Supplementary Information

Horizontal transfer of DNA methylation patterns into bacterial chromosomes

Jung-Eun Shin[1], Chris Lin[1] and Han N. Lim[1, 2]

[1] Department of Integrative Biology, University of California Berkeley

[2] Corresponding author: drhanlim@gmail.com

CONTENTS:

1. SUPPLEMENTARY METHODS

1.1. Construction of strains and plasmids

Additional details of strains, plasmids, and oligonucleotides are provided in the **Tables S1** and **S2** and **Fig. S1**. Strains and plasmids have been submitted to Addgene for distribution. Note that strains generated in replicate transduction and transformation experiments were given different strain numbers and had the same strain number but had a different numbered suffix (*e.g.* #1, #2 and #3) depending on whether they were generated on different days or the same day respectively. The plasmid origin and terminators were obtained from the pZ system (1). The kanamycin resistance gene with flanking FRT sites was obtained from pKD13 (2). The genes for monomeric CFP and YFP (*cfp* and *yfp* respectively) were obtained from R. Tsien (University of California, San Diego). The PT7 promoter, T7 10 sequence and T7 terminator were obtained from pHL32 (3) and *sgrS* was PCR amplified from MG1655 genomic DNA. The PT7 promoter with lac operator sequence was synthesized by PCR using oligonucleotides pt7lacoidaatf and pt7lacoidxmar (**Table S2**). The *dam*, *oxyR* and *sgrS* genes were deleted using the lambda Red method (2) creating strains HL444, HL372and HL752 respectively with the kanamycin resistance gene in place of these genes. The antibiotic resistance gene and hence the deletion was then moved to reporter strains by bacteriophage P1 transduction (2).

MG1655, which was the recipient strain for the transduction and transformation experiments, has several restriction-modification (RM) systems (4). It contains a type I RM system (*Eco*KI system) and type IV RM systems (McrA, McrBC and Mrr). MC4100, which was the donor strain for the transduction and transformation experiments, has the same RM systems as MG1655 except McrA (5), which is a 5-methylcytosine DNA binding protein that is involved in cleaving methylated bacteriophage lambda DNA. Bacteriophage P1, which was used in the transduction experiments, has a type III RM system (6).

DNA from randomly selected OFF and ON colonies that were generated by the transduction and transformation experiments were sequenced to confirm HGT and that there were no differences in the *agn43* region.

1.2. Colony measurements to determine the percentage of OFF and ON colonies

Colonies were visualized with bright field (with phase condenser annulus 3) and fluorescence using a Nikon TE2000E inverted microscope with 4 × objective, X-cite 120PC lamp (Exfo) and images were captured using a Pixus 1024 CCD camera (Princeton Instruments) and Metamorph 7.0 imaging software (Molecular Devices). Excitation filter/dichroic mirror/emission filter sets were 436 ± 10 nm/455 nm/480 ± 20 nm (CFP) and 500 ± 10 nm/515 nm/535 ± 15 nm (YFP) (7).

Images of colonies were initially scored as OFF, ON or mixed by two observers. Scoring was based on the estimated percentage of area in each colony having ON and OFF cells, which could be clearly distinguished in colonies because they had very different levels of fluorescence. ON colonies had >70% ON cells, OFF colonies had >70% OFF cells and mixed colonies had <70% OFF and ON cells (**Fig. S4A**). Because the difference in the OFF and ON colonies were usually very clear there was high concordance in the scoring by two observers: 97.7% agreement in three biological replicates for the transduction experiments and 98.4% agreement in two biological replicates for the transformation experiments. Most OFF colonies also had a distinct “fringe” around the periphery which distinguished them from ON colonies (**Fig. S4B**). Because of the high concordance in scoring between observers only one scorer was used for subsequent experiments.

1.3. General protocol for bacteriophage P1 transduction

Donor phage were prepared by adding 50 µL of overnight culture (HL23 and HL24) to 2 mL of lysis media (LB media with a final concentration of 100 mM $MgCl_2$ and 5 mM $CaCl_2$) and 10 µl of 1M glucose. The mix was incubated for 1 hour at 37^{o}C and 200 rpm. 100 µl of P1 bacteriophage was then added to each flask and the culture was grown at 37^{o}C at 200 rpm until the cells lysed. Two mL of lysed cells were added to a 2 mL microcentrifuge tube and 50 µL of chloroform was added. The mix was vortexed and centrifuged at 16, 100 RCF for 2 minutes and the supernatant transferred to a fresh microcentrifuge tube with 20 µL of chloroform. The phage lysate was stored at 4^{o}C.

One mL of overnight culture of recipient cells was centrifuged at 16, 100 g and the cell pellet resuspended in 200 µL of lysis media. The cells were then divided into two 100 µL aliquots and then 100 µL of donor phage from the Δ*dam* or Δ*oxyR* background was added to each aliquot and incubated at 37°C for 30 minutes at 200 rpm. 1 mL of sodium citrate-2 × YT media mix, which was made by combining 0.5 M sodium citrate at pH 5.5 and 2 × YT media (16 g tryptone, 10 g yeast extract and 5 g sodium chloride in 1000 mL at pH 7.0) in a 2:3 ratio, was added to the cells. The cells were incubated for 2 hours at 37°C and 200 rpm. The cells were centrifuged at 16, 100 RCF for 1 min. The cell pellet was resuspended in 100 µL sodium citrate-2 × YT media mix and then plated on lysogeny broth (LB) media agar with 34 $\mu g \cdot mL^{-1}$ chloramphenicol. The plates were incubated overnight at 37°C.

1.4. General protocol for transformation

Recipient cells with the pKD46 plasmid were grown overnight in LB media with 100 $\mu g \cdot mL^{-1}$ ampicillin at 30°C and shaking at 200 rpm. The next morning the cultures were inoculated into 100 mL LB media (4 flasks of 25 mL) with final concentrations of 100 $\mu g \cdot mL^{-1}$ ampicillin and 10 mM L-arabinose. The initial A_{600nm} (optical absorption at a wavelength of 600 nm using the Cary 3E UV/Vis Spectrophotometer (Varian, Victoria, Australia) was ~0.025 and the culture was grown at 30°C with shaking at 200 rpm until it reached A_{600nm} ~0.6. The culture was then centrifuged for 15 minutes at 3650 RCF in a Sorvall Legend RT with rotor 6441 (brake speed 3) at 4°C. The supernatant was removed and the pellet was resuspended in ice-cold 10% glycerol. The resuspended cells were centrifuged and the 10% glycerol wash was performed three more times. The final cell pellet was resuspended in 100 to 600 µL 10% glycerol and stored at -80°C.

Transformations were performed by mixing recipient cells, PCR product purified using the QIAquick gel extraction, QIAquick PCR purification or QIAquick nucleotide removal kits (Qiagen, Hilden, Germany) and 10% glycerol (the latter was added to make a final volume of 42 µL). Cells and reagents were all ice cold and inserted into a pre-chilled electroporation cuvette with 0.1 cm gap. The mix was stored on ice until electroporation. Electroporation was performed using the Bio-Rad MicroPulser with Ec1 setting for *E. coli* (single 1.80 kV pulse for 0.1 cm cuvette). One mL of room temperature SOC media (0.5% w/v yeast extract,

2% w/v tryptone, 10 mM NaCl, 2.5 mM KCl, 20 mM $MgSO_4$ and 20 mM glucose) was added immediately after electroporation and then the resuspended cells were removed from the cuvette and placed into a 1.5 mL microcentrifuge tube and incubated at 30°C for 4 hours or at 30°C for 2 hours and then at room temperature overnight. After incubation the cells were plated on LB media agar with 34 $\mu g \cdot mL^{-1}$ chloramphenicol or 50 $\mu g \cdot mL^{-1}$ kanamycin and incubated at 30°C overnight.

1.5. Flow cytometry to measure OFF and ON expression in single cells

We evaluated whether flow cytometry, which can measure fluorescence in single cells, could be used as an alternative to the colony counting method.

Our first experiments simply examined whether we could distinguish: (i) CFP and YFP fluorescence; (ii) OFF and ON expression states (CFP fluorescence); and (iii) the presence and absence of constitutive YFP expression in the recipient and donor strains respectively. The experiment was performed with the following strains: donor strain (HL23, which has no CFP fluorescence and no YFP fluorescence); recipient strain (HL5786, which has no CFP fluorescence but has YFP fluorescence); wild-type recipient strain (which has Dam and OxyR) transduced with methylated DNA donor phage (HL6487, which has ON CFP fluorescence and also YFP fluorescence); and wild-type recipient strain transduced with unmethylated DNA donor phage (HL6486, which has OFF CFP fluorescence and has YFP fluorescence). We used fluorescence microscopy to select two colonies with ON CFP fluorescence for HL6487 and two colonies with OFF CFP fluorescence for HL6486. The colonies were inoculated into 3 mL LB with the appropriate antibiotics and grown for 4 hours at 37°C at 200 rpm. The cells were placed on ice and measured by flow cytometry using BD Bioscience LSR Fortessa X20 cell analyzers with a 488 nm laser, 505 nm long-pass dichroic mirror and 530/30 nm band-pass filter (for YFP) or a 405 nm laser and 450/50 nm band-pass filter (for CFP).

The flow cytometry measurements showed low CFP and YFP fluorescence levels in the donor strain, which was expected because it does not have either of the fluorescent reporter genes (**Fig. S3A**). The recipient strain had low CFP and high YFP fluorescence levels, which is consistent with it having no *cfp* gene and constitutive transcription of *yfp* (**Fig. S3A**). Cultures of wild-type recipient cells transduced with donor phage

with methylated DNA (HL6487) had two distinct CFP fluorescence levels consistent with the OFF and ON expression states and YFP fluorescence levels were similar to recipient cells (indicating transcription of *yfp*) (**Fig. S3B**). Cultures of wild-type recipient cells transduced with unmethylated DNA donor phage (HL6486) had primarily low CFP fluorescence levels consistent with the OFF state and YFP fluorescence levels that were much higher than in the donor cell (indicating transcription of *yfp*) (**Fig. S3B**). The YFP fluorescence levels (and also to some extent the OFF CFP fluorescence levels) in the wild-type recipient cells transduced with unmethylated donor phage were lower than when transduced with methylated donor phage; the reasons for this difference are not known and they did not occur in subsequent experiments. Together the results demonstrate that flow cytometry is able to distinguish (i) CFP and YFP fluorescence; (ii) OFF and ON CFP fluorescence states; and (iii) the presence and absence of constitutive YFP expression in recipient and donor strains respectively.

1.6. Optimization of flow cytometry protocol for transduction experiments

Having established that flow cytometry could distinguish OFF and ON CFP expression and distinguish CFP and YFP expression we sought to determine whether we could measure the percentage of successfully transduced cells with methylated and unmethylated donor DNA (with ON and OFF expression respectively), and if not, to optimize the protocol to do so.

In the first set of experiments the transduction protocol was the same as the general protocol described above with wild-type recipient cells (HL5786) and donor phage (HL23 and HL24) except that following transduction the cell pellet was resuspended in 500 µL of sodium citrate-2x YT media mix. We inoculated 50 µL of the 500 µL of resuspended transduced cells into 10 mL LB media with 17 $\mu g \cdot mL^{-1}$ chloramphenicol and 25 $\mu g \cdot mL^{-1}$ of kanamycin and grew them for 4-7 hours at 37^{o}C and shaking at 200 rpm (note: to allow cells time to recover from the transduction and synthesize proteins for antibiotic resistance the concentration of antibiotics was half that used for the plates). At 0, 4, 5, 6 and 7 hour time points we removed 1 mL of culture and placed it on ice. After 10-20 minutes CFP and YFP fluorescence were measured in the samples by flow cytometry as described above. Approximately 300 µL from the initial 500 µL of resuspended transduced cells was plated as described for the transduction experiments as a control to

determine if transduction itself had been successful. The entire experiment was performed on four separate cultures (biological replicates).

We found the cultures had very low cell density at all time points. The distribution of YFP fluorescence was unimodal and the level was moderately high at all time points and in all strains; this result was consistent with constitutive expression of YFP in recipient and transduced cells (right, **Fig. S3C**). Initially there was no detectable CFP expression and the distribution was almost identical for the cultures of recipient cells transduced with methylated and unmethylated DNA donor phage (left, **Fig. S3C**). However, after 7 hours there was a shift to a higher level of CFP fluorescence in cultures transduced with methylated DNA donor phage and in cultures transduced with unmethylated DNA donor phage (to a lesser extent). However, we still could not clearly distinguish the OFF and ON expression states within cultures or distinguish the cultures derived from methylated and unmethylated DNA donor phage.

In contrast to the flow cytometry measurements, colony counting was able to be used to determine the percentage of OFF and ON cells (from the percentage of ON and OFF colonies) arising from the above transductions. We found that transduction of the unmethylated DNA donor phage into the wild-type recipient strain (generating HL6715) resulted in 67.9 ± 25.3%, 0.0 ± 0.0% and 32.1 ± 25.3% of colonies that were OFF, ON and mixed respectively (n = 13). In contrast, transduction of methylated DNA donor phage into the wild-type recipient strain (generating HL6716) resulted in 11.7 ± 1.2%, 71.6 ± 10.8% and 16.8 ± 4.6% of colonies that were OFF, ON and mixed respectively (n = 77). While the numbers of colonies were relatively small they were sufficient to rule out the possibility that the transduction had failed. Furthermore, it demonstrated that a low number of successful transductions could be detected by the colony counting method but not by the flow cytometry protocol. We concluded that under the conditions of the experiment, seven hours was insufficient time to increase the population of successfully transduced cells through antibiotic selection and growth so that they could be measured and to decrease the population of recipient cells which had not been successfully transduced so that they were not counted as OFF expression cells.

In a second set of experiments we made the following changes: (i) increased the concentration of antibiotics to the levels used in the plates to increase selection against the non-transduced recipient cells (34 $\mu g \cdot mL^{-1}$ chloramphenicol and 50 $\mu g \cdot mL^{-1}$ of kanamycin); and (ii) increased the initial inoculate from 50 µL to 100 µL of the initial 500 µL of resuspended transduced cells. The flow cytometry experiments were performed as described above except cells were harvested at 4 and 8 hours. Approximately 300 µL of resuspended transduced cells was plated as described previously. The results from the flow cytometry measurements were very similar to the previous experiment (**Fig. S3D**); the transduction of methylated and unmethylated donor phage resulted in very similar distributions of CFP and YFP expression. There was no clear distinction between cells with OFF and ON expression and it appears that most of the population was again recipient cells that had not been successfully transduced. Colony counting showed again that the transduction was successful and that methylated and unmethylated DNA donor phage resulted in different fractions of OFF, ON and mixed colonies. Transduction of the unmethylated DNA donor phage into the wild-type recipient strain (generating HL6718) resulted in 100.0 ± 0.0% of colonies that were OFF (n = 183). Transduction of the methylated DNA donor phage into the wild-type recipient strain (generating HL6719) resulted in 13.9 ± 1.0%, 60.2 ± 0.4% and 25.9 ± 1.4% of colonies that were OFF, ON and mixed respectively (n = 730).

In the third set of experiments we made two further changes to the protocol: (i) the cell density of the culture was increased by inoculating 100 µL of the 500 µL of resuspended transduced cells into 5 mL instead of 10 mL of LB (with 34 $\mu g \cdot mL^{-1}$ chloramphenicol and 50 $\mu g \cdot mL^{-1}$ of kanamycin); and (ii) the growth period for the transduced cells was increased to 16 and 20 hours. The 20 hour growth period was obtained by taking 30 µL of the 16 hour culture and inoculating it into 3 mL of fresh LB with 34 $\mu g \cdot mL^{-1}$ chloramphenicol and 50 $\mu g \cdot mL^{-1}$ of kanamycin and growing the culture for an additional 4 hours at 37^{o}C and shaking at 200 rpm. These experiments were otherwise the same as the previous sets of experiments except the remaining 400 µL of resuspended transduced cells was centrifuged at 16,100 RCF for 1 min, the supernatant was removed and the pellet was resuspended in 100 µL LB and plated.

With this new protocol we were able to distinguish OFF and ON expression states and to distinguish the effects of transducing methylated and unmethylated donor DNA by flow cytometry. Transduction of wild-type recipient cells with unmethylated DNA donor phage resulted in cells (HL6720 and HL6744) that predominantly had low CFP fluorescence levels, consistent with the OFF state (**Fig. S3E**), and a small fraction of cells with higher fluorescence levels, consistent with the ON state (which are difficult to see in the histograms because they are a small fraction of the population). Transduction of wild-type recipient cells with methylated DNA donor phage resulted in cells (HL6721 and HL6745) that had two distinct levels of CFP expression (**Fig. S3F**), consistent with the OFF and ON expression states. All cells had YFP expression indicating they were derived from recipient cells and were not donor cell carry-through (**Fig. S3E**, **F**). Note: we cannot be certain that some of the OFF cells are not residual recipients that were not successfully transduced; however, because the percentage of OFF cells at 20 hours increased substantially from the percentage at 16 hours (see below) it suggests that most of the cells are actively dividing and therefore are successful transductants.

Because the peaks in CFP fluorescence level for the OFF and ON states were well separated it was possible to use the antimode (the lowest fraction between the two peaks) and count the number of cells below and above it to determine the number of OFF and ON cells respectively. This was performed by exporting the flow cytometry data in .fcs format using BD FACSDiva 3.0 (BD Biosciences, San Jose, CA, USA). The raw fluorescence intensity values were then extracted and saved as a text file using FlowPy 4.0 (written by Revanth Sai Kumar, Tejas Mehta, and Biplab Bose, Department of Biotechnology, Indian Institute of Technology Guwahati). The text files were analyzed using custom code written in Matlab (R2013a, Natick, MA, USA) that defined the gate (*i.e.* ranges of forward and side scatter values for selecting cells in the analysis) and counted the number of OFF and ON cells. The percentage of OFF cells was calculated by dividing the number of cells in the OFF state by the total number of cells and multiplying by 100.

The percentage of OFF cells measured by flow cytometry was compared to that obtained by plating and colony counting (**Fig. S3G**). Note: the two protocols are comparing measurements for the same

transduction experiments. Most of the mixed colonies arising from transduction are believed to be due to switching from ON to OFF during the growth of colonies on the plate (see main text) therefore in one of the plots mixed colonies were not considered to be OFF colonies (left, **Fig. S3G**). This plot showed that the percentage of OFF cells is much greater when measured by flow cytometry instead of colony counting (**Fig. S3G**). Even if mixed colonies are categorized as OFF colonies, the percentage of OFF colonies measured by flow cytometry is greater than measured by colony counting (right, **Fig. S3G**). Furthermore, the percentage of OFF cells measured by flow cytometry was much more variable (**Fig. S3F**, **G**). The greater percentage of OFF cells measured with the flow cytometry protocol is due to the long growth period that is required, which increases the relative number of OFF cells because they grow faster than the ON cells due to their lower metabolic burden associated with lower T7 RNA polymerase and/or CFP production (see growth rate section). In support of this, an additional 4 hours of growth (from 16 to 20 hours) increased the percentage of OFF cells in cultures arising from the transduction of wild-type recipient cells with methylated DNA donor phage (HL6721) (**Fig. S3G**).

In summary, flow cytometry can be used to accurately distinguish CFP and YFP fluorescence signals and OFF and ON CFP fluorescence levels in single cells. This was a useful tool for some experiments (see section on growth rate measurements). However, it was unsuitable for our transduction experiments (and transformation experiments) because it requires extended growth periods which bias the measurement towards OFF cells. The faster growth rate of OFF cells was less of an issue with the colony counting method because each colony starts from a single cell and we count the number of colonies with primarily OFF and ON cells rather than the actual number of cells. That is, OFF colonies may have more cells than ON colonies because of their faster growth but this does not alter the numbers of OFF and ON colonies. Therefore plating the cultures and counting the colonies with primarily OFF and ON expression provides a more accurate measure (and also a more precise measure based on the variation) of the percentage of OFF and ON cells respectively after recovery from transduction or transformation.

1.7. Growth rate measurements

Fluorescence microscopy was used to identify 10 colonies of HL6487 (wild-type recipient cells transduced with methylated DNA donor phage) that were primarily ON and 10 colonies of HL6486 (wild-type recipient cells transduced with unmethylated DNA donor phage) that were primarily OFF. The colonies were inoculated into 5 mL of LB with 34 $\mu g \cdot mL^{-1}$ chloramphenicol and 50 $\mu g \cdot mL^{-1}$ of kanamycin and grown for 3 hours at 37°C and shaking at 200 rpm. After this growth period the optical density of the culture was measured by the absorbance of light at 600 nm (A_{600nm}; further defined in the section describing the general protocol for transformation). In addition, some cells from the cultures were placed on ice to measure the initial percentage of OFF and ON cells in the cultures by flow cytometry. Cells from the cultures were diluted in 10 mL of LB with 34 $\mu g \cdot mL^{-1}$ chloramphenicol and 50 $\mu g \cdot mL^{-1}$ of kanamycin so the initial A_{600nm} was 1×10^{-2}. The cultures were grown at 37°C with shaking at 200 rpm and sampled every 40 minutes to measure the A_{600nm} (**Fig. S5A, B**). This set of cultures is termed the "Sampling Protocol" because they were sampled every 40 minutes. After the completion of the experiment the percentage of OFF and ON cells was measured by flow cytometry (**Fig. S5C**).

Exponential growth, which is a linear function on a plot with a logarithmic y-axis and linear x-axis occurred in the range of A_{600nm} values from ~3×10^{-2} to ~3×10^{-1} (grey shaded area in **Fig. S5A**). Therefore an exponential fit was only performed on measurements that fell within this range, which was 80 to 160 minutes for the OFF colony cultures and 120 to 200 minutes for the ON colony cultures. This subset of the data that was fitted to an exponential function is shown in **Fig. S5B**. To make it easier to visually compare the slopes we overlaid them by subtracting the period before they entered exponential growth; this corrected time therefore begins at zero for both OFF and ON cultures. It should be noted that the ~40 minute time delay observed in the ON cultures before exponential growth occurred was believed to be due to technical errors in the measurements of the first few time points and this was confirmed in subsequent experiments.

The data was fitted to an exponential equation ($A_{600nm} = a \cdot e^{-k \cdot time}$) by a nonlinear least squares method with bisquare weighting using the Levenberg-Marquardt algorithm in Matlab (R2013a, Mathworks, Natick, MA, USA). The constant *k* from this equation specifies the slope of the fit, a is the A_{600nm} at the beginning of the exponential growth, and $\log_e(2)/k$ is the doubling time. These fits yielded an average doubling time of 24.3

± 1.2 minutes for the OFF cultures and 32.1 ± 5.5 minutes for the ON cultures. The relatively large error for the ON cultures is due to the different percentages of ON cells in the ON cultures, which ranged from >80% to ~50% in the final measurements (**Fig. S5C**). The percentage of ON cells in all the cultures derived from ON colonies decreased from the initial measurements to the final measurement 4 hours later, which is consistent with the faster growth rate of the OFF cells (*i.e.* shorter doubling time).

We also performed a "Continuous Protocol", where the culture was grown for an extended period without regular sampling that might have decreased the growth rate for the Sampling Protocol. Five single colonies that were primarily ON (HL6487) and five single colonies that were primarily OFF (HL6486) were identified by fluorescence microscopy and inoculated into 5 mL of LB with 34 $\mu g \cdot mL^{-1}$ chloramphenicol and 50 $\mu g \cdot mL^{-1}$ of kanamycin and grown for 5 hours (ON colonies) and 4 hours (OFF colonies) at 37°C with 200 rpm shaking. The cultures derived from ON colonies were grown for 5 hours instead of 4 hours so that they had approximately the same starting density for the next step as the cultures derived from OFF colonies. The A_{600nm} was then measured and the cultures were inoculated into 10 mL of fresh LB with appropriate antibiotics to an initial A_{600nm} of 2×10^{-2}. The initial densities were confirmed by measuring them and samples were also measured by flow cytometry to determine the initial percentage of ON cells (**Fig. S5D, E**). The diluted cultures were grown for 100 minutes at 37°C with 200 rpm shaking and then the final A_{600nm} and the percentage of ON cells were measured by flow cytometry (**Fig. S5D, E**). The doubling time was calculated by the formula: Doubling time = $100 \cdot \log_e(2)/\log_e(\text{Final } A_{600nm}/\text{Initial } A_{600nm})$.

The percentages of ON cells in cultures derived from ON colonies were variable and some of the cultures had a low percentage of ON cells after the initial growth period (**Fig. S5D**). We selected cultures with ~70% or greater ON cells (indicated by horizontal dash line) to determine the average doubling rate of cells in the ON expression state. We found the average doubling rates to be 25.44 ± 0.24 minutes (n = 5) for the OFF cultures and 29.18 ± 0.22 minutes (n = 3) for the ON cultures (**Fig. S5E**). The latter was considered to be an underestimate of the doubling time for ON cells because these cultures contained some OFF cells with shorter doubling times. We therefore plotted the doubling times for all cultures in the experiments as a function of percent ON cells (**Fig. S5F**). A linear fit of the data (Doubling time = m*Fraction ON + c) yielded

a slope (*m*) of 4.50 ± 0.32 minutes/100% percent ON cells and a y-intercept of 25.42 ± 0.16 minutes (R^2 = 0.9619; P<0.0001; *n* = 10). The doubling time for OFF cells is the y-intercept. The doubling time for 100% ON cells is 25.42 ± 0.16 minutes plus 4.50 ± 0.32 minutes, which is equal to 29.92 ± 0.36 minutes. This value was considered to be the best estimate for the doubling time of ON cells, which could not be measured experimentally in a wild-type background because of switching between the ON and OFF states and the faster growth rate of OFF cells.

1.8. SgrS streak plate protocol and measurements

For the preliminary streak plate experiments (**Fig. S6**), frozen culture was diluted 1/100 in 1× M9 minimal media (without any carbon source) and this dilution was used for streaking on the appropriate plate (see below). The dilution was performed to decrease the amount of LB media present in the frozen cultures which can provide alternate carbon sources for cells on M9 minimal media with glucose. We also found that for strains with SgrS and the *agn43* region regulating T7 RNA polymerase there was very little difference in colony size with *oxyR* or *dam* deleted when grown on LB media + 0.5% w/v α-MG. Colonies were grown at 37°C for approximately 16 hours for LB, 36 hours for M9 minimal media + 0.2% w/v glucose, and 48 hours for M9 minimal media + 0.5% w/v α-MG + 2% v/v glycerol; the lengths of time depended on the growth rate of the cells and the points at which differences in colony size were most pronounced. All plates had antibiotic and bovine liver catalase as described above.

With this system, DNA methylation at the *agn43* region should cause SgrS transcription which acts to decrease glucose uptake. Consequently, DNA methylation at the *agn43* region should result in slower growth of colonies on plates with glucose. We tested this in pilot experiments (prior to performing HGT) by comparing the ratio (as a percent) of the average area of colonies of strains with DNA methylation (Δ*oxyR*) divided by the average area of colonies of strains with no DNA methylation (Δ*dam*) on M9 minimal media with 0.2% w/v glucose. We confirmed our prediction; colonies with DNA methylation had only 15.9 ± 1.6% (*n* = 141) of the area of colonies with no DNA methylation (**Fig. S6A**). Note the colonies in the upper right quadrant (with DNA methylation at the *agn43* region) are barely visible compared to colonies in the upper left quadrant which have SgrS but it is not induced (no DNA methylation at the *agn43* region) and compared

to colonies in strains without SgrS in the lower left and right panels (**Fig. S6A**). The global loss of DNA methylation did have some effect on colony growth in strains that had a plasmid without SgrS and were grown on M9 minimal media with 0.2% w/v glucose but the effect was much less than observed above; colonies with DNA methylation (Δ*oxyR*) were 44.9 ± 3.7% (*n* = 139) of the area of colonies with no DNA methylation (Δ*dam*). Similarly, global loss of DNA methylation also had some effect on colony growth in strains with SgrS that were grown on LB, which has carbon sources other than glucose; colonies with DNA methylation (Δ*oxyR*) had 41.8 ± 3.9% (*n* = 86) of the area of colonies with no DNA methylation (Δ*dam*). In both sets of controls the ratio was closer to 100% than in our experimental system indicating there is less effect on colony size when there are alternate carbon sources or there is no SgrS. Our findings are consistent with regulation of SgrS transcription in our system altering cell fitness through its effect on glucose permease. However, there is also clearly an independent effect of the deletion of *oxyR* or *dam* on cell fitness. Therefore cell fitness was only compared in subsequent experiments in strains with the same genetic background and under the same growth conditions.

We expected to see the opposite effects on fitness when cells with our SgrS system were grown on media with 0.5% w/v α-MG. DNA methylation at the *agn43* region should cause SgrS transcription resulting in decreased uptake of toxic α-MG. This was found to be the case; strains with DNA methylation (Δ*oxyR*) grew on M9 minimal media and 0.5% w/v α-MG (plus 2% v/v glycerol as a carbon source) whereas strains without DNA methylation (Δ*dam*) did not (upper right and upper left panels respectively, **Fig. S6B**). Also, strains with an empty plasmid did not grow on M9 minimal media + 0.5% w/v α-MG + 2% v/v glycerol (lower right and lower left panels, **Fig. S6B**), and as expected the DNA methylation state at the *agn43* region had no effect on colony size. The effects of SgrS regulation on colony growth were more dramatic in these experiments than in the previous set because uptake of α-MG can occur only via glucose permease (8), whereas glucose not only enters cells by glucose permease but also via the mannose, galactose and maltose transport systems which diminish the impact of SgrS regulation (9).

1.9. Measuring colony area

It is important to stress that measurements of colony size will depend on how long the plates are incubated. The time period was chosen to maximize the ratio of the colony sizes of strains with induction of SgrS transcription (methylated *agn43* region) and the strains without induction of SgrS transcription (unmethylated *agn43* region). If the plates are removed after too short a period then the colonies are too small to measure accurately and if the plates are left in the incubator for too long a period then the colonies will touch one another making sizing difficult and it is theoretically possible that the slower growing colonies will catch-up with the faster growing colonies. The latter will cause the sizes of colonies with and without SgrS transcription to be similar (*i.e.* the ratio of the sizes of colonies with and without SgrS transcription will be ~1). The approximate lengths of time the plates were in the incubator depended on the media and these are provided in the section describing the SgrS streak plate protocol and measurements. It must also be stressed that under all circumstances the plates with and without induction of SgrS are plated together and placed in and out of the incubator at the same time to ensure they have identical growth periods and conditions.

Plates were placed on a black cloth background and images were captured using the GelDoc system (Bio-Rad). The images were imported into ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA) and a region encompassing most of the plate was selected (the edges of the plate were removed because reflections from the plate edges prevented thresholding for the colonies). The region was then thresholded for light objects at a level that identified all colonies (allowing for ≤ 5 colonies to not be identified). Thresholding by itself sometimes could not distinguish between colonies and precipitate particles that sometimes formed in M9 minimal media. To eliminate the latter from the analysis we used automated selection criteria for circularity to 0.7-1 (note: circularity is a unitless metric calculated by $4\pi \times \text{area}/\text{perimeter}^2$ and a value of 1 is a perfect circle) and also manually identified the particles (they were easy to distinguish visually). Having identified all the colonies on the plate, ImageJ was used to determine the area of each colony in pixels.

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3. SUPPLEMENTARY FIGURE AND TABLE LEGENDS

Fig. S1. Plasmid maps for pHL1477, pHL1701 and pHL2147. These plasmids were created in this study. Other plasmids that were used in this study are reported elsewhere (see **Supplementary Table S1**).

Fig. S2. DNA methylation, restriction endonuclease cleavage and/or agarose gel electrophoresis of PCR products for the transformation experiments. The PCR products were amplified with yeepupf and t7seqr991 oligonucleotides and HL210 as template which contains the *agn43 cis*-regulatory sequence with three GATC sequences that are near each other in the switch region. (**A**) *Dpn*I treatment of methylated PCR product. *Dpn*I cuts fully methylated GATC sequences (note: according to the manufacturer, New England Biolabs, it cuts hemi-methylated DNA with 60-fold less efficiency). The first lane shows the unmethylated PCR product with no treatment and the fourth lane shows the same PCR product following *in vitro* DNA methylation with Dam and cleavage with the *Dpn*I restriction endonuclease. The gel is 1.2% w/v agarose with 1 $\mu g \cdot mL^{-1}$ ethidium bromide and the total amount of 2-log DNA ladder (New England Biolabs) is 1 µg per lane. DNA fragment sizes in the ladder are shown in panel **B**. These results demonstrate that Dam has methylated one or more GATC sequences within the *agn43 cis*-regulatory sequence and this sequence is probably fully methylated. (**B**, **C**) *Mbo*I treatment of unmethylated (panel **B**) and methylated (panel **C**) PCR products. *Mbo*I cuts unmethylated GATC sequences but not fully methylated GATC sequence (and probably not hemi-methylated GATC sequences according to the manufacturer). This experiment demonstrates Dam has methylated all three sites in the *agn43 cis*-regulatory sequence because if any one of the GATC sequences was not methylated at the adenine then it would be cleaved resulting in partial length fragments. Panel **B** shows the PCR products without Dam methylation and Panel **C** shows the same PCR products after Dam treatment. The methylated and unmethylated PCR products were incubated with *Mbo*I or in an identical reaction mix with water instead of *Mbo*I. The full length PCR product following Dam treatment and *Mbo*I digestion was gel extracted and used as the methylated DNA for the transformation experiments. The full length PCR product that was not treated with Dam or with *Mbo*I was gel extracted and used as the unmethylated DNA for the transformation experiments.

Fig. S3. Flow cytometry measurements. Error bars are the SEM. (**A**, **B**) Experiments were performed as described in the **Supplementary Methods** section on *Flow cytometry to measure OFF and ON expression in single cells*. Representative histograms of CFP and YFP fluorescence in the donor (HL23) and recipient (HL5786) strains are shown in panel A. Histograms from unmethylated (HL6486) and methylated (HL6487) transduced colonies are shown in panel B (each curve is a normalized histogram with an average distribution obtained from duplicate cultures). (**C**) The first set of optimization experiments described in the **Supplementary Methods** section *Optimization of flow cytometry protocol for transduction experiments*. In the left plot, the distribution of the unmethylated transduction cultures (HL6715) at 0 hours (orange curve) lies under the methylated transduction cultures (HL6716) at 0 hours (cyan curve). In the right plot, all four cultures have distributions that overlie one another. Each curve is a normalized histogram with an average distribution obtained from four replicate cultures. (**D**) The second set of optimization experiments described in the **Supplementary Methods** section *Optimization of flow cytometry protocol for transduction experiments.* In the left and right plots, all four cultures have similar distributions. Each curve is a normalized histogram with an average distribution obtained from four replicate cultures. (**E**-**G**) Experiments were performed as described in the **Supplementary Methods** (third set of experiments in *Optimization of flow cytometry protocol for transduction experiments*). Panel **E** shows the normalized histogram for four replicate cultures with unmethylated donor DNA. Strains: HL6720A-B (replicates #1 and #2) and HL6744A-B (replicates #3 and #4). Panel **F** shows the normalized histogram for four replicate cultures with methylated donor DNA. Strains: HL6721A-B (replicates #1 and #2) and HL6745A-B (replicates #3 and #4). Panel **G** shows the percent OFF cells measured by flow cytometry as a function of percent OFF cells measured by plating and colony counting. Mixed colonies were categorized as ON (left plot) or OFF (right plot). The data in panel **G** is the same as that shown in panels **E** and **F**.

Fig. S4. Brightfield (with phase condenser annulus 3) and fluorescence microscopy of OFF, ON and mixed colonies (HL6487). (**A**) Comparison of OFF, ON and mixed colonies. The top row shows a mixed colony (red arrow) with a diffuse mix of OFF and ON cells. The bottom row shows a mixed colony (red arrow) with segments of OFF and ON cells. Scale bars are 500 μm. (**B**) Detailed image of a typical OFF

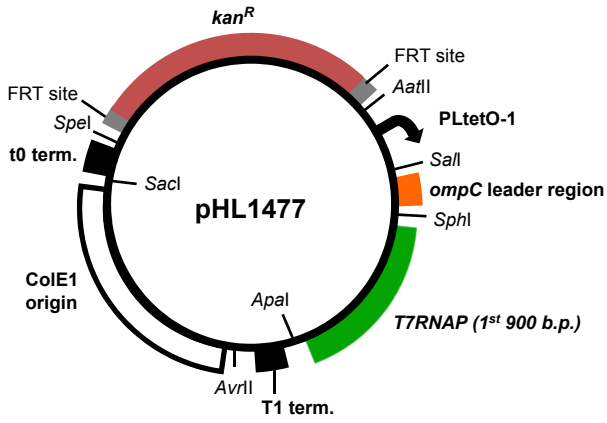
colony with a longer exposure time showing a fluorescent fringe at the periphery (red arrow). Scale bar is 500 µm.

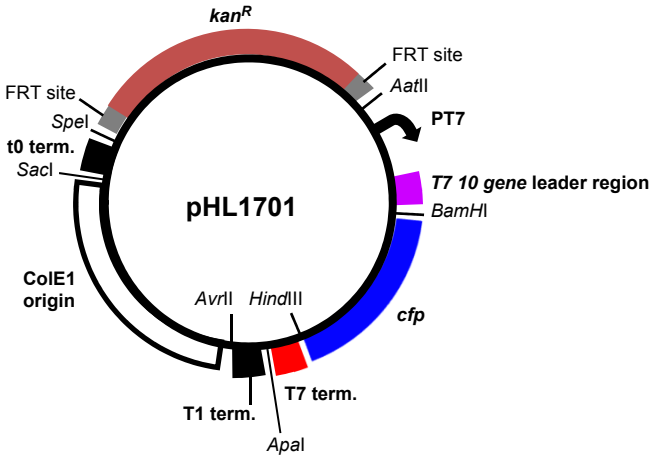
Fig. S5. Growth rate measurement for OFF and ON expression cells. Two sets of experiments were performed as described in the **Supplementary Methods** (Sampling Protocol and Continuous Protocol). All error bars are the SEM. SSE is the sum of squares error. R^2 is the coefficient of determination. *n* is the total number of measurements in each plot. (**A**-**C**) Growth measurements with "Sampling Protocol" in which 10 OFF colonies and 10 ON colonies were grown at 37^{o}C and cells were harvested every 40 minutes to measure the density of the culture. Panel **A** shows the average optical density of cultures as a function of time. The grey shaded region indicates where the spectrophotometer measurements were most accurate and the culture was exponentially growing. The asterisk indicates the initial measurement were calculated from the dilution and not measured. Panel **B** shows individual measurements in the grey shaded region as a function of corrected time, which subtracts the period that occurs before cells enter the grey shaded region. Exponential fits were performed as described. Panel **C** shows the initial and final (after 4 hours) percentage of ON cells determined by flow cytometry for all the OFF and ON cultures. (**D**-**F**). Growth measurements with the Continuous Protocol, which involved growing cultures for 4 hours without any sampling. Panel **D** shows the initial and final (after 4 hours) percentage of ON cells determined by flow cytometry for all the OFF and ON cultures. The grey dash line in the latter indicates the 70% ON cells used as a cut-off to select the samples for calculating the ON cell doubling time. Panel **E** shows individual doubling times for cultures of OFF colonies and ON colonies with equal to or greater than 70% ON cells. Panel **F** shows all the cultures in the Continuous Protocol experiment with their calculated doubling times as a function of the initial percentage of ON cells.

Fig. S6. Effect of SgrS regulation on colony growth. Colony growth of strains with SgrS on streak plates with M9 minimal media + 0.2% w/v glucose (**A**) or M9 minimal media + 0.5% w/v α-MG + 2% v/v glycerol (**B**). Streak plates were performed in duplicate. Strains with unmethylated donor DNA (Δ*dam*) and SgrS or no SgrS were HL6862 and HL6880 respectively. Strains with methylated donor DNA (Δ*oxyR*) and SgrS or no SgrS were HL6864 and HL6881 respectively.

Table S1. Strains and plasmids. Amp = ampicillin, kan = kanamycin and cam = chloramphenicol.

Table S2. Oligonucleotides.


kanR
FRT site
AatII
PLtetO-1
SalI
ompC leader region
SphI
T7RNAP (1st 900 b.p.)
T1 term.
AvrII
ApaI
ColE1 origin
t0 term.
SacI
SpeI
FRT site
pHL1477


kanR
FRT site
AatII
PT7
T7 10 gene leader region
BamHI
cfp
T7 term.
T1 term.
ApaI
AvrII
HindIII
ColE1 origin
t0 term.
SacI
SpeI
FRT site
pHL1701

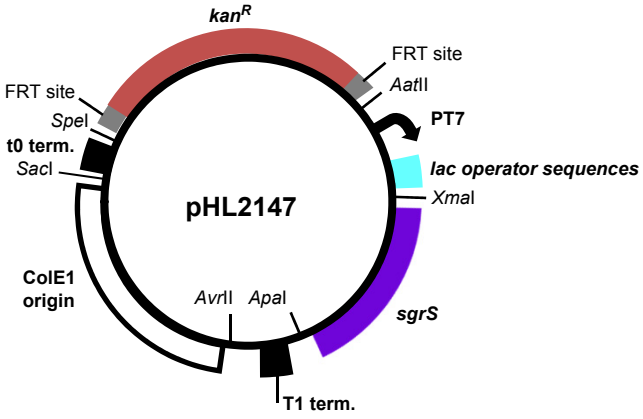

kanR
FRT site
AatII
PT7
lac operator sequences
XmaI
sgrS
T1 term.
AvrII
ApaI
ColE1 origin
t0 term.
SacI
SpeI
FRT site
pHL2147

Fig. S1

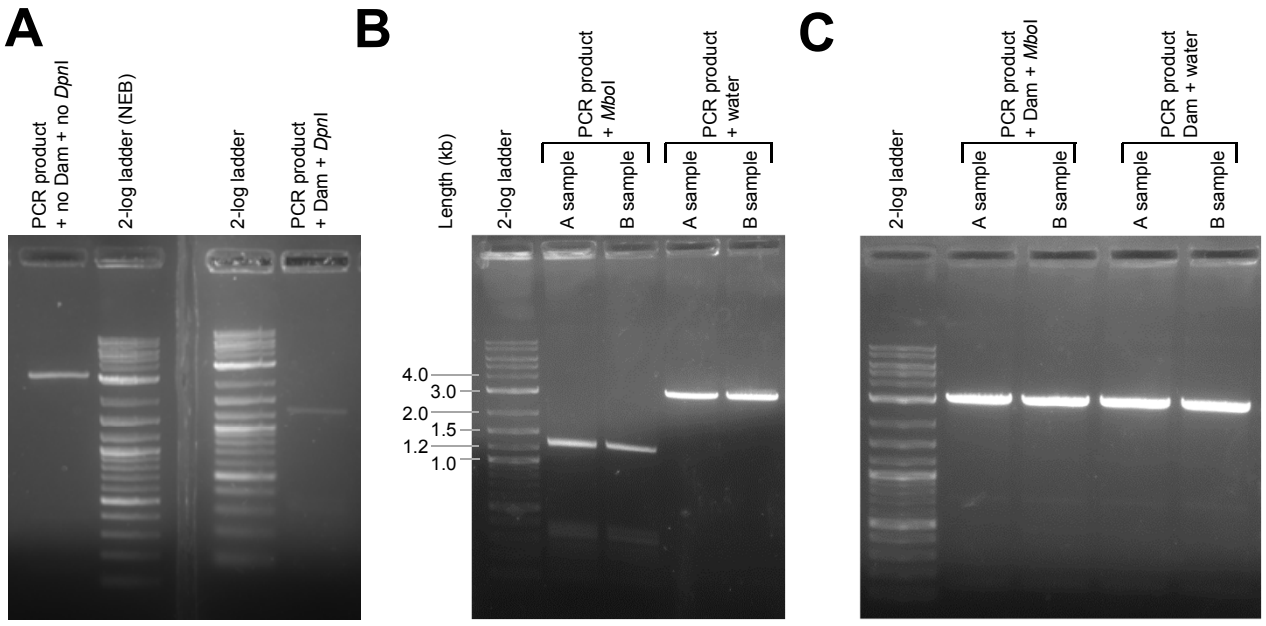

A
PCR product
+ no Dam + no DpnI
2-log ladder (NEB)
2-log ladder
PCR product
+ Dam + DpnI
B
Length (kb)
2-log ladder
PCR product
+ MboI
A sample
B sample
PCR product
+ water
A sample
B sample
4.0
3.0
2.0
1.5
1.2
1.0
C
2-log ladder
PCR product
+ Dam + MboI
A sample
B sample
PCR product
Dam + water
A sample
B sample

Fig. S2

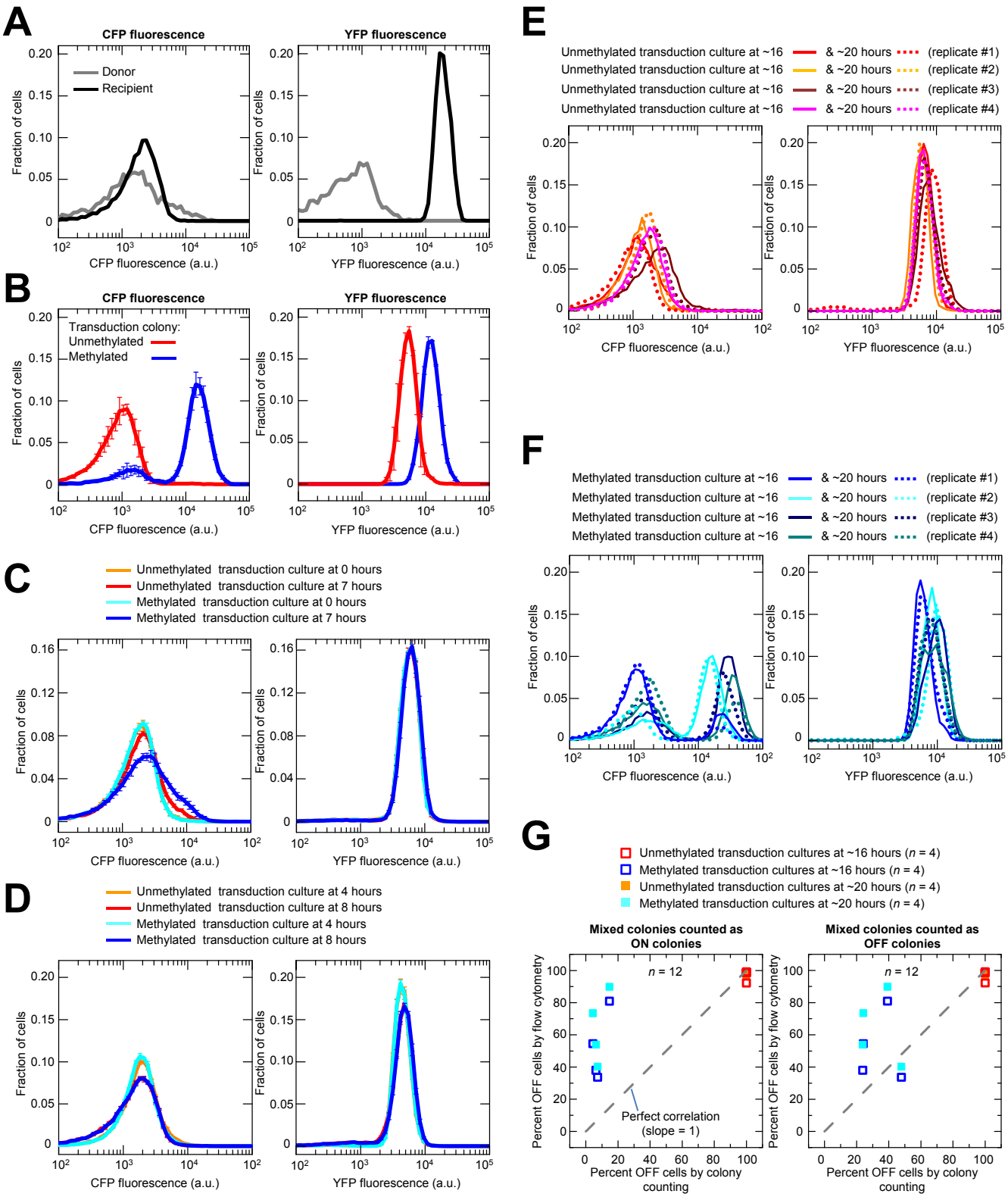

A
CFP fluorescence
YFP fluorescence
Donor
Recipient
Fraction of cells
CFP fluorescence (a.u.)
YFP fluorescence (a.u.)
B
Transduction colony:
Unmethylated
Methylated
C
Unmethylated transduction culture at 0 hours
Unmethylated transduction culture at 7 hours
Methylated transduction culture at 0 hours
Methylated transduction culture at 7 hours
D
Unmethylated transduction culture at 4 hours
Unmethylated transduction culture at 8 hours
Methylated transduction culture at 4 hours
Methylated transduction culture at 8 hours
E
Unmethylated transduction culture at ~16 hours & ~20 hours (replicate #1)
Unmethylated transduction culture at ~16 hours & ~20 hours (replicate #2)
Unmethylated transduction culture at ~16 hours & ~20 hours (replicate #3)
Unmethylated transduction culture at ~16 hours & ~20 hours (replicate #4)
F
Methylated transduction culture at ~16 hours & ~20 hours (replicate #1)
Methylated transduction culture at ~16 hours & ~20 hours (replicate #2)
Methylated transduction culture at ~16 hours & ~20 hours (replicate #3)
Methylated transduction culture at ~16 hours & ~20 hours (replicate #4)
G
Unmethylated transduction cultures at ~16 hours (n = 4)
Methylated transduction cultures at ~16 hours (n = 4)
Unmethylated transduction cultures at ~20 hours (n = 4)
Methylated transduction cultures at ~20 hours (n = 4)
Mixed colonies counted as ON colonies
Mixed colonies counted as OFF colonies
n = 12
Perfect correlation (slope = 1)
Percent OFF cells by flow cytometry
Percent OFF cells by colony counting

Fig. S3

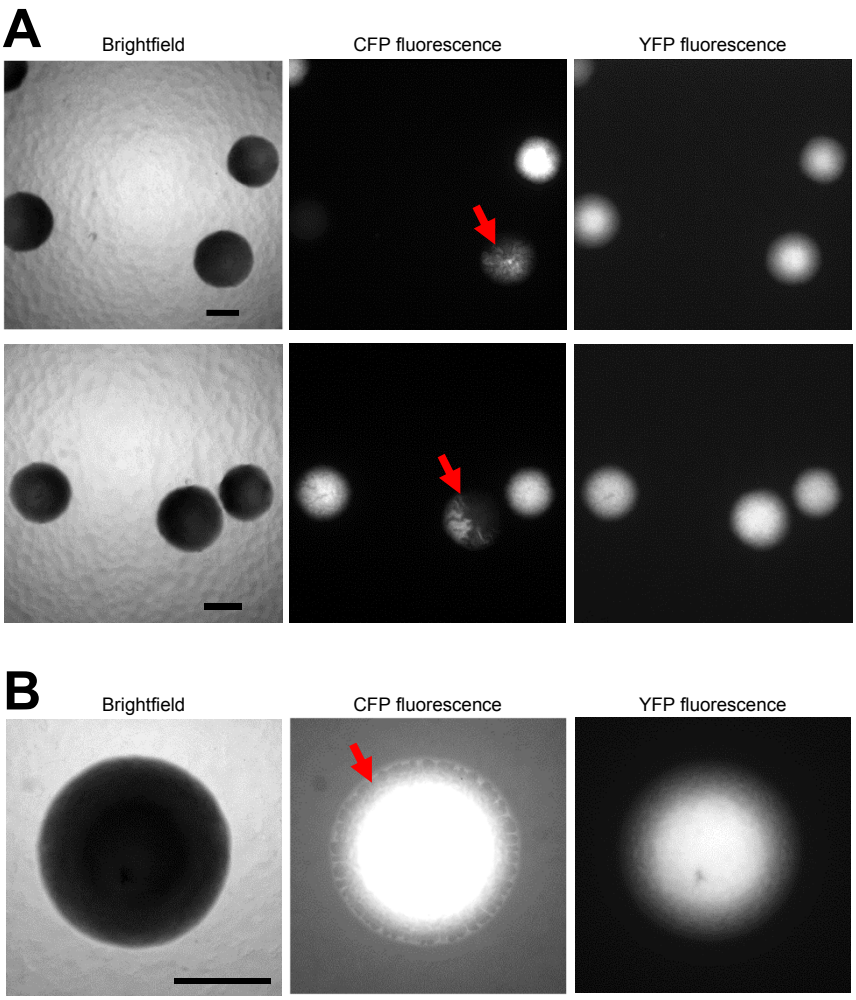

A
Brightfield
CFP fluorescence
YFP fluorescence
B
Brightfield
CFP fluorescence
YFP fluorescence

Fig. S4

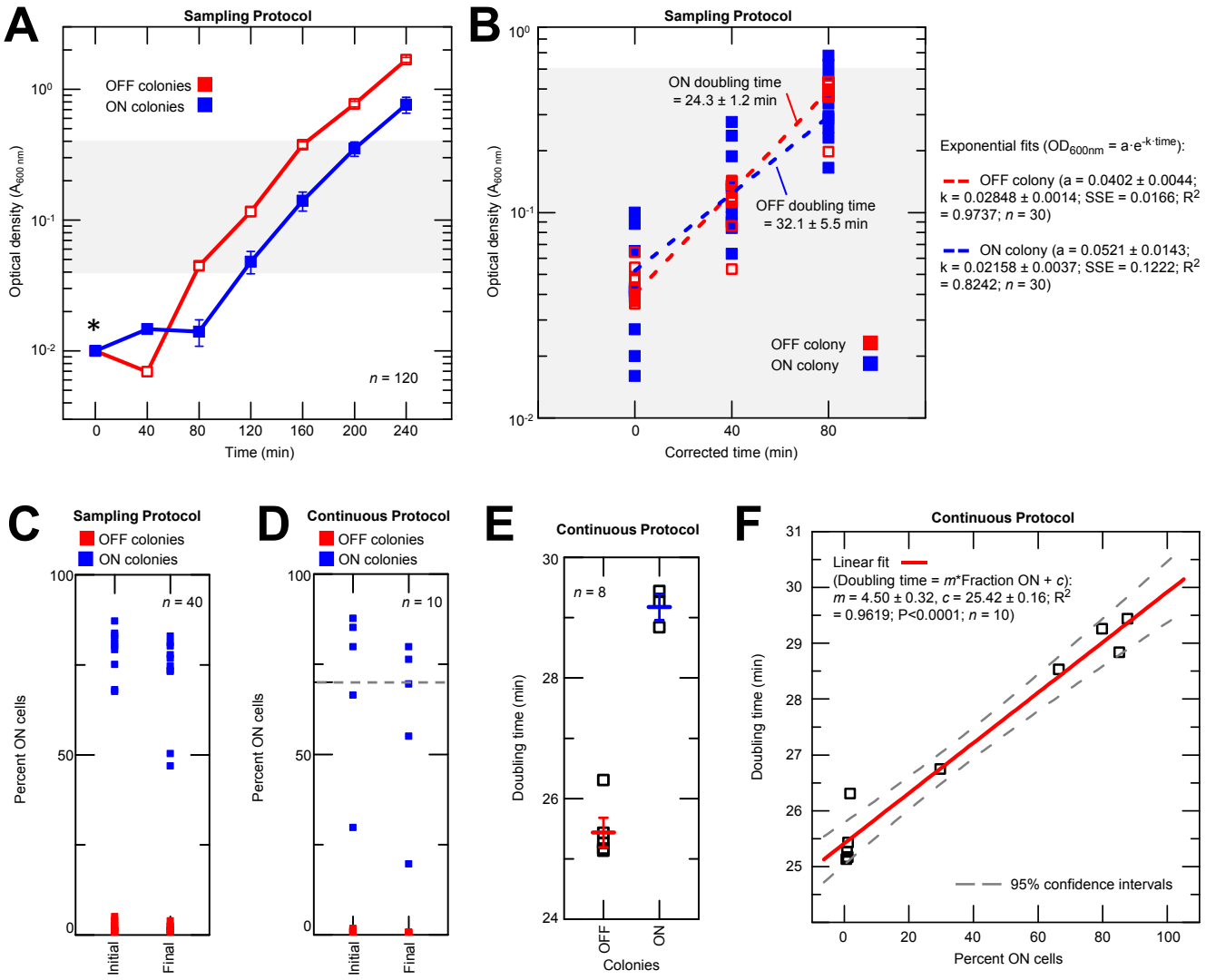

A
Sampling Protocol
OFF colonies
ON colonies
Optical density (A600 nm)
Time (min)
n = 120
B
Sampling Protocol
ON doubling time
= 24.3 ± 1.2 min
OFF doubling time
= 32.1 ± 5.5 min
OFF colony
ON colony
Corrected time (min)
Exponential fits (OD600nm = a·e-k·time):
OFF colony (a = 0.0402 ± 0.0044; k = 0.02848 ± 0.0014; SSE = 0.0166; R2 = 0.9737; n = 30)
ON colony (a = 0.0521 ± 0.0143; k = 0.02158 ± 0.0037; SSE = 0.1222; R2 = 0.8242; n = 30)
C
Sampling Protocol
OFF colonies
ON colonies
n = 40
Percent ON cells
Initial
Final
D
Continuous Protocol
OFF colonies
ON colonies
n = 10
Percent ON cells
Initial
Final
E
Continuous Protocol
n = 8
Doubling time (min)
OFF
ON
Colonies
F
Continuous Protocol
Linear fit
(Doubling time = m*Fraction ON + c):
m = 4.50 ± 0.32, c = 25.42 ± 0.16; R2 = 0.9619; P<0.0001; n = 10)
95% confidence intervals
Doubling time (min)
Percent ON cells

Fig. S5

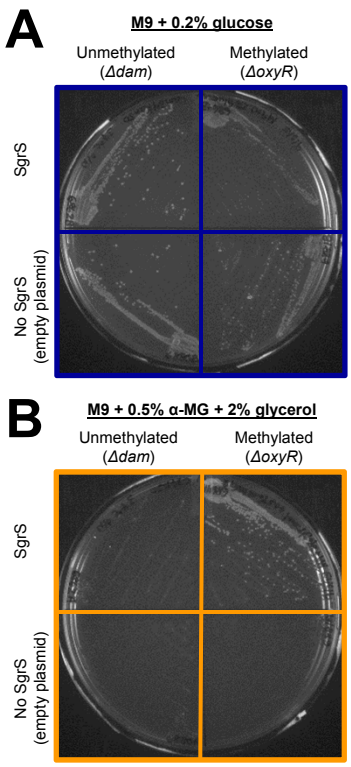

A
M9 + 0.2% glucose
Unmethylated
(Δdam)
Methylated
(ΔoxyR)
SgrS
No SgrS
(empty plasmid)
B
M9 + 0.5% α-MG + 2% glycerol
Unmethylated
(Δdam)
Methylated
(ΔoxyR)
SgrS
No SgrS
(empty plasmid)

Fig. S6

Table S1. Strains and plasmids.

Strain	Description	Source	Antibiotic Resistance
HL1	MG1655 + pKD46	(10)	amp
HL13	MC4100 + *agn43* coding sequence (except first 48 b.p.) replaced by *T7RNAP* and *camR*	(3)	cam
HL23	MC4100 + *agn43* coding sequence (except first 48 b.p.) replaced by *T7RNAP* and *camR* + Δ*dam*	This study	cam
HL24	MC4100 + *agn43* coding sequence (except first 48 b.p.) replaced by *T7RNAP* and *camR* + Δ*oxyR*	This study	cam
HL147	HL13 + pKD46	(3)	cam, amp
HL210	HL147 with deletion of *agn43 cis*-regulatory sequence (-1032 to -526 b.p. relative to start codon) using yeeppkd1f and yeep2ko4r oligonucleotides and pKD13 as template	This study	cam, kan
HL337	HL147 + with insertion of FRT "scar" (2) 51 b.p. downstream of *agn43* switch (and 136 b.p. upstream of the start codon) using pKD13 as template and oligonucleotides agko1f and agko10pkd4r. The scar is simply a genetic marker and this modified *agn43* sequence is not used to control CFP expression in any of the experiments.	This study	cam, kan
HL341	HL147 + deletion of the *agn43* switch and other cis-regulatory sequences (-221 b.p to -19 b.p. was deleted relative to start codon leaving FRT scar sequence) using pKD13 as template and oligonucleotides aggatckopkd1f and agko8pk4r.	This study	cam, kan
HL343	HL337 + pCP20	This study	cam
HL347	HL341 + pCP20	This study	cam
HL365	HL343 + pKD46	This study	cam, amp
HL372	HL365 + Δ*oxyR* using pKD13 as template and oligonucleotides oxpkd1f and oxpkd4r	This study	cam, kan
HL432	HL347 + pKD46	This study	cam, amp
HL444	HL432 + Δ*dam* using pKD13 as template and oligonucleotides dampkd1f and dampkd4r	This study	cam, kan
HL713	HL1 + integration at *intS* of PCR product *kanR::lacIq* amplified from pHL67 using oligonucleotides intspkd1f and laciqints	This study	kan
HL716	HL713 + pCP20 and then cured	(10)	none
HL744	HL716 + pKD46	This study	amp
HL752	HL744 + Δ*sgrS* using pKD13 as template and oligonucleotides sgrsko1pkd1f and sgrsko2pkd4r	This study	kan
HL1951	HL1 + integration at *galK* iof PCR product PLlacO-1::T710::*yfp* from pHL582	(11)	None
HL2846	HL1951 + pKD46	This study	amp
HL3438	HL2846 + Δ*lacI* using pKD13 as template and oligonucleotides lacikopkd1f and lacikopkd4r	This study	kan
HL3500	HL3438 + pCP20 then cured	This study	none
HL4722	HL147 + integration at *agn43* of PCR product *kanR* (with FRT sites)::PLtetO-1::*ompC*::T7RNAP (first 897 b.p.) amplified from pHL1477 using oligonucleotides yeeppkd1f and t7stop900apar	This study	cam, kan
HL4770	HL4722 + pCP20 and then cured of *kanR*	This study	cam, amp
HL5056	MG1655 + integration at *gtrAB* of PLlacO-1::T710::*cfp*::T1 terminator	(12)	None
HL5067	HL5056 + pKD46	This study	amp
HL5085	HL5067 + integration at *galK* of PCR product *camR*::T1 terminator::PLtetO-1::T710::*yfp*::Asp terminator amplified from pHL1274 using oligonucleotides pkd1fgalkf and galkcoler	This study	cam
HL5433	HL713 + *camR*::PLtetO-1::*yfp*::Asp terminator via transduction from HL5085	This study	cam,kan
HL5454	HL4770 + pKD46	This study	cam, amp
HL5475	HL5454 + integration at *yfjV'* of PCR product *kanR*::PT7::T710::*cfp* using pHL1701 as template and oligonucleotides yfjvpkd1f and yfjvcoler	This study	cam, kan
HL5483	HL5433 + pCP20 and cured	This study	none
HL5508	HL5483 + *kanR*::PT7::T710::*cfp* via transduction from HL5475	This study	kan
HL5530	HL5508 + integration at *agn43* of PLtetO-1::*ompC*::*T7RNAP*::*camR* via transduction from HL4770	This study	kan, cam
HL5557	HL5530 + pCP20 and then cured	This study	cam
HL5786	HL3500 + pHL1701	This study	kan
HL6486	HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6487	HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6494	HL5557 + pKD46	This study	cam, amp
HL6537	HL6494 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified using HL210 as template and oligonucleotides yeepupf and t7seqr991. Identical to HL6538.	This study	cam, kan
HL6538	HL6494 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified using HL210 as template and oligonucleotides yeepupf and t7seqr991. Identical to HL6537.	This study	cam, kan
HL6539	HL6494 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified using HL210 as template and oligonucleotides yeepupf and t7seqr991. Identical to HL6540.	This study	cam, kan
HL6540	HL6494 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified using HL210 as template and oligonucleotides yeepupf and t7seqr991. Identical to HL6539.	This study	cam, kan

HL6644	HL3500 + Δ*oxyR* using pKD13 as template and oligonucleotides oxpkd1F and oxpkd4r	This study	kan
HL6648	HL5557 + Δ*oxyR* using pKD13 as template and oligonucleotides oxpkd1F and oxpkd4r	This study	cam, kan
HL6661	HL3500 + Δ*dam* using pKD13 as template and oligonucleotides dampkd1F and dampkd4r	This study	kan
HL6662	HL5557 + Δ*dam* using pKD13 as template and oligonucleotides dampkd1F and dampkd4r	This study	cam, kan
HL6684	HL6644 + pCP20 and then cured	This study	none
HL6686	HL6661 + pCP20 and then cured	This study	none
HL6692	HL6648 + pCP20 and then cured	This study	cam
HL6693	HL6662 + pCP20 and then cured	This study	cam
HL6697	HL6684 + Δ*dam* using pKD13 as template and oligonucleotides dampkd1F and dampkd4r	This study	kan
HL6702	HL6692 + Δ*dam* using pKD13 as template and oligonucleotides dampkd1F and dampkd4r	This study	cam, kan
HL6703	HL6697 + pCP20 and then cured	This study	none
HL6712	HL6686 + pHL1701	This study	kan
HL6713	HL6703 + pHL1701	This study	kan
HL6715	Replicate of HL6486. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6716	Replicate of HL6487. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6718	Replicate of HL6486. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6719	Replicate of HL6487. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6720	Replicate of HL6486. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6721	Replicate of HL6487.HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6730	HL6702 + pCP20 and then cured	This study	cam
HL6744	Replicate of HL6486. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6745	Replicate of HL6487. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6746	HL3500 + Δ*sgrS* using pKD13 as template and oligonucleotides sgrsko1pkd1f and sgrsko2pkd4r	This study	kan
HL6747	HL1951 + Δ*sgrS* using pKD13 as template and oligonucleotides sgrsko1pkd1f and sgrsko2pkd4r	This study	kan
HL6749	HL6686 + Δ*sgrS* using pKD13 as template and oligonucleotides sgrsko1pkd1f and sgrsko2pkd4r	This study	kan
HL6750	Replicate of HL6486. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6751	Replicate of HL6487. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6754	HL6712 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6755	HL6712 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6756	HL6713 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6757	HL6713 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6759	HL3500 + Δ*oxyR* using pKD13 as template and oligonucleotides oxpkd1F and oxpkd4r	This study	kan
HL6773	HL6692 + pKD46	This study	amp, cam
HL6775	HL6730 + pKD46	This study	amp, cam
HL6776	HL6746 + pCP20 and then cured	This study	none
HL6777	HL6747 + pCP20 and then cured	This study	none
HL6778	HL6749 + pCP20 and then cured	This study	none
HL6779	HL6759 + pCP20 and then cured	This study	none
HL6794	HL6779 + pHL1701	This study	kan
HL6796	HL6693 + pKD46	This study	amp, cam
HL6812	Replicate of HL6486. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6813	Replicate of HL6487. HL5786 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6814	HL6794 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6815	HL6794 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6816	Replicate of HL6754. HL6712 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6817	Replicate of HL6755. HL6712 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6818	Replicate of HL6756.HL6713 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan

HL6819	Replicate of HL6757. HL6713 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6822	HL6779 + Δ*sgrS* using pKD13 as template and oligonucleotides sgrsko1pkd1f and sgrsko2pkd4r	This study	kan
HL6825	HL6822 + pCP20 and then cured	This study	none
HL6828	HL6494 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product not treated with Dam or *Mbo*I	This study	cam, kan
HL6829	HL6494 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product treated with Dam and *Mbo*I	This study	cam, kan
HL6832	HL6773 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product not treated with Dam or *Mbo*I	This study	cam, kan
HL6833	HL6773 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product treated with Dam and *Mbo*I	This study	cam, kan
HL6834	HL6796 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product not treated with Dam or *Mbo*I	This study	cam, kan
HL6835	HL6796 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product treated with Dam and *Mbo*I	This study	cam, kan
HL6836	HL6775 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product not treated with Dam or *Mbo*I	This study	cam, kan
HL6837	HL6775 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product treated with Dam and *Mbo*I	This study	cam, kan
HL6842	HL23 + Δ*sgrS* using pKD13 as template and oligonucleotides sgrsko1pkd1f and sgrsko2pkd4r	This study	cam, kan
HL6843	HL24 + Δ*sgrS* using pKD13 as template and oligonucleotides sgrsko1pkd1f and sgrsko2pkd4r	This study	cam, kan
HL6845	HL6842 + pCP20 and then cured	This study	cam
HL6846	HL6843 + pCP20 and then cured	This study	cam
HL6847	HL6494 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product not treated with Dam or *Mbo*I	This study	cam, kan
HL6848	HL6494 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product treated with Dam and *Mbo*I	This study	cam, kan
HL6849	HL6773 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product not treated with Dam or *Mbo*I	This study	cam, kan
HL6850	HL6773 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product treated with Dam and *Mbo*I	This study	cam, kan
HL6851	HL6796 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product not treated with Dam or *Mbo*I	This study	cam, kan
HL6852	HL6796 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product treated with Dam and *Mbo*I	This study	cam, kan
HL6853	HL6775 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product not treated with Dam or *Mbo*I	This study	cam, kan
HL6854	HL6775 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product treated with Dam and *Mbo*I	This study	cam, kan
HL6862	HL6845 + pHL2147	This study	cam, kan
HL6864	HL6846 + pHL2147	This study	cam, kan
HL6866	HL6776 + pHL2147	This study	kan
HL6868	HL6778 + pHL2147	This study	kan
HL6869	HL6825 + pHL2147	This study	kan
HL6870	HL6866 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6871	HL6866 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6874	HL6868 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6875	HL6868 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6876	HL6869 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL23	This study	cam, kan
HL6877	HL6869 + *agn43 cis*-regulatory sequence::T7RNAP + *camR* via transduction from HL24	This study	cam, kan
HL6880	HL6845 + pZE21	This study	cam, kan
HL6881	HL6846 + pZE21	This study	cam, kan

HL6886	HL6494 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product not treated with Dam or *Mbo*I	This study	cam, kan
HL6887	HL6494 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product treated with Dam and *Mbo*I	This study	cam, kan
HL6888	Replicate of HL6886. HL6494 + integration at *agn43* of PCR product *kanR*::unmethylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product not treated with Dam or *Mbo*I	This study	cam, kan
HL6889	Replicate of HL6887. HL6494 + integration at *agn43* of PCR product *kanR*::methylated *agn43 cis*-regulatory sequence::T7RNAP (first 1014 b.p.) amplified from HL210 using oligonucleotides yeepupf and t7seqr991. PCR product treated with Dam and *Mbo*I	This study	cam, kan
pCP20	Plasmid with FLP recombinase for excision of *kanR* or *camR* cassette flanked by FRT sites	(2)	amp
pHL67	Plasmid with *lacIq* from pTrc99a + ColE1 from pZE21 + *kanR* cassette flanked by FRT sites from pKD13	(10)	kan
pHL582	Plasmid with *kanR* flanked by FRT sites + PLlacO-1::T710::yfp::T7 terminator::T1 terminator + ColE1	(7)	amp
pHL1274	Plasmid with *camR* flanked by FRT sites + T1 term + PLtetO-1::T710::*yfp*::Asp term +ColE1	(12)	cam
pHL1477	Plasmid with *kanR* flanked by FRT sites + PLtetO-1::*ompC*::T7RNAP::T1 terminator + ColE1	This study	kan
pHL1701	Plasmid with *kanR* flanked by FRT sites + PT7::T710::*cfp*::T7 terminator::T1 terminator + ColE1	This study	kan
pHL2147	Plasmid with *kanR* flanked by FRT sites + PT7::lac operator::*sgrS*::T1 terminator + ColE1	This study	kan
pKD13	Plasmid with *kanR* flanked by FRT sites for lambda Red deletion	(2)	amp
pKD46	Plasmid with inducible lambda Red recombinase	(2)	amp
pZE21	Plasmid with *kanR* + ColE1 origin	(1)	kan

Table S2. Oligonucleotides.

Forward	Reverse	Sequence	Function (strain created)
ag9f	t7seqr1531	ctcaggcccgtgaacagtttacc	PCR check for correct integration of *agn43* sequence
aggatckopkd1f	agko8pk4r	ttcgttactgtctctcttgtccgtgcaatagctcaataatagaataaa acgtgtaggctggagctgcttc	deletion of all three GATC methylation sites in the *agn43* coding region to the ribosomal binding site
agko8pk4r	aggatckopkd1f	gcctgtagcaggtattcagatgtcgtttcatcagcttttccttagattg aattccggggatccgtcgacc	deletion of all three GATC methylation sites in the *agn43* coding sequence to the ribosomal binding site
agko1f	agko10pkd4r	gataagctaataataacctttgtcagtaacatgcacagatacgta cagaagtgtaggctggagctgcttc	deletion of the *agn43* gene and the three GATC methylation sites
agko10pkd4r	agko1f	tccggctgtgggagtttctgaattgtggttctgttgttccctgaatgtct attccggggatccgtcgacc	deletion of the ag43 gene and the three GATC methylation sites
cyfpgalkr	pkd1fgalkf	gtttgcgcgcagtcagcgatatccattttcgcgaatccggagtgta agaattacttgtacagctcgtccatgcc	PCR amplify PLlacO-1::T710::*yfp* using pHL582 as template to integrate at *galK*
dampkd1f	dampkd4r	aattagttagtcagcatgaagaaaaatcgcgctttttgaagtggg cagggtgtaggctggagctgcttc	delete *dam* using pKD13 as template in HL444
dampkd4r	dampkd1f	ttatttttcgcgggtgaaacgactcctggtttgtacaaagccagca gttattccggggatccgtcgacc	delete *dam* using pKD13 as template in HL444
damecor1f	damkpn1r	ccggaattcatgaagaaaaatcgcgcttttttg	PCR check for random *dam* transduction
damkpn1r	damecor1f	cgcggtaccttatttttcgcgggtgaaacgac	PCR check for random *dam* transduction
galkcoler	pkd1fgalkf	gtttgcgcgcagtcagcgatatccattttcgcgaatccggagtgta agaaagctgataccgctcgccgcagccgaacg	PCR amplify *camR*::T1 terminator::PLtetO-1::T710::*yfp*::Asp terminator using pHL1274 as template for integration at *galK* in HL5067 (HL5085)
intspkd1f	laciqints	ccgtagatttacagttcgtcatggttcgcttcagatcgttgacagcc gcagtgtaggctggagctgcttc	PCR amplify *kanR*::*lacIq* using pHL67 as template and inserted at *intS* in HL1 (HL713)
lacikopkd1f	lacikopkd4r	gcggtatggcatgatagcgcccggaagagagtcaattcagggt ggtgaatgtgtaggctggagctgcttc	delete *lacI* using pKD13 as template in HL2846 (HL3438)
lacikopkd4r	lacikopkd1f	cctggggtgcctaatgagtgagctaactcacattaattgcgttgcg ctcaattccggggatccgtcgacc	delete *lacI* using pKD13 as template in HL2846 (HL3438)
laciqints	intspkd1f	atagttgttaaggtcgctcactccaccttctcatcaagccagtccgc ccagctaactcacattaattgcgttgc	PCR amplify *kanR*::*lacIq* using pHL67 as template and inserted at *intS* in HL1 (HL713)
oxpkd1f	oxpkd4r	atgaatattcgtgatcttgagtacctggtggcattggctgaacacc gccagtgtaggctggagctgcttc	delete *oxyR* using pKD13 as template in HL372
oxpkd4r	oxpkd1f	ttaaaccgcctgttttaaaactttatcgaaatggccatccattcttgc gcattccggggatccgtcgacc	delete *oxyR* using pKD13 as template in HL372
pkd1fgalkf	cyfpgalkr or galkcoler	ttcatattgttcagcgacagcttgctgtacggcaggcaccagctctt ccggtgtaggctggagctgcttc	PCR amplification with sequences for integration at *galK*
pompc1apaf	t7seqr1531	catgggcccttgccgactgattaatgagggtta	PCR check for carry through of recipient cells
pt7lacoidaatf	pt7lacoidxmar or sgrsrevapaIr	cgcgacgtctaatacgactcactataggggaattgtgagcg	PCR amplify PT7::lac operator for pHL2147
pt7lacoidxmar	pt7lacoidaatf	cctcccgggaattgtgagcgctcacaattcccctatagtg	PCR amplify PT7::lac operator for pHL2147
sgrsko1pkd1f	sgrsko2pkd4r	gcaaaagacagcaattttattttccctatattaagtcaataattccta acgtgtaggctggagctgcttc	delete *sgrS* using pKD13 as template in HL752
sgrsko2pkd4r	sgrsko1pkd1f	gccatcgtcattatccagatcatacgttccctttttagcgcggcgag aatattccggggatccgtcgacc	delete *sgrS* using pKD13 as template in HL752
sgrsrevapaIr	pt7lacoidaatf	catgggcccaagctttttagcgcggcgagaataaaaaaaac	PCR amplify *sgrS* for pHL2147
t7seqr1531	ag9f	gtactcaaagcagaacgcaaggaa	PCR check for correct integration of *agn43* sequence
t7seqr991	yeepupf	ggcgaccgctaggactttcttgtt	PCR amplify kanR::*agn43* regulatory sequence::T7RNAP (first 1014 b.p.) using HL210 as template for integration at *agn43* in HL6494 (HL6537, HL6538, HL6539, HL6540)
t7stop900apar	yeeppkd1f	catagggcccttaagtacgcaccagcgccagaggacg	PCR amplify *kanR*::PLtetO-1::*ompC*::T7RNAP (first 897 b.p.) using pHL1477 as template for integration at *agn43* in HL147 (HL4722). Note: stop codon in the oligonucleotide is not in recombined sequence in the recipient cell chromosome.
yeeppkd1f	yeep2ko4r or t7stop900apar	atcaccaaaaagggtgaatctccggactccctatatcacttaaatt gatagtgtaggctggagctgcttc	PCR amplification with sequences for integration at *yeeP'*
yeep2ko4r	yeeppkd1f	gcctgagaccggacagattccgtgcgcagctcgtcctgcagtcg ggtcatattccggggatccgtcgacc	PCR amplify product to delete *agn43 cis*-regulatory sequence (-1032 to -526 b.p. relative to start codon) using pKD13 as template in HL147 (HL210)
yeepupf	t7seqr991	gatgagtctgcgctggagcgtgtt	PCR amplify *kanR*::*agn43* regulatory sequence::T7RNAP (first 1014 b.p.) using HL210 as template for integration at *agn43* in HL6494 (HL6537, HL6538, HL6539, HL6540)
yfjvpkd1f	yfjvcoler	ccacaggccgggttgtgatagatggtaatgttgctcatatcagtat ctaagtgtaggctggagctgcttc	PCR amplify *kanR*::PT7::T710::*cfp* using pHL1701 as template for integration at *yfjV'* in HL5454 (HL5475)
yfjvcoler	yfjvpkd1f	cgatacagtgttgatggctgaagactctatcaacgacaaagttcc tacctagctgataccgctcgccgca	PCR amplify *kanR*::PT7::T710::*cfp* using pHL1701 as template for integration at *yfjV'* in HL5454 (HL5475)