Chen & Gottesman Hfq links translation repression to stress-induced mutagenesis in E. coli

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Supplemental Materials and Methods

Strain Construction:

The $P_{BAD}$-mutS-lacZ translational fusion reporter strain (JC1006) was generated by $\lambda$ Red recombineering (Court et al. 2003) in PM1805, using a PCR product containing the mutS leader (74 bp) plus the 1st 10 codons, amplified using primers JC29/JC30; recombinants were selected on M63 minimal sucrose agar (Mandin and Gottesman 2009). The specificity mutant $P_{BAD}^{ArcZ*}$-lacZ (JC1008) was generated by $\lambda$ Red recombineering using gblocks gene fragment JC45 (IDT, Coralville IA) in PM1805. The $CP12b$-mutS-lacZ translational fusion reporter strain (JC1060), where mutS-lacZ is driven by a synthetic constitutive promoter (Jensen and Hammer 1998), was generated by replacing the $P_{BAD}$ promoter in JC1006 via $\lambda$ Red recombineering with the $kan$-$CP12b$ fragment amplified from JC1051, using primers JC63/JC72 and selected on LB+Kan agar. The ARN1* (JC1111), ARN3* (JC1112) and ARN1*+3* (JC1113) mutants of $CP12b$-mutS-lacZ were generated by $\lambda$ Red recombineering in JC1006 using PCR fragments amplified from JC1060 using primers JC63/JC105, JC63/JC106 and JC63/JC107 respectively.

To construct $mutS^{ArcZ*}$ (JC1083), ARN1* (JC1129) and $mutS^{ArcZ*}$ARN1* (JC1130) mutants in the native mutS leader, a $P_{BAD}$-ccdB-kan cassette was amplified from JC1078 using primers JC83/JC84 and inserted into the mutS leader by $\lambda$ Red recombineering in NM541 to generate JC1074. gblocks gene fragments containing WT (JC111), $mutS^{ArcZ*}$ (JC85), ARN1* (JC112) and $mutS^{ArcZ*}$ARN1* (JC113) were recombineered into JC1074, selecting recombinants resistant to arabinose induction of the CcdB toxin (Battesti et al. 2015); thus an isogenic WT as well as mutant version was generated, and used as the isogenic parent for experiments with these strains. When generating the intermediate JC1074 strain, the $kan$ gene promoter was designed to read through into the downstream mutS gene, allowing MutS production, to avoid increased mutagenesis during the construction (Supplemental Fig. S8B). All constructs were verified by PCR and sequencing. hfq or sRNA deletion strains were constructed by standard P1vir transduction; lacZ ochre mutants were generated by a two-step P1vir transduction, first introducing a closely linked auxotrophic marker, proC::kan, and then introducing the mutation of interest (lacZ C70T) by a second transduction, selecting for loss of the auxotrophy by growth on glucose M63 minimal agar. P1 transductants were verified by PCR. The sRNA plasmid library was expressed from IPTG-inducible pBRplac plasmids, which were described in detail previously (Mandin and Gottesman 2010).

All strains used for adaptive mutagenesis are derivatives of P90C or a rifampicin-resistant (Rif$^R$) isolate of P90C (FC36) (Cairns and Foster 1991). All derivatives were constructed by first moving chromosomal mutations into the P90C or FC36 strains using P1vir transduction; then an episome containing lacI33-lacZ proAB$^+$ or $\Delta$ (lacIZ) proAB$^+$ was mated into FC36 or P90C variants respectively, selecting for the proline prototrophy on M63 glucose agar. The $\Delta$mutS::kan, $\Delta$hfq::cat, $\Delta$arcZ::tet, $\Delta$sdsR::kan, $\Delta$rpoS::kan alleles were moved using P1vir transduction and selected on LB agar containing appropriate antibiotics. The various mutS alleles (WT, mutS$^{ArcZ*}$, ARN1*, mutS$^{ArcZ*}$ARN1*) were transduced via linkage to cysC::kan, and cysC::kan was removed by subsequent transduction to cysC$^+$, selecting on M63 glucose agar.
containing Proline (100 µg/mL). All transductions were verified by PCR and sequencing analysis.

**RNA radiolabeling**

Various T7-*mutS* alleles were PCR amplified from strain JC1060 or JC1111 using primers JC108/JC109 and from strain JC1112 or JC1113 using primers JC108/JC110 and *in vitro* transcribed using MEGAscript T7 Transcription Kit (Ambion, Austin TX) according to the manufacturer’s instructions. Transcribed RNAs were treated with RQ1 DNase (Promega, Madison WI), phenol chloroform extracted and ethanol precipitated. Purified RNAs were then treated with calf intestinal phosphatase (CIP) (BioLabs Inc.) and radiolabeled with γ[³²P]-ATP at the 5’ end using T4 polynucleotide kinase (T4 PNK) (BioLabs Inc.). Free labeled ³²P was removed through G50 columns (GE Healthcare). ³²P-labeled RNAs were resolved on 6% TBE-urea gel (Invitrogen) and RNA products were gel purified. Purified RNA samples were diluted to a stock concentration of 10 nM in DEPC water, and denatured at 95°C for 1 minute then cooled to room temperature over 8 minutes.
Figure S1. Overexpression of ArcZ or SdsR sRNA efficiently downregulates mutS expression.

A. A mutS-lacZ translational reporter strain deleted for arcZ and sdsR (JC1073) was transformed with pArcZ or pSdsR, or the vector control (pBRplac), and grown at 37 °C for 6 hrs in LB-ampicillin (–OD600 3.5) with 100 µM IPTG for sRNA induction before assay of β-galactosidase activities. Note that this fusion is under the control of the constitutive CP12b promoter (Jensen and Hammer 1998), rather than the PBad promoter used in Fig. 1.

B. Whole cell lysates were prepared in parallel with samples used for Fig. S1A, and analyzed by Western blot using antibodies to MutS and EF-Tu. MutS levels relative to the wild-type cells (set to 1) were quantified after normalizing to EF-Tu loading control (mean ± SEM).

C. Upper panel, putative base pairing predicted by TargetRNA2 and tested mutants; lower panel, examination of direct base pairing between SdsR and mutS leader. WT (mutS-lacZ; JC1060) or the compensatory mutant (mutSsdsR* -lacZ; JC1101) were transformed with pSdsR, pSdsR* or vector control (pBRplac), and grown in LB-Ampicillin plus 100 µM IPTG at 37 °C for 6 hrs before assay of β-galactosidase activities.

D. Northern blot to determine SdsR* accumulation. JC1069 (NM525 ΔarcZ ΔsdsR) was transformed with pSdsR, pSdsR* or vector control (pBRplac), and grown in LB-Ampicillin plus 100 µM IPTG at 37 °C for 6 hrs before RNA extraction for northern analysis using SdsR and SsrA northern probes.

Three independent cultures were assayed for β-galactosidase activities, and data are plotted as mean ± SEM. For Northern blot, representative images from two experiments are shown.

Figure S1.
Figure S2. ArcZ-mediated mutS-lacZ repression relies on the proximal and lateral faces of Hfq.
A. mutS-lacZ translational reporter strains harboring various hfq alleles on the chromosome (WT: JC1103; Δhfq: JC1104; Q8A: JC1105; Y25D: JC1106; R16A: JC1107) were transformed with the pArcZ, pArcZ* plasmids or the vector control (pBRplac), and induced in LB with 100 µM IPTG for 6 hrs before assaying β-galactosidase activities in Miller units. Three independent cultures were assayed for each strain, and data are plotted as mean ± SEM.
B. Total RNAs were extracted from the same set of experiments as S2A and analyzed by Northern blot using ArcZ and SsrA probes. The ArcZ probe detects both the primary transcript (ArcZ long) and the mature ArcZ sRNA (ArcZ short), but covers the region of the ArcZ* mutation, and this is likely to account for the apparent lower detection of ArcZ*. VA: vector alone.

Figure S2.

A. mutS-lacZ

B. WT Δhfq Q8A Y25D R16A

100 nt - 300 nt -
Figure S3. ArcZ can affect SdsR expression through RpoS.
A. Schematic representation of ArcZ and SdsR regulatory networks, both of which are shown in this paper to downregulate MutS levels.
B. WT (NM525) and ΔarcZ (JC1042) cells were grown in LB at 37 °C for 16 hrs before RNA extraction for Northern blot analysis of SdsR levels in cells. SsrA serves as a RNA loading control.
C. WT (NM525) cells were grown at 37 °C, and whole cell lysates as well as total RNAs were prepared at OD_{600} of 0.5, 1.0, 2.0, 4.0 and 6.0. Proteins were analyzed by Western blot using antibodies against MutS, RpoS, RpoD and EF-Tu, and RNAs were analyzed by Northern blot using SdsR, ArcZ and SsrA probes.
Figure S4. Roles of Hfq-bound RNA sequence motifs and Hfq faces on sRNA-mediated mutS-lacZ repression in vivo.

A. Schematic representations of sequence elements (ARN1, ARN3, ArcZ pairing site, putative SdsR site and RBS) in mutS leader and their mutants, highlighted with changes shown above sequence (ΔS', ARN1*, mutSArcZ*, mutSSdsR*, ARN3*).

B. mutS-lacZ translational fusion reporter variants (WT: JC1060; ARN1*: JC1111; ARN3*: JC1112; ARN1*+3*: JC1113; ΔS*: JC1169) were transformed with the pArcZ or pArcZ* plasmid or the pBRplac vector control, and induced with 100 μM IPTG for 6 hrs before assaying β-galactosidase activities (in Miller units).

C. mutS allele reporter strains (WT: JC1060, WT Δhfq: JC1072; ARN1*: JC1111, ARN1* Δhfq: JC1116; ARN3*: JC1112; ARN3* Δhfq: JC1114; ARN1*+3*: JC1113, ARN1*+3* Δhfq: JC1115; Δ5*: JC1169, Δ5'Δhfq: JC1170) were grown for 6 hrs to OD₆₀₀ of ~3.0 before assaying β-galactosidase activities.

D. mutS-lacZ translational reporter strains harboring various hfq alleles on the chromosome (WT: JC1103; Δhfq: JC1104; Q8A: JC1105; Y25D: JC1106; R16A: JC1107) were transformed with pSdsR or the vector control (pBRplac), and induced with 100 μM IPTG for 6 hrs before assaying β-galactosidase activities.

E. mutS-lacZ translational fusion reporter variants (WT: JC1060; ARN1*: JC1111; ARN3*: JC1112; ARN1*+3*: JC1113) were transformed with pSdsR or the vector control (pBRplac), and induced with 100 μM IPTG for 6 hrs before assaying β-galactosidase activities.

Three independent cultures were assayed for each strain, and data are plotted as mean ± SEM.
Figure S4.

A. *E. coli* mutS leader

\[
\text{TGCCTATGGTATTACGAAAATAAAAAACATCACACCCATTTTAATATCGGGATCCGACATAACCC}
\]

\[
\text{mutS}^\text{ArcZ*} \quad \text{mutS}^\text{SdsR*} \\
\text{ArcZ pairing site} \quad \text{RBS}
\]

\[
\text{TT.GGA mutS}^\text{ArcZ*} \quad \text{CAC mutS}^\text{SdsR*}
\]

\[
\text{mutS}^\text{ArcZ*} \quad \text{mutS}^\text{SdsR*}
\]

\[
\text{CG..T.T.. ARN1*} \\
\text{ARN1}
\]

\[
\text{C..C..G.. ARN3*} \\
\text{atg AGT GCA ATA GAA AAT TTC GAC GCC CAT}
\]

\[
\text{ARN3}
\]

B. mutS-lacZ

\[
\begin{array}{|c|c|c|c|c|c|c|c|}
\hline
\text{mutS alleles:} & WT & ARN1* & ARN3* & ARN1*+3* & \Delta 5' & \text{\beta-galactosidase activity} \\
\hline
1 & 0.38 & 0.88 & 1 & 1 & 0.81 & 1 \\
2 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
3 & 0.89 & 0.88 & 0.98 & 1.06 & 0.96 & \\
4 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
5 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
6 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
7 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
8 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
9 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
10 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
\hline
\end{array}
\]

C. mutS-lacZ

\[
\begin{array}{|c|c|c|c|c|c|c|c|}
\hline
\text{mutS alleles:} & WT & ARN1* & ARN3* & ARN1*+3* & \Delta 5' & \text{\beta-galactosidase activity} \\
\hline
1 & 0.38 & 0.88 & 1 & 1 & 0.81 & 1 \\
2 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
3 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
4 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
5 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
6 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
7 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
8 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
9 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
10 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
\hline
\end{array}
\]

D. mutS-lacZ

\[
\begin{array}{|c|c|c|c|c|c|c|}
\hline
\text{hfq alleles:} & WT & \Delta hfq & Q8A & R16A & Y25D & \text{\beta-galactosidase activity} \\
\hline
1 & 0.38 & 0.88 & 1 & 1 & 0.81 & 1 \\
2 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
3 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
4 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
5 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
6 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
7 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
8 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
9 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
10 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
\hline
\end{array}
\]

E. mutS-lacZ

\[
\begin{array}{|c|c|c|c|c|c|c|}
\hline
\text{mutS alleles:} & WT & ARN1* & ARN3* & ARN1*+3* & \Delta 5' & \text{\beta-galactosidase activity} \\
\hline
1 & 0.38 & 0.88 & 1 & 1 & 0.81 & 1 \\
2 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
3 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
4 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
5 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
6 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
7 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
8 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
9 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
10 & 0.89 & 0.95 & 0.98 & 1.06 & 0.96 & \\
\hline
\end{array}
\]
**Figure S5.** Hfq dose-dependent inhibition of *mutS* translation *in vitro*.

**A.** Schematic diagram showing *in vitro* transcribed RNA containing the 76 nt long 5’UTR, 900 nt *mutS* coding sequence (ORF900) and 27 nt *flag* sequence with stop codon. 

**B.** *in vitro* translation of *mutS’-flag* mRNA in the presence of increasing amount of Hfq proteins. A 1003 nt long *mutS’-flag* mRNA was *in vitro* transcribed, and 2.5 pmol of purified WT *mutS’-flag* RNA was incubated with 0, 1.875 and 2.5 pmol of Hfq at room temperature for 5 minutes, then *in vitro* translated using the PURExpress *in vitro* protein synthesis kit. The translated MutS’-FLAG proteins were detected by Western blot using anti-FLAG antibodies. The same protein samples were stained with InstantBlue to show total proteins in each sample.
Figure S6. Modulation of *E. coli* spontaneous mutagenesis, measured by rifampicin resistance and *lac* ochre reversion.

A. WT (NM525) and ΔmutS (JC1065) strains were grown in LB for 16 hrs at 37 °C and plated on LB plates containing rifampicin (50 µg/ml) to count RifR mutants, or on nonselective LB agar after serial dilution to count total viable colonies. RifR frequencies were determined by normalizing mean of RifR colonies to that of total viable colonies. Three independent experiments were conducted.

B. WT (JC1156) and ΔmutS (JC1160) strains in a *lacZ* ochre mutation background were grown in LB for 6 hrs at 37 °C and plated on M63 minimal lactose selection agar or nonselective M63 glucose agar after serial dilution. Lac+ ochre reversion frequencies were determined by normalizing mean number of CFUs on selective lactose agar to that on nonselective glucose agar. Four independent experiments were conducted.

C. WT (NM525) cells were grown to OD600 of 0.6 (exponential) and 4.5 (stationary) in LB, and assayed for rifampicin resistance as described above. Three independent experiments were conducted.

D. WT (NM525), ΔmutS (JC1065), ΔarcZ (JC1042), ΔsdsR (JC1021) or ΔarcZ ΔsdsR (JC1069) were analyzed by the rifampicin resistance assay as for Fig. S6A.

E. WT (JC1156) and Δhfq (JC1171) strains in a *lacZ* ochre mutation background were grown in LB for 6 hrs at 37 °C and assayed for frequencies of Lac+ reversion as described for Fig. S6B. Four independent experiments were conducted. Data were plotted as mean ± SEM. Unpaired student t-test was used to calculate the significance (***: p<0.001; ****: p<0.0001) using GraphPad Prism 5.

**Figure S6.**
Figure S7. Adaptive mutagenesis in strains deleted for *hfq*, *rpoS*, *mutS*, *arcZ* or *sdsR*.

**A.** Mutations were introduced into both a revertible Lac- frameshift strain and into a Δlac scavenger strain (Revertible strains: WT: JC1215; Δhfq: JC1222; ΔrpoS: JC1223; ΔmutS: JC1221; ΔarcZ: JC1224; ΔsdsR: JC1225 or the scavengers: WT: JC1214; Δhfq: JC1217; ΔrpoS: JC1218; ΔmutS: JC1216; ΔarcZ: JC1219; ΔsdsR: JC1220). These strains were grown to saturation in M9-0.1% glycerol, and ~0.6×10^8 revertible cells (~30 µl saturation cultures) mixed with 1.6×10^9 scavenger cells (~800 µl saturation cultures) were spread on M9-lactose agar. The number of Lac^+ colonies were counted from day 1 to 6, and the adaptive mutation frequencies to Lac^+ were calculated as the mean of Lac^+ colonies each day from days 2 to 6, normalized to 10^8 total viable cells. Note, ΔmutS showed much higher mutation rates (~40-fold higher than WT) and are not shown on this graph.

**B, C.** Dot plot of total number of Lac^+ colonies per 10^8 viable cells on day 6 (B) and day 2 (C) from the set of experiments in Fig. S7A.

**D.** Dot plot of total number of Lac^+ colonies per 10^8 viable cells on day 2 from the set of experiments in Fig. 6D.

Two independent isolates (5 cultures each) for each construct were assayed and data were plotted as mean plus standard error (SEM). Unpaired student t test (two-tail) was used to calculate statistical significance (ns: non-significant, p>0.05; *: p<0.05; ***: p<0.001).
Figure S8. *mutS* expression in various mutants and during *E. coli* growth.

A. WT *E. coli* cells (WT: JC1081) were grown in LB at 37 °C, and cells were collected at OD$_{600}$ of 0.19, 0.66, 1.45, 2.54, +3 h and 16 h to analyze protein amounts of MutS and Hfq by Western blot.

B. Bacterial cells (WT: JC1081; *mutSArcZ* Arn1*: JC1130; Arn1*: JC1129; ccdB-kan-*mutS*: JC1074) were grown for 6 hrs in LB at 37 °C, and then analyzed by Western blot for MutS production. EF-Tu is a protein loading control.

C. Bacterial cells (WT: JC1081, Δhfq: JC1090, *mutSArcZ*: JC1083, Arn1*: JC1129, *mutSArcZ* Arn1*: JC1130) were inoculated into LB to an initial OD$_{600}$ of 0.02 and grown for 10 hrs in 24-well plates. Cell growth was monitored using the TECAN 10M plate reader every 15 minutes. Two independent experiments were conducted and data were plotted as the mean value with standard error. Note that because this growth curve is in a 24-well plate, ODs as a function of time are not comparable to growth in other experiments.

D. Same set of bacterial strains as in Fig. S8C were grown in LB and samples taken at OD$_{600}$ of 0.2, 0.6, 1.4, 2.5, +3 h and 16 h to measure MutS levels by Western blot. EF-Tu serves as a protein loading control. Western blot band signals were quantified using ImageStudio software and normalized to EF-Tu at each sample. All values were then normalized to a value set to 1 for the normalized MutS in WT cells at OD$_{600}$ of 0.2 in WT. Experiments were conducted twice and all graphs were plotted as mean ± SEM.
Figure S9: Proposed secondary structure of mutS leader upon Hfq binding
The alternative RNA folding was predicted by RNAStructure AllSub server (http://rna.urmc.rochester.edu/RNAstructureWeb/Servers/AllSub/AllSub.html) using a shorter mutS leader (-41 to 30) truncated for the Hfq bound region and then re-joined with the 5’ end sequences. The selected structure is the top prediction with the lowest free energy and best fit to our structure probing data. The final RNA secondary structure is drawn by using the forna tool (http://rna.tbi.univie.ac.at/forna/).
Table S1: Strains and plasmids used in this study

| Strain or plasmid | Description | Reference or source |
|-------------------|-------------|---------------------|
| **Strains**       |             |                     |
| MG1655            | Wild type E. coli K-12 |                     |
| PM1805            | MG1655 mal::lacO AraBAD araC+ lacI::PBAD-cat-sacB-lacZ, mini-z-tetR | Lab strain collection (Lee and Gottesman 2016) |
| NM525             | MG1655 lacO FLP-scar | Lab strain collection |
| NM541             | MG1655 lacO FLP-scar mini-z-tetR | Lab strain collection (De Lay and Gottesman 2012) |
| NRD750            | PM1205 ΔasDS::zeo |                     |
| PM1442            | MG1655 ΔarcZ::tet | (De Lay and Gottesman 2012) |
| KMT12055          | DJ480 Δhfr::cat ryB::lacZ |                     |
| DJS22942          | PBAD-ompX-lacZ hfr-Y25D |                     |
| DJS23162          | PBAD-ompX-lacZ Δhfr::cat-sacB |                     |
| DJS23172          | PBAD-ompX-lacZ wild-type hfr |                     |
| DJS23182          | PBAD-ompX-lacZ hfr-Q8A |                     |
| DJS23192          | PBAD-ompX-lacZ hfr-R16A |                     |
| DDS41             | MC4100 rpsS::kan |                     |
| JW2703            | BW25113 AmuS738::kan (Keio Collection) | Sankar Adhya lab |
| SA751             | lacZ ochre mutation C70T |                     |
| JC1006            | MG1655 mal::lacO AraBAD araC+ lacI::PBADmutS-lacZ | This study³ |
| JC1008            | MG1655 mal::lacO AraBAD araC+ lacI::PBADmutSlsigZ-lacZ | This study³ |
| JC1021            | MG1655 lacO FLP-scar, ΔasDS::zeo | NM525+P1(NRD750) |
| JC1042            | MG1655 lacO FLP-scar, ΔarcZ::tet | NM525+P1(PM1442) |
| JC1051            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-whyN-lacZ | (Chen and Gottesman 2016) |
| JC1060            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutS-lacZ | This study³ |
| JC1061            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutS-lacZ, ΔarcZ::tet | JC1060+P1(PM1442) |
| JC1065            | MG1655 lacO FLP-scar, ΔmutS::kan | NM525+P1(Keio:JW270 3) |
| JC1069            | MG1655 lacO FLP-scar, ΔarcZ::tet, ΔasDS::zeo |                     |
| JC1071            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutS-lacZ, ΔasDS::zeo |                     |
| JC1072            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutS-lacZ, Δhfr::cat | JC1060+P1(KMT12055) |
| JC1073            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutS-lacZ, ΔarcZ::tet | JC1061+P1(NRD750) |
| JC1074            | MG1655 lacO FLP-scar, mutS::PBAD-cddB-kan, miniz-tetR |                     |
| JC1081            | MG1655 lacO FLP-scar, WT mutSleader |                     |
| JC1083            | MG1655 lacO FLP-scar, mutSlsigZ leader |                     |
| JC1090            | MG1655 lacO FLP-scar, Δhfr::cat |                     |
| JC1101            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutSlsigZ-lacZ | NM525+P1(KMT12055) |
| JC11032           | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutS-lacZ, WT |                     |
| JC11042           | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutS-lacZ, Δhfr::cat-sacB |                     |
| JC11052           | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutS-lacZ, Δhfr::q8A | DJS2318+P1(JC1060) |
| JC11062           | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutS-lacZ, Δhfr::q25D | DJS2329+P1(JC1060) |
| JC11072           | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutS-lacZ, Δhfr::q16A | DJS2319+P1(JC1060) |
| JC1111            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutSlsigZ-lacZ | This study³ |
| JC1112            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutSlsigZ-lacZ | This study³ |
| JC1113            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutSlsigZ-lacZ | This study³ |
| JC1114            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutSlsigZ-lacZ, Δhfr::cat | JC1112+P1(KMT12055) |
| JC1115            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutSlsigZ-lacZ, Δhfr::cat | JC1113+P1(KMT12055) |
| JC1116            | MG1655 mal::lacO AraBAD araC+ lacI::kan-CP12b-mutSlsigZ-lacZ, Δhfr::cat | JC1111+P1(KMT12055) |
| JC1129            | MG1655 lacO FLP-scar, mutSlsigZ leader |                     |
| JC1130            | MG1655 lacO FLP-scar, mutSlsigZlsigZ leader |                     |
| JC11352           | MG1655 lacO FLP-scar, mutSlsigZ leader, Δarc70T ochre mutation |                     |
| JC11357           | MG1655 lacO FLP-scar, mutSlsigZ leader, Δarc70T ochre mutation |                     |
| JC11358           | MG1655 lacO FLP-scar, mutSlsigZ leader, Δarc70T ochre mutation |                     |

³ This study refers to the study by Zhang et al. (2013).
| JC1159 | MG1655 lacI33 FLP-scar, mutS\textsuperscript{arcZ-arn1*} leader, lacZ C70T ochre mutation | This study\textsuperscript{1} |
| JC1160 | MG1655 lacI33 FLP-scar, AmutS:::kan, lacZ C70T ochre mutation | JC1159+P1(Keio:JW2703) |
| JC1169 | MG1655 mal::lacI33 AaraBAD araC+:lacI33-P12b-mutS\textsuperscript{arcZ-lacZ} | This study\textsuperscript{1} |
| JC1170 | MG1655 mal::lacI33 AaraBAD araC+:kan-P12b-mutS\textsuperscript{arcZ-lacZ} Δhfq::cat | JC1169+P1(KMT12055) |
| JC1171 | MG1655 lacI33 FLP-scar, Δhfq::cat, lacZ C70T ochre mutation | JC1090+P1(Keio:JW0377, SA751) |

**P90C** recipient of scavenger episome, F' ara Δ(lac-proB) \textit{XIII} thi

**FC36** recipient of revertible episome, P90C Rf\textsuperscript{P1(PM1442)}

**FC33** donor of scavenger episome, XA105/F' Δ(lacIZ) Pro\textsuperscript{+}

**FC34** donor of revertible episome, XA105/F' Φ(lacI33-lacZ) Pro\textsuperscript{+}

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| Vector | Parental plasmid containing Plac promoter, pBR322 origin, Ampr | Lab plasmid collection |
| ArcZ | Wild type arcZ gene cloned into pBRplac AattII/EcoRI | Lab plasmid collection |
| ArcZ* | mutant arcZ gene (G73TG74CT75CT77AT78A) on pBRplac vector | (De Lay and Gottesman 2012) |
| SdsR | Wild type sdsR gene cloned into pBRplac AattII/EcoRI | Lab plasmid collection |
| SdsR* | mutant sdsR gene (C66GC67TC68G) on pBRplac vector | This study |

\textsuperscript{1} Described in Supplemental Materials and Methods

\textsuperscript{2} Strain is a φ80 bacteriophage lysogen, inadvertently introduced into the parent strain. This lysogen has no known effects on experiments presented here.
Table S2: Primers, probes and synthetic gene fragments used in this study

| Primers, probes and synthetic gene fragments | DNA sequence (40 nt homologous sequence for recombineering in bold) |
|---------------------------------------------|---------------------------------------------------------------|
| **Primers**                                 |                                                               |
| JC30                                        | TAACGCCAGGGTTTTCACCATGACAGGTTTACAAACGACATGGCGCTCGGAAAAATTTTCTA |
| JC34                                        | ACCGTACGCTTTTACGCAACTCTCTACTGGTTCTCCATTTGCCCTATTATGTTATGTAACATC |
| JC63                                        | AACCCCGCTTTAATAAAGCATCTCTGTAACAAAAACGGGCAACAAAGCCCACAGT         |
| JC72                                        | TGGTAATGGTTTATTTCCGTTGTAATCACATAGGCACATCGAGATTCTT |
| JC83                                        | ATGTTGATCCAAGCAATAAAAACCATCACACCCCATTTTTATATTTTCCTGACAAGGAACCGG |
| JC84                                        | AAATTTTCTATTTGACATGACATGGGTATGCGTTTTCTCCTTTTATAGAGAAGACGTCAGAAG |
| JC102                                       | CTCCTAAAGCAGATTCGATGCGTCCTGTGATAGGCTGACAGTGTGATGTAACGACAGG |
| JC103                                       | GTCCACAAATTTGCTCGTGGAGAGCGACATCGCGTTCTCTAATAGTTGGAACG |
| JC104                                       | TACACCGAGGTTTTCACATGACACGTGTTTATGCGCTCTTCTCCATTTTATAGAGGCTGAG |
| JC105                                       | TACACGGGTTTTCTACATGACACGTGTTTATGCGCTCTTCTCCATTTTATAGAGGCTGAG |
| JC106                                       | TACACGGGTTTTCTACATGACACGTGTTTATGCGCTCTTCTCCATTTTATAGAGGCTGAG |
| JC107                                       | TACACGGGTTTTCTACATGACACGTGTTTATGCGCTCTTCTCCATTTTATAGAGGCTGAG |
| JC108                                       | ACGCACCCTATAGCTGATACTCAGTAC AG ACTATAAG |
| JC109                                       | TGGCGCTATGTGAAATACAGC |
| JC110                                       | ATAGGGGCTCAAAATTTTCCTAGT |
| JC116                                       | TACACTGTGCATCGCTTTTGTGATGCGCAGCTCAAGGAGAGGAC |
| **Probes**                                  |                                                               |
| ArcZ NB4†                                   | GGCTAGACGGGGTGCAGAATCTGCGCAGCACCCGAGG |
| SdsR1†                                      | GGCCTCGTGGAGAGAC |
| SsrA1†                                      | CGCCACTACAAACACTGCTGATAGTCAAGGTTTTAAGC |
| **Synthetic gene fragments**                 |                                                               |
| P_{BAD^{mutS}}-lacZ                         | ACCTGACGCTTTTATCGCAACTCTCTACTGTTCCTCATTTGCCCTATTAGT |
| P_{BAD^{mutS}}-lacZ                         | GATTTACAAAGGAAATAAAAAACATCACACCACCTTTTTGGAAAGGGACAG |
| P_{BAD^{mutS}}-lacZ                         | ACAATAACCCCATAGTGACTAGAAATCCGACGCGCAATGTCGTTTTTACAA |
| P_{BAD^{mutS}}-lacZ                         | CGTCTGCTGAGGAAAAACCGCTTGCTTTG |
| JC85                                        | ATGTTGATTACAAAGGAAAAATAAAAACATCACACCCCATTTTTGGAAGG |
| JC111                                       | ACGCGAGCATAACCCCATGAGTCAATAGAATAATTT |
| JC112                                       | GTCTTCCTGGGAAATGTTAACTCCTGGTATCATGTCGCTCTATTATG |
| JC113                                       | CTCAAAAGGACTTAATACGTCACACACCCTTTAAATACGAGGAAACCCGGAC |
| JC113                                       | ATACCCGCTAATGCGCATAGAAATCCGACGCGCAATGCGAGAC |
| JC113                                       | ATACCCGCTAATGCGCATAGAAATCCGACGCGCAATGCGAGAC |

†. Probes reported previously (Mandin and Gottesman 2010)
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