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 λ-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 (MathWorks). 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 (≈0.3 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 anti-rabbit IgG HRP (GE Healthcare Life Sciences) and donkey anti-goat 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:

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

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_{rrfB}) from five samples at five different dilutions. Measurements were performed in at least triplicate.

1. Urban JH, Vogel J (2007) Translational control and target recognition by Escherichia coli small RNAs in vivo. *Nucleic Acids Res* 35:1018–1037.
2. Argaman L, Altuvia S (2000) fhlA repression by OxyS RNA: Kissing complex formation at two sites results in a stable antisense-target RNA complex. *J Mol Biol* 300: 1101–1112.
3. Gardner TS, Cantor CR, Collins JJ (2000) Construction of a genetic toggle switch in Escherichia coli. *Nature* 403:339–342.
4. Shaner NC, et al. (2004) Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat Biotechnol* 22:1567–1572.
5. Lutz R, Bujard H (1997) Independent and tight regulation of transcriptional units in Escherichia coli via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements. *Nucleic Acids Res* 25:1203–1210.
6. Lanzer M, Bujard H (1988) Promoters largely determine the efficiency of repressor action. *Proc Natl Acad Sci USA* 85:8973–8977.

7. Vellanoweth RL, Rabinowitz JC (1992) The influence of ribosome-binding-site elements on translational efficiency in Bacillus subtilis and Escherichia coli in vivo. *Mol Microbiol* 6:1105–1114.

8. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proc Natl Acad Sci USA* 97:6640–6645.

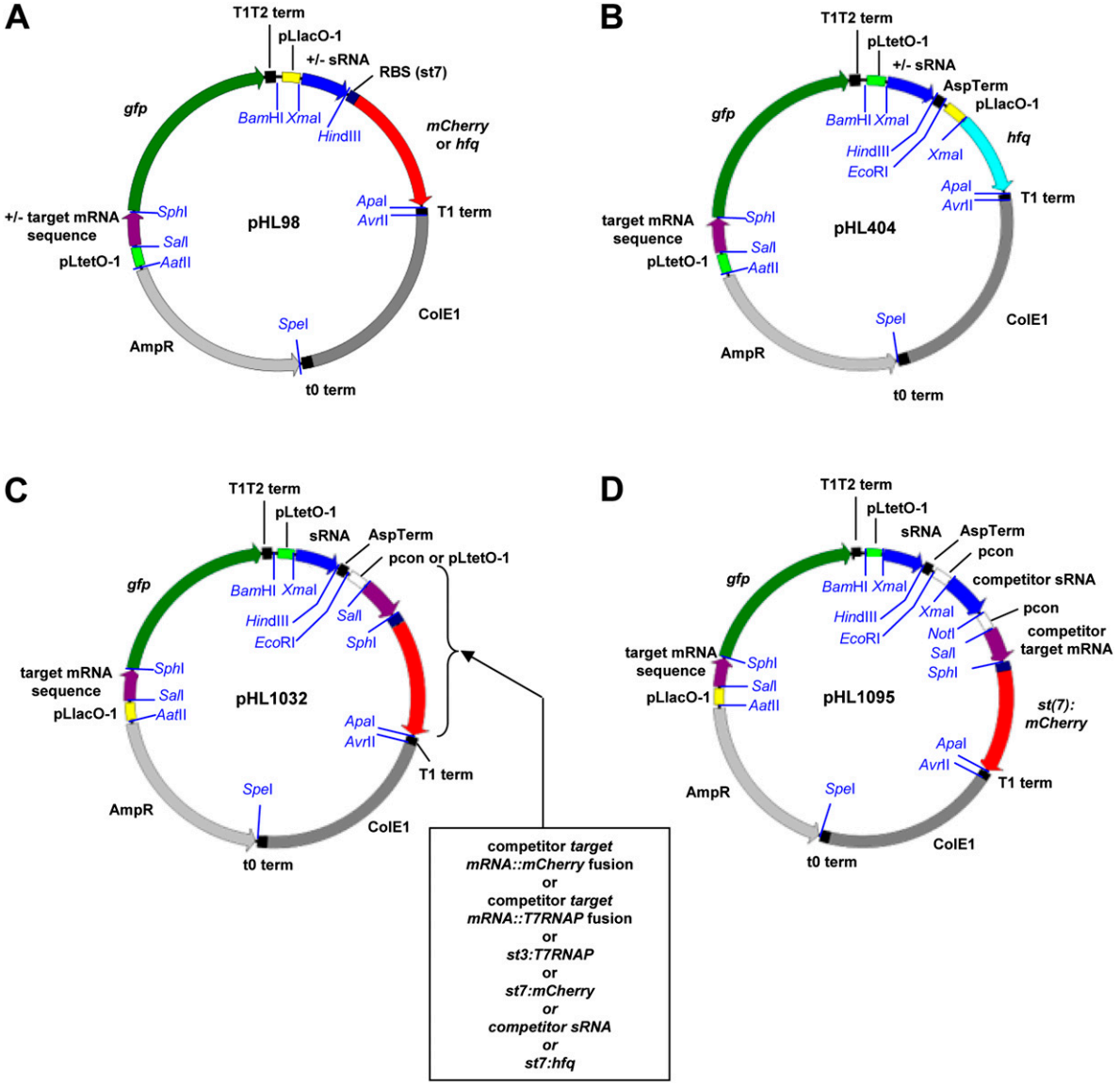

Fig. S1. Plasmid construction. The general construction of the plasmids used for the experiments is shown. (*A*) Plasmids shown in Figs. 1 *A–C*, 2*E*, and 3. (*B*) Plasmids shown in Figs. 1*D* and 2 *B–D*. (*C*) Plasmids shown in Fig. 4. (*D*) Plasmids shown in Fig. 5.

Table S1. Strains

Strain	Description
MG1655	Yale *E. coli* genetic stock center (CGSC#7740)
HL 1	MG1655 + pkd46
HL 716	HL 1 + *lacIq* inserted into the chromosome at the *int*S site
HL 770	HL 716 + Δ*hfq*
HL 818	HL 716 + pHL 98
HL 822	HL 716 + pHL 100
HL 839	HL 716 + pHL 108
HL 862	HL 716 + Δ*micC*
HL 865	HL 716 + Δ*dsrA*
HL 1128	HL 862 + Δ*hfq*
HL 1178	HL 862 + pHL 282
HL 1188	HL 865 + Δ*hfq*
HL 1329	HL 865 + pHL 344
HL 1474	HL 862A + pHL 404
HL 1543	HL 1188 + pHL 344
HL 2300	HL 716 + pHL 745
HL 2301	HL 716 + pHL 772
HL 2304	HL 716 + pHL 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 716 + pHL 908
HL 2627	HL 770 + pHL 908
HL 2752	HL 716 + Δ*rhyB*
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 716 + pHL 1070
HL 3089	HL 716 + pHL 1074
HL 3090	HL 716 + pHL 1035
HL 3091	HL 716 + pHL 1075
HL 3098	HL 716 + pHL 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 716 + pHL 1057
HL 3176	HL 716 + pHL 1039
HL 3186	HL 716 + pHL 282
HL 3187	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 + Δ*oxyS*
HL 3270	HL 716 + pHL 1158
HL 3326	HL 716 + pHL 1008
HL 3327	HL 716 + pHL 1009
HL 3328	HL 716 + pHL 1011
HL 3329	HL 770 + pHL 1008

Table S1. Cont.

Strain	Description
HL 3330	HL 770 + pHL 1009
HL 3331	HL 770 + pHL 1011
HL 3338	HL 770 + Δ*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 + Δ*oxyS*
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

Table S2. Plasmids

Plasmid	Description
pHL 67	*lacIq* from pTrc99a + ColE1 from pZE21 + KanR cassette from pKD13 (including the P1 and P4 oligonucleotide sites). Template for *lacIq* insertion into the genome.
pHL 98	pLlacO-1:DsrA-mCherry, pLtetO-1:*rpoS::gfp*
pHL 100	pLlacO-1:OxyS-mCherry, pLtetO-1:*fhlA::gfp*
pHL 108	pLlacO-1:MicC-mCherry, pLtetO-1:*ompC::gfp*
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:*rpoS::gfp*
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::gfp*
pHL 772	pLtetO-1:RhyB, pLtetO-1:*hfq*, pLlacO-1:*sodB::gfp*
pHL 777	pLtetO-1:MicC, pLtetO-1:RhyB, pLlacO-1:*ompC::gfp*
pHL 787	pLtetO-1:MicC, pLtetO-1:*rpoS*::mCherry, pLlacO-1:*ompC::gfp*
pHL 802	pLtetO-1:OxyS-mCherry, pLlacO-1:*ompC::gfp*
pHL 814	pLtetO-1:MicC, pLtetO-1:OxyS, pLlacO-1:*ompC::gfp*
pHL 818	pLtetO-1:MicC, pLtetO-1:*fhlA*::mCherry, pLlacO-1:*ompC::gfp*
pHL 841	pLtetO-1:RhyB-mCherry, pLlacO-1:*ompC::gfp*
pHL 908	pLlacO-1:RhyB::mCherry, pLtetO-1m9:*sodB*::gfp
pHL 990	pLtetO-1:DsrA, pcon:RhyB, pLlacO-1:*rpoS::gfp*
pHL 991	pLtetO-1:RhyB, pLlacO-1:*hfq*, pLtetO-1m9:*sodB::gfp*
pHL 1008	pLlacO-1:DsrA-mCherry, pLtetO-1:RBS (st7) gfp
pHL 1009	pLlacO-1:MicC-mCherry, pLtetO-1:RBS (st7) gfp
pHL 1011	pLlacO-1:RhyB-mCherry, pLtetO-1:RBS (st7) gfp
pHL 1019	pLtetO-1:RhyB, 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:*rpoS::gfp*
pHL 1057	pcon:RhyB, pLlacO-1:*rpoS::gfp*
pHL 1069	pcon:MicC, pLlacO-1:*sodB::gfp*
pHL 1070	pcon:DsrA, pLlacO-1:*sodB::gfp*
pHL 1073	pcon:*ompC*::mCherry, pLlacO-1:*sodB::gfp*
pHL 1074	pcon:*rpoS*::mCherry, pLlacO-1:*sodB::gfp*
pHL 1075	pcon:*fhlA*::mCherry, pLlacO-1:*sodB::gfp*
pHL 1095	pLtetO-1:RhyB, pcon:MicC, pcon:*ompC*::mCherry, pLlacO-1:*sodB::gfp*
pHL 1096	pLtetO-1:RhyB, pcon:DsrA, pcon:*rpoS*::mCherry, pLlacO-1:*sodB::gfp*
pHL 1147	pcon:OxyS, pLlacO-1:*rpoS::gfp*
pHL 1148	pcon:OxyS, pLlacO-1:*sodB::gfp*
pHL 1158	pLtetO-1:RhyB, pcon:DsrA, pcon:*ompC*::mCherry, pLlacO-1:*sodB::gfp*
pHL 1179	pLtetO-1:mCherry, pLlacO-1:*fhlA::gfp*
pHL 1191	pLlacO-1:OxyS-mCherry, pLtetO-1:RBS (st7) *gfp*
pHL 1213	pLlacO-1:*hfq*, pLtetO-1:RBS (st7) *gfp*
pHL 1228	pLtetO-1:DsrA-mCherry, pLlacO-1:*ompC::gfp*
pHL 1303	pLtetO-1:*sodB*::mCherry, pLlacO-1:*ompC::gfp*
pHL 1304	pLtetO-1:*rpoS*::mCherry, pLlacO-1:*ompC::gfp*
pHL 1305	pLtetO-1:*fhlA*::mCherry, pLlacO-1:*ompC::gfp*
pHL 1358	pLlacO-1:*hfq*, pLtetO-1m9:*sodB*::gfp
pHL 1360	pLlacO-1:*hfq*, pLtetO-1:*ompC::gfp*
pHL 1370	pLtetO-1:DsrA, pcon:T7(st3) (900bps), pLlacO-1:*rpoS::gfp*
pHL 1381	pLlacO-1:*hfq*, pLtetO-1:*rpoS::gfp*
pHL 1389	pLtetO-1:DsrA, pLlacO-1:*rpoS::gfp*
pHL 1391	pLlacO-1:*rpoS::gfp*
pHL 1394	pLtetO-1:RhyB, pcon:*T7*(st3) (133bps)::mCherry, pLlacO-1:*sodB::gfp*

Table S2. Cont.

Plasmid	Description
pHL 1405	pLtetO-1:RhyB, pcon:DsrA, pLtetO-1:*hfq*, pLlacO-1:*sodB::gfp*
pHL 1410	pLtetO-1:DsrA, pcon:*ompC::*T7noRBS(900bps), pLlacO-1:*rpoS::gfp*
pHL 1411	pLtetO-1:DsrA, pcon:*fhlA:*T7noRBS(900bps), pLlacO-1:*rpoS::gfp*
pHL 1413	pcon:*ompC::*T7noRBS(900bps), pLlacO-1:*rpoS::gfp*
pHL 1414	pcon:*fhlA::*T7noRBS(900bps), pLlacO-1:*rpoS::gfp*
pHL 1416	pcon:T7 (st3) (900bps), pLlacO-1:*rpoS::gfp*

Table S3. Oligonucleotides

Name	Function	Sequence
AatpLacSalF	Fuses pLac directly to *gfp*, with *Aat*II *and Sal*I sites	ctagacgtcataaatgtgagcggataacattgacattgtgagcgga taacaagatactgt
AspTermBamF	Clones Asp terminator from pLex	ggccggatcctttaatcgtacagggtagtacaaata
AspTermHindIIIF	Clones Asp terminator from pLex	ggccaagctttaatcgtacagggtagtacaaata
AspTermEcoRIR	Clones Asp terminator from pLex	ccggaattcactgctcacaagaaaaaaggcacg
ColEApaIF	Adds *Apa*I restriction site to ColE 1 origin	gacgggcccatggtacgcgtgctagagg
ColEBamHIF	Amplifies ColE origin with terminators from pZE21	cgcggatcccatggtacgcgtgctaga
ColeAatIIR	Amplifies ColE origin with terminators from pZE21	ctagacgtcgttcgagagctcgcttggactcct
DsrA1XmaIF	PCR amplifies DsrA with native terminator	tcctcccgggaacacatcagatttcctggtgtaa
DsrA2ApaR	PCR amplifies DsrA with native terminator	catgggcccagcgtctctgaagtgaatcgttga
DsrA3NoTermHindIIIR	RT-PCR amplifies DsrA without native terminator	ggccaagcttcccgaccctgaggggtcgggat
DsrAKO1pkD1F	Deletes chromosomal DsrA using pKD13 and the λ-Red method	atatggcgaatattttcttgtcagcgaaaaaaattgcggataaggtgatg gtgtaggctggagctgcttc
DsrAKO2pkD4R	Deletes chromosomal DsrA using pKD13 and the λ-Red method	tattcatgacttcagcgtctctgaagtgaatcgttgaatgcacaataa aaattccggggatccgtcgacc
DsrA2HindIIIR	PCR amplifies DsrA with native terminator	ggccaagcttagcgtctctgaagtgaatcgttga
DsrA2NotIR	PCR amplifies DsrA with native terminator	tcctgcggccgcagcgtctctgaagtgaatcgttga
FhlA1SalIF	PCR amplifies *fhlA* sequence to fuse to *gfp*	tactgtcgacgcgaattgctgggactggacgccc
FhlA3fusionSphIR	PCR amplifies *fhlA* sequence to fuse to *gfp*	tacgcatgctctcacacagcgaggccagatcggg
GfpRBSSalF	PCR amplifies *gfp* with *Sal*I site and RBS (st7)	cctgtcgactaaggaggaaaaaaaatgcgtaaaggagaagaacttttc
GFPseqR	RT-PCR amplifies mRNA sequences fused to *gfp*	gtatgttgcatcaccttcaccctctccactgacag
HfqNoStopSphR	PCR amplifies *hfq* sequence to fuse to *gfp*	ttacgcatgctttcggtttcttcgctgtcctgttg
HfqRBSXmaIF	PCR amplifies *hfq* with RBS (st7)	cctcccgggtaaggaggaaaaaaaatggctaaggggcaatctttacaag
HfqPromAatF	PCR amplifies *hfq,* native *hfq* promoter, and *hfq* leader region	cgcgacgtcgcgtgacgaagtattacaggttgtt
HfqApaIR	PCR amplifies *hfq* with RBS (st7)	gacgggcccttattcggtttcttcgctgtcctg
HfqHindR	PCR amplifies *hfq* with RBS (st7)	ggccaagcttattcggtttcttcgctgtcctg
HfqpkD1F	Deletes chromosomal *hfq* using pKD13 and the λ-Red method	tcagaatcgaaaggttcaaagtacaaataagcatataaggaaaagagag agtgtaggctggagctgcttc
HfqpkD4R	Deletes chromosomal *hfq* using pKD13 and the λ-Red method	ggaacgcaggatcgctggctccccgtgtaaaaaaacagcccgaaacctta attccggggatccgtcgacc
IntSpKD1F	PCR amplifies *lacIq* and the KanR cassette from pHL67 to insert at the *intS* site	ccgtagatttacagttcgtcatggttcgcttcagatcgttgacagccgcag tgtaggctggagctgcttc
LacIQIntS	PCR amplifies *lacIq* and KanR cassette from pHL67 to insert at *intS* site	atagttgttaaggtcgctcactccaccttctcatcaagccagtccgccc agctaactcacattaattgcgttgc
mCherryRBSBsiWIHindIIIF	PCR amplifies mCherry with RBS (st7)	tcttaaaagcttattaaagaggagaaacgtacgatggtgagcaagggc gaggagg
mCherryCYFPRBSSphXmaIF	Clones mCherry in the absence of an sRNA with *Xma*I and *Apa*I sites	tacgcatgccctcccgggtaaggaggaaaaaaaatggtgagcaaggg cgaggag
mCherryCYFPRBSXmaSphIF	Clones mCherry fused to target mRNA with *Sph*I and *Apa*I sites	cctcccgggtacgcatgctaaggaggaaaaaaaatggtgagcaagg gcgaggag
mCherryApaIR	PCR amplifies mCherry with RBS (st7)	catgggcccttacttgtacagctcgtccatgcc
MicC1XmaIF	PCR amplifies MicC with native terminator	tcctcccgggttatatgcctttattgtcacaga
MicC2ApaR	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	atacaaaataaaaattatactttaatttgctatacgttattctgcgcgggtgtagg ctggagctgcttc
MicCKO2pkD4R	Deletes chromosomal MicC using pKD13 and the λ-Red method	aaaaagcaacaccgattaaatgctctggataaggattatccaattctaaaattccg gggatccgtcgacc
OxySXmaIF	PCR amplifies OxyS with native terminator	tcctcccgggaaacggagcggcacctcttttaa
OxySApaR	PCR amplifies OxyS with native terminator	taagggccctttgagcctggcttatcgccgggc
OxySHindIIR	PCR amplifies OxyS with native terminator	ggccaagctttgagcctggcttatcgccgggc
OxySNotIR	PCR amplifies OxyS with native terminator	tcctgcggccgctgagcctggcttatcgccgggc
OxySNoTermHindIIIR	RT-PCR amplifies OxyS without native terminator	ggccaagcttgcggatcctggagatccgcaaaag
OxyS1pkD1F	Deletes chromosomal OxyS using pKD13 and the λ-Red method	agcaatgaacgattatccctatcaagcattctgactgataattgctcacagtgta ggctggagctgcttc
OxyS2pkD4R	Deletes chromosomal OxyS using pKD13 and the λ-Red method	atttatatgtataaatttgagcctggcttatcgccgggctttttttatggc attccggggatccgtcgacc
partpTetOMicCXmaF	First round PCR cloning of pLtetO-1 upstream of MicC	gattgacatccctatcagtgatagagatactcccgggttatatgcctttat tgtcacaga
PconBamF	PCR synthesis of pCon promoter	cgcggatcctcgagcaccgtcgttgttgacatttttaagcttggcggttataat

Table S3. Cont.

Name	Function	Sequence
PconEcoRF	PCR synthesis of pCon promoter	ccggaattctcgagcaccgtcgttgttgacatttttaagcttggcggttataat
PconNoHindBamHF	PCR synthesis of pCon promoter with no *Hin*dIII site	cgcggatcctcgagcaccgtcgttgttgacatttttatgcttggcggttataat
PconNoHindEcoRF	PCR synthesis of pCon promoter with no *Hin*dIII site	ccggaattctcgagcaccgtcgttgttgacatttttatgcttggcggttataat
PconNoHindXmaR	PCR synthesis of pCon promoter with no *Hin*dIII site	cctcccgggtgtgtggaatccattataaccgccaagcataaaaaatgtcaacaac
PconNoHindNotIHF	PCR synthesis of pCon promoter with no *Hin*dIII site	tcctgcggccgctcgagcaccgtcgttgttgacatttttatgcttggcggttataat
PconXmaR	PCR synthesis of pCon promoter	cctcccgggtgtgtggaatccattataaccgccaagcttaaaaatgtcaacaac
pLacEcoRF	PCR synthesis of pLlacO-1 promoter with *Eco*RI	ccggaattcgatccataaatgtgagcggataacattgacattg
pLacXmaBamHT1T2R	PCR synthesis of pLlacO-1 promoter with *Bam*HI and *Xma*I sites	tcctcccgggagtatcttgttatccgctcacaatgtcaatgttatccgctcacattta tggatcccctaggtctagggcggcggatttg
pTetO-1SalIR	PCR synthesis of pLtetO-1 promoter with *Sal*I	tccctatcagtgatagagattgacatccctatcagtgatagagatactgtcgac
pLtetO1m9SalR	Mutates pLtetO-1 promoter by PCR	caagtcgacagtctctctatcactgatagggatgtcaatct
ptetOBamHIF	PCR synthesis of pLtetO-1 promoter with *Bam*HI	cgcggatcctccctatcagtgatagagattgacatccctatcagtgatag
pTetOXmaIR	PCR synthesis of pLtetO-1 promoter with *Xma*I	cctcccgggagtatctctatcactgatagggatgtcaatctctatcactg
pOmpC1SalIF	PCR amplifies *ompC* sequence to fuse to *gfp*	tactgtcgacttgccgactgattaatgagggtta
pOmpC2SphIR	PCR amplifies *ompC* sequence to fuse to *gfp*	tacgcatgctagctgggaccaggagggacagtac
PtsGgfpSphISalIF	PCR amplifies *gfp* (*ptsg* sequence in oligo removed with *Sal*I and *Sph*I)	tactgtcgaccacgcgtgagaacgtaaaaaaagcacccatactcaggagcactct caagcatgcgtaaaggagaagaacttttcact
RpoS-150SalIF	PCR amplifies *rpoS* sequence to fuse to *gfp*	tactgtcgacaccggaaccagttcaacacgcttgcatttttg
RpoS30SphIR	PCR amplifies *rpoS* sequence to fuse to *gfp*	tacgcatgctatcatgaactttcagcgtattctg
rhyBXmaF	PCR amplifies RyhB with native terminator	cctcccggggcgatcaggaagaccctcgcggag
rhyBApaR	PCR amplifies RyhB with native terminator	taagggcccgtggataaattgagaacgaaagat
rhyBHindR	PCR amplifies RyhB with native terminator	ggccaagcttgtggataaattgagaacgaaagat
rhyBNotIR	PCR amplifies RyhB with native terminator	tcctgcggccgcgtggataaattgagaacgaaagat
ryhBnotermR	RT-PCR amplifies RyhB without native terminator	ctaagtaatactggaagcaatgtg
ryhBpkD1F	Deletes chromosomal RyhB using pKD13 and the λ-Red method	taacgaacacaagcactcccgtggataaattgagaacgaaagatcaaaa agtgtaggctggagctgcttc
ryhbBpkD4R	Deletes chromosomal RyhB using pKD13 and the λ-Red method	tttgcaaaaagtgttggacaagtgcgaatgagaatgattattattgtc tcattccggggatccgtcgacc
rrnB1361F	RT-PCR amplifies the control, *rrfB* sequences	gaatgccacggtgaatacgtt
rrnB1475R	RT-PCR amplifies the control, *rrfB* sequences	cacaaagtggtaagcgccct
sodBSalF	PCR amplifies *sodB* sequence to fuse to *gfp*	caagtcgaccatacgcacaataaggctattgtacg
sodBSphR	PCR amplifies *sodB* sequence to fuse to *gfp*	tacgcatgctcgcggtacctttaatcaggttgtt
3T7RBS3XmaF	PCR amplifies T7 RNA polymerase with an RBS (st3)	cctcccgggtaaggaggaaaatgaacacgattaacatcgctaagaa
3T7RBS3SalF	PCR amplifies T7 RNA polymerase with an RBS (st3)	ttagtcgactaaggaggaaaatgaacacgattaacatcgctaagaa
T7SphIF	PCR amplifies T7 RNA polymerase with no RBS	acatgcatgcacacgattaacatcgctaagaac
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