# Supporting Information

## Hussain et al. 10.1073/pnas.1316298111

### Plasmid Assembly

The wild-type dual-feedback oscillator was obtained from the Jeff Hasty laboratory at University of California, San Diego (1). The pJS167NheI plasmid has the gene sequences for activator, *araC*, and reporter, *gfp*, fused to the carboxy-terminus *ssrA* degradation tag, which translates into the amino acid sequence, TS**AANDENYALAA** (TS residues act as linker). This *ssrA* tag specifically targets proteins to the ClpXP degradation pathway in *Escherichia coli*. The repressor plasmid, pZA14LacI, has the wild-type (wt) lacI gene fused to the *ssrA* degradation tag. All three genes were cloned under the transcriptional control of the $P_{lac/ara}$ hybrid promoter which has the $I_1$—$I_2$ binding sites for AraC as well as two *lacO1* operator sites with the proximal operator overlapping the transcriptional start site and a distal site that is ∼500 bp upstream of the proximal operator site. The $P_{lac/ara}$ hybrid promoter is activated by AraC in the presence of arabinose and repressed by LacI in the absence of isopropyl β-D-1-thiogalactopyranoside (IPTG). The repressor and activator plasmids carried ampicillin and kanamycin resistance, respectively. The repressor module contains the p15A origin of replication (*ori*), whereas the activator module has the ColE1-based *ori*.

The two plasmid-based induction system was created with the reporter gene, *gfp*, fused to the *ssrA* degradation tag under transcriptional control of the $P_{LlacO1}$ promoter (pGFPKan). On a separate plasmid, the repressor gene (wt or ts), *lacI*, was placed under the constitutive *I* promoter (pZSLacI). To maintain plasmid stability, the reporter plasmid contained the medium copy number *ori*, p15A, and the repressor plasmid contained a variant of the pSC101 *ori*. The reporter and repressor plasmids carried kanamycin and ampicillin resistance, respectively.

$P_{LlacO1}$ promoter sequence: The two operator sites are in bold, and the transcription start site is underlined. An additional operator sequence is located 500 bp upstream of the transcriptional start site.

**GTGAGCGGATAACAA**TTGACATT **GTGAGCGGATAACAA**GATACTGAGCAC<u>A</u>TCAGCAGGACGCACTGACC

*I* promoter sequence:

GACACCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATTCAGG

Maps of each plasmid used in this study are shown in Fig. S3. The specifics of each plasmid are given in Table S2.

### Mutagenesis

The QuikChange site-directed mutagenesis kit (Agilent) was used to generate the tsLacI variant in pZA14LacI and pZSLacI. For each mutagenesis reaction, wild-type LacI gene was used as the parental template, and two synthetic complementary primers containing the desired mutation were obtained from Sigma-Aldrich. Standard mutagenesis protocol from Agilent was used to create the variant LacI species, and each variant gene was confirmed by primer extension sequencing analysis (LoneStar Labs).

### Cell-Viability Assay

To ensure tsLacI is not lethal to *E. coli* cells, JS006 cells were transformed with either the wt or ts oscillator. Cells were grown overnight at 30 °C in Luria Bertani (LB) media supplemented with antibiotics. The next morning, fresh LB cultures were inoculated with the overnight culture at a 1:100 dilution and grown in an incubator/shaker at 37 °C for 2 h before being plated in a 96-well plate (BD Falcon) in triplicates. At this point, the plate was placed in a Tecan plate reader preset to 37 °C to measure optical density every 15 min for 6 h with intermittent orbital shaking. Cells with either type of the oscillator grew at a similar rate as shown by the overlapping growth curves (Fig. S2).

### Induction Assay

The *lacI* gene (wt or ts) was cloned into the pZSlacI plasmid under control of the constitutive *I* promoter. The fluorescent reporter gene, *gfp*, was cloned into the pGFPkan plasmid under the transcriptional control of the $P_{LlacO1}$ promoter. The plasmids were cotransformed into the Δ*araC*Δ*lacI E. coli* strain, JS006, and plated on agar plates supplemented with 100 μg/mL ampicillin (amp) and 50 μg/mL kanamycin (kan). Plates were incubated at 37 °C overnight and placed at 4 °C until inoculation of liquid cultures. Single colonies were picked for inoculation of 5 mL LB supplemented with 100 μg/mL ampicillin and 50 μg/mL kanamycin and grown overnight at 30 °C in an incubator/shaker. Fresh 5 mL LB with amp/kan was inoculated with 50 μL of the overnight culture and grown in the incubator/shaker preset to the desired temperature. For wtLacI at 37 °C, increasing concentrations of IPTG were added to the media. After three hours of growth, 200 μL of the cell cultures were plated in triplicate in a 96-well plate. The optical density (600 nm) and GFP fluorescence ($\lambda_{ex} = 488$ nm and $\lambda_{em} = 509$ nm) of the cell cultures was measured using a plate reader (Infinite M1000 TECAN).

### Photolithography

The microfluidic device was manufactured as described in Stricker et al. (1). Briefly, a 4-inch silicon wafer (Silicon Quest) was cleaned with acetone, isopropyl alcohol, and water. The wafer was coated with SU-8 2000 series photoresist (MicroChem) and spun for 30 seconds in a spin coater (Brewer Instruments) to evenly distribute the resist. The resist was baked at 95 °C, and cooled to room temperature. The wafer was mounted to the chuck of a mask aligner (SUSS). The photomask (CAD/Art Services) was mounted, and the wafer was aligned to the photomask. Resist was exposed with UV light for cross-linking. The wafer was baked at 95 °C to optimize cross-linking. Undeveloped resist was removed with SU-8 developer (MicroChem). The process was repeated until all layers were completed. The wafer was hard-baked at 150 °C to solidify all of the layers. Finally, the wafer was coated with release agent [((tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane); Pfaltz & Bauer] for 10 min under vacuum.

### Soft Lithography

Polydimethylsiloxane (PDMS) polymer (Sylgard 184; Dow Corning) was mixed in a weigh boat with curing agent in a 10:1 mass ratio until thoroughly combined (∼5 min). The mixture was degassed under vacuum until all air bubbles were cleared. The wafer for the selected device was wrapped in aluminum foil to hold the PDMS. PDMS was poured onto the foil-wrapped wafer and degassed under vacuum to remove all air bubbles. The wafer and PDMS were baked at 80 °C for 1 h. The monolith was removed from the wafer and trimmed. Ports for fluidic connections were punched with a 0.5-mm biopsy punch (GE Healthcare). Individual chips were diced from the monolith. Chips were submerged in methanol and sonicated for 8 min. A second round of sonication with fresh methanol was conducted for 8 min. The chips were baked at 80 °C for 30 min. Chips were cleaned with tape (3M), and no. 1.5 coverslips (VWR) were cleaned with isopropyl alcohol and dried with compressed nitrogen. Chips and coverslips were cleaned for 3 min in a UV/ozone oven (Jelight Co.). Chips were inverted onto the coverslips for binding, and the assembled devices were baked overnight at 80 °C.

## Model Parameters

Certain parameters such as β were fit directly from experimental observation. The dilution rate β can be computed as $\ln(2)/\tau_{cc}$ where $\tau_{cc}$ is the cell-cycle time. Because the temperature affects the cell-cycle time precisely in the same manner as it does all other biochemical processes, the appropriate dilution rate at a temperature $T$ can be obtained as $\beta \cdot A(T)$, where β is the dilution rate at $T = 37$ °C and $A(T)$ is the Arrhenius scaling; $\tau_{cc}$ was experimentally observed to be ∼25 min at 37 °C. Therefore, we set $\beta = 0.0275$ min$^{-1}$.

Parameters such as θ and $T_{ref}$ were obtained from existing literature (1). Other parameters such as $\tau_{a,r}$, $\alpha_{a,r}$, $C_{r,max}$, $C_{r,min}$, $T_0$, $b$, $\gamma_{a,r}$, and $R_0$ are difficult to measure in vivo. Initial values for these parameters were obtained by specifying biologically feasible protein levels, and then computing the parameter values that would lead to those levels.

The initial estimates of the model parameters were then fine-tuned by hand so that the model accurately reproduced the data obtained from three types of experiments: (*i*) the oscillation period data for the wild-type cells, (*ii*) the oscillation period for the temperature-sensitive mutant, and (*iii*) the relative induction as a function of temperature for the temperature-sensitive mutant. The final values of all parameter used in the computational model are given in Table S1.

## Numerical Simulation of the Model

The model was simulated using the delayed differential-equation function *dde23* in MATLAB. An initial history $a(t) = r(t) = 1,300$ molecules/cell was chosen for $t \leq 0$ min, following which a transient of ∼20 oscillation cycles was computed. The results of that integration were then used as the initial history for another integration of ∼20 oscillations. Following the completion of both transients, data were gathered for at least 40 oscillation cycles and the period was computed as the average of these oscillations.

In some cases, an accumulation of numerical errors in *dde23* causes oscillations to cease in the numerical simulations. We verified that such cases were indeed numerical artifacts introduced by the integrator by decreasing the maximum allowed step size by a factor of 10, which restored oscillations. However, oscillations can also be restored not by changing the step size, but by using a precalculated oscillatory history instead of the constant history described above. Using the oscillatory history not only restores oscillations, but also allows for much faster integration of the model.

In addition, small numerical inaccuracies can cause the time series for AraC and LacI to become negative. A simple verification shows that the positive quadrant is invariant in the mathematical model, and negative solutions are therefore a numerical artifact. We have therefore introduced soft boundaries at $a = 0$ and $r = 0$ to prevent the accumulation of such errors.

## Period Determination of the Mathematical Model

The period was determined using two methods. First, a zero-crossing method was implemented, and the period was computed as the time between two consecutive 0 up-crossings of the mean-centered time series. The obtained periods were validated by using the *findpeaks* function of MATLAB. The averaged period obtained from both methods were very comparable.

## Robustness to Parameter Variation

The model exhibits the same qualitative behavior for a range of parameter values. To test the robustness of the model, we perturbed the parameters by adding normally distributed increments. The noise was added to all parameters except $N$, $T_{ref}$, and θ. For each parameter, the size of the noise was taken to 10% of its mean value. The parameters were sampled 110 times, and three types of datasets were gathered: (*i*) the period curve for the wild-type cells, (*ii*) the period curve for the temperature sensitive mutant, and (*iii*) the relative induction curve for the temperature-sensitive mutant. The results are shown in Fig. S1.

1. Stricker J, et al. (2008) A fast, robust and tunable synthetic gene oscillator. *Nature* 456: 516–519.

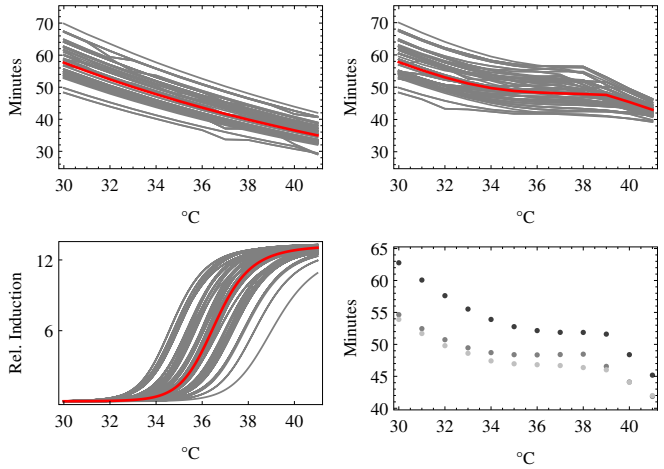



**Fig. S1.** Robustness of the model to parameter variability. Gray curves correspond to the period data for parameters perturbed by normally distributed noise. The red curve is the plot obtained for the parameters presented in Table S1. The model displays the same qualitative behavior over the entire range of parameter values tested. (*Upper Left*) Period data for wild-type cells. (*Upper Right*) Period data for the temperature-sensitive mutant. (*Lower Left*) Induction curve for the temperature sensitive mutant. (*Bottom Right*) Three sample curves from the data displayed in the *Upper Right*.

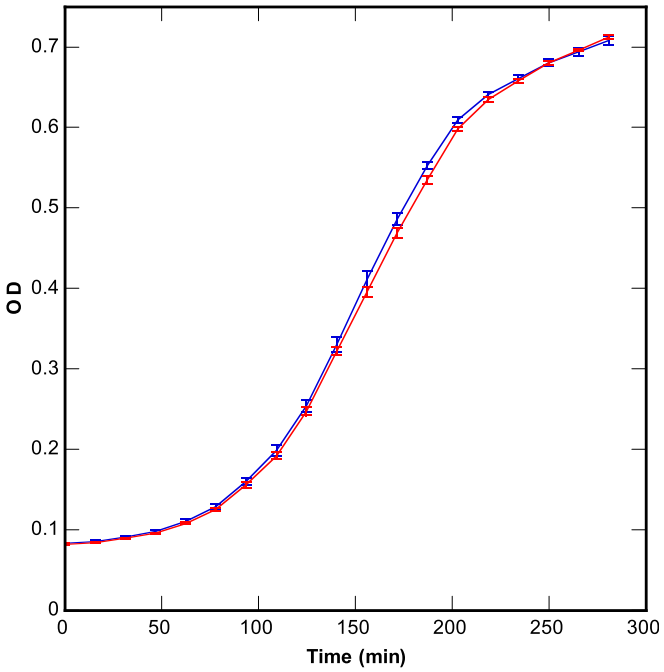



**Fig. S2.** Growth curve of JS006 cells with either wtLacI (blue) or tsLacI (red) oscillator systems measured as optical density at 600 nm over time. Error bars represent SD.

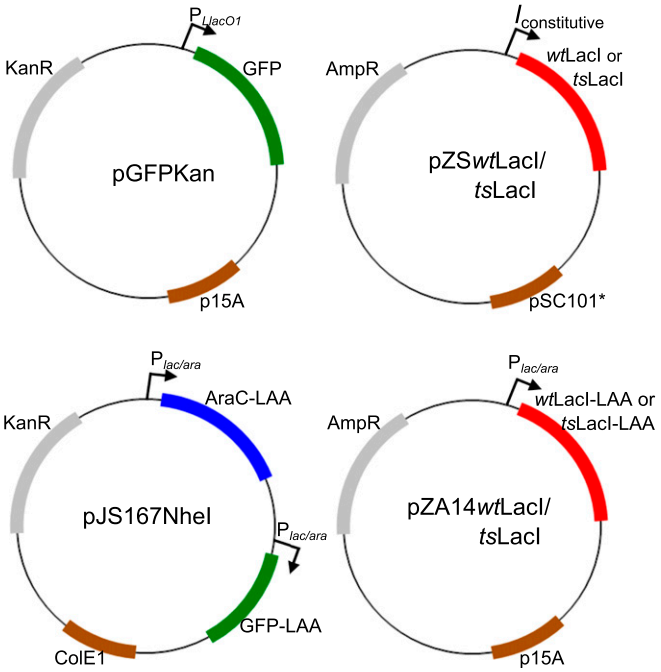


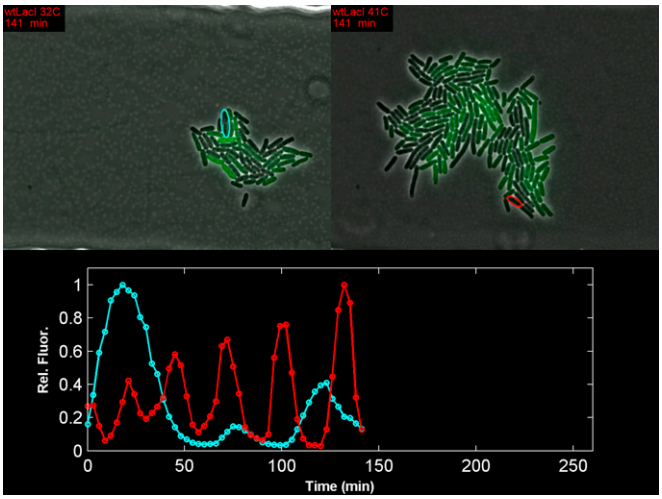

**Fig. S3.** Maps of the plasmids used in this study.

**Table S1. Parameter values of the model obtained from fitting**

| Parameter | Value | Units |
|---|---|---|
| $\tau_r$ | 13.5 | min |
| $\tau_a$ | 15 | min |
| $\beta$ | 0.0275 | $min^{-1}$ |
| $\gamma_r$ | 76 | (molecules/cell) $min^{-1}$ |
| $\gamma_a$ | 76 | (molecules/cell) $min^{-1}$ |
| $R_0$ | 1.8 | molecules/cell |
| $N$ | 4 | (unitless) |
| $f$ | 2 | (unitless) |
| $C_a$ | 5 | molecules/cell |
| $\alpha_r$ | 265 | (molecules/cell) $min^{-1}$ |
| $\alpha_a$ | 92.75 | (molecules/cell) $min^{-1}$ |
| $\theta$ | 4,500 | K |
| $T_{ref}$ | 37 | °C |
| $C_{r,max}$ | 830 | molecules/cell |
| $C_{r,min}$ | 50 | molecules/cell |
| $T_0$ | 38 | °C |
| $b$ | 20 | (unitless) |

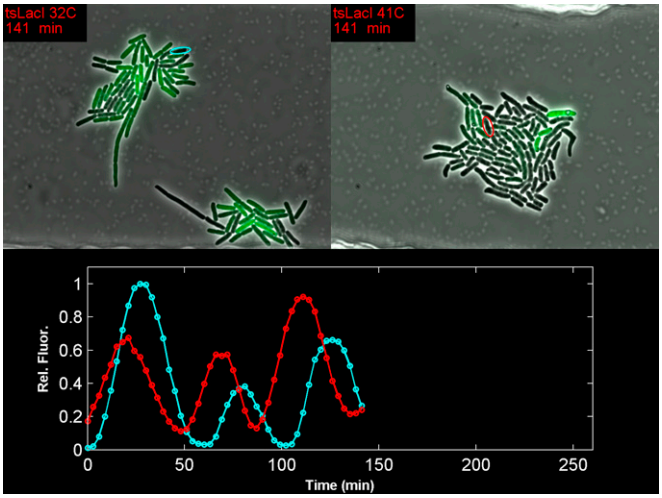
**Table S2. Plasmids used in this study**

| Plasmid name | ORFs | ori/resistance |
|---|---|---|
| pZSwtLacI | $P_I$:wtLacI | pSC101*/ampicillin |
| pZStsLacI | $P_I$:tsLacI | pSC101*/ampicillin |
| pGFPKan | $P_{LlacO1}$:GFP | p15A/kanamycin |
| pZA14wtLacI | $P_{lac/ara}$:wtLacI-LAA | p15A/ampicillin |
| pZA14tsLacI | $P_{lac/ara}$:tsLacI-LAA | p15A/ampicillin |
| pJS167NheI | $P_{lac/ara}$:AraC-LAA, $P_{lac/ara}$:GFP-LAA | ColE1/kanamycin |




**Movie S1.** *E. coli* cells containing the dual-feedback oscillator with wtLacI grown in a microfluidic device at 32 ° (*Upper Left*) and 41 °C (*Upper Right*). Tracked cells are outlined in cyan and red, respectively. Relative GFP fluorescence of the tracked cells as a function of time is shown in *Lower*.

Movie S1




**Movie S2.** *E. coli* cells containing the dual-feedback oscillator with tsLacI grown in a microfluidic device at 32 °C (*Upper Left*) and 41 °C (*Upper Right*). Tracked cells are outlined in cyan and red, respectively. Relative GFP fluorescence of the tracked cells as a function of time is shown in *Lower*.

Movie S2