCR amplifies fp (ptsy sequence in oligo removed with Sal and SphI)tacgcatgctagctgaagaagtacagttcaatRpoS150SallFPCR amplifies rpoS sequence to fuse to gfptacgcatgctagcgatagaagagaagtttcaatRpoS30SphIRPCR amplifies rpoS sequence to fuse to gfptacgcatgctagagaggatagatrhyBXmaFPCR amplifies RyhB with native terminatorccacgcatggatagaagagaccttcgggagrhyBApaRPCR amplifies RyhB with native terminatortacgcagctgggataaattgagaacgaaagatrhyBNotlRPCR amplifies RyhB with native terminatortacgcagctgggataaattgagaacgaaagatryhBotIRPCR amplifies RyhB with native terminatortacgagcagcgggggataaattgagaacgaaagatryhBotIRPCR amplifies RyhB with native terminatortacgagcagcgggggataaattgagaacgaaagatryhBotIRPCR amplifies RyhB with native terminatortacgagacgacggtggataattgagaacgaaagatryhBotIRPCR amplifies RyhB with native terminatortacgagacgacgatggatggatggatggatggatggatgg	pTetOXmalR	PCR synthesis of pLtetO-1 promoter with XmaI	cctcccgggagtatctctatcactgatagggatgtcaatctctatcactg
pOmpC2SphIRPCR amplifies ompC sequence to fuse to gfptacgcatgctagctaggaccaggaggacagtacPtsGgfpSphISallFPCR amplifies ompC sequence in oligotactgtcgaccaggagaccagtcaaaaaaagcaccatctacgaggagcagtacRpoS-150SallFPCR amplifies rpoS sequence to fuse to gfptactgtcgaccaggaagaactacagttgaattgagaaggattttactRpoS-150SallFPCR amplifies rpoS sequence to fuse to gfptactgtcgacacggagaaccagttcaacagcttgcatttgrhyBAmaFPCR amplifies RyhB with native terminatorccagcagctggagaagaactttcaggaagaagtrhyBApaRPCR amplifies RyhB with native terminatortaaggacccgtggataaattgagaacgaaagatrhyBNotlRPCR amplifies RyhB with native terminatortcaggaaccagttggataaattgagaacgaaagatryhBNotPMRRT-PCR amplifies RyhB with native terminatortcaggaacacagtcggggacagtacagtagaagatryhBNotRRRT-PCR amplifies RyhB with native terminatortcaggaacacagtcgggagacagtaagaagatryhBNotFDeletes chromosomal RyhB using pKD13 andtaacgaacacagcactcccgtggataaattgagaacgaaagatrrmB1361FRT-PCR amplifies the control, rrfB sequencesgaatgcacagtggaatggtattattgtctrattcgggatcggtggataggatggtatggatggaatgtatttatgtctactgcacggtggataggatggtsodBSalFPCR amplifies sodB sequence to fuse to gfpcaagtcgctgcggtaaaggagaatgtacagtgtsodBSalFPCR amplifies T7 RNA polymerase with an RBS (st3)tactgcacaggtaggaaaatgaacagttaacatcgttaagaastrRsS3SmaFPCR amplifies T7 RNA polymerase and addscatcgcacgtatacatcgctaagaacagtstop codon at 133 bpPCR amplifies T7 RNA polymerase and addscataggaggagaaatgaacagtatactgtcaagaagatrrmB1361FPCR amplifies T7 RNA polymeras	pOmpC1SallF	PCR amplifies ompC sequence to fuse to gfp	tactgtcgacttgccgactgattaatgagggtta
PtsGgfpSphISallFPCR amplifies gfp (ptg sequence in oligo removed with Sal and Sphi)tactgtcgaccagcgtgagaacgtaaaaaaagcacccatactcaggagcactat caagcatgcgtaaaggagaagaacttttcatRpoS-150SallFPCR amplifies rpoS sequence to fuse to gfp PCR amplifies RyhB with native terminatortactgtcgaccaggtagaagaactttcagcgtgat tacgcatggagaagaactttcaggagtrhyBApaRPCR amplifies RyhB with native terminatortacggccgtggataattgagaagaagatrhyBApaRPCR amplifies RyhB with native terminatortagggccgtggataattgagaagaagatrhyBNotIRPCR amplifies RyhB with native terminatortggccagcttgggataattgagaacgaagatryhBnotermRRT-PCR amplifies RyhB with native terminatortcctggggcgctcgggataattgagaacgaagatryhBpkD1FDeletes chromosomal RyhB using pKD13 and the λ-Red methodtttgcaaaagtgtggacgtaaatggaacgaagatgataattggrrmB1361FRT-PCR amplifies the control, rrfB sequencesgaatgcacgtggataatggsodBSphRPCR amplifies roB sequence to fuse to gfpcaagtgcgtggadacgtaaatggatsodBSphRPCR amplifies T7 RNA polymerase with an RSS (st3)tacgcatgcggagaagaacgataacagt3T7RBS3XmaFPCR amplifies T7 RNA polymerase and addscatgcatgcagatgaaagatgaacagataacagtaagaaT7ShPFPCR amplifies T7 RNA polymerase and addscatgcatgcgatagagacgaagatT7RNAP133stopSphRPCR amplifies T7 RNA polymerase and addscatgcatgctgtagaaggagacgaagata stop codon at 133 bptacgcatgccttagtagagcacagatgacagatgaacagatagaagat	pOmpC2SphIR	PCR amplifies ompC sequence to fuse to gfp	tacgcatgctagctgggaccaggagggacagtac
removed with Sall and Sphil)caagcatgcgtaaaggagaagaacttttcactRpoS-150SallFPCR amplifies rpoS sequence to fuse to gfptactgtcgacaccggttcaacgcgtgctttaatcacgcttgttttgRpoS30SphiRPCR amplifies rpoS sequence to fuse to gfptacgcatgctataggaactttcagcgtatttgrhyBXmaFPCR amplifies RyhB with native terminatortacgcatgctaaggaagaactaggaaggaagaatrhyBHindRPCR amplifies RyhB with native terminatorggccaagcttgtggataaattgagaacgaaagatrhyBNotlRPCR amplifies RyhB with native terminatortcctccgggcgctggataaattgagaacgaaagatryhBnotermRRT-PCR amplifies RyhB with native terminatortcaggaaccaagtgaacgatggagaagaagaagaagaagaagaagaagaagaagaag	PtsGgfpSphISalIF	PCR amplifies gfp (ptsg sequence in oligo	tactgtcgaccacgcgtgagaacgtaaaaaaagcacccatactcaggagcactct
Rpo5-150SallFPCR amplifies rpoS sequence to fuse to gfptactgtcgacaccggaaccagttcaacacgcttgcattttgRpo530SphIRPCR amplifies rpoS sequence to fuse to gfptacgcatgctacaggaagaccagttcaacacgcttgcatttgrhyBAmaFPCR amplifies rpoS sequence to fuse to gfptacgcatgctacaggaagaccctgggagrhyBApaRPCR amplifies RyhB with native terminatortacgggccgtggataattgagaacgaaagatrhyBNotIRPCR amplifies RyhB with native terminatorggccaagcttgtggataattgagaacgaaagatrhyBNotIRPCR amplifies RyhB with native terminatortcctgcggccgtggataattgagaacgaaagatryhBnotermRRT-PCR amplifies RyhB with native terminatortcatgtaatctggaagcaagtggaacgaaagatryhBpkD1FDeletes chromosomal RyhB using pKD13 andtaacgaacacagcattccgtggataaattgagaacgaaagatrmB1361FRT-PCR amplifies the control, rrfB sequencesgaatgcaagtggaacgtagggtggtggatcagtggagtggtrrnB1361FPCR amplifies sodB sequence to fuse to gfpcacaaagtggtaaggccccasodBSalFPCR amplifies T7 RNA polymerase with an RBS (st3)tacgcatgcaggaagatgaacagatagaa3T7RB53SalFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtcgataggaagaagatgaacagatagaaT7Stop900ApaRPCR amplifies T7 RNA polymerase and addscatgcatgctaggagaatgaacagatagaagata stop codon at 1930 bptacgcatgctagtagacccaggtagaacagatgaagatgaacagatgaagatgaa		removed with Sall and SphI)	caagcatgcgtaaaggagaagaacttttcact
RpoS30SphIRPCR amplifies rpoS sequence to fuse to gfptacgcatgctactaggacttaggacgtacggagagrhyBXmaFPCR amplifies RyhB with native terminatorccttccggggggtagagagagagagagagagagagagaga	RpoS-150SallF	PCR amplifies rpoS sequence to fuse to gfp	tactgtcgacaccggaaccagttcaacacgcttgcattttg
rhyBXmaFPCR amplifies RyhB with native terminatorcctcccgggcgatcaggagagccctgggagrhyBApaRPCR amplifies RyhB with native terminatortaagggccgtggataattgagaacgaagatrhyBHindRPCR amplifies RyhB with native terminatorggccaagcttgtggataattgagaacgaagatrhyBNotIRPCR amplifies RyhB with native terminatortcctgcggcggtaaattgagaacgaagatryhBnotermRRT-PCR amplifies RyhB without native terminatortcctgcggccgtggataattgagaacgaagatryhBokD1FDeletes chromosomal RyhB using pKD13 andtaacgaaccaagcactcccgtggataaattgagaacgaagatryhBpkD4RDeletes chromosomal RyhB using pKD13 andtttgcaaaagtgttggacagtggatgattattattgtcthe $\lambda$ -Red methodtattccggggtgataactggacgcccrrnB1361FRT-PCR amplifies the control, rrfB sequencesgaatgccacgtggataactggtsodBSalFPCR amplifies sodB sequence to fuse to gfpcaagtggacacatagggaagaacacgattacatgaasodBSphRPCR amplifies T7 RNA polymerase with an RBS (st3)tctcccgggtgaaatgaacgaaagaacacgattaacatgcaagaaT7SphIFPCR amplifies T7 RNA polymerase and addscatagcactagcaccacgcccacgacgacacggacgaagaaT7RNAP133stopSphRPCR amplifies T7 RNA polymerase and addscatagcactgctgaagaccacggtccaa stop codon at 133 bptacgcatgctctagtagaagcaccacggccaa	RpoS30SphIR	PCR amplifies <i>rpoS</i> sequence to fuse to <i>gfp</i>	tacgcatgctatcatgaactttcagcgtattctg
rhyBApaRPCR amplifies RyhB with native terminatortaagggcccgtggataaattgagaacgaaagatrhyBHindRPCR amplifies RyhB with native terminatorggccaagttgtggataaattgagaacgaaagatrhyBNotIRPCR amplifies RyhB with native terminatortcctgcggccgtggataaattgagaacgaaagatryhBnotermRRT-PCR amplifies RyhB without native terminatortcctgcggccgtggataaattgagaacgaaagatryhBkD1FDeletes chromosomal RyhB using pKD13 andtaacgaacacaagcatccccgtggataaattgagaacgaaagatcaaaathe λ-Red methodagtgtaggctggagctgcttcryhBbpkD4RDeletes chromosomal RyhB using pKD13 andtttgcaaaaagtgttggacaagtggaatgatattattgtcthe λ-Red methodtcattccggggatccgtcgaccrrnB1361FRT-PCR amplifies the control, <i>rrfB</i> sequencesgaatgccaggtgaatagttgacagtggatagtsodBSalFPCR amplifies sodB sequence to fuse to gfpcaagtgcacactacgcacaataaggttgtsodBSalFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtgactagagagaaatgatcaatgtagaaT7Sbp900ApaRPCR amplifies T7 RNA polymerase with no RBSacatgcaccacggtaaaatgagacagaagatT7RNAP133stopSphRPCR amplifies T7 RNA polymerase and addsacatgggccctaagtagcaccaggccagagagatT7RNAP133stopSphRPCR amplifies T7 RNA polymerase and addsacatggaccacaggccacagggccadagagacagaacaga	rhyBXmaF	PCR amplifies RyhB with native terminator	cctcccggggcgatcaggaagaccctcgcggag
rhyBHindRPCR amplifies RyhB with native terminatorggccaagcttgtggataaattgagaacgaagatrhyBNotIRPCR amplifies RyhB with native terminatortcctgcggccgtggataaattgagaacgaagatryhBnotermRRT-PCR amplifies RyhB without native terminatorctaagtaatactggaagcaatgtgryhBpkD1FDeletes chromosomal RyhB using pKD13 and the λ-Red methodtaacgaaccaagcatcccgtggataaattgagaacgaaagatryhBpkD4RDeletes chromosomal RyhB using pKD13 and the λ-Red methodttgcaaaagtgtggaccgatggagatgagaagatgatattattgtcrrnB1361FRT-PCR amplifies the control, rrfB sequences sodBSalFgaatgccagtggataagtgtagcgccttrrnB1475RRT-PCR amplifies the control, rrfB sequences sodBSalFcaaagtgtgagcgaccagtgagaaagatgaacgaagaPCR amplifies 77 RNA polymerase with an RBS (st3)cctcccgggtaagagaagaacgaaagat tagtgaggagaaaaggaacgaagaagaa3T7RB53SalFPCR amplifies 77 RNA polymerase with no RBS a stop codon at 133 bpttagcaatgcaccagcgccaggggacgT/RNAP133stopSphRPCR amplifies 77 RNA polymerase and adds a stop codon at 133 bpcacgatgccttagtaagaccagggccaa	rhyBApaR	PCR amplifies RyhB with native terminator	taagggcccgtggataaattgagaacgaaagat
rhyBNotIRPCR amplifies RyhB with native terminatortcctgcggcgcgtggataaattgagaacgaagatryhBnotermRRT-PCR amplifies RyhB without native terminatorctaagtaatactggaagcaatgtgryhBpkD1FDeletes chromosomal RyhB using pKD13 and the λ-Red methodtaacgaacacaagcactcccgtggataaattgagaacgaaagatcaaaa agtgtaggctggactggttcryhBpkD4RDeletes chromosomal RyhB using pKD13 and the λ-Red methodtttgcaaaaagtgtggacaagtgggaatgatattattattgtcrrnB1361FRT-PCR amplifies the control, <i>rrfB</i> sequencesgaatgccacggtgaatacgttrrnB1475RRT-PCR amplifies the control, <i>rrfB</i> sequencescaagtgtggtagcgcctsodBSalFPCR amplifies sodB sequence to fuse to <i>gfp</i> caagtgcactatggcaaatgatgtgtgtsodBSphRPCR amplifies T7 RNA polymerase with an RBS (st3)ctccccgggtaagaagatgaacacagatgaagaa3T7RB53SalFPCR amplifies T7 RNA polymerase and addscatagggccctaaggagaaaatgaacacgattaacatcgctaagaa77Stop900ApaRPCR amplifies T7 RNA polymerase and addscataggcccttagtaagaaccacggccaagagac77RNAP133stopSphRPCR amplifies T7 RNA polymerase and addscataggaccatagcacaggccaagagaca stop codon at 133 bpbptacgcatgcttgtaagagcccaggccaagagac	rhyBHindR	PCR amplifies RyhB with native terminator	ggccaagcttgtggataaattgagaacgaaagat
ryhBnotermRRT-PCR amplifies RyhB without native terminatorctaagtaatactggaagcaatgtgryhBpkD1FDeletes chromosomal RyhB using pKD13 and the λ-Red methodtaacgaacacaagcactcccgtggataaattgagaacgaaagatcaaaa agtgtaggctggagctgcttcryhBpkD4RDeletes chromosomal RyhB using pKD13 and the λ-Red methodtttgcaaaaagtgttggacaagtgcgaatgagaatgattattattgtc tcattccggggatccgtcgaccrrnB1361FRT-PCR amplifies the control, <i>rrfB</i> sequencesgaatgccacggtgaatacgttsodBSalFPCR amplifies the control, <i>rrfB</i> sequencescacagtggaccaatgaggaaagtgattattattgtcsodBSphRPCR amplifies sodB sequence to fuse to gfptacgcatgctggaccaataggtgttggacaagtgatgattattattgtc3T7RBS3XmaFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtgactaggaggaaatgaacagaagaaT7SphIFPCR amplifies T7 RNA polymerase and addsacatgcatgcacagtgcacagtgcgaatgagaacT7RNAP133stopSphRPCR amplifies T7 RNA polymerase and addstacgcatgctctagtaagaccgagggccaa a stop codon at 133 bp	rhyBNotIR	PCR amplifies RyhB with native terminator	tcctgcggccgcgtggataaattgagaacgaaagat
ryhBpkD1FDeletes chromosomal RyhB using pKD13 and the λ-Red methodtaacgaacacaagcactcccgtggataaattgagaacgaaagatcaaaa agtgtaggctggagctgcttcryhBpkD4RDeletes chromosomal RyhB using pKD13 and the λ-Red methodtttgcaaaaagtgttggacaagtgcgaatgagaatgattattattgtc tcattccggggatccgtcgaccrrnB1361FRT-PCR amplifies the control, <i>rrfB</i> sequencesgaatgccacggtgaatacgttsodBSalFPCR amplifies the control, <i>rrfB</i> sequencescacaaagtgttaggcgcctsodBSphRPCR amplifies sodB sequence to fuse to <i>gfp</i> caagtcgactgcggacaataggtgtggacaagtgctatgtgt3T7RBS3XmaFPCR amplifies T7 RNA polymerase with an RBS (st3)cctcccgggtaaggagaaatgaacagatagaaa3T7SphIFPCR amplifies T7 RNA polymerase with no RBSacatgcacacgattaacatcgctaagaacT7Stop900ApaRPCR amplifies T7 RNA polymerase and addsacatgcaccacgatcagcccaggggacga stop codon at 133 bptacgcatgccttagtaagaccgaaggccaa	ryhBnotermR	RT-PCR amplifies RyhB without native terminator	ctaagtaatactggaagcaatgtg
ryhbBpkD4RDeletes chromosomal RyhB using pKD13 and the λ-Red methodtttgcaaaagtgttggacaagtgcgaatgagaatgattattattgtc tcattccggggatccgtcgaccrrnB1361FRT-PCR amplifies the control, <i>rrfB</i> sequencesgaatgccacggtgaatacgttrrnB1475RRT-PCR amplifies the control, <i>rrfB</i> sequencescacaaagtggtaaggcactatgtacgtsodBSalFPCR amplifies sodB sequence to fuse to gfpcaagtcgaccatacgcacaataaggctattgtacgsodBSphRPCR amplifies T7 RNA polymerase with an RBS (st3)ctccccgggtaaggagaaatgaacacgattaacatcgctaagaa3T7RBS3SalFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaaT7SphIFPCR amplifies T7 RNA polymerase with no RBSacatgcagcacacgattaacatcgctaagaacT7Stop900ApaRPCR amplifies T7 RNA polymerase and addscatagggcccttagtaaggacgacgaggacga stop codon at 900 bptacgcatgccttagtaagacccatgccaagggccaaT7RNAP133stopSphRPCR amplifies T7 RNA polymerase and addstacgcatgccttagtaagactcatgctcaagggccaaa stop codon at 133 bptacgcatgccttagtaagactcatgctcaagggccaa	ryhBpkD1F	Deletes chromosomal RyhB using pKD13 and the λ-Red method	taacgaacacaagcactcccgtggataaattgagaacgaaagatcaaaa agtgtaggctggagctgcttc
the λ-Red methodtcattccggggatccgtcgaccrrnB1361FRT-PCR amplifies the control, rrfB sequencesgaatgccacggtgaatacgttrrnB1475RRT-PCR amplifies the control, rrfB sequencescacaaagtggtaagcgcctsodBSalFPCR amplifies sodB sequence to fuse to gfpcaagtcgaccatacgcacaataaggctattgtacgsodBSphRPCR amplifies T7 RNA polymerase with an RBS (st3)cctcccgggtaaggagaaaatgaacacgattaacatcgctaagaa3T7RB53SalFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaa3T7RB53SalFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaaT7SphIFPCR amplifies T7 RNA polymerase with no RBSacatgcatgcacacgattaacatcgctaagaacT7Stop900ApaRPCR amplifies T7 RNA polymerase and addscatagggcccttagtaaggaccacgatgaccagggacgacga stop codon at 900 bpT7RNAP133stopSphRPCR amplifies T7 RNA polymerase and addstacgcatgccttagtaagactcatgctcaagggccaaa stop codon at 133 bptacgcatgccttagtaagactcatgctcaagggccaaastop codon at 133 bp	ryhbBpkD4R	Deletes chromosomal RyhB using pKD13 and	tttgcaaaaagtgttggacaagtgcgaatgagaatgattattattgtc
rrnB1361FRT-PCR amplifies the control, rrfB sequencesgaatgccacggtgaatacgttrrnB1475RRT-PCR amplifies the control, rrfB sequencescacaagtggtaagcgccctsodBSalFPCR amplifies sodB sequence to fuse to gfpcagtcgaccatacgcacaataaggctattgtacgsodBSphRPCR amplifies 77 RNA polymerase with an RBS (st3)cctcccgggtaaggagaaaatgaacacgattaacatcgctaagaa3T7RB53SalFPCR amplifies 77 RNA polymerase with an RBS (st3)ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaa3T7RB53SalFPCR amplifies 77 RNA polymerase with an RBS (st3)ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaaT7SphIFPCR amplifies 77 RNA polymerase with no RBSacatgcatgcacacgattaacatcgctaagaacT7Stop900ApaRPCR amplifies 77 RNA polymerase and addscatagggcccttagtaaggaccagatgacgacgacgacgacgacgacgacgacgaccaggtcaaggaggacaatgaccaggtcaagggccaaT7RNAP133stopSphRPCR amplifies 77 RNA polymerase and addstacgcatgcttagtaagactcatgctcaagggccaaa stop codon at 133 bptacgcatgccttagtaagactcatgctcaagggccaa		the $\lambda$ -Red method	tcattccggggatccgtcgacc
rrnB1475RRT-PCR amplifies the control, rrfB sequencescacaaagtggtaagcgcctsodBSalFPCR amplifies sodB sequence to fuse to gfpcaagtcgaccatacgcacaataaggctattgtacgsodBSphRPCR amplifies sodB sequence to fuse to gfptacgcatgctgcggtacctttaatcaggttgtt3T7RB53XmaFPCR amplifies T7 RNA polymerase with an RBS (st3)cctcccgggtaaggaggaaaatgaacacgattaacatcgctaagaa3T7RB53SalFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaaT7SphIFPCR amplifies T7 RNA polymerase with no RBSacatgcatgcacacgattaacatcgctaagaacT7Stop900ApaRPCR amplifies T7 RNA polymerase and addscatagggcccttaagtaagcaccgatgaggagaaggagaaggagaaggagagaga	rrnB1361F	RT-PCR amplifies the control, <i>rrfB</i> sequences	gaatgccacggtgaatacgtt
sodBSalFPCR amplifies sodB sequence to fuse to gfpcaagtcgaccatacgcacaataaggctattgtacgsodBSphRPCR amplifies sodB sequence to fuse to gfptacgcatgctgcggtacctttaatcaggttgtt3T7RBS3XmaFPCR amplifies T7 RNA polymerase with an RBS (st3)cctcccgggtaaggaggaaaatgaacacgattaacatcgctaagaa3T7RBS3SalFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaaT7SphIFPCR amplifies T7 RNA polymerase with no RBSacatgcatgcacacgattaacatcgctaagaacT7Stop900ApaRPCR amplifies T7 RNA polymerase and addscatagggccctaagtacgcacaggggagga stop codon at 900 bptacgcatgcttagtaagactcatgctcaagggccaaT7RNAP133stopSphRPCR amplifies T7 RNA polymerase and addstacgcatgcttagtaagactcatgctcaagggccaaa stop codon at 133 bptacgcatgccttagtaagactcatgctcaagggccaatacgcatgcttagtaagactcatgctcaagggccaa	rrnB1475R	RT-PCR amplifies the control, <i>rrfB</i> sequences	cacaaagtggtaagcgccct
sodBSphRPCR amplifies sodB sequence to fuse to gfptacgcatgctcgcggtacctttaatcaggttgtt3T7RB53XmaFPCR amplifies T7 RNA polymerase with an RBS (st3)cctcccgggtaaggaggaaaatgaacacgattaacatcgctaagaa3T7RB53SalFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaaT7SphIFPCR amplifies T7 RNA polymerase with no RBSacatgcatgcacacgattaacatcgctaagaacT7Stop900ApaRPCR amplifies T7 RNA polymerase and addscatagggccctaagtacgcacaggggaggaggagggaggg	sodBSalF	PCR amplifies sodB sequence to fuse to gfp	caagtcgaccatacgcacaataaggctattgtacg
3T7RBS3XmaFPCR amplifies T7 RNA polymerase with an RBS (st3)cctcccggtaaggaggaaaatgaacacgattaacatcgctaagaa3T7RBS3SalFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaaT7SphIFPCR amplifies T7 RNA polymerase with no RBSacatgcatgcacacgattaacatcgctaagaacT7Stop900ApaRPCR amplifies T7 RNA polymerase and addscatagggcccttaagtacgcaccagggggaggaggagggggggg	sodBSphR	PCR amplifies sodB sequence to fuse to gfp	tacgcatgctcgcggtacctttaatcaggttgtt
3T7RBS3SalFPCR amplifies T7 RNA polymerase with an RBS (st3)ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaaT7SphIFPCR amplifies T7 RNA polymerase with no RBSacatgcatgcacacgattaacatcgctaagaacT7Stop900ApaRPCR amplifies T7 RNA polymerase and addscatagggcccttaagtacgcacaggggaggaggggggggg	3T7RBS3XmaF	PCR amplifies T7 RNA polymerase with an RBS (st3)	cctcccgggtaaggaggaaaatgaacacgattaacatcgctaagaa
T7SphIFPCR amplifies T7 RNA polymerase with no RBSacatgcatgcacacgattaacatcgctaagaacT7Stop900ApaRPCR amplifies T7 RNA polymerase and addscatagggcccttaagtacgcacaggggacga stop codon at 900 bpT7RNAP133stopSphRPCR amplifies T7 RNA polymerase and addstacgcatgcatgcacagggccaaa stop codon at 133 bp	3T7RBS3SalF	PCR amplifies T7 RNA polymerase with an RBS (st3)	ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaa
T7Stop900ApaR PCR amplifies T7 RNA polymerase and adds a stop codon at 900 bp catagggcccttaagtacgcaccagcgccagaggacg   T7RNAP133stopSphR PCR amplifies T7 RNA polymerase and adds a stop codon at 133 bp tacgcatgccttagtaagactcatgctcaagggccaa	T7SphIF	PCR amplifies T7 RNA polymerase with no RBS	acatgcatgcacacgattaacatcgctaagaac
T7RNAP133stopSphR PCR amplifies T7 RNA polymerase and adds tacgcatgccttagtaagactcatgctcaagggccaa a stop codon at 133 bp	T7Stop900ApaR	PCR amplifies T7 RNA polymerase and adds a stop codon at 900 bp	catagggcccttaagtacgcaccagcgccagaggacg
the second se	T7RNAP133stopSphR	PCR amplifies T7 RNA polymerase and adds a stop codon at 133 bp	tacgcatgccttagtaagactcatgctcaagggccaa