# Supporting Information

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### SI Materials and Methods

**Bacterial Strains and Plasmids.** Bacterial strains and plasmids are listed in Table S2, and DNA primers are listed in Table S3.

**Strain and Plasmid Construction.** ***Construction of the Δ10TA strains.*** We previously developed in our laboratory a parent strain deleted of five TA loci (*relBE*, *chpB*, *mazF*, *dinJ/yafQ*, and *yefM/yoeB*) (11). Here, we deleted the five newly described TA pairs of *E. coli* K-12 (*yafNO*, *hicAB*, *higBA, prlf/yhaV*, and *MqsRA*) (2, 5, 6). To minimize deleterious effects of leaving an Flippase Recognition Target scar-sequence at each deletion, we used a marker- and scarless-deletion procedure also developed by the Wanner laboratory (12). First, the TA pair to be deleted was replaced by an *aphA* (KanR)-encoding gene cassette. The cassette also contained the toxin gene *parE* of RK2 (13) under the control of a rhamnose-inducible promoter. The *aphA-parE* cassette was then removed by using a combined PCR product of the flanking regions of the integrated cassette. By counterselection on rhamnose-containing plates, where the toxic gene *parE* is induced, we were able to select for positive marker and scarless transformants. After deletion of one TA pair, we sequentially removed the remaining TA systems in the same manner, resulting in the Δ*10TA* strain (MG1655 Δ*mazF* Δ*chpB* Δ*relBE* Δ*(dinJ-yafQ)* Δ(*yefM-yoeB*) Δ*higBA* Δ(*prlF-yhaV*) Δ*yafNO* Δ*mqsRA* Δ*hicAB*). We next describe in detail how one TA locus (*higBA*) was deleted by the marker and scarless method.

Briefly, cells were transformed with plasmid pKD46 carrying the arabinose-inducible λ*red* gene. pKD46-containing cells were grown in LB at 30 °C to an $OD_{450}$ of 0.4. The λ*red* was induced by addition of 0.2% arabinose for 20 min. Cells were made electrocompetent by several wash steps with ice-cold $H_2O$. The cells were electroporated with a purified PCR product amplified from pKD267 containing homologous ends flanking the *higBA* operon and plated on solid medium containing kanamycin (25 μg/mL). Kanamycin-resistant colonies were retransformed with pKD46 and made electrocompetent. Cells were electroporated with a purified PCR product containing flanking regions of the *higBA* operon. The PCR product was made in two steps in the following way. A first PCR product was generated using primers higBA-up-f and higBA-up-r, and a second PCR product was then generated using primers higBA-down-f and higBA-down-r. The two products were purified and mixed 1:1 in a second round of PCR using primers higBA up-f and higBA-down-r. Electroporated cells were plated on M9 minimal plates containing 0.5% rhamnose as the only carbon source.

***Construction of strain EJM46.*** A P1 lysate was made from JW2990 (BW25113 Δ*mqsR::kan*), and the *kan* allele was transduced into MG1655 Δ*hicAB::frt*. The kanamycin resistance cassette was removed using the pCP20 plasmid (14).

***Construction of EJM467.*** A P1 lysate was made from JW0223 (BW25113 Δ*yafO::kan*), and the *kan* allele was transduced into EJM46. The kanamycin resistance cassette was removed using the pCP20 plasmid (14).

***Construction of EJM4678.*** A P1 lysate was made from JW3099 (BW25113 Δ*yhaV::kan*), and the *kan* allele was transduced into EJM467. The kanamycin resistance cassette was removed using the pCP20 plasmid (14).

***Construction of EJM46789.*** A P1 lysate was made from JW3054 (BW25113 Δ*higB::kan*), and the *kan* allele was transduced into EJM4678. The kanamycin resistance cassette was removed using the pCP20 plasmid (14).

***Construction of MO11.*** An EcoRI-SalI fragment from pMO227 plasmid carrying the *relB-relE*$^{R81A}$*::DsRed2* gene fusion was ligated with an EcoRI-SalI fragment of plasmid pTAC3590 containing the *aphA* ($Kan^R$) gene and λ *attP*, thereby generating circular DNA substrates for integration into the chromosome at *attB* (15).

***Construction of pCA24N-lon*$^{K362Q}$ *and pCA24N-lon*$^{S679A}$.** The GFP gene from pCA24N (16) has been removed by NotI digestion, followed by self-ligation. The *lon* gene was amplified from pBAD::*lon*$^{K362Q}$ or pBAD::*lon*$^{S679A}$ (17) with primers lon-f and lon-r (Table S3). Primers were designed as described by Kitagawa et al. (16) and were phosphorylated before being used in PCR. The PCR products were individually ligated with vector pCA24N (GFP-free) that had been digested with StuI and dephosphorylated.

***Construction of plasmid pMO227.*** Plasmid pMGJ4004 was digested with BamHI and StuI to generate an ∼8.5-kb fragment, including R1 origin and *bla*. pMO229 was then used as a PCR template, together with DsRed2-BamHI-SD8-f (Table S3); BamHI-tailed forward primer, which places a strong SD8 Ribosome Binding Site in front of DsRed2 (Clontech); and DsRed2-EcoRV-r (Table S3) EcoRV-tailed reverse primer for DsRed2. The PCR product was combined with the BamHI-StuI fragment of pMGJ4004, leading to pMO227 (*relB-relE*$^{R81A}$*::DsRed2*).

***Construction of plasmid pEJM10.*** A DNA fragment encoding *yefM* and six histidine codons at the 5′ end of *yefM* was generated by PCR using *yefM-f* and yefM-r (Table S3) as oligonucleotides. The fragment was cut with EcoRI and BamH1 and ligated with pMG25 digested with the same enzymes.

**Growth Conditions and Media.** Cells were grown in LB at 37 °C with shaking. When appropriate, the medium was supplemented with chloramphenicol (50 μg/mL; Sigma). The $P_{T5\text{-}lac}$ promoter was induced by the addition of 100 μM isopropyl β-D-1-thiogalactopyranoside (IPTG), and the $P_{BAD}$ promoter was induced by the addition of 0.2% arabinose.

**Persister Cell Assay.** Persistence was measured by determining the number of cfu/mL on exposure to 1 μg/mL ciprofloxacin or 100 μg/mL ampicillin (Sigma). Overnight cultures were diluted 100-fold in 10 mL of fresh medium in a 100-mL flask and incubated for 2.5 h at 37 °C with shaking (typically reaching ∼$2 \times 10^8$ cfu/mL). Aliquots of 5 mL were then transferred in 28 × 114-mm Sarstedt canonical polypropylene tubes, and antibiotics were added at the indicated concentrations. Tubes were placed at an inclination of 45 °C with shaking at 37 °C for 5 h. For determination of cfu counts, 1-mL aliquots were removed at the indicated time and the cells were harvested, resuspended in fresh medium, serially diluted, and plated on solid medium. Persisters were calculated as the surviving fraction by dividing the number of cfu/mL in the culture after 5 h of incubation with the antibiotic by the number of cfu/mL in the culture before adding the antibiotic.

**Determination of Persister in Cells Overexpressing mRNases.** To determine the number of persisters formed by cells expressing mRNases from plasmid pBAD33, cells were grown in rich medium for 1.5 h and mRNase production was induced by the addition of 0.2% of arabinose for 45 min. Then, 0.2% of glucose was added to repress pBAD promoter, and cells were grown for an additional 15 min. Next, 5-mL aliquots were subjected to antibiotic (ampicillin or ciprofloxacin) and persister cell formation determined as described above, except that the cells were plated on solid medium 0.2% glucose (without antibiotics).

**Determination of Persisters in Cells Overexpressing Lon.** To determine the number of persisters formed by cells overexpressing Lon or its mutant forms ($Lon^{K362Q}$ or $Lon^{S679A}$) from pAC24N, cells were grown in rich medium as described above but with the addition of 100 μM IPTG for 2.5 h. At this relatively low level of induction, cell growth was indistinguishable from that of the uninduced culture. Then, 5-mL aliquots were subjected to antibiotic (ampicillin or ciprofloxacin) and persister cell formation was determined as described above.

**Microscopy.** For phase-contrast and fluorescence microscopy, 1-mL culture samples were taken at each time point, centrifuged for 5 min at 1,200 × *g*, and then resuspended in 100 μL of LB. Next, 1–3 μL of the resuspended culture was placed on a microscope slide coated with a thin LB agarose (1%) layer and then covered with a coverslip. Prepared microscope slides were warmed before use to minimize shock to the cells. Images were acquired with a Cool-Snap HQ cooled CCD camera (Roper Scientific) attached to a DeltaVision microscope (Applied Precision). The images were acquired and analyzed with the softWoRx (IMSOL) computer program using a bright-field channel first and then a red fluorescence filter channel. Final image preparation was performed in ImageJ (National Institutes of Health).

**MIC Determination.** MICs for ampicillin and ciprofloxacin were determined by the agar dilution method (18). Briefly, inoculums of ~$10^4$ cfu were spotted by micropipette, delivering 3 μL to the LB-agar plates containing a range of antibiotic concentrations. The MIC was the lowest concentration of drug that prevented visible growth after 24 h of incubation at 37 °C.

**In Vivo YefM Degradation and Western Blot Analysis.** The degradation rate of His6-YefM was determined using samples from exponentially growing cells. Expression of His6-YefM protein from pEJM10 was induced by the addition of 1 mM IPTG at an $OD_{600}$ of 0.4. After 30 min of induction, protein synthesis was stopped by addition of 100 μg/mL chloramphenicol, and samples were removed at the indicated time points. His6-YefM protein was detected by Western blotting using a monoclonal His-tag antibody (Qiagen) and a polyclonal goat-anti mouse IgG HRP conjugate (Sigma–Aldrich).


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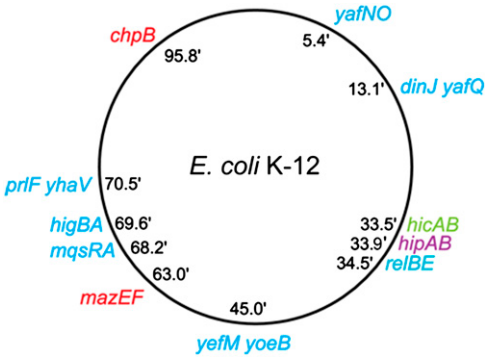


**Fig. S1.** Map positions of the 11 known type II TA loci of *E. coli* K-12. The 7 TA loci encoding mRNases belonging to the RelE superfamily are shown in blue, those belonging to the MazF family are shown in red, and those belonging to the HicA family are shown in green. Most RelE-like mRNases (RelE, YoeB, HigB, YhaV, YafO, and YafQ) cleave mRNAs positioned at the ribosome, between the second and third A-site bases (1–5). MazF, ChpB, MqsR, and HicA cleave mRNAs site-specifically, independent of the ribosomes (6–8). The *hipAB* locus that encodes HipA, an inhibitor of Elongation Factor Tu (9), is shown in magenta. In all cases, transcription of the TA operons is regulated by the antitoxins that bind to operator sequences in the promoter regions. The mRNases act as corepressors of transcription. Moreover, excess mRNase relative to antitoxin derepresses TA operon transcription by destabilizing the binding of the TA complexes to the promoter regions (10).

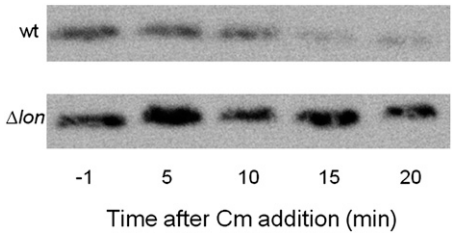


**Fig. S2.** Lon protease degrades antitoxin YefM. N-terminal hexa-histidine (his6)-tagged YefM was expressed from pEJM10 in the *wt* strain and its Δ*lon* derivative. Cells were grown in LB, and at an $OD_{600}$ of 0.4, 1 mM IPTG was added. After 30 min of induction, protein synthesis was stopped by the addition of 100 μg/mL chloramphenicol, and samples for Western blotting were withdrawn over the course of 20 min. Cm, chloramphenicol.

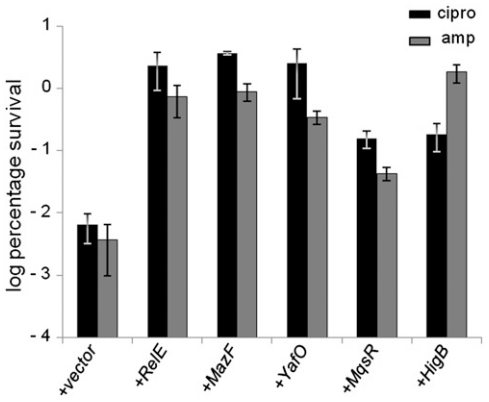


**Fig. S3.** Toxin overexpression induces persisters. Each mRNase was expressed independent of a plasmid with a tightly regulated arabinose-inducible promoter (using vector plasmid pBAD33), and the levels of persister cells generated by exponentially growing cultures were measured. MG1655 (*wt*) carrying the vector plasmid or one of the mRNase-encoding plasmids was grown exponentially and exposed to 1 μg/mL ciprofloxacin (black bars) or 100 μg/mL ampicillin (gray bars) after 45 min of induction by arabinose (0.2%), followed by quenching of induction by glucose (0.2%) (details are provided in *SI Materials and Methods*). The percentage of survival after 5 h was compared with that of the control strain carrying the empty vector. The graph shows averages of four independent experiments; error bars indicate the SD. amp, ampicillin; cipro, ciprofloxacin.

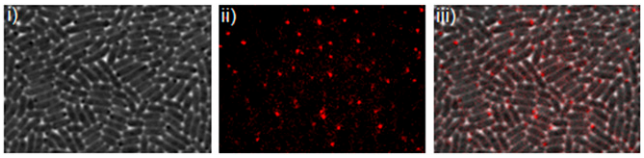


**Fig. S4.** Titration of RelB by RelE$^{cs6}$ in a strain lacking *wt relBE* (MO12). RelE$^{cs6}$ overexpression from plasmid pKP3103 in strain MO12 (Δ*relBE relBE*$^{R81A}$*::dsRed2* ≡ MO11Δ*relBE*) was induced by arabinose (0.2%) for 2 h, and cells were analyzed by microscopy. As seen, more than 50% of the cells of MO12 became fluorescent. This high fraction should be compared with the much lower fraction (0.083%) seen with strain MO11, supporting the interpretation that endogenous RelE encoded by *relBE* in MO11 is activated and inhibits translation and formation of DsRed2; hence, there is a very low fraction of fluorescent cells in MO11 but a high fraction in MO12. Images represent phase contrast (i; *Left*), fluorescence (ii, *Center*), and merged (iii; *Right*) images, respectively.

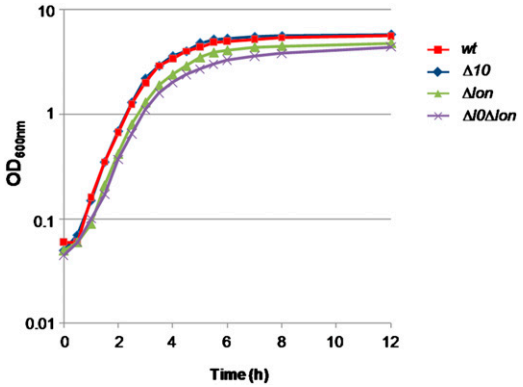


**Fig. S5.** Growth curves of strains MG1655 (*wt*) and its Δ*10TA,* Δ*lon*, and Δ*10TA*Δ*lon* derivatives. Cells were grown in LB at 37 °C for more than 12 h.

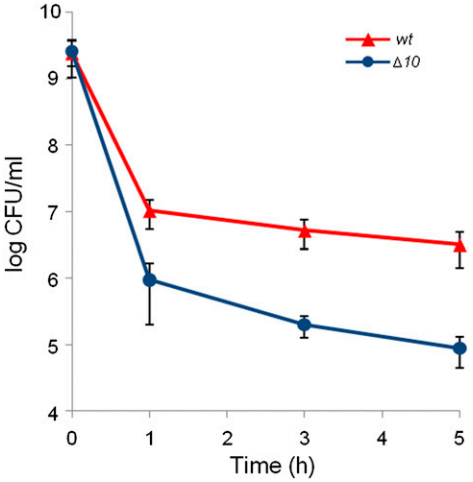


**Fig. S6.** Persisters generated by *wt* and Δ*10TA* strains in stationary phase. Stationary phase cultures (12 h) of MG1655 (*wt*) and its Δ*10TA* derivative were exposed to 1 μg/mL ciprofloxacin. Numbers of surviving cells were determined by plating on solid medium. The graphs show averages of three independent experiments; error bars indicate the SD.

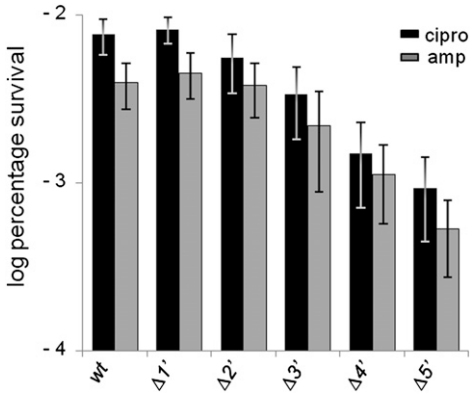


**Fig. S7.** Cumulative contribution of mRNases to persister cell formation. Exponentially growing cells of MG1655 carrying increasing numbers of deletions of TA genes encoding mRNases (the antitoxin genes were not deleted except in the case of *hicB*; in such case, both *hicA* and *hicB* were deleted) were exposed to 1 μg/mL ciprofloxacin (black bars) or 100 μg/mL ampicillin (gray bars). The percentage of survival after 5 h of antibiotic treatment was compared with that of the *wt* strain (log scale). The bars show averages of at least three independent experiments; error bars indicate the SD. The genotypes of Δ*1′ to* Δ*5′* strains are listed in Table S2. amp, ampicillin; cipro, ciprofloxacin.

**Table S1. MICs**

| | MICs* (mean ± SD) | |
|---|---|---|
| Strain | Ciprofloxacin, ng/mL | Ampicillin, μg/mL |
| *wt* | 5.3 ± 0.45 | 3.4 ± 0.42 |
| Δ*10* | 5.0 ± 0.35 | 3.2 ± 0.27 |
| Δ*lon* | 2.8 ± 0.27 | 5.7 ± 0.75 |
| Δ*10* Δ*lon* | 2.5 ± 0.35 | 5.4 ± 0.42 |

*MICs were determined as described in *SI Materials and Methods*.

**Table S2. Strains and plasmids**

| Strains/plasmids | Genotype/plasmid properties | Source |
|---|---|---|
| MG1655 | *E. coli* K-12 (*wt*); *rph1* | 19 |
| TB28 | MG1655 Δ*lac* | 20 |
| SC30 | MG1655 Δ*mazF** | 21 |
| SC31 (*Δ1TA*) | MG1655 Δ*chpB* | 21 |
| SC34 | MG1655 Δ*relBE* | 21 |
| SC36 | MG1655 Δ(*yefM-yoeB*) | 11 |
| SC37 | MG1655 Δ(*dinJ-yafQ*) | 11 |
| MCD2801 | TB28Δ*yafNO*::*kan* | 2 |
| MCD2802 | TB28Δ*higAB*::*kan* | 2 |
| MCD2803 | TB28Δ*mqsRA*::*kan* | 2 |
| MG1655 Δ*yafNO* | MG1655 Δ*yafNO*::*kan* | P1 MCD2801 x MG1655 |
| MG1655 Δ*higAB* | MG1655 Δ*higAB*::*kan* | P1 MCD2802 x MG1655 |
| MG1655 Δ*mqsRA* | MG1655 Δ*mqsRA*::*kan* | P1 MCD2803 x MG1655 |
| MG1655 Δ*hicAB (Δ1′)* | MG1655 Δ*hicAB*::*frt* | 6 |
| MG1655 Δ*yhaV* | MG1655 Δ*yhaV*::*kan* | P1 JW3099 x MG1655 |
| SC301 (*Δ2TA*) | MG1655 Δ*mazF** Δ*chpB* | 11 |
| SC3410 (*Δ3TA*) | MG1655 Δ*mazF** Δ*chpB* Δ*relBE* | 11 |
| SC30146 (*Δ4TA*) | MG1655 Δ*mazF** Δ*chpB* Δ*relBE* Δ*yefM/yoeB* | 11 |
| SC301467 (*Δ5TA*) | MG1655 Δ*mazF**Δ*chpB*Δ*relBE*Δ(*dinJ-yafQ*) Δ(*yefM-yoeB*) | 11 |
| MGJ59 (*Δ6TA*) | MG1655 Δ*mazF** Δ*chpB* Δ*relBE* Δ(*dinJ-yafQ*) Δ(*yefM-yoeB*) Δ*higBA* | This work |
| MGJ598 (*Δ7TA*) | MG1655 Δ*mazF** Δ*chpB* Δ*relBE* Δ(*dinJ-yafQ*) Δ(*yefM-yoeB*) Δ*higBA* Δ(*prlF-yhaV*) | This work |
| MGJ5987 (*Δ8TA*) | MG1655 Δ*mazF** Δ*chpB* Δ*relBE* Δ(*dinJ-yafQ*) Δ(*yefM-yoeB*) Δ*higBA* Δ(*prlF-yhaV*) Δ*yafNO* | This work |
| MGJ5987 (*Δ9TA*) | MG1655 Δ*mazF** Δ*chpB* Δ*relBE* Δ(*dinJ-yafQ*) Δ(*yefM-yoeB*) Δ*higBA* Δ(*prlF-yhaV*) Δ*yafNO* Δ*mqsRA* | This work |
| MGJ5987 (*Δ10TA*) | MG1655 Δ*mazF** Δ*chpB* Δ*relBE* Δ(*dinJ-yafQ*) Δ(*yefM-yoeB*) Δ*higBA* Δ(*prlF-yhaV*) Δ*yafNO* Δ*mqsRA* Δ*hicAB* | This work |
| JW0428 | BW25113 Δ*clpX*::*kan* | 14 |
| JW0427 | BW25113 Δ*clpP*::*kan* | 14 |
| JW0866 | BW25113 Δ*clpA*::*kan* | 14 |
| JW3903 | BW25113 Δ*hslV*::*kan* | 14 |
| JW3902 | BW25113 Δ*hslU*::*kan* | 14 |
| JW3099 | BW25113 Δ*yhaV*::*kan* | 14 |
| JW2990 | BW25113 Δ*mqsR*::*kan* | 14 |
| JW0223 | BW25113 Δ*yafO*::*kan* | 14 |
| JW3054 | BW25113 Δ*higB*::*kan* | 14 |
| MG1655 Δ*clpX* | MG1655 Δ*clpX*::*kan* | P1 JW0428 × MG1655 |
| MG1655 Δ*clpP* | MG1655 Δ*clpP*::*kan* | P1 JW0427 × MG1655 |
| MG1655 Δ*clpA* | MG1655 Δ*clpA*::*kan* | P1 JW0866 × MG1655 |
| MG1655 Δ*hslV* | MG1655 Δ*hslV*::*kan* | P1 JW3903 × MG1655 |
| MG1655 Δ*hslU* | MG1655 Δ*hslU*::*kan* | P1 JW3902 × MG1655 |
| MG1655 Δ*lon* | MG1655 Δ*lon*::*tet* | 22 |
| *Δ10*Δ*lon* | MG1655 Δ*mazF** Δ*chpB* Δ*relBE* Δ(*dinJ-yafQ*) Δ(*yefM-yoeB*) Δ*higBA* Δ(*prlF-yhaV*) Δ*yafNO* Δ*mqsRA* Δ*hicAB* Δ*lon*::*tet* | P1 MG1655 Δ*lon* × MGJ5987 |
| EJM46 (*Δ2′*) | MG1655 Δ*hicAB*::*frt* Δ*mqsR*::*frt* | This work |
| EJM467 (*Δ3′*) | MG1655 Δ*hicAB*::*frt* Δ*mqsR*::*frt* Δ*yafO*::*frt* | This work |
| EJM4678 (*Δ4′*) | MG1655 Δ*hicAB*::*frt* Δ*mqsR*::*frt* Δ*yafO*::*frt* Δ*yhaV*::*frt* | This work |
| EJM46789 (*Δ5′*) | MG1655 Δ*hicAB*::*frt* Δ*mqsR*::*frt* Δ*yafO*::*frt* Δ*yhaV*::*frt* Δ*higB*::*frt* | This work |
| MO11 | MG1655 *attB*:: *relB-relE*$^{R81A}$::*DsRed2* | This work |
| MO12 | MG1655Δ*relBE attB*:: *relB-relE*$^{R81A}$::*DsRed2* | This work |
| pBAD33 | p15A; *cat*, pBAD promoter | 23 |
| pKP3035 | pBAD33; pBAD::*relE* | 24 |
| pKP3103 | pBAD33; pBAD::*his6::relE*$^{cs6}$ | 24 |
| pMCD3326 | pBAD33; pBAD::SD$_{opt}$::*mazF* | 1 |
| pMCD3312 | pBAD33; pBAD::SD::*mqsR* | 2 |
| pMCD3306 | pBAD33; pBAD::SD::*yafO* | 2 |
| pMCD3310 | pBAD33; pBAD::SD::*higB* | 2 |
| pCA24N | *cat*; *lacI*$^{q}$, pCA24N** | 16 |
| pCA24N::*lon* | *cat*; *lacI*$^{q}$, pCA24N P$_{T5\text{-}lac}$::*lon*$^{\dagger}$ | This work |
| pCA24N::*lonK362Q* | *cat*; *lacI*$^{q}$, pCA24N P$_{T5\text{-}lac}$::*lon*$^{K362Q\dagger}$ | This work |
| pCA24N::*lonS679A* | *cat*; *lacI*$^{q}$, pCA24N P$_{T5\text{-}lac}$::*lon*$^{S679A\dagger}$ | This work |
| pTAC3590 | pBR322; *attλ* | 15 |

**Table S2. Cont.**

| Strains/plasmids | Genotype/plasmid properties | Source |
|---|---|---|
| pMGJ4004 | pOU254, *bla*, *relBE*$^{R81A}$::*lacZYA* | 10 |
| pMO229 | pBlueScript SK$^{-}$ *DsRed2* | Laboratory collection |
| pMO227 | pMGJ4004 *relB-relE*$^{R81A}$::*DsRed2* | This work |
| pMG25 | pUC *bla lacI*$^{q}$ pA1/O4/O3 | Laboratory collection |
| pEJM10 | pMG25 SD$_{OPT}$::*his*$_6$::*yefM* | This work |

*In the case of the *mazEF* locus, we only deleted the *mazF* gene because we found that our *mazEF* deletion strain exhibited a partially relaxed phenotype, probably attributable to an effect on expression of *relA* that is located 80 bp upstream of *mazE*.
†GFP-free.

**Table S3. DNA primers**

| | |
|---|---|
| higBA deletion-f | 5′ ACATTCTCTTGTTTAGCGTTTTTCTACGTTTATTCTTCCGTCACACAGATCTCTACGCCGGACGCATCGTG |
| higBA deletion-r | 5′ GGAGATTTAAATCGTTATTTGAAGCGCCGGATGCAACGCATCCGGCACGTACTGATCAGTGATAAGCTGTC |
| higBA up-f | 5′ ACCGATAACGTCGCCTGGGA |
| higBA up-r | 5′ GAAGCGCCGGATGCAACGCATCCGTAGAAAAACGCTAAACAAGAG |
| higBA down-f | 5′ CTCTTGTTTAGCGTTTTTCTACGGATGCGTTGCATCCGGCGCTTC |
| higBA down-r | 5′ CATCGGTTGTGGCGGGATTG |
| yafNO deletion-f | 5′ GTATACTATTATGTATATTCTGGTGTGCATTATTATGAGGGTATCACTGTCTCTACGCCGGACGCATCGTG |
| yafNO deletion-r | 5′ GCTGAAAATCGCCAGGCTGATAGTTTCTTATTTGTATGTTATTCATAATATAAAACTGATCAGTGATAAGCTGTC |
| yafNO up-f | 5′ GGAGCGTAGTCAGGGGATTG |
| yafNO up-r | 5′ CTTATTTGTATGTTATTCATAATAAATAATGCACACCAGAATATACAT |
| yafNO down-f | 5′ ATGTATATTCTGGTGTGCATTATTTATTATGAATAACATACAAATAAGAAAC |
| yafNO down-r | 5′ ACCAGGCGGGCGTTATTTTC |
| mqsRA deletion-f | 5′ ATACGTTTTGTGTGGTCACTATCTCCGTACATCTAACTAACCTTTTAGGTCTCTACGCCGGACGCATCGTG |
| mqsRA deletion-r | 5′ TACGCCTGTGGCATTGTTCGCTCAAACTTATCGCGAGTGATTTGGCTCACACTGATCAGTGATAAGCTGTC |
| mqsRA up-f | 5′ GCGTCGCCTGGGACGACCCT |
| mqsRA up-r | 5′ AGTGATTTGGCTCACAGGTTAGTTAGATGTACGGA |
| mqsRA down-f | 5′ TCCGTACATCTAACTAACCTGTGAGCCAAATCACTCGCGA |
| mqsRA down-r | 5′ GAGCGGGCTGCACCTGGCCT |
| prlf/yhaV deletion-f | 5′ GCCATGTTTTTATTGTTTAAAGCCCCCACGTCCATTAATAATGCATTTGCCTCTACGCCGGACGCATCGTG |
| prlF/yhaV deletion-r | 5′ AAGGCTGGGGTTGAAGTGATTCTGGTCGGGGAGTGAGAAAGGATGCCCGCACTGATCAGTGATAAGCTGTC |
| prlF/yhaV up-f | 5′ TTGTCTGTGGCACGCAACAG |
| prlF/yhaV up-r | 5′ TGGTCGGGGAGTGAGAAAGGGCAAATGCATTATTAATGGAC |
| prlF/yhaV down-f | 5′ GTCCATTAATAATGCATTTGCCCTTTCTCACTCCCCGACCA |
| prlF/yhaV down-r | 5′ CTCGTCTGATGCGCAAGCAC |
| hicAB deletion-f | 5′ GTTATTATTCAGTTTTGCAAATTAGCGCAAAGAAATTCTGGAATCTTCCCTCTACGCCGGACGCATCGTG |
| hicAB deletion-r | 5′ TCATTATCGAATCGTAATTATGTGCAGATGATTCGGCAGTCTATATCAGTACTGATCAGTGATAAGCTGTC |
| hicAB up-f | 5′ GTAAGTCCGGATGAGCTGCTG |
| hicAB up-r | 5′ CAGATGATTCGGCAGGCTAATTTGCAAAACTGAATAATAAC |
| hicAB down-f | 5′ GTTTTGCAAATTAGCGGCAAAGAGTTATCGCTGGTG |
| hicAB down-r | 5′ CTGCGTTTCCTGGCGGTAAGT |
| lon-f | GCCAATCCTGAGCGTTCTGAA |
| lon-r | CCTTTTGCAGTCACAACCTG |
| DsRed2-BamHI-SD8-f | CCCCGGATCCTAAGGAGGAAAAAAAAAATGGCCTCCTCCGAGAACGTC |
| DsRed2-EcoRV-r | CCCCCCGATATCCTACAGGAACAGGTGGTGGCG |
| *yefM-f* | CCCCCGAATTCAAGGAGTTTTATAAATGCACCACCACCACCACCACCGTACAATTAGCTACAGCGAAGCG |
| *yefM-r* | CCCCGGATCCTCACTCAATGATGTCCTTTTCCGT |