

Table of contents

- Appendix Information I: Mathematical model of tandem promoters**
- Appendix Information II: Model of gate dynamics**
- Appendix Information III: Model of circuit dynamics**
- Appendix Figure S1: Biophysical model of tandem promoters**
- Appendix Figure S2: Gate modifications to improve performance**
- Appendix Figure S3: Modification of the OC6 sensor**
- Appendix Figure S4: Evaluation of tandem promoters: experiments versus the predictions**
- Appendix Figure S5: Circuit prediction using UCF Eco1C1G1T1 (first UCF)**
- Appendix Figure S6: Roadblocking assays for the gate output promoters**
- Appendix Figure S7: Activity of sensor output promoters in tandem**
- Appendix Figure S8: Characterization of gates**
- Appendix Figure S9: Characterization of genetic sensors**
- Appendix Figure S10: Detailed designs and data for the 7-segment circuits**
- Appendix Figure S11: Response and growth impact of the 7-segment circuits**
- Appendix Figure S12: Fit of promoter models to response data**
- Appendix Figure S13: Analysis of Segment F (low copy plasmid) after the 88-hour continuous switching experiment**
- Appendix Figure S14: Characterization and stability of the first set of 7-segment designs carried on a higher-copy plasmid**
- Appendix Figure S15: Analysis of Segment F and G (on high copy plasmids) after the 88-hour continuous switching experiment.**
- Appendix Figure S16: 3D printed device and seven segment display**
- Appendix Figure S17: Plasmid backbones**
- Appendix Figure S18: High copy plasmid backbone**
- Appendix Figure S19: Plasmid maps for the sensor characterization**
- Appendix Figure S20: Plasmid maps for the sensor output tandem promoter characterization**
- Appendix Figure S21: Plasmid maps for the gate characterization**
- Appendix Figure S22: Plasmid maps for the gate output tandem promoter characterization**
- Appendix Figure S23: Plasmid maps for the tandem promoter evaluation**
- Appendix Figure S24: Plasmid maps of the 7-segment circuits**
- Appendix Figure S25: Plasmid maps for the YFP expression from a constitutive promoter in two p15A backbones**
- Appendix Table S1: Parameterization of sensors**
- Appendix Table S2: List of plasmids used in each figure**
- Appendix Table S3: Complete annotated sequences of gates and sensors**
- Appendix Table S4: Genetic part sequences used in this work**
- Appendix Table S5: Sequences of the 7-segment circuits**
- Appendix Reference**

Appendix Information I.

Mathematical model of tandem promoters

Non-additive model

Consider two promoters oriented in series whose RNAP fluxes are y_1 (upstream) and y_2 (downstream), respectively. If there is no interaction between the RNAP fluxes originating from the two promoters, they can additively contribute to the total RNAP flux,

$$y_{tot} = y_1 + y_2 \quad . \quad (S1)$$

In this case, the empirical response functions (Equation 1) for each promoter could be substituted for y_1 and y_2 .

To demonstrate how non-additivity impacts Appendix Equation S1, we derive the promoter activities using a Shea-Ackers formalism (Ackers et al, 1982) and a simple biophysical model where a single repressor binds non-cooperatively to each promoter. All of the possible promoter states are enumerated in Appendix Figure S1 when repressor R₁ binds to input promoter 1 with affinity K_{R,1}, repressor R₂ binds to input promoter 2 with affinity K_{R,2}, and RNAP can bind to either promoter with affinities K_{RNAP,1} and K_{RNAP,2}. First, for the additive case, the ensemble of all possible states is given by

$$Z = 1 + K_{RNAP,1}[RNAP] + K_{RNAP,2}[RNAP] + K_{R,1}[R_1] + K_{R,2}[R_2] + K_{R,1}K_{R,2}[R_1][R_2] + K_{R,1}K_{RNAP,2}[R_1][RNAP] + K_{RNAP,1}K_{R,2}[RNAP][R_2] + K_{RNAP,1}K_{RNAP,2}[RNAP]^2 \quad , \quad (S2)$$

which can be simply factored into the sum of the individual contributions from each promoter. The probability P that either promoter is in a state where RNAP is binding and initiating transcription is

$$P = Z^{-1}(K_{RNAP,1}[RNAP] + K_{RNAP,2}[RNAP] + K_{RNAP,1}K_{R,2}[RNAP][R_2] + K_{RNAP,2}K_{R,1}[RNAP][R_1] + K_{RNAP,1}K_{RNAP,2}[RNAP]^2) \quad . \quad (S3)$$

This can be factored and the total transcription rate from the two promoters calculated as

$$\frac{x_{Tot}}{[DNA]} = \frac{x_1}{[DNA]} \frac{K_{RNAP,1}[RNAP]}{1+K_{RNAP,1}[RNAP]+K_{R,1}[R_1]} + \frac{x_2}{[DNA]} \frac{K_{RNAP,2}[RNAP]}{1+K_{RNAP,2}[RNAP]+K_{R,2}[R_2]} \quad , \quad (S4)$$

where x₁/[DNA] and x₂/[DNA] are the RNA fluxes from the two promoters.

Appendix Equations S3 and S4 are based on the complete binding polynomial that results from the consideration of all binding states to the promoters. The gate response function (Equation 1) represents an empirical form of this equation. This empirical form does not account for all of the binding states of the promoter, but allows for cooperativity (not necessarily due to multimer formation), and the maximum and minimum promoter activities are provided by sets of experiments (measured in RPU). When these equations are substituted into Equation 1, this yields

$$y = [y_{min,1} + (y_{max,1} - y_{min,1}) \frac{K_1^{n_1}}{K_1^{n_1} + x_1^{n_1}}] + [y_{min,2} + (y_{max,2} - y_{min,2}) \frac{K_2^{n_2}}{K_2^{n_2} + x_2^{n_2}}] \quad , \quad (S5)$$

where the parameters are defined in the main text. When compared to Appendix Equation S4, the RNAP binding, unbound DNA state, and transcription rates are captured by y_{min} and y_{max}. The fractions are related to the binding of each repressor to its cognate promoter.

Non-additivity due to roadblocking only impacts the upstream promoter (first term of Appendix

Equation S4). Within this term, roadblocking only affects the promoter state when RNAP is bound to the upstream promoter and R₂ is bound to the downstream promoter (Appendix Figure S1). This does not have the effect of completely blocking transcription. Instead, there is a probability that R₂ blocks transcription, thus reducing the contribution of transcription from the upstream promoter to the total transcription rate by a term β . To calculate the rate from the first promoter, the corresponding portion of Appendix Equation S3 can be re-written as

$$P_1 = Z^{-1} (K_{RNAP,1}[RNAP] + \beta K_{RNAP,1} K_{R,2}[RNAP][R_2] + K_{RNAP,1} K_{RNAP,2}[RNAP]^2) . \quad (S6)$$

With this modified form, the first term of Appendix Equation S4 can be simplified to

$$\frac{x_1}{[DNA]} \left(\frac{1+K_{RNAP,2}[RNAP]+\beta K_{R,2}[R_2]}{1+K_{RNAP,2}[RNAP]+K_{R,2}[R_2]} \right) \left(\frac{K_{RNAP,1}[RNAP]}{1+K_{RNAP,1}[RNAP]+K_{R,1}[R_1]} \right) , \quad (S7)$$

where the second term is simply the probability of transcription occurring from promoter 1 and the first term is a correction due to R₂ blocking transcription.

Finally, an additional term α is included to capture all of the non-additive effects due to the existence of a downstream promoter. This includes interference due to pause sites, antisense transcription, binding of other proteins, and other mechanisms (Brophy & Voigt, 2016; Dahirel et al, 2009; Roberts, 2014). The addition of this parameter to Equation S7 yields

$$\frac{x_1}{[DNA]} \alpha \left(\frac{1+K_{RNAP,2}[RNAP]+\beta K_{R,2}[R_2]}{1+K_{RNAP,2}[RNAP]+K_{R,2}[R_2]} \right) \left(\frac{K_{RNAP,1}[RNAP]}{1+K_{RNAP,1}[RNAP]+K_{R,1}[R_1]} \right) . \quad (S8)$$

These effects are assumed to uniformly reduce transcription from the upstream promoter. In other words, it neither depends on the state of the downstream promoter nor the level of transcription from the upstream one.

As above, the equivalent of Appendix Equation S8 can be derived for the empirical response functions. This results in the form

$$y = y_{min,2} + (y_{max,2} - y_{min,2}) \frac{K_2^{n_2}}{K_2^{n_2} + x_2^{n_2}} + \alpha \left(\frac{K_2^{n_2} + \beta x_2^{n_2}}{K_2^{n_2} + x_2^{n_2}} \right) \left[y_{min,1} + (y_{max,1} - y_{min,1}) \frac{K_1^{n_1}}{K_1^{n_1} + x_1^{n_1}} \right] , \quad (S9)$$

where α and β are parameters associated with promoter 2 and capture its non-additive impact on an upstream promoter. Smaller values of both indicate more interference and as α and β approach unity, the interference effects go to zero.

Simplified model for characterizing sensors

A sensor's output promoter can also cause roadblocking. The response function of a sensor captures how the output promoter changes as a function of the stimulus (*e.g.*, the concentration of inducer). If the response function is known, then the equations from the previous section can be applied with some modifications, described in this section. The input of a sensor's response function (x-axis) is the concentration of the inducer c , as opposed to a promoter activity in RPU. Also, the response function turns on as a function of c , which is the opposite of a gate where the output promoter turns off as a function of the input promoter. Thus, the form of the response follows that of an activator (regardless of whether the inducer activates an activator or derepresses a repressor), written by

$$y = y_{min} + (y_{max} - y_{min}) \frac{c^n}{K^n + c^n} . \quad (S10)$$

where c is the concentration of inducers. With these modifications, the equation describing roadblocking by an input promoter at position 2 is

$$y = y_{min,2} + (y_{max,2} - y_{min,2}) \frac{c_2^{n_2}}{K_2^{n_2} + c_2^{n_2}} + \alpha \left(\frac{\beta K_2^{n_2} + c_2^{n_2}}{K_2^{n_2} + c_2^{n_2}} \right) \left[y_{min,1} + (y_{max,1} - y_{min,1}) \frac{K_1^{n_1}}{K_1^{n_1} + x_1^{n_1}} \right] . \quad (S11)$$

A simpler form can be used because the inputs to Cello are only the RNAP flux of the output promoter for two states, for example the absence or presence of a defined concentration of inducer (OFF or ON). Considering this, the interference term of Appendix Equation S11 can be rearranged as follows

$$\frac{\beta K_2^{n_2} + c_2^{n_2}}{K_2^{n_2} + c_2^{n_2}} = \beta + (1 - \beta) \left(\frac{c_2^{n_2}}{K_2^{n_2} + c_2^{n_2}} \right) . \quad (S12)$$

Note that the fraction is the same in the input term (position 2) in Appendix Equation S11. When the sensor output is H, this fraction goes to 1 and $y_2 = y_{max,2}$. When the sensor output is OFF, this fraction term goes to 0 and $y_2 = y_{min,2}$. Here, $y_{min,2}$ and $y_{max,2}$ represent the OFF and ON values of the sensor in Cello. When the sensor is ON, Appendix Equation S12 goes to 1 and when it is OFF, it goes to β . This simplifies the impact of the interference parameters and Appendix Equation S11 becomes

$$y = \alpha \beta^{(1-q)} \left[y_{min,1} + (y_{max,1} - y_{min,1}) \frac{K_1^{n_1}}{K_1^{n_1} + x_1^{n_1}} \right] + \delta(1-q)(y_{max,2} - y_{min,2}) + y_{min,2}, \quad (S13)$$

where q is 0 when the sensor is OFF or 1 when the sensor is ON. Here, the delta function $\delta(x)$ is defined as 1 when x is zero, and zero otherwise.

Appendix Information II. Model of gate dynamics

A gate model is developed that captures the speed by which the gate reaches its steady-state value after a change in the input. This model does not require the multitude of hard-to-measure kinetic parameters required by a detailed biophysical model. Rather, we simply consider characteristic times for the circuit to adjust after the perturbation to the input. We consider two parameters: one that captures the response to go to a steady-state that is higher than the current output τ_y^{ON} and one to go to a steady-state that is lower τ_y^{OFF} . The values of these parameters are expected to be different because the underlying biophysics determining the timescale differs. For each gate, the corresponding conditional ordinary differential equation is

$$\frac{dy}{dt} = \begin{cases} \tau_y^{ON}(y_{ss} - y) & \text{if } y < y_{ss} \\ \tau_y^{OFF}(y_{ss} - y) & \text{otherwise} \end{cases}, \quad (S14)$$

where y_{ss} is the steady state of the gate given its input sequences, as calculated using the response function (Equation 1).

Experiments were then designed to extract the two characteristic times for each gate. To measure the response of each NOT gate, a sensor is connected to serve as the input and the output is measured by connecting the output promoter to the transcription of *yfp*. Cells carrying this circuit are grown in the presence of inducer until reaching steady-state, then switched into media lacking inducer and fluorescence is measured as the gate turns on. The same is done in reverse, where cells are grown in the absence of inducer, then switched into media containing it, and the loss of fluorescence is measured over time until steady-state is reached. However, using only these data is problematic as the sensor (addition/removal of inducer to the turning on of the sensor output promoter) and the expression/degradation of YFP both have induction and relaxation timescales that need to be separated from those of the gate.

We use data from the sensor to estimate these parameters. First, the sensor is grown under conditions with inducer and then moved to conditions lacking inducer and the loss of YFP fluorescence is measured over time (Appendix Figure S9). The time for the sensor to turn off (the binding of the repressor without inducer to the promoter) is fast with respect to YFP degradation, whose half-life is expected to be about the cell doubling time. This simple exponential decay can be modeled by

$$\frac{d[YFP]}{dt} = -\tau_{YFP}^{OFF} ([YFP] - [YFP]_{min}), \quad (S15)$$

where $[YFP]$ is the fluorescence as measured by cytometry and min refers to the steady-state value in the absence of inducer. This is fit to the data and $\tau_{YFP}^{OFF} = 1.06 \text{ hr}^{-1}$. The timescale of the off rate of the sensor is selected to be as slow as possible without impacting the above fit. This leads to $\tau_x^{OFF} = 4.0 \text{ hr}^{-1}$, which is held constant for all of the sensors.

The next step is to obtain the YFP production rate. This is calculated by considering the equation for the production and degradation of YFP

$$\frac{d[YFP]}{dt} = \tau_{YFP}^{ON} y - \tau_{YFP}^{OFF} [YFP], \quad (S16)$$

where y is the RNAP flux from gate's and sensor's output promoter reported in RPU (the units of $[YFP]$ are the fluorescence in au). At steady-state, Appendix Equation S16 becomes

$$\tau_{YFP}^{ON} = \tau_{YFP}^{OFF} \frac{[YFP]_{max}}{y_{max}}, \quad (S17)$$

where $[YFP]_{max}$ is the highest fluorescence measured when uninduced (for gates) or fully induced (for sensors), and y_{max} is the maximum RPU from the gate and sensor output promoter. This leads to τ_{YFP}^{ON} , which differs for each gate and sensor ($1163 \text{ au-RPU}^{-1}\text{hr}^{-1}$). The following equations were used to extract τ_x^{ON} from data from turning the sensor on (OFF to ON),

$$\frac{dx}{dt} = \tau_x^{ON} (x_{ss} - x) \quad \text{and} \quad (S18)$$

$$\frac{d[YFP]}{dt} = \tau_{YFP}^{ON} x - \tau_{YFP}^{OFF} [YFP], \quad (S19)$$

where x is the RNAP flux from sensor's output promoter reported in RPU, and τ_x^{ON} , τ_{YFP}^{ON} , and τ_{YFP}^{OFF} were determined above.

Using these parameters, equations can be written to extract on- and off- rates for a gate. For the case when cells are grown in the presence of inducer and then switched to media lacking inducer (the gate output goes from OFF to ON),

$$\frac{dx}{dt} = \tau_x^{OFF} (x_{ss} - x), \quad (S20)$$

$$\frac{dy}{dt} = \tau_y^{ON} (y_{ss} - y), \quad \text{and} \quad (S21)$$

$$\frac{d[YFP]}{dt} = \tau_{YFP}^{ON} y - \tau_{YFP}^{OFF} [YFP], \quad (S22)$$

where the only unknown parameter is τ_y^{ON} . These equations are solved and fit to the data (Appendix Figure S8) to obtain the characteristic on time for each gate (Table 1).

Finally, the off-times of the gates can be determined by growing the cells in the absence of inducer and then switching them into media containing the inducer (Methods). Modeling this change results in the following equations

$$\frac{dx}{dt} = \tau_x^{ON} (x_{ss} - x), \quad (S23)$$

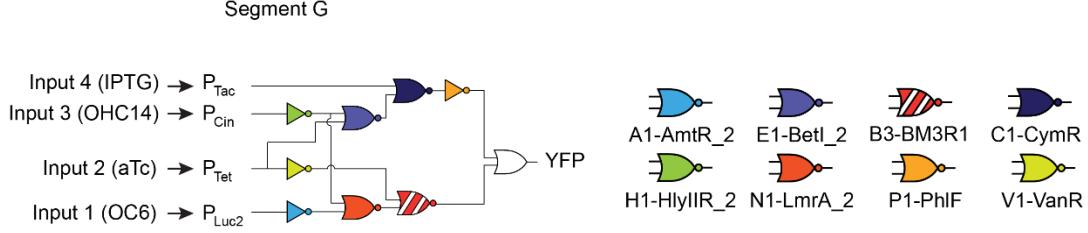
$$\frac{dy}{dt} = \tau_y^{OFF} (y_{ss} - y), \quad \text{and} \quad (S24)$$

$$\frac{d[YFP]}{dt} = \tau_{YFP}^{ON} y - \tau_{YFP}^{OFF} [YFP], \quad (S25)$$

where τ_y^{OFF} is the only unknown parameter. These equations are solved and fit to the data for each gate (Appendix Figure S8) and the resulting parameters are shown in Table 1.

Appendix Information III. Model of circuit dynamics

The prediction of the dynamic behavior of circuits requires the time-dependent response that is accumulated from all gates. The following is the equation set for Segment G.



For the sensors:

$$\frac{dx_{Lux2}}{dt} = \begin{cases} \tau_{Lux2}^{ON} (x_{Lux2,SS} - x_{Lux2}) & \text{if } x_{Lux2} < x_{Lux2,SS} \\ \tau_{Lux2}^{OFF} (x_{Lux2,SS} - x_{Lux2}) & \text{otherwise} \end{cases}, \quad (S26)$$

$$\frac{dx_{Tet}}{dt} = \begin{cases} \tau_{Tet}^{ON} (x_{Tet,SS} - x_{Tet}) & \text{if } x_{Tet} < x_{Tet,SS} \\ \tau_{Tet}^{OFF} (x_{Tet,SS} - x_{Tet}) & \text{otherwise} \end{cases}, \quad (S27)$$

$$\frac{dx_{Cin}}{dt} = \begin{cases} \tau_{Cin}^{ON} (x_{Cin,SS} - x_{Cin}) & \text{if } x_{Cin} < x_{Cin,SS} \\ \tau_{Cin}^{OFF} (x_{Cin,SS} - x_{Cin}) & \text{otherwise} \end{cases}, \quad (S28)$$

$$\frac{dx_{Tac}}{dt} = \begin{cases} \tau_{Tac}^{ON} (x_{Tac,SS} - x_{Tac}) & \text{if } x_{Tac} < x_{Tac,SS} \\ \tau_{Tac}^{OFF} (x_{Tac,SS} - x_{Tac}) & \text{otherwise} \end{cases}, \quad (S29)$$

For the gates:

$$\frac{dy_{VanR}}{dt} = \begin{cases} \tau_{VanR}^{ON} \left(y_{VanR,min} + (y_{VanR,max} - y_{VanR,min}) \frac{K_{VanR} n_{VanR}}{K_{VanR} n_{VanR} + x_{Tet} n_{VanR}} - y_{VanR} \right) & \text{if } y_{VanR} < y_{VanR,SS} \\ \tau_{VanR}^{OFF} \left(y_{VanR,min} + (y_{VanR,max} - y_{VanR,min}) \frac{K_{VanR} n_{VanR}}{K_{VanR} n_{VanR} + x_{Tet} n_{VanR}} - y_{VanR} \right) & \text{otherwise} \end{cases}, \quad (S30)$$

$$\frac{dy_{PhIF}}{dt} = \begin{cases} \tau_{PhIF}^{ON} \left(y_{PhIF,min} + (y_{PhIF,max} - y_{PhIF,min}) \frac{K_{PhIF} n_{PhIF}}{K_{PhIF} n_{PhIF} + y_{CymR} n_{PhIF}} - y_{PhIF} \right) & \text{if } y_{PhIF} < y_{PhIF,SS} \\ \tau_{PhIF}^{OFF} \left(y_{PhIF,min} + (y_{PhIF,max} - y_{PhIF,min}) \frac{K_{PhIF} n_{PhIF}}{K_{PhIF} n_{PhIF} + y_{CymR} n_{PhIF}} - y_{PhIF} \right) & \text{otherwise} \end{cases}, \quad (S31)$$

$$\frac{dy_{AmtR2}}{dt} = \begin{cases} \tau_{AmtR2}^{ON} \left(y_{AmtR2,min} + (y_{AmtR2,max} - y_{AmtR2,min}) \frac{K_{AmtR2} n_{AmtR2}}{K_{AmtR2} n_{AmtR2} + x_{Lux2} n_{AmtR2}} - y_{AmtR2} \right) & \text{if } y_{AmtR2} < y_{AmtR2,SS} \\ \tau_{AmtR2}^{OFF} \left(y_{AmtR2,min} + (y_{AmtR2,max} - y_{AmtR2,min}) \frac{K_{AmtR2} n_{AmtR2}}{K_{AmtR2} n_{AmtR2} + x_{Lux2} n_{AmtR2}} - y_{AmtR2} \right) & \text{otherwise} \end{cases}, \quad (S32)$$

$$\frac{dy_{BM3R1}}{dt} = \begin{cases} \tau_{BM3R1}^{ON} \left(y_{BM3R1,min} + (y_{BM3R1,max} - y_{BM3R1,min}) \frac{K_{BM3R1} n_{BM3R1}}{K_{BM3R1} n_{BM3R1} + f(y_{VanR}, y_{LmrA2}) n_{BM3R1}} - y_{BM3R1} \right) & \text{if } y_{BM3R1} < y_{BM3R1,SS} \\ \tau_{BM3R1}^{OFF} \left(y_{BM3R1,min} + (y_{BM3R1,max} - y_{BM3R1,min}) \frac{K_{BM3R1} n_{BM3R1}}{K_{BM3R1} n_{BM3R1} + f(y_{VanR}, y_{LmrA2}) n_{BM3R1}} - y_{BM3R1} \right) & \text{otherwise} \end{cases}, \quad (S33)$$

$$\frac{dy_{LmrA2}}{dt} = \begin{cases} \tau_{LmrA2}^{ON} \left(y_{LmrA2,min} + (y_{LmrA2,max} - y_{LmrA2,min}) \frac{K_{LmrA2} n_{LmrA2}}{K_{LmrA2} n_{LmrA2} + f(y_{HlyIIR2}, y_{AmtR2}) n_{LmrA2}} - y_{LmrA2} \right) & \text{if } y_{LmrA2} < y_{LmrA2,SS} \\ \tau_{LmrA2}^{OFF} \left(y_{LmrA2,min} + (y_{LmrA2,max} - y_{LmrA2,min}) \frac{K_{LmrA2} n_{LmrA2}}{K_{LmrA2} n_{LmrA2} + f(y_{HlyIIR2}, y_{AmtR2}) n_{LmrA2}} - y_{LmrA2} \right) & \text{otherwise} \end{cases}, \quad (S34)$$

$$\frac{dy_{HlyIIR2}}{dt} = \begin{cases} \tau_{HlyIIR2}^{ON} \left(y_{HlyIIR2,min} + (y_{HlyIIR2,max} - y_{HlyIIR2,min}) \frac{K_{HlyIIR2}^{nHlyIIR2}}{K_{HlyIIR2}^{nHlyIIR2} + x_{Cin}^{nHlyIIR2}} - y_{HlyIIR2} \right) & \text{if } y_{HlyIIR2} < y_{HlyIIR2,SS} \\ \tau_{HlyIIR2}^{OFF} \left(y_{HlyIIR2,min} + (y_{HlyIIR2,max} - y_{HlyIIR2,min}) \frac{K_{HlyIIR2}^{nHlyIIR2}}{K_{HlyIIR2}^{nHlyIIR2} + x_{Cin}^{nHlyIIR2}} - y_{HlyIIR2} \right) & \text{otherwise} \end{cases}, \quad (S35)$$

$$\frac{dy_{BetI2}}{dt} = \begin{cases} \tau_{BetI2}^{ON} \left(y_{BetI2,min} + (y_{BetI2,max} - y_{BetI2,min}) \frac{K_{BetI2}^{nBetI2}}{K_{BetI2}^{nBetI2} + f(y_{HlyIIR2} \cdot x_{Tet})^{nBetI2}} - y_{BetI2} \right) & \text{if } y_{BetI2} < y_{BetI2,SS} \\ \tau_{BetI2}^{OFF} \left(y_{BetI2,min} + (y_{BetI2,max} - y_{BetI2,min}) \frac{K_{BetI2}^{nBetI2}}{K_{BetI2}^{nBetI2} + f(y_{HlyIIR2} \cdot x_{Tet})^{nBetI2}} - y_{BetI2} \right) & \text{otherwise} \end{cases}, \quad (S36)$$

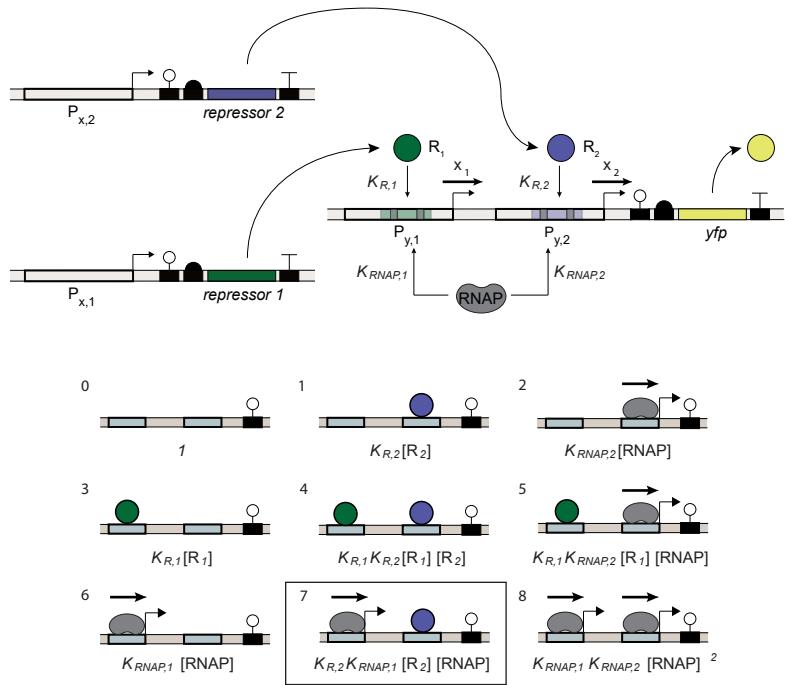
$$\frac{dy_{CymR}}{dt} = \begin{cases} \tau_{CymR}^{ON} \left(y_{CymR,min} + (y_{CymR,max} - y_{CymR,min}) \frac{K_{CymR}^{nCymR}}{K_{CymR}^{nCymR} + f(x_{Tac} \cdot y_{BetI2})^{nCymR}} - y_{CymR} \right) & \text{if } y_{CymR} < y_{CymR,SS} \\ \tau_{CymR}^{OFF} \left(y_{CymR,min} + (y_{CymR,max} - y_{CymR,min}) \frac{K_{CymR}^{nCymR}}{K_{CymR}^{nCymR} + f(x_{Tac} \cdot y_{BetI2})^{nCymR}} - y_{CymR} \right) & \text{otherwise} \end{cases}, \quad (S37)$$

$$\frac{dYFP}{dt} = \tau_{YFP}^{ON} f(y_{PhlF}, y_{BM3R1}) - \tau_{YFP}^{OFF} YFP \quad . \quad (S38)$$

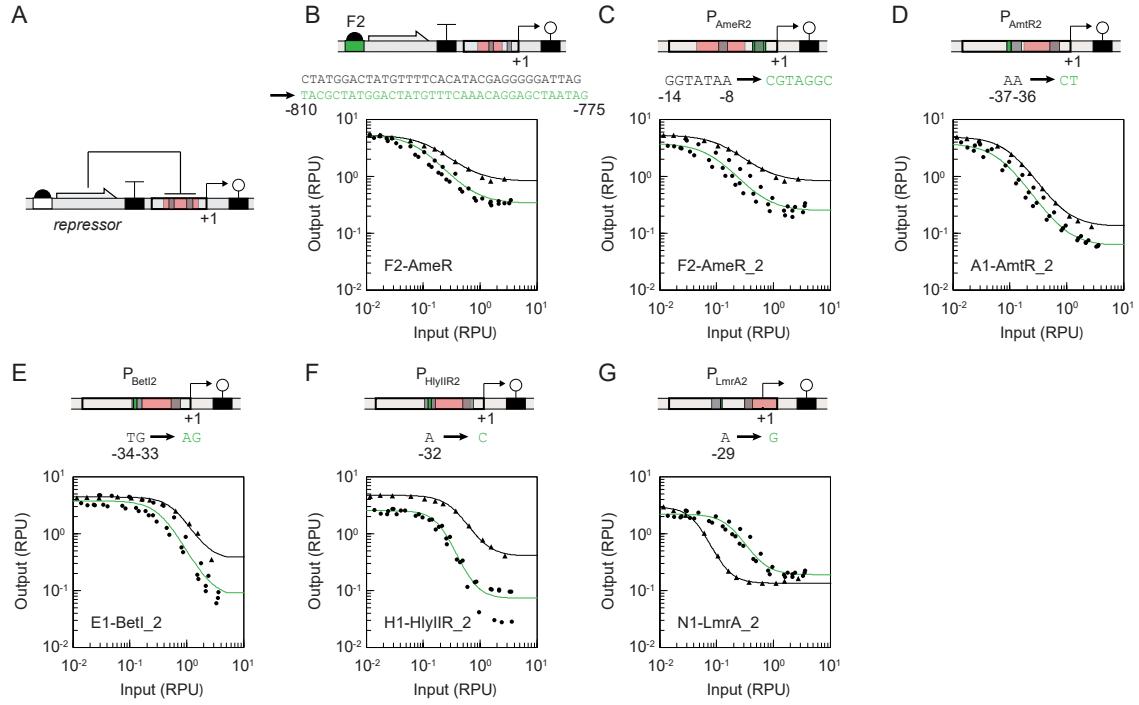
In the above equations, roadblocking is accounted for by the function

$$f(u, d) = y_u \alpha \left(\frac{y_d - y_{d,min} + \beta (y_{d,max} - y_d)}{y_{d,max} - y_{d,min}} \right) + y_d \quad , \quad (S39)$$

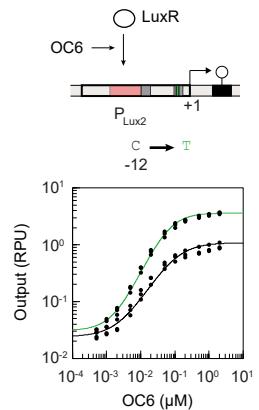
where u and d in $f(u, d)$ capture the upstream and downstream promoter in a tandem promoter (Appendix Equation S9 and S13).



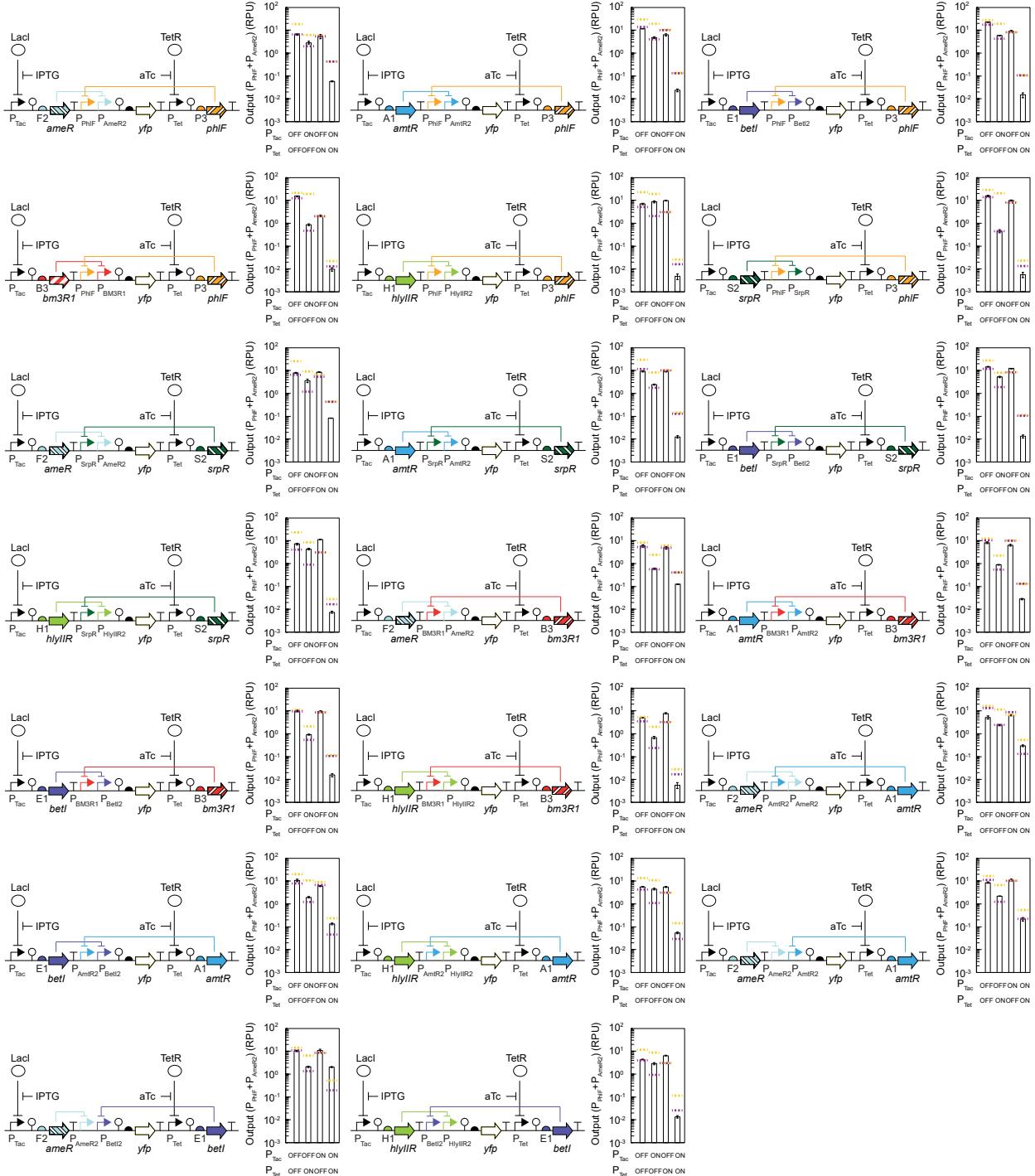
Appendix Figure S1: Biophysical model of tandem promoter. This diagram corresponds to the derivation of the non-additive promoter model in Appendix Information I (the parameters are defined in that section). The schematic at the top shows two tandem promoters that are the output promoters from two upstream gates. Roadblocking can occur when the repressor bound to the downstream promoter (R_2) interferes with transcription of the upstream promoter. The binding of the repressors and RNAP to the promoters are assumed to be mutually exclusive, as indicated with overlapping operators. The enumeration of binding states to the tandem promoters is shown, following a Shea-Ackers formalism (Ackers et al, 1982). The arrows above the promoters demarcate the states where transcription is active. The boxed state is when roadblocking potentially occurs.



Appendix Figure S2: Gate modifications to improve performance. **(A)** Schematic diagram of a NOT gate. **(B - G)** Comparison of parent gates and modified gates. The black solid line shows the response function of parent gate and the green lines are the response functions of the improved gates. The mutations to either the RBS or promoter are shown, corresponding to the line color. Dark grey indicates -10 and -35 boxes and red represents the operator. The gate in **(B)** involved a replacement of the RBS driving repressor expression and **(C to G)** are modifications to the output promoter. The data correspond to three experiments performed on different days. The lines are the best fit to Equation 1 (Table 1).



Appendix Figure S3: Modification of the OC6 sensor. A single mutation was made at the -10 region of the P_{Lux}^* promoter (black) to make $P_{\text{Lux}2}$ (green). The solid lines show the response functions before (black) and after (green) the mutation. Dark grey indicates -10 and -35 boxes and red represents the operator. The fit lines are the best fit to Appendix Equation S10 (Appendix Table S1). The data points correspond to three experiments performed on different days.



Appendix Figure S4: Evaluation of tandem promoters, experiments versus the predictions. These data correspond to the dot plots shown in Figure 1D, E. Each schematic shows the circuits diagram for a NAND gate, designed to have the output of two NOT gates serve as the input to an OR gate (two promoters in series). The bar graphs show the steady-state response for combinations of inducers (5 ng/mL aTc, 1 mM IPTG); error bars are the standard deviation from three experiments performed on different days. The colored lines on the bar graphs are the two models for promoters in series: additive (yellow) and non-additive (purple). Note that the high-copy mutant of the p15a plasmids were used for this analysis, including the parameterization of the model for combining the NOT gate response functions to determine the predicted output (plasmid maps in Appendix Fig S23).

A

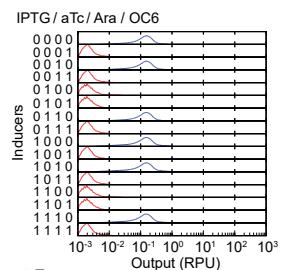
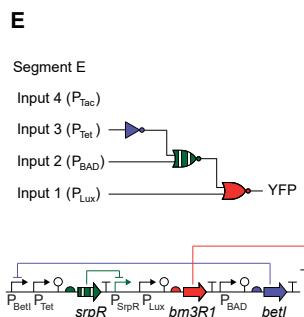
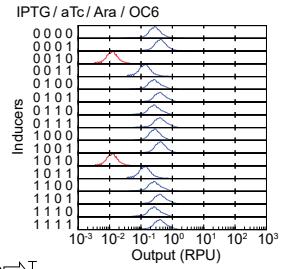
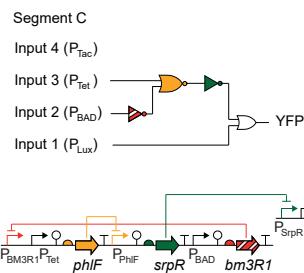
```
===== Assigned circuits =====
assigned lcs: 0
Total elapsed time for assignment algorithm: 115818 milliseconds

||||||| No assignments found. Exiting Cello. ||||||
|||||||
```

B

```
===== Assigned circuits =====
assigned lcs: 0
Total elapsed time for assignment algorithm: 115818 milliseconds

||||||| No assignments found. Exiting Cello. ||||||
|||||||
```

C**G**

```
===== Assigned circuits =====
assigned lcs: 0
Total elapsed time for assignment algorithm: 115818 milliseconds

||||||| No assignments found. Exiting Cello. ||||||
|||||||
```

D

```
===== Assigned circuits =====
assigned lcs: 0
Total elapsed time for assignment algorithm: 115818 milliseconds

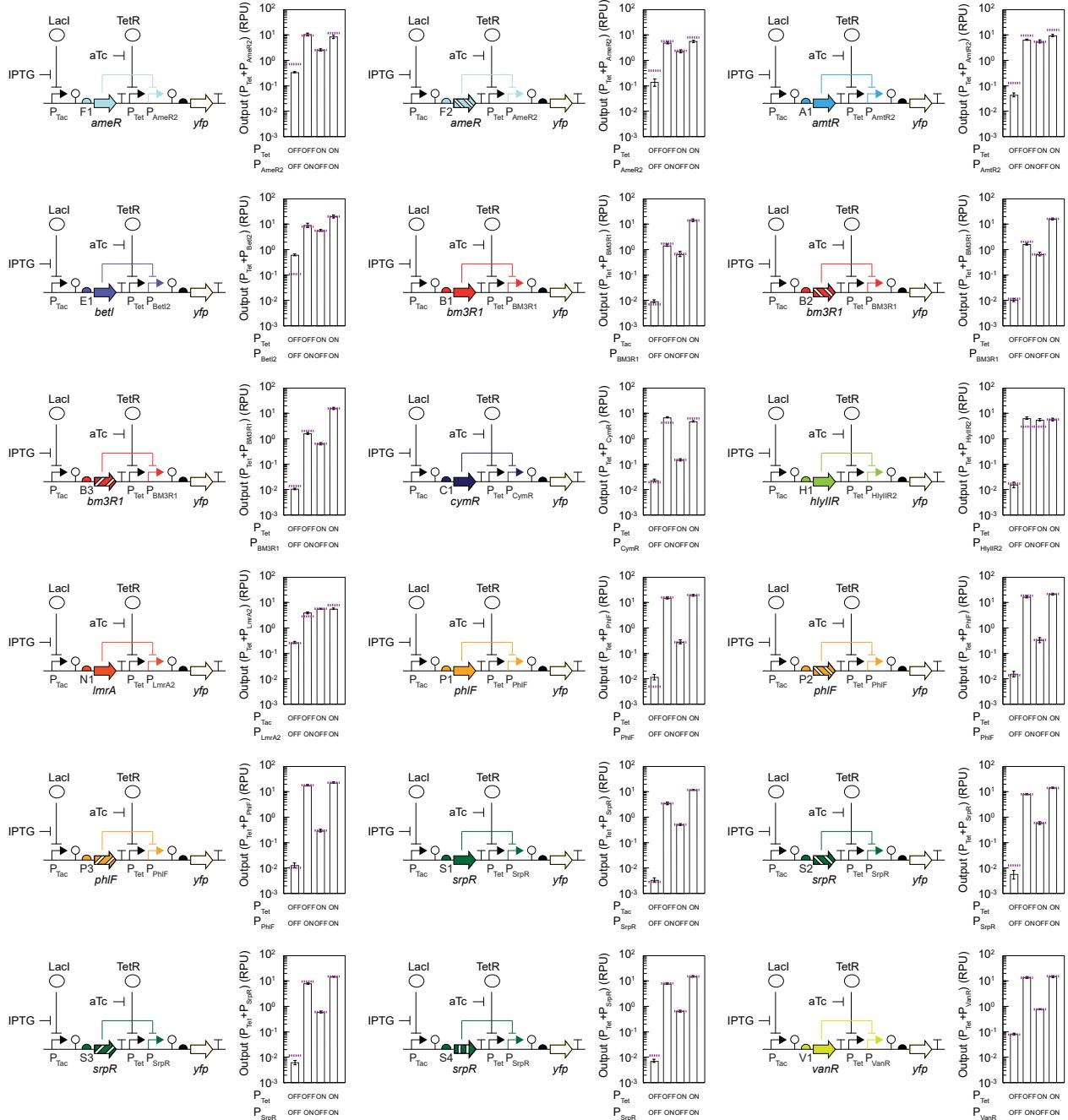
||||||| No assignments found. Exiting Cello. ||||||
|||||||
```

F

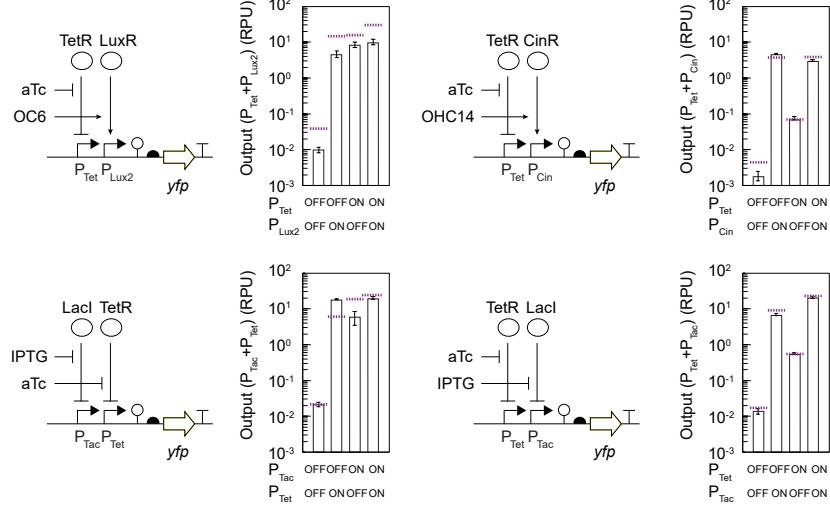
```
===== Assigned circuits =====
assigned lcs: 0
Total elapsed time for assignment algorithm: 115818 milliseconds

||||||| No assignments found. Exiting Cello. ||||||
|||||||
```

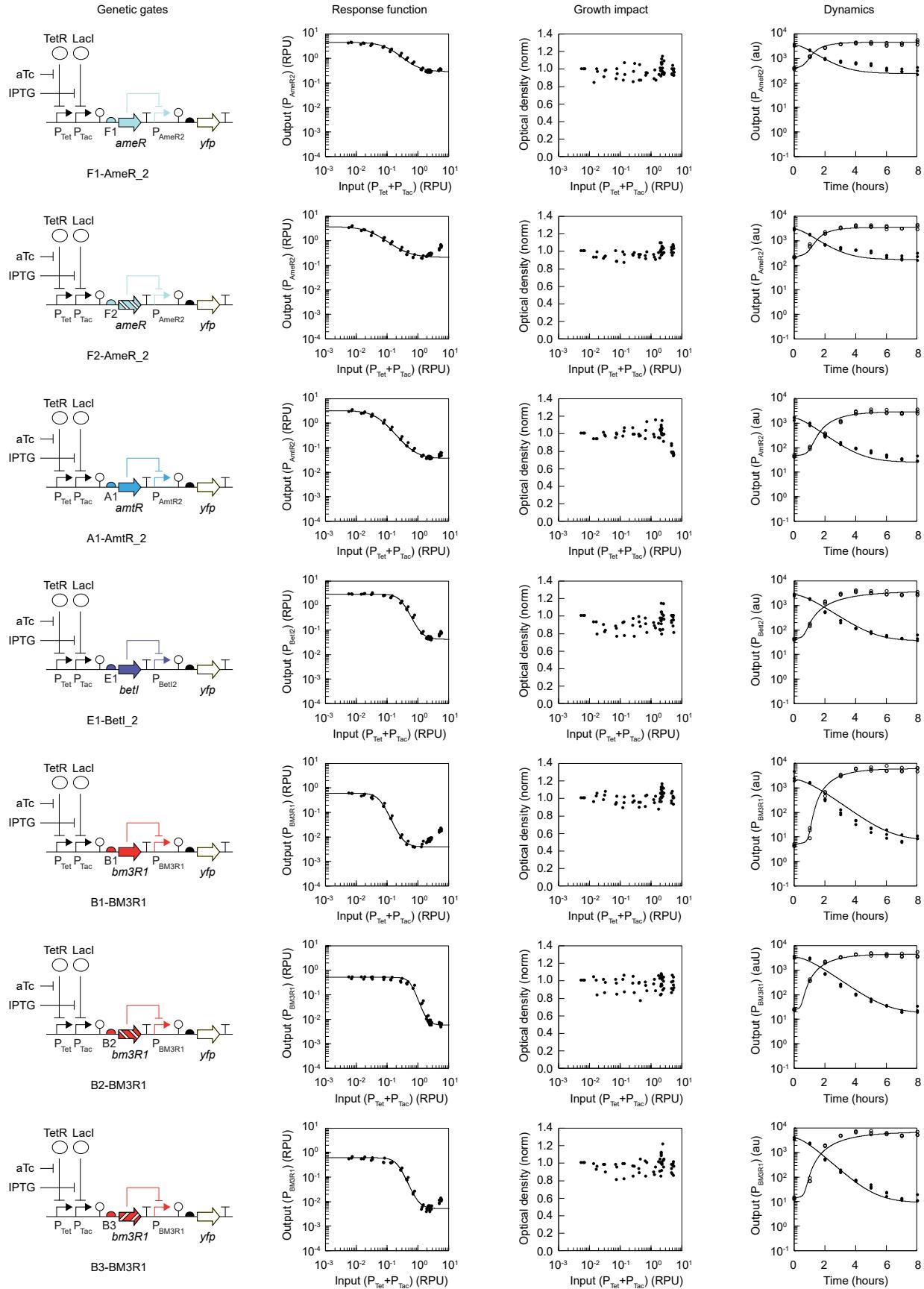
Appendix Figure S5: Circuit prediction using UCF Eco1C1G1T1 (first UCF). Cello predictions are shown using the previously published version of the UCF (cellocad.org using UCF, Eco1C1G1T1), which excludes promoters that roadblock (according to a threshold) from the downstream position (Nielsen et al, 2016). Only the circuits for Segment C and E, notably the smallest circuits (containing three gates), could be designed. No solutions for the other circuits were found (the error message is shown). Blue and red contour lines indicate the predicted ON and OFF, respectively (C and E).

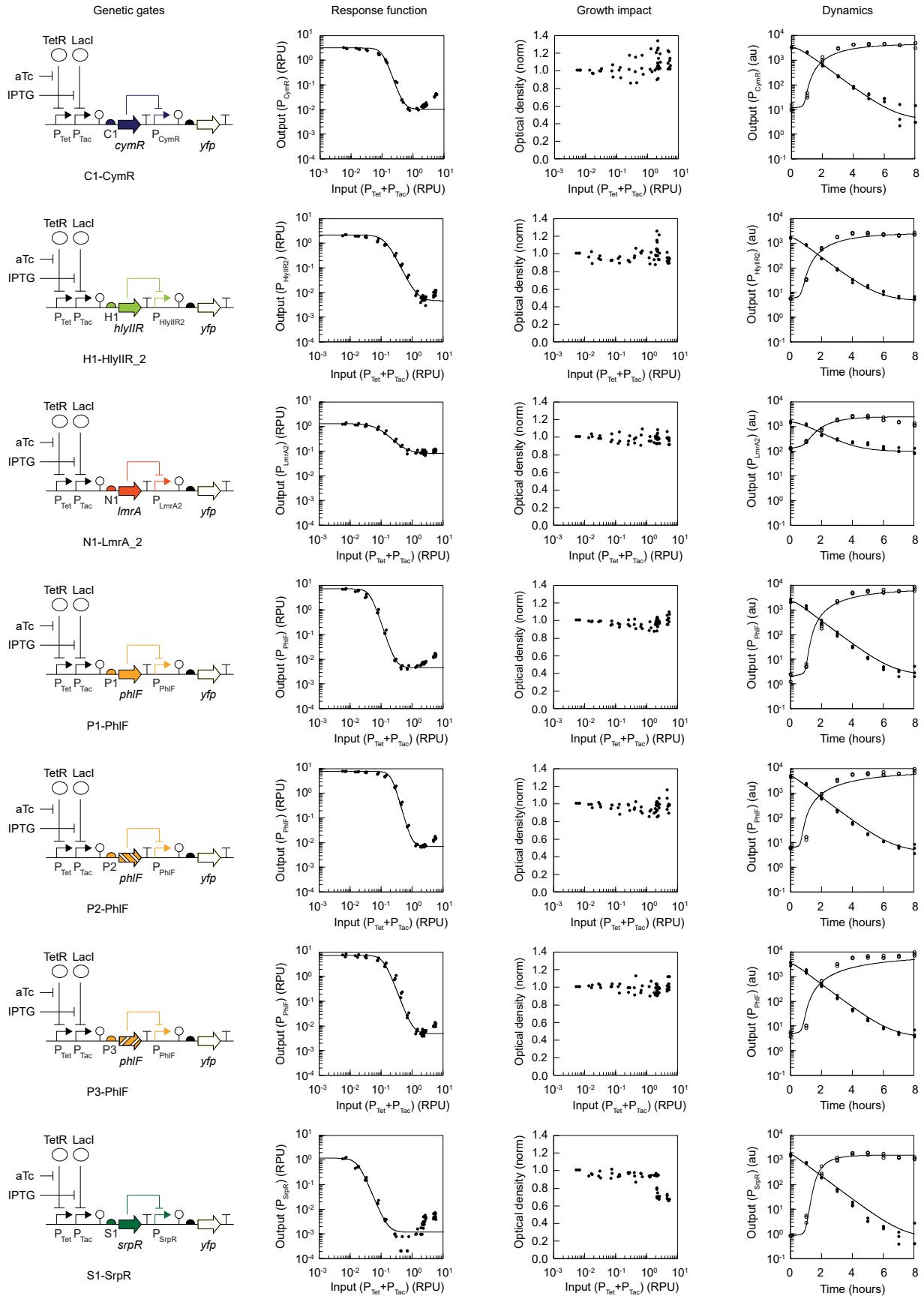


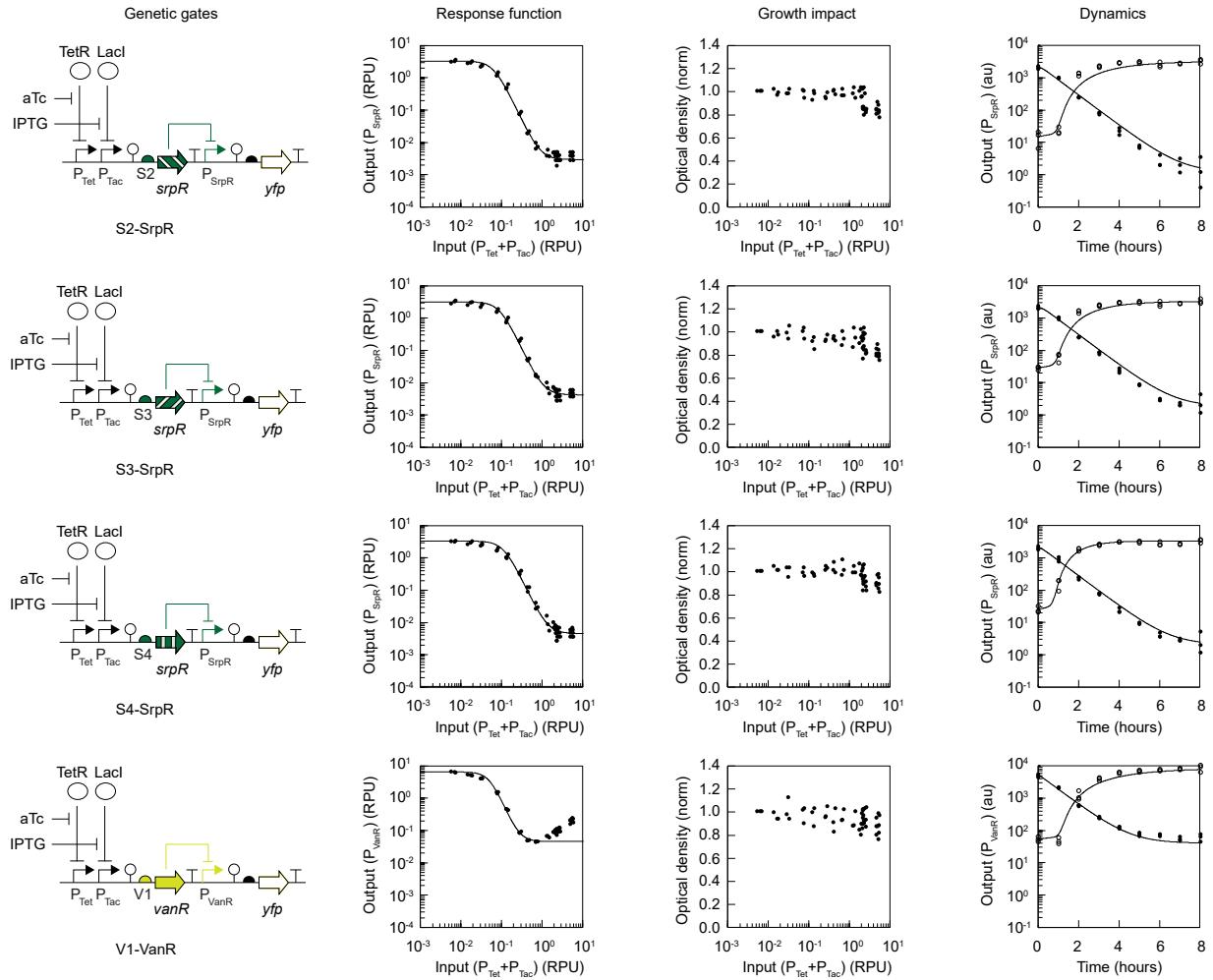
Appendix Figure S6: **Roadblocking assays for the gate output promoters.** The schematics show the gate diagrams, with the output promoter in the downstream position in front of *yfp*. By fitting these data to Appendix Equation S9, the parameters for roadblocking were extracted (Table 1). The non-additive model fit is shown as the purple lines. The OFF states are always the absence of inducer and the ON states are the presence of either 20 ng/mL or 1 mM IPTG. The error bars represent the standard deviation (SD) from three experiments performed on different days.



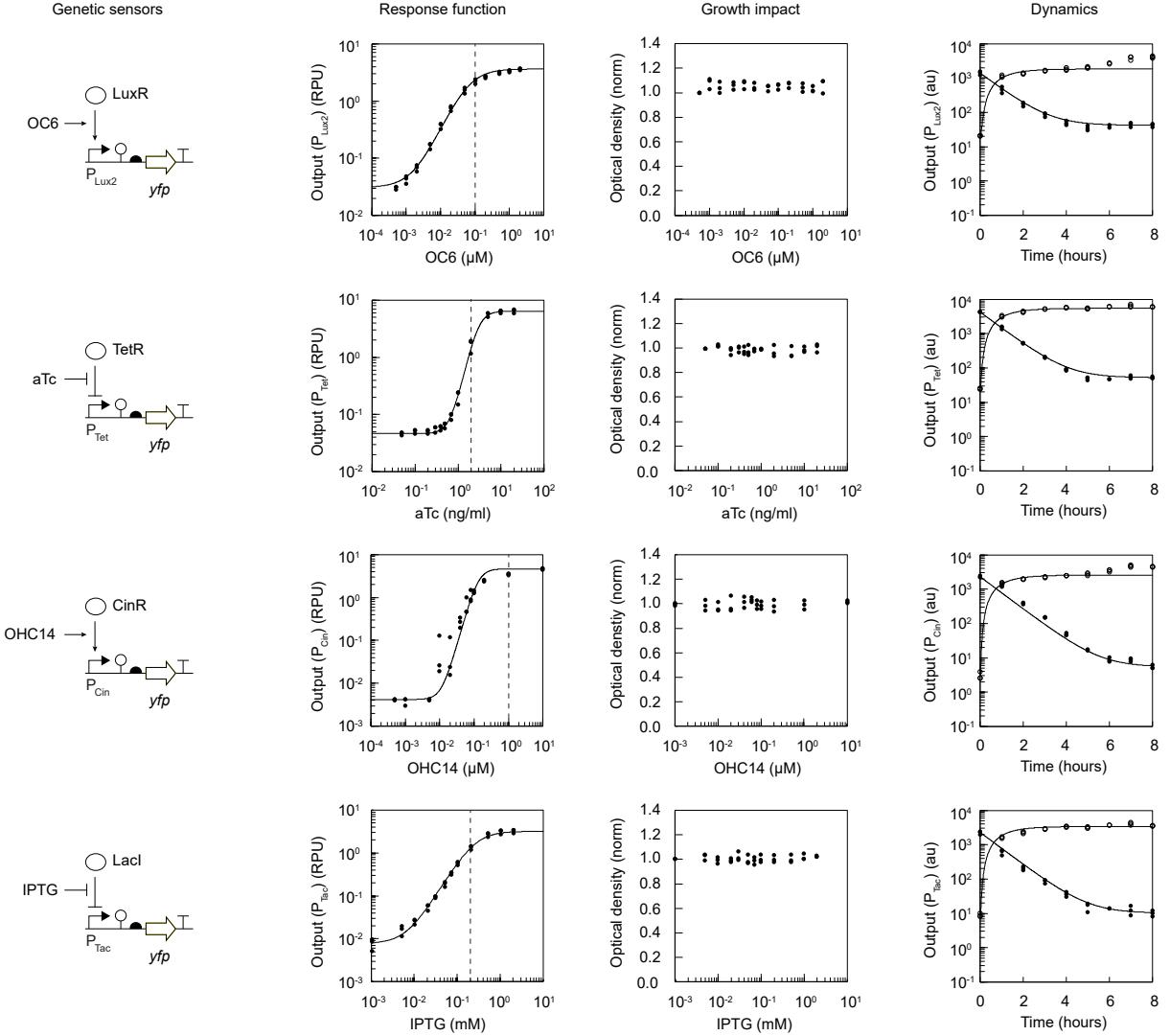
Appendix Figure S7: Activity of sensor output promoters in tandem. The schematics show the dual promoters evaluated in series (complete plasmid maps are shown in Appendix Fig S20). The combined output from the promoters is shown as measured in RPU. The purple lines show the predictions from the non-additive model (Appendix Equation S13). The OFF state for the sensors is always the absence of inducer. The ON states are 20 ng/ml aTc (P_{Tet}), 2 μ M OC6 (P_{Lux2}), 2 μ M OHC14 (P_{Cin}), 1 mM IPTG (P_{Tac}). The error bars represent the standard deviation (SD) from three experiments performed on different days.



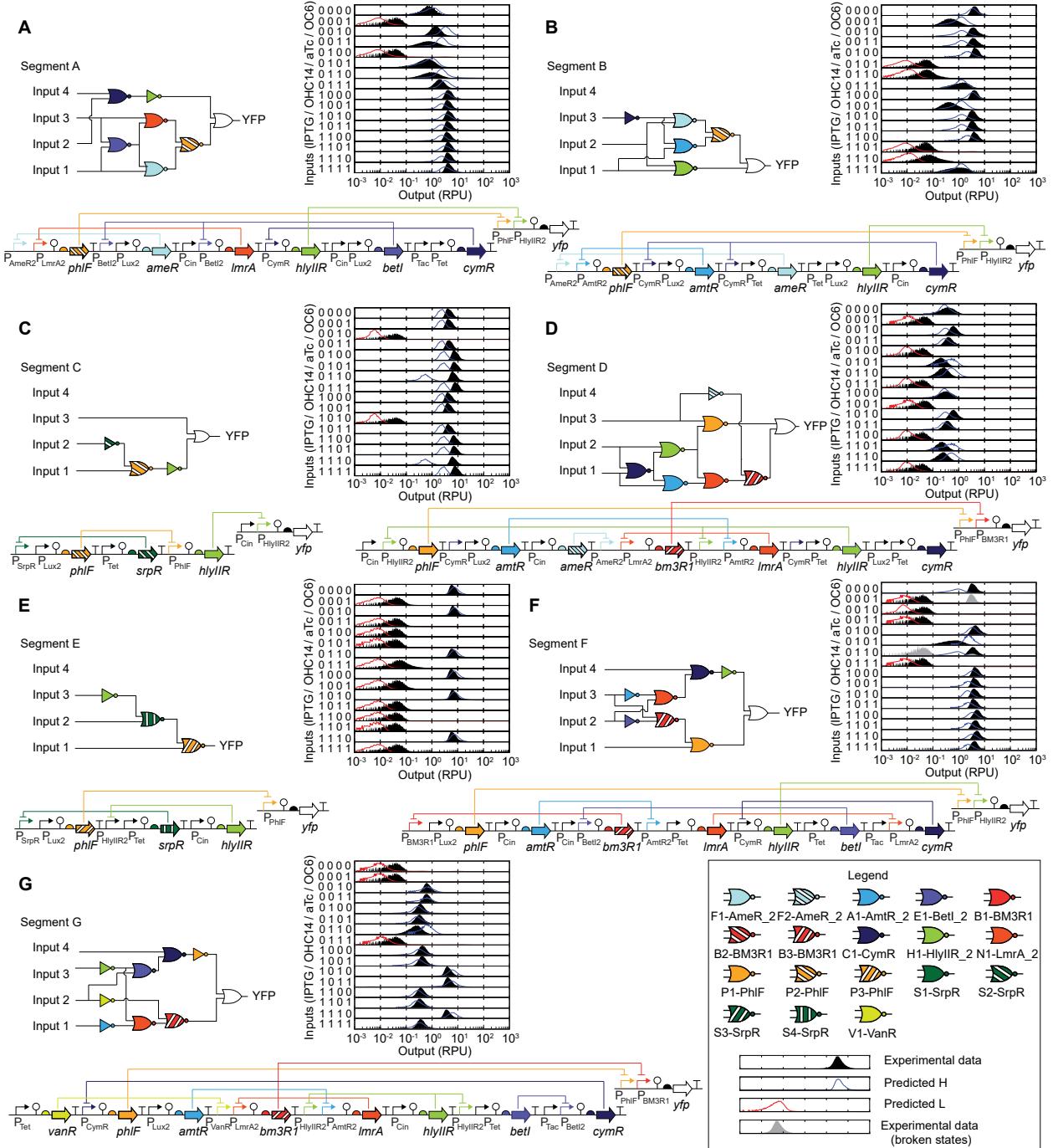




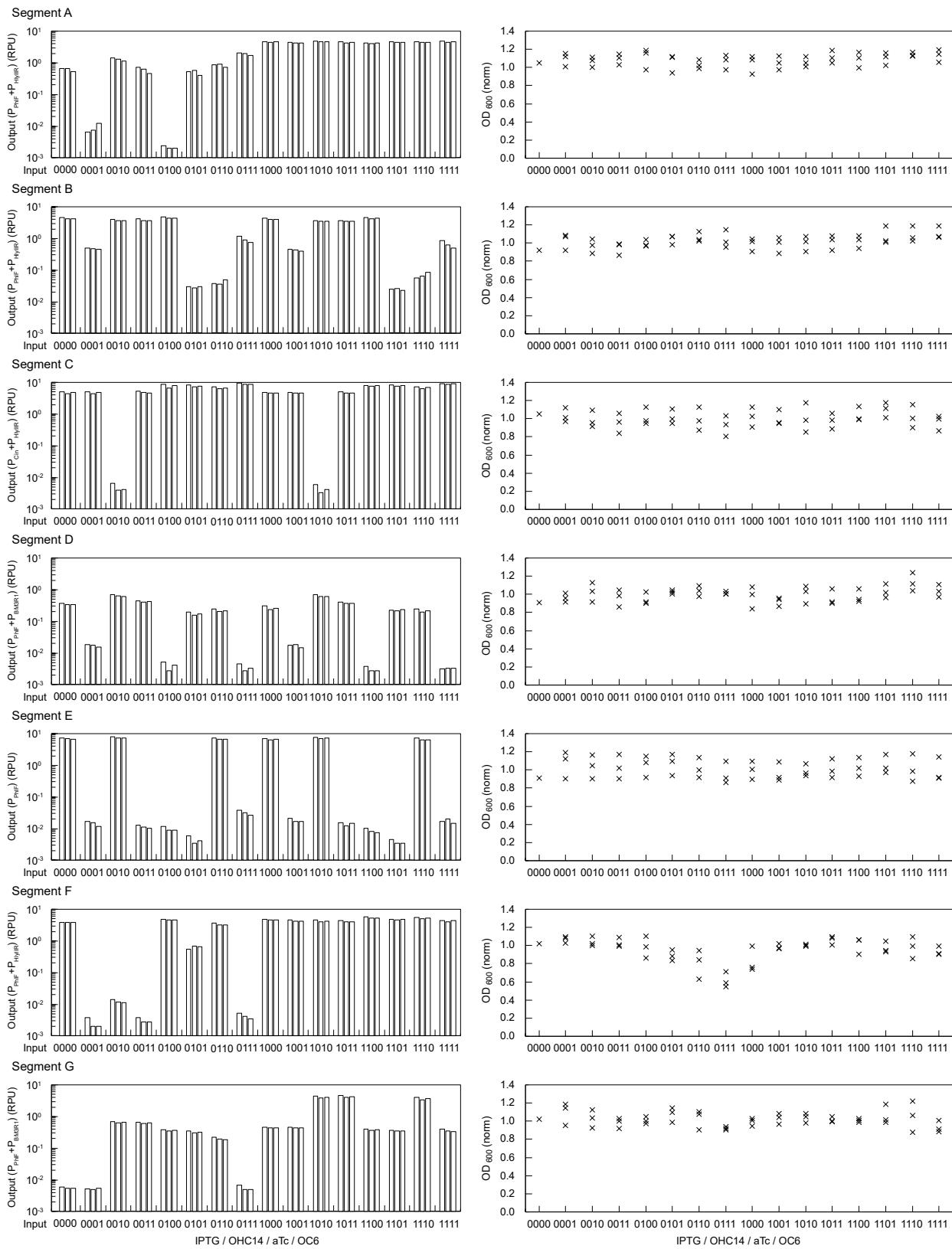
Appendix Figure S8: Characterization of gates. Schematics for the gate are shown to the left; the full sequences, parts, and plasmids are provided in Appendix Fig S21, Appendix Table S3 and S4. Data for the response functions are shown. The solid lines are fits to Equation 1 and the resulting fit parameters are shown in Table 1. The growth impact is the OD₆₀₀ normalized by the uninduced sample. The dynamic experiments are fit to numerical solutions of the ODEs in Appendix Equations S20 to S25. The fit parameters are provided in Table 1. Experimental details, including the combinations of inducers used, are provided in the Materials and Methods. The data represent three replicates performed on different days.



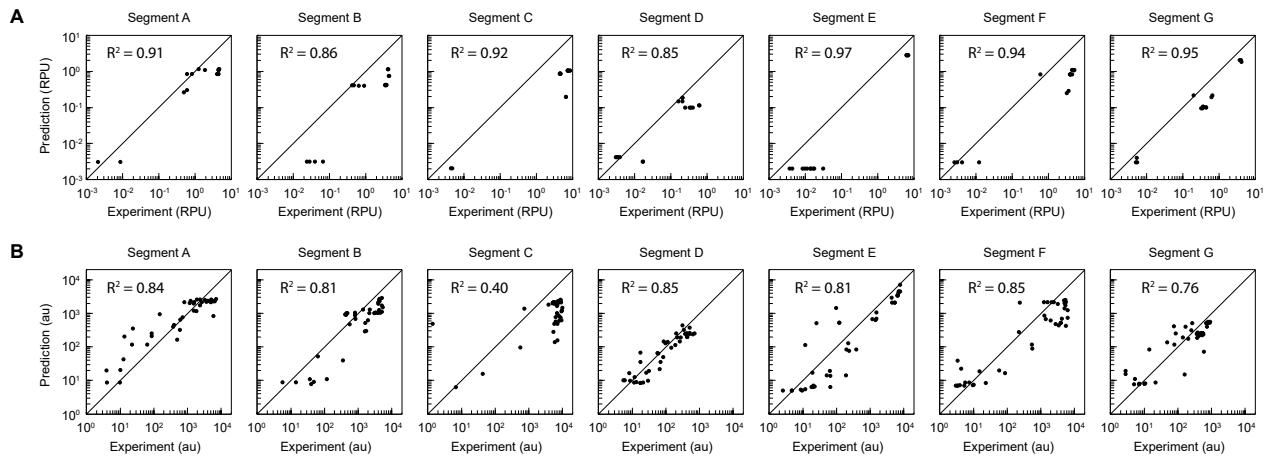
Appendix Figure S9: Characterization of genetic sensors. Schematics for the genetic sensors are shown on the left. The complete sensor sequences are provided in Appendix Table S3, genetic parts are in Appendix Table S4, and plasmid maps in Appendix Fig S19. The response functions for the sensors are shown. The data were collected over three days and the line is the fit to Appendix Equation S10. The dashed line indicates the concentration of inducer used for the ON state in Cello (the OFF state is for no inducer). The impact on growth is shown (the data are normalized by the OD_{600} of uninduced sample). The dynamic experiments are fit to numerical solutions of the ODEs in Appendix Equations S18 and S19. The fit parameters are provided in Appendix Table S1. Experiments are described in the Materials and Methods.



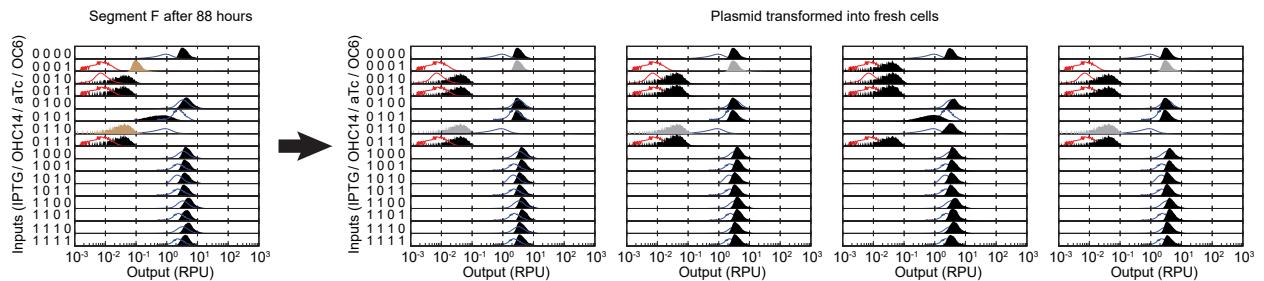
Appendix Figure S10: Detailed designs and data for the 7-segment circuits. The complete genetic designs for each circuit are shown. The full sequences are provided in Appendix Table S5 and the plasmid maps are in Appendix Fig S24. The predicted and measured responses are shown for each combination of inputs (0 is absence of inducer and 1 is its presence). The order is (from left to right): 0.2 mM IPTG, 1 μ M OHC14, 2 ng/ml aTc, 0.1 μ M OC6. The grey distributions for Segment F show the two failed states for circuits recovered after the 88-hour time course (Appendix Fig S13). The median values from the black distributions corresponds to one replicate of three. Additional replicates are shown in Appendix Fig S11 (Input 1: OC6, Input 2: aTc, Input 3: OHC14, Input 4: IPTG).



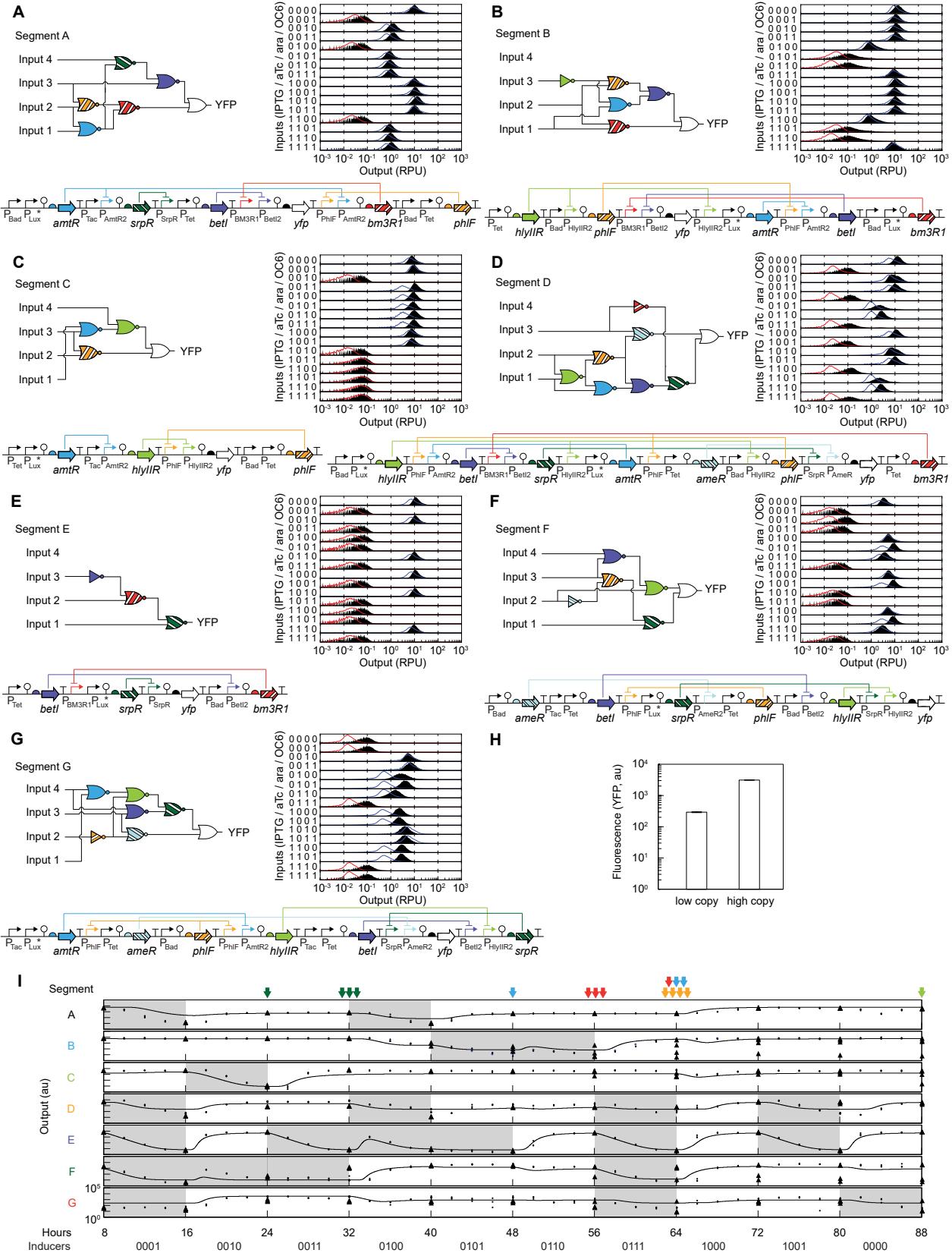
Appendix Figure S11: Response and growth impact of the 7-segment circuits. The intent of these graphs is to show the reproducibility of the experiments for the cytometry plots in Appendix Fig S10. Inducer concentrations of inputs (P_{Tac} , P_{Cin} , P_{Tet} , P_{Lux2}) were 0.2 mM IPTG, 1 μ M OHC14, 2 ng/ml aTc, and 0.1 μ M OC6. The bars and data points show the results of three experiments performed on different days. The optical densities are normalized by the uninduced sample. The bars show the median of the fluorescence distribution measured via cytometry, scaled by the RPU standard plasmid (Materials and Methods).



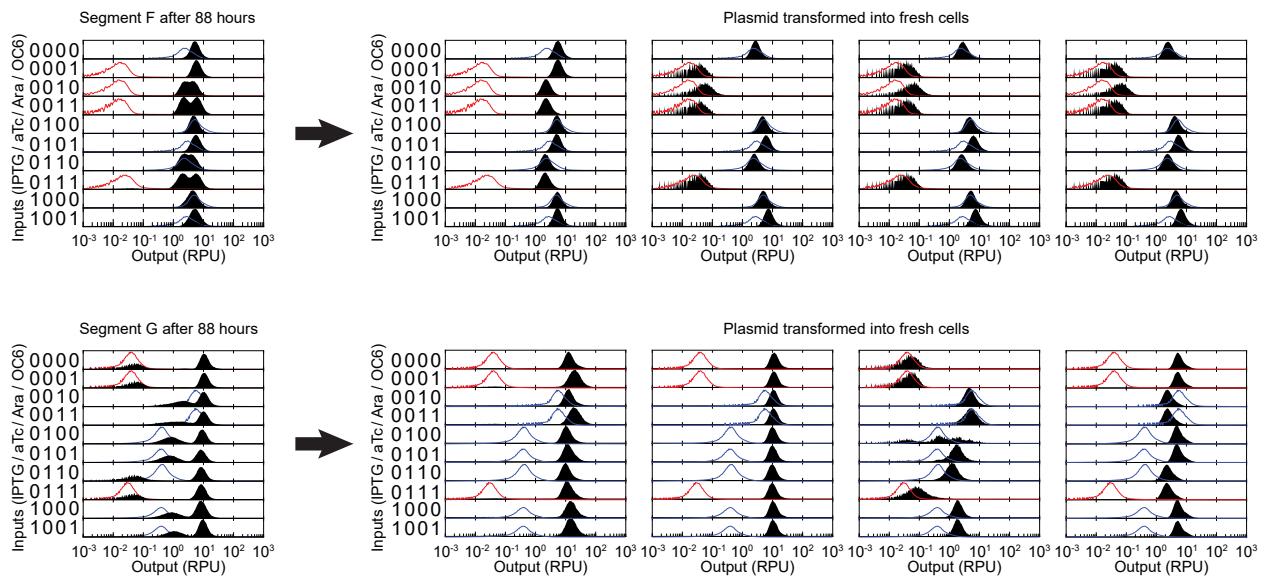
Appendix Figure S12: **Fit of promoter models to response data.** **(A)** Response of seven-segment circuits shown in Appendix Fig S11 is compared to Cello prediction. **(B)** Response of seven-segment circuits measured every 2 hours (Figure 3B) is compared to model prediction.



Appendix Figure S13: Analysis of Segment F (low copy plasmid) after the 88-hour continuous switching experiment. While the Segment F circuit worked for the states required to display the digit, it failed in some of the 16 possible states (brown distributions, far left). Plasmids for Segment F were recovered after 88-hour time course and retransformed into fresh cells. Four colonies were picked and tested. The predicted and measured responses are shown for each combination of inputs (0 is absence of inducer and 1 is its presence). The order is (from left to right): 0.2 mM IPTG, 1 μ M OHC14, 2 ng/ml aTc, 0.1 μ M OC6. The correct circuit response as predicted by Cello (blue and red) is compared to the measured distributions (black). The grey distributions for Segment F show the two failed states for circuits recovered after the 88-hour time course.



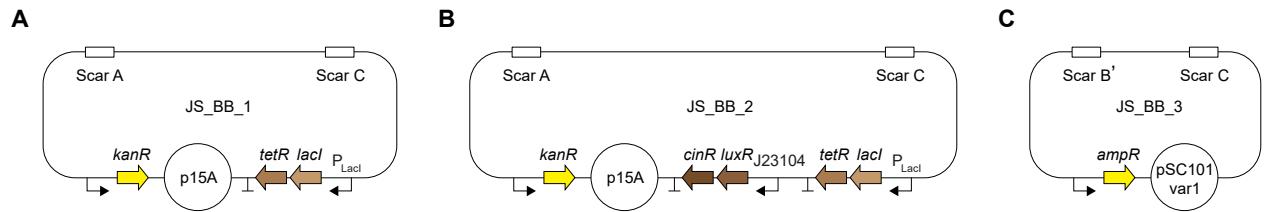
Appendix Figure S14: Characterization and stability of the first set of 7-segment designs carried on a higher-copy plasmid. The gates were originally designed on a higher copy p15a backbone (see text and Materials and Methods for details). These response functions were then used to build a UCF for Cello and the 7-segment circuits were designed. Their DNA was constructed and carried on the same higher copy plasmid. **(A - G)** These circuits performed as expected for all states. The notation, symbols and experimental conditions are identical to those described for Appendix Fig S10. **(H)** The expression of *yfp* from a constitutive promoter is compared for the lower and higher copy plasmids (1 and 2, respectively). Plasmid maps are shown in Appendix Fig S25. **(I)** The time trajectories for the circuits are shown from experiments performed identically to those used to make Fig 3B (Materials and Methods). These experiments were repeated four times on different days and each colored arrow indicates where a circuit failed (defined as producing the wrong output after that time point). The solid line shows the predicted behavior obtained from the set of ODEs described in Appendix Information II, but with parameters that were obtained by fitting equivalent dynamic experiments performed for each gate on the higher-copy plasmid (Materials and Methods).



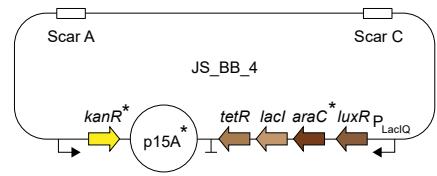
Supplementary Figure S15: Analysis of Segment F and G (on high copy plasmids) after the 88-hour continuous switching experiment. Plasmids were recovered from the cells and then transformed into fresh cells. Four colonies were picked and were tested for the circuit response. The correct circuit response as predicted by Cello (blue and red) is compared to the measured distributions (black).



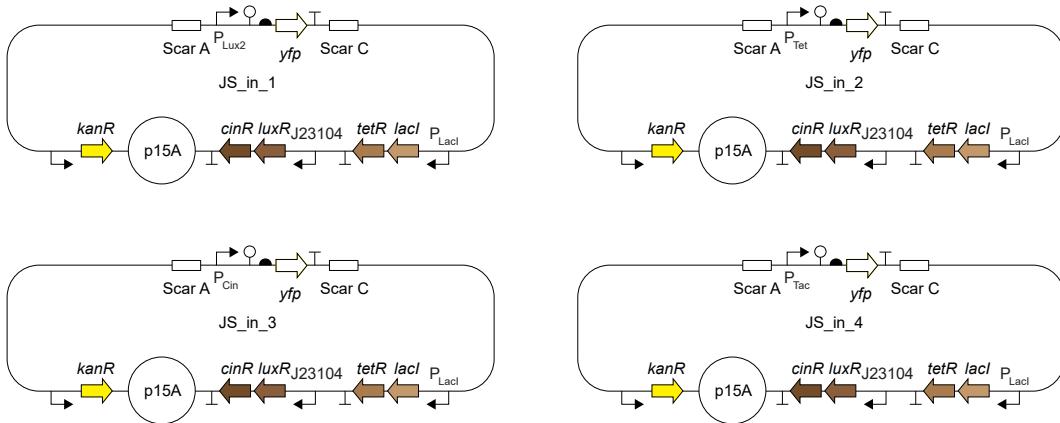
Appendix Figure S16: 3D printed device and seven segment display. Decimal numbers are shown in Chemidoc using CCD camera with 530/28 filter. Figures taken from Chemidoc under white light (top) and with 530/28 filter (middle) were shown. The image was adjusted using Photoshop CC 2019 (bottom).



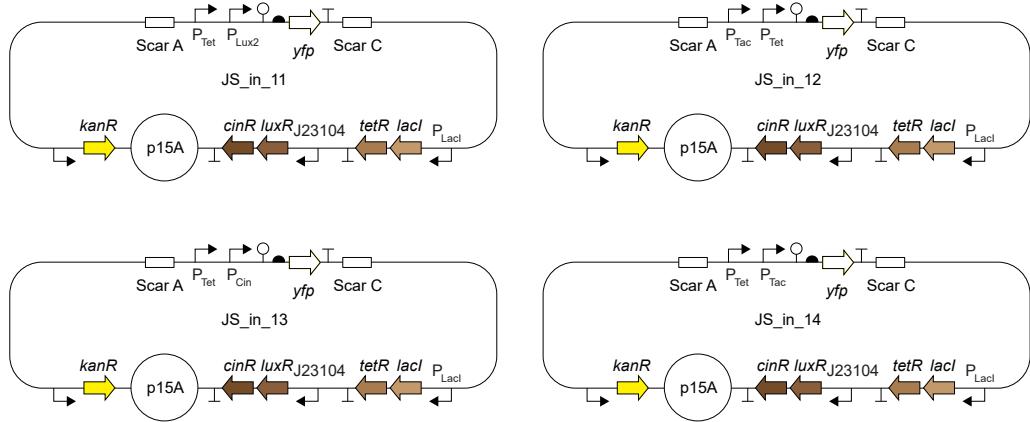
Appendix Figure S17: **Plasmid backbones.** **(A)** Plasmid backbone used for gate characterization. **(B)** Plasmid backbone used for sensor characterization and seven segment circuits for the second design (low copy). **(C)** Plasmid backbone used for reporters of seven segment circuits for the second design (low copy).



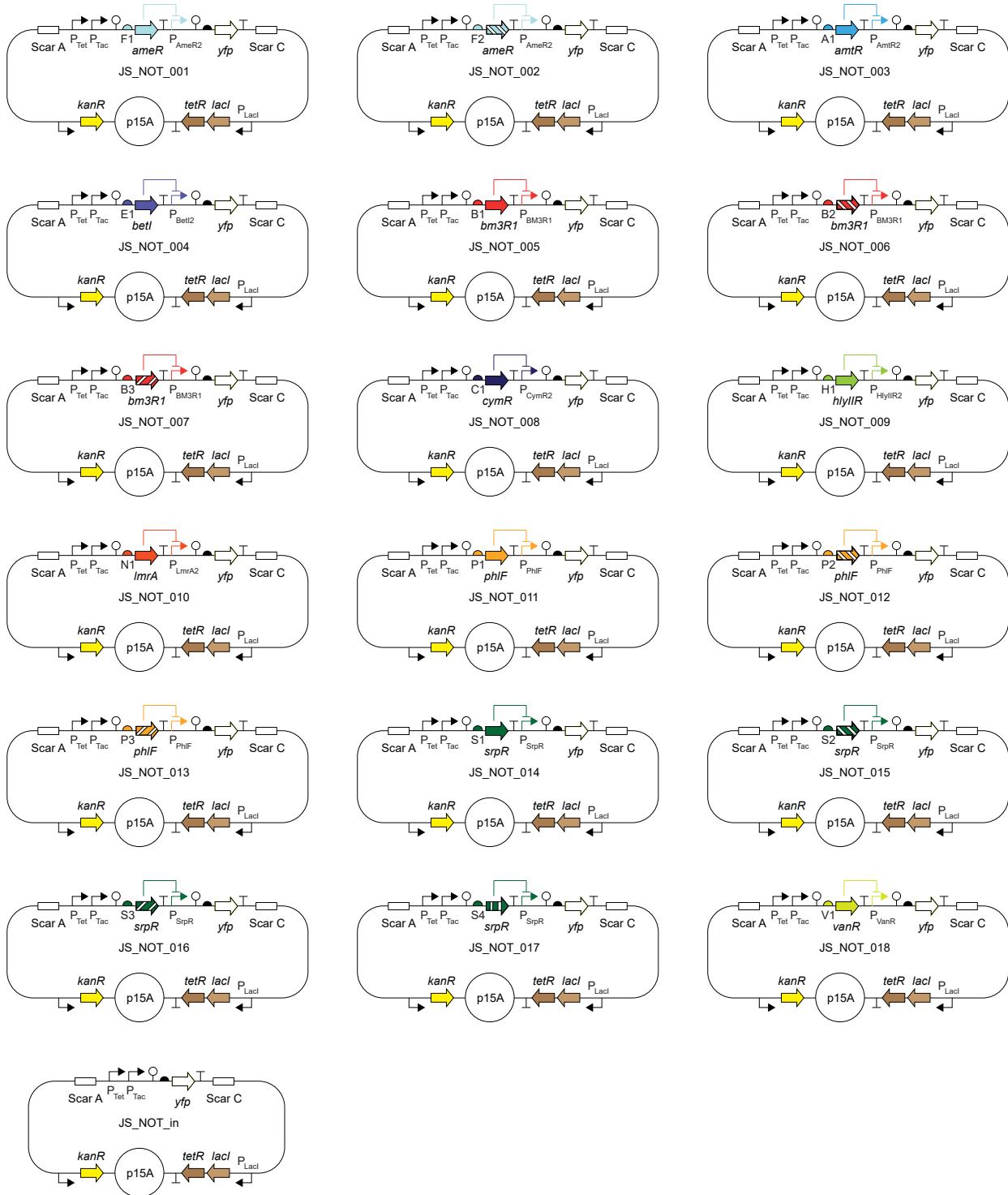
Appendix Figure S18: High copy plasmid backbone. This plasmid backbone used for the tandem promoter characterization and the seven segment circuits for the first design (high copy).



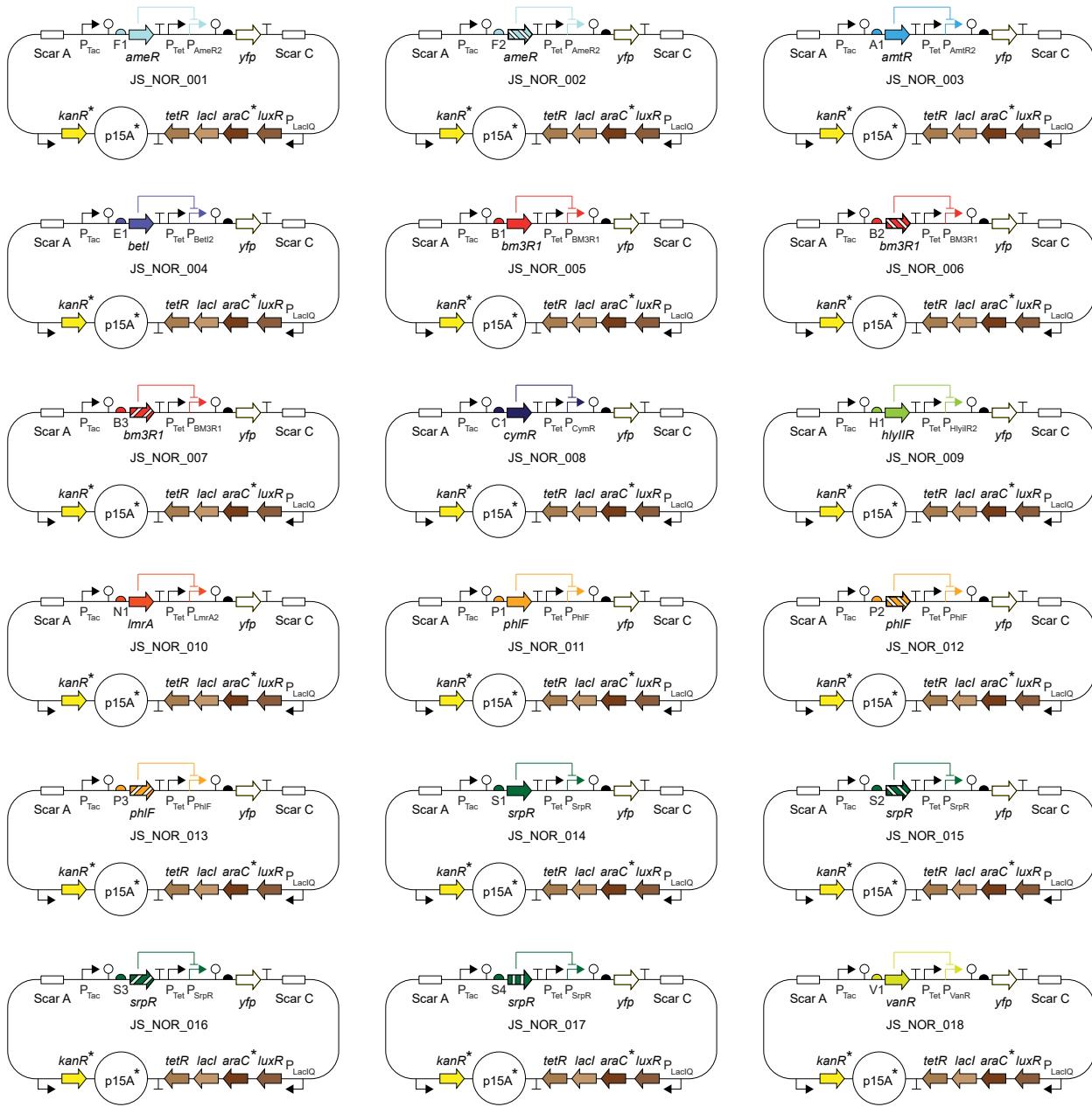
Appendix Figure S19: Plasmid maps for the sensor characterization. These plasmids were used for Appendix Fig S9.



Appendix Figure S20: Plasmid maps for the sensor output tandem promoter characterization. These plasmids were used for Appendix Fig S7.



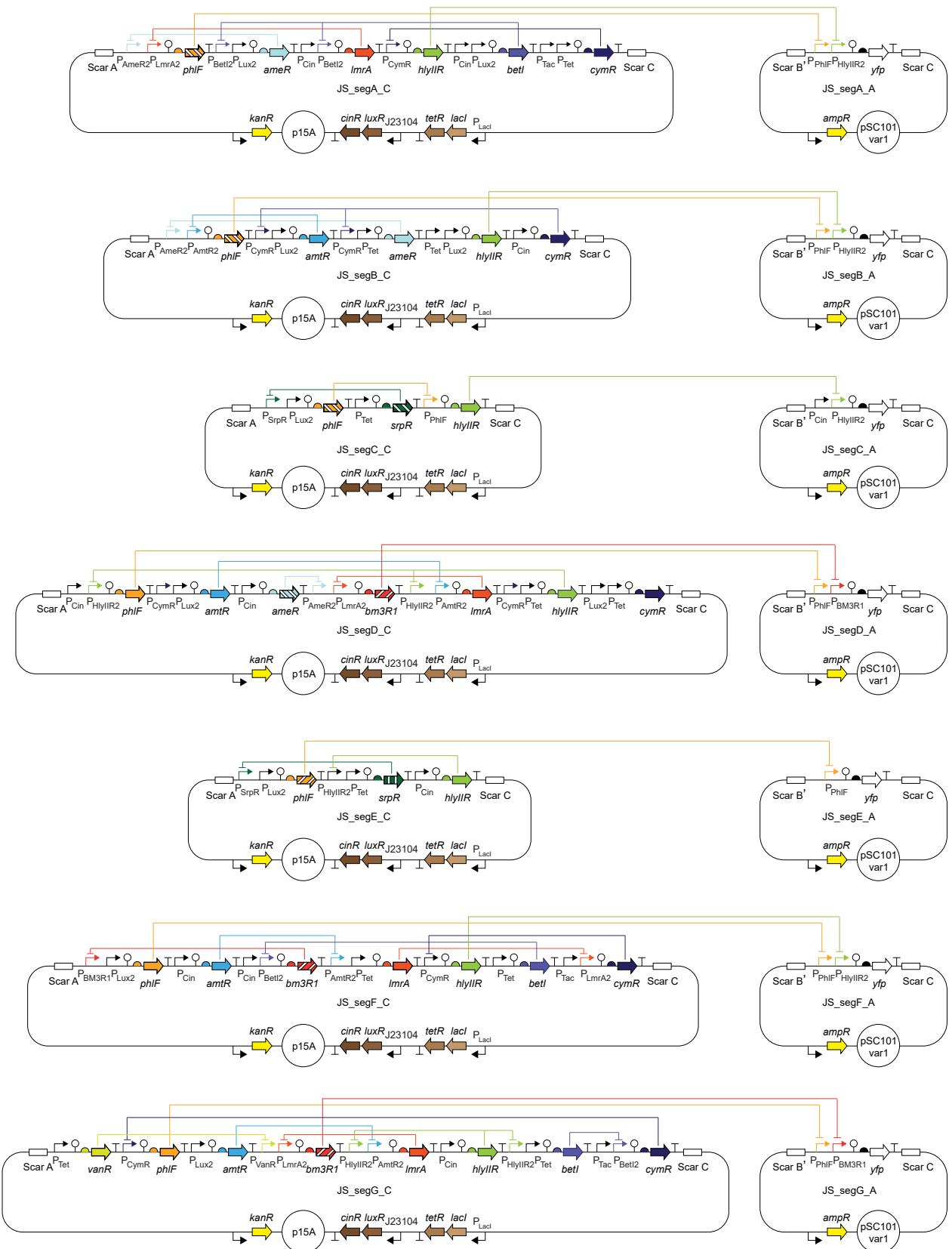
Appendix Figure S21: Plasmid maps for the gate characterization. These plasmids were used for Appendix Fig S8.



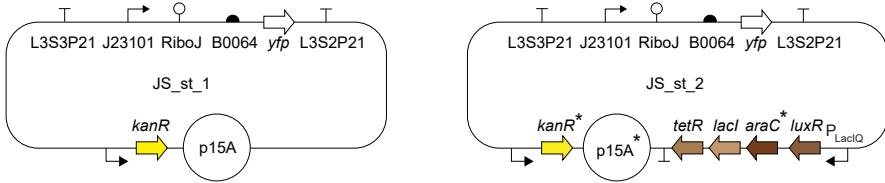
Appendix Figure S22: Plasmid maps for the gate output tandem promoter characterization. These plasmids were used for Appendix Fig S6.



Appendix Figure S23: Plasmid maps for the tandem promoter evaluation. These plasmids were used for Fig 1C, 1D and 1E.



Appendix Figure S24: Plasmid maps of the 7-segment circuits. Circuit and reporter plasmids are shown.



Appendix Figure S25: Plasmid maps for the YFP expression from a constitutive promoter in two p15A backbones. Two plasmids maps used for Appendix Fig S14H are shown. Sequences are provided in Appendix Table S4.

Appendix Table S1: Parametrization of sensors

	Response Function			Promoter Interference		Kinetics		
	γ_{\max}	γ_{\min}^a	K	n	Non-specific (α)	Roadblocking (β) ^b	Induction (τ_y^{ON})	Relaxation (τ_y^{OFF})
OHC14(P_{Cin})	4.652	0.004			0.01	0.99	2.70	4.00
OC6 (P_{LuxZ})	3.630	0.031			0.80	1.00	2.30	4.00
aTc (P_{Tet}) ^c	6.406	0.046			0.69	1.00	3.50	4.00
IPTG (P_{Tac})	3.194	0.008			0.73	0.04	3.50	4.00

a. The sensor output promoter (in RPU) in the presence and absence of the inducer. The ON state was measured for the following concentrations of inducer: 2 μM OHC14, 2 μM OC6, 20 ng/ml aTc, and 1 mM IPTG.

b. The maximum allowed value is one during fitting.

c. To evaluate the P_{Tet} promoter for promoter interference, this promoter in an upstream position from the circuit in Appendix Fig S6 is replaced with P_{Tac} .

Appendix Table S2: List of plasmids used in each figure

Figures	Plasmids
Figure 1 C	JS_NOR_102
Figure 1 D and E	JS_NOR_101 to JS_NOR_120
Figure 1 F and G	JS_NOT_001 to JS_NOT_018
Figure 2	JS_segA to JS_segG
Figure 3	JS_segA to JS_segG
Appendix Figure S2	JS_NOT_002, 003, 004, 009, and 010
Appendix Figure S3	JS_in_1
Appendix Figure S4	JS_NOR_101 to JS_NOR_120
Appendix Figure S6	JS_NOR_001 to JS_NOR_018
Appendix Figure S7	JS_in_11 to JS_in_14
Appendix Figure S8	JS_NOT_001 to JS_NOT_018
Appendix Figure S9	JS_in_1 to JS_in_4
Appendix Figure S10	JS_segA to JS_segG
Appendix Figure S11	JS_segA to JS_segG
Appendix Figure S12	JS_segA to JS_segG
Appendix Figure S13	JS_segF
Appendix Figure S16	JS_segA to JS_segG

Appendix Table S3: Complete annotated sequences of gates and sensors

Part name	Type	DNA sequence ^a
YFP	Reporter	<pre> AGCTGTACCGGATGTGCTTCGGTCTGATGAGTCGTGAGGACGAAACAGCCTACAAATAATTGTTAATACTAGAGAAAGAGGGAAAT ACTAGATGGTGAGCAAGGGCAGGGAGCTGTCACCGGGTGGTGCACCATCTGGTCGAGCTGGACGCCAACGGCACAAGTCAGCGTGT CCGGCAGGGCGAGGGCATGCCAACCTGGCAAGCTGGACCTGAGGTCATCTGGTCGACGCCAACGGCAGGGCTGGCCACCCCTGGCGTGA CCACCTTCGGCTACGGGCCATGGTCTCCGGCATACGGCACCTGGTCGAGCTGGACGCCAACGGCAGGGCTGGCCACCCCTGGCGTGA AGGAGCGCACCCATCTTCAAGGACGACGGCAACTACAAAGGCCGGAGGTGAAGTGAGGGCGACACCTGGTAACCGCAGTCAGCTGA AGGGCATTGACCTCAAGGAGGACCGAACATCTGGGCAAAAGCTGGAGTAACAACTACAAAGGCCAACCTCTATATCTGGCCACAAAGCAGA AGAACGGCCTGCTGCTGGCCGACACCCACTTCTGGTACCTGGTACCTGGACGCCAGCTGGCGAGCTGGCCGACCAACTGGCGAC ACGGCCGGTGTGGCTGGCCGACACCCACTTCTGGTACCTGGTACCTGGTACCTGGACGCCAGCTGGCGAGCTGGCCGACCAACTGGCG AGTCGTGACCGCCGGGATCACTCTGGCATGGACAGCTGTACAAGTAACCTGGTACCAAAATTCCAGAAAAGAGGCCCTGGGAAAGGGGGC CTTTTCGTTGGTCC </pre>
F1-AmeR_2	Gate	<pre> AGGGGTCAAGTTGATGTGCTTCACCTGATGAGTCAGTGATGACGAAACCCCTACAAATAATTGTTAATCTGGACTATGTTACATGGACTATGTTACAT ACGAGGGGATTAGATGAACAAAACCATTGATCAGGTGCTAAAGGTGATCGTAAAGCGATCTGCCGGTCTGCTGCGCCTGCTAGTGGCC AAGAAACCCCTGGTATTCGGCAAAGGCCAGAACTGTTCTGGCAAGCTGGTAAATGCACTGGTCCATTGGCATATTGCAAGCCACTGTA ATATGAGTCGGCAAATGTTAAACATTCTGGCAGAAACCGCAGTGGTATGCAATTGGTTGGTCAAGTGGTTGGTCAAGCTGGTCTGGCAGA TTCTGGCTGGATAAAAGCCATGACCCCTGGATCTGGCTCATCTGGCACCTAATCTGATGGAACCATCATCAGGATCATTCAACACAC TACGGGTTTATTAGATCTGATGACGCCAACAGGATATGAAATGGCGATTATTACAAAGCGTGTGAAACTGCTGGCGAACATTCATCCGG TTCTGATGTTGAAGCAGGTCTGTATATTGCAACCGATATTCCGGTCTGGCAGAACCGTCTGGCATCTGACTGACCGCTTATTCCGG TTCTGATGCAAAAGAGATATTGTAATCTGGCAACCCGGTGTGATCAGCTGGTGTGATCTGATTGATGCCAGGTCTGGTAATCCGCTGGAAAT AACCAATTATTGACACCCCTAACGGGTGTTTTTTGGTCTCCCTCGTCACTAGAGGGCATAGTGACAAACTCATCATTCC TACGTAGGCTGCTAGC </pre>
F2-AmeR_2	Gate	<pre> AGGGGTCAAGTTGATGTGCTTCACCTGATGAGTCAGTGATGACGAAACCCCTACAAATAATTGTTAATACGCTATGGACTATGTTCA AACAGGAGCTAATAGATGAACAAAACCATTGATCAGGTGCTAAAGGTGATCGTAAAGCGATCTGCCGGTCTGCTGCGCCTGCTAGTGGC GAAGAAACCCCTGGTATTCGGCAAAGCGAAGAACTGTTCTGGTACACCGTGTGTTAAATGCACTGGCATTGGCAGATATTGCAAGCCACTG AATATGAGTCGGCAAATGTTAAACATTCTGGCAGAAACCGCAGTGGTATGCAATTGGTTGGTCAAGTGGTTGGTCAAGCTGGCAG ATTGGCTGGTGTGATAAAAGCCATTGACCCCTGGATCTGGCTCATCTGGCACCTAATCTGATGCCAACCATCATCAGGATCATTCAACAC ATACGGGTTTATTAGATCTGATGACGCCAACAGGATATGAAATGGCGATTATTACAAAGCGTGTGAAACTGCTGGCGAACATTCCGG ATTCTGATGTTGAAGCAGGTCTGTATATTGCAACCGATATTCCGGTCTGGCAGAACCGTCTGGCATCTGCACTGACCGCCTTATTCTGG TTCTGATGTCACAGGAAAGATATTGTAATCTGGCAACCCGGTGTGATCAGCTGGTGTGATCTGATTGATGCCAGGTCTGGTAATCCGCTGGAAA TAACCAATTATTGACACCCCTAACGGGTGTTTTTTGGTCTCCCTCGTCACTAGAGGGCATAGTGACAAACTCATCATTCTC CTACGTAGGCTGCTAGC </pre>
A1-AmrT_2	Gate	<pre> AGGGTCTCAAGGTGCTACCTGACTGATGAGTCGGAAAGGACGAAACCCCTACAAATAATTGTTAAATGCTTAATAATCAGC AAAGAGGTTACTAGATGGCAGGGCAGTGGTCTCCGCTGCTAGTGACCCGCTGCTGAGGTTAAACCGCTGTAAGAAATTCTGGATGCAA GGCAGAAACTGTTACCGCTCAGGGTTTGCACCAACCAAGTACCCGCTGAGGTTCTGGTATCTGCTGAGGCAACCCCTGTTATTCTATT TCCGACAAAGGAAACATTCTGCAACCCCTGGTCAAAGGAGCTGGTGTGAAACCGGCTGAGGAGCAGCCTGGCAGGAAAGTGGACCTGGATGCGCTG CGGAAATGGCTCTGGGCAATTGGTCAAGGCAACTGGTCTGCTGCTGAGGCAACCCGGTGTGAGCTGGTGTGATCTGCTGAGGCTGATTGTTG GTAGCGAGAAATTGCGAGAATATCATAGCCAGCGTGAAGCACTGACCAATGTTCTGGTGTGATCTGGCAACCGAAATTGGTGTGATCTGGCTG CAGAAACTGCCGTTTACATTACATGAGGGTTATTGAAATGGCTCCAAATGTTGAAATGGTAAATTCCGAGTCCGCTGAGGCCAGATGCCGCGGAA CCGAAATTCTGGCGACATGCAAGCTGGCAGTCTGGGTGCAACCCGGTGTGAGCTGGTGTGAAAGGGGAAACTGTTGGTGTGAAACCTGG ATGCAAAACTGGTACCCAAAGACGAACAATAGACGCTGAAAGCGTCTTCTGGTGTGCTGAGCTGGTGTGAAAGGGGAAACTGTTGGTCCCTGCTC TTGACAGTTCTATGATCTATAGATAATGCTAGC </pre>
E1-BetI_2	Gate	<pre> AGAAGTCATTAATGTCCTTAACTCTGATGAGTCGGTACGGACGACAAACCCCTACAAATAATTGTTAAACCCCGAGGGTAGCACATG CCGAAACTGGTATGCAGAGCATTGCTCGTCAGCTGATGTCAGCAACCTGGAAAGCAATTAATGAGATTGTTGATGATGCAACCATGCA CAGATTGCACTGCTGCGCTGTTAGCACCGTTATTAGCATTTCGGCAGATAAAACCGTCTGGTGAACGCAACCATGCGTGTATATTAC AGCCAGCTGCTGATGCACTGGTCTGCACTGCGCAGGGTGGCAGGACAGCCTGCTGAGGCCAGATGCCGCGGAA GAAAGGCTGGTGTGAGCCAGGAAAGCATGGCTGGCATTTGGGCAAGCAGCATGTCAGCCGATCTGCTGAGCTGGTGTGAAATGGTGTGAAATGGTGT AGTCGTGCTGCTGAGCAATCTGGTACGGTAAACCCCTGGATAAAACCGTGGCAATAGCCCTGACCCGGTCTGGTGTGAGCTGGCAGACTGATT GATGGTCTGTCGCTGCGTGCAGCACTGAGGGTAAACCCCTGGATAAAACCGTGGCAATAGCCCTGACCCGGTCTGGTGTGAGCTGGCAGACTGATT ACCGATTAACCAATTATTGACACCCCTCGGGGTGTTTTGGTCTCCCTGGCGGGGATTCGTTACCAATAGACAATTGAT TGGACGTTCAATATAATGCTAGC </pre>
B1-BM3R1	Gate	<pre> AGACTGTGCGGGGATGTGATCCGACCTGACGATGGCCAAAAGGGCGGAAACAGCTCTACAAATAATTGTTAACTATGGACTATGTTA ACTAGATGGAAGCACCCGACCAACAGAAAGCAATTTCAGCGCAAGCCTGCTGCTGTTGCAAGCTGGTTGGTATGCAACCCACATGC CGATGATTGCGAAAGTGGTCAAGGCTGACCTTATCCATTCTGAAACAAAGGCTGGTGAAGCAACTGTTCTGAGCAGCTG TTAATGAACTTCTGCACTGGTGTATTGAAAGCGGTCTGGCAATGAGCTGATGGTTCTGGTGTGATGGCTTCTACACATTGAGGTTGGTACCT TTCAACAAAATCTGGCTGACTGGTGTGTTTCTGCAAGGCTGAGGAGGGACGGCTGGCAGGAAAGGGCTGGTGTGAACTGAGGTTGGTACCT TTGAAATGGTGTGACCTTCTGGTCAAGGCTGAGGAGGGACGGCTGGTGTGAACTGAGGTTGGTGTGAGGAGGGATTCGTTACCAATAGACAATTGAT TGGACGTTCAATATAATGCTAGC </pre>

GTCAGAGCTAACTCGGTACCAAATCCAGAAAAGAGACGCTTCGAGCGTCTTTTGCTTTGGTCC**AATCCGCGTGATAGGCTGATTGTTAC**
CAATTGACGGAATGAACGTTCATCCGATAATGCTAGC

P2-PhIF	Gate	<p>AGCGCTCAACGGCATGTCCTTGCCTCTGATGAGACAGTGATGTCGAAACGCCCTACAATAATTGTTAAGAGCTATGGACTATGTTGA AAGGCTGAAATACAGATGGCACGTACCCGAGCGTAGCAGCATTGGTAGCTGCTGAGCATCCATAAACGAACTCTGACCCAGCACCAT TGAAATCCTGAAAAGATGTTATAGCGCTGAGCATGAAAGCGTAGCTGCTGAGCATCCATAAACGAACTCTGACCCAGCACCAT CAATAAAGCAGCACTGATGCCCAGTGTGAAATGAAAGCGAACAGGTGGTAAATTCCGGATCTGGTAGCTTAAAGCCGATCTGGATT TCTGCTGCGTAATCTGTTGGAAAGTTGGCTGAAACCATTTGTTGGTAGACGATTTCTGTTGGTAGACGATCTGACAGCTGGACCCCTGCAACCC GACCAGCTGAAAGATCAGTTTATGACAGCTGCTGAGATGCCAAAAACTGTTGAAATGCAATGTTGAACTGCGCAAAGTAC CAATCGTGAACCTGCTGCGATGATTGTTGGTTGCTGGTACACAGCTTAAGGAAACACAGAAAAAGCCCGCACCTGACAGTGC AAGGCGACGACGGTGAATCTGATTGCTTACCAATTGACATGACGAAACGCTACCGTATCGTTAAGG TCTCCTGCTGATTAATGTTGTTGCTGGTACACAGCTTAAGGAAACACAGAAAAAGCCCGCACCTGACAGTGC AAGGCGACGACGGTGAATCTGATTGCTTACCAATTGACATGACGAAACGCTACCGTATCGTTAAGG</p>
P3-PhIF	Gate	<p>AGCGCTCAACGGCATGTCCTTGCCTCTGATGAGACAGTGATGTCGAAACGCCCTACAATAATTGTTAAGAGCTATGGACTATGTTGA GCACGCTACCCGAGCGTAGCAGTGTGAAAGCGTAGCTGCTGAGCATCCATAAACGAACTCTGACCCAGCACCATGAAATCTG GGTATAGCGGTCTGAGCATGAAAGCGTAGCTGCTGAGCATCCATAAACGAACTCTGCTGTTGGGACCAATAAGCAGCACTGATT GCCGAAGTGTGAAATGAAAGCGAACAGGTGGTAAATTCCGGATCTGGTAGCTTAAAGCCGATCTGATTTCTGCTGCTGAAATCTG AAAGGTTGGCTGAAACCATTTGTTGGTAGACGATTTGCTGTTGGTAGACGATCTGACCCAGCTGCAACCCGACCCAGCTGAAAGATCAG TTTGGGAAAGCTGCTGCTGAGTGGTAAAGGCTGATTAGCAGTGTGAAAGTACCTGGAACGACTGCGAAAGATACCAATCTG GATATGATTGTTGTTGTTGTTGCTGCTGCTGACCGGAGCTGACGGTAAACGGATATTGAAAGATTTACCTTCTGCTGATTAATG GTTTGTCCGGGTACACAGCTTAAGGAAACACAGAAAAAGCCCGCACCTGACAGTGC GGGGTCCGGGTACACAGCTTAAGGAAACACAGAAAAAGCCCGCACCTGACAGTGC TCTGATTGCTTACCAATTGACATGACGAAACGCTACCGTATCGTTAAGG</p>
S1-SrpR	Gate	<p>AGCGCTCAACGGGTGCTCCGCTCTGATGAGTCGTGAGGACAAAGCCCTACAATAATTGTTAAGAGCTATGGACTATGTTTC ACAGAGGAGTACCAAGGATGGCACGTAACCGCAGCAGAGAACCCGTCAGCAGTATTGATGACGACTGGAAGTTTGTGAC AGGGTTAGTGTGATGCAACCCGATCAGATTGCACTGAAAGCCGGTTACCCGTTGAGTTTACCGGATTTAATGTAACCTGGAAGTTC TGCAGGGCAGTTCTGGCAACCCGTCAGCATCCGCTGGAACCTGATTACCCGATCTGGTATTGAACTGAGCTGGGAAGCAGTTGTTGCAA TCTGAGATGCACTGATGAGTCAGACAGAACAGTTACCGGAAATTCTGATTATCAGGGCTGAGAAAGCCGCTGATTGATGATCTGAGACCA TTCAGGCAAGCGATGTTCTGCGATGATTATCATCAGGGCTGCGATCAGTTACCCAGGGTAACGCGGATAATCTGGATCTGAGACCA GCATTGGTTTTAAAGGTTGATTAACCGGCTGCTGTTGAGGTTGCTGAGCAAAGTACAGCAGGACAGATTATCAAAGTTGCACTGGGTA GCTTTGGGCACTGCTGCTGAGACCCGCTGTTCTGCTGTTGAGGAAAGACAGATTAAACAGGTGAAATCTCGAATAATTCA CTTAAGACCGCGGTCTGTCACACTTCCAGTAAATGGGTGAGCAGGATGGGGTTTCTTCTCTCAA CTATGATTGTTGCTCAGATT CGTTACCAATTGACAGCTAGCTCAGTCAGTCTAGGTATATACATACATGCTTGTGTTGAAAC</p>
S2-SrpR	Gate	<p>AGCGCTCAACGGGTGCTCCGCTCTGATGAGTCGTGAGGACAAAGCCCTACAATAATTGTTAAGAGCTATGGACTATGTTTC ACATGAGATACCAAGGATGGCACGTAACCGCAGCAGAGAACCCGTCAGCAGTATTGATGACGACTGGAAGTTTGTGAC AGGGTTAGTGTGATGCAACCCGATCAGATTGCACTGAAAGCCGGTTACCCGTTGAGTTTACCGGATTTAATGTAACCTGGAAGTTC TGCAGGGCAGTTCTGGCAACCCGTCAGCATCCGCTGGAACCTGATTACCCGATCTGGTATTGAACTGAGCTGGGAAGCAGTTGTTGCAA TCTGAGATGCACTGATGAGTCAGACAGAACAGTTACCGGAAATTCTGATTATCAGGGCTGAGAAAGCCGCTGATTGATGATCTGAGACCA TTCAGGCAAGCGATGTTCTGCGATGATTATCATCAGGGCTGCGATCAGTTACCCAGGGTAACGCGGATAATCTGGATCTGAGACCA GCATTGGTTTTAAAGGTTGATTAACCGGCTGCTGTTGAGGTTGCTGAGCAAAGTACAGCAGGACAGATTATCAAAGTTGCACTGGGTA GCTTTGGGCACTGCTGCTGAGACCCGCTGTTCTGCTGTTGAGGAAAGACAGATTAAACAGGTGAAATCTCGAATAATTCA CTTAAGACCGCGGTCTGTCACACTTCCAGTAAATGGGTGAGCAGGATGGGGTTTCTTCTCTCAA CTATGATTGTTGCTCAGATT CGTTACCAATTGACAGCTAGCTCAGTCAGTCTAGGTATATACATACATGCTTGTGTTGAAAC</p>
S3-SrpR	Gate	<p>AGCGCTCAACGGGTGCTCCGCTCTGATGAGTCGTGAGGACAAAGCCCTACAATAATTGTTAAGAGCTATGGACTATGTTTC ACAAAGGAAGTACCAAGGATGGCACGTAACCGCAGCAGAGAACCCGTCAGCAGTATTGATGACGACTGGAAGTTTGTGAC AGGGTTAGTGTGATGCAACCCGATCAGATTGCACTGAAAGCCGGTTACCCGTTGAGTTTACCGGATTTAATGTAACCTGGAAGTTC TGCAGGGCAGTTCTGGCAACCCGTCAGCATCCGCTGGAACCTGATTACCCGATCTGGTATTGAACTGAGCTGGGAAGCAGTTGTTGCAA TCTGAGATGCACTGATGAGTCAGACAGAACAGTTACCGGAAATTCTGATTATCAGGGCTGAGAAAGCCGCTGATTGATGATCTGAGACCA TTCAGGCAAGCGATGTTCTGCGATGATTATCATCAGGGCTGCGATCAGTTACCCAGGGTAACGCGGATAATCTGGATCTGAGACCA GCATTGGTTTTAAAGGTTGATTAACCGGCTGCTGTTGAGGTTGCTGAGCAAAGTACAGCAGGACAGATTATCAAAGTTGCACTGGGTA GCTTTGGGCACTGCTGCTGAGACCCGCTGTTCTGCTGTTGAGGAAAGACAGATTAAACAGGTGAAATCTCGAATAATTCA CTTAAGACCGCGGTCTGTCACACTTCCAGTAAATGGGTGAGCAGGATGGGGTTTCTTCTCTCAA CTATGATTGTTGCTCAGATT CGTTACCAATTGACAGCTAGCTCAGTCAGTCTAGGTATATACATACATGCTTGTGTTGAAAC</p>
S4-SrpR	Gate	<p>AGCGCTCAACGGGTGCTCCGCTCTGATGAGTCGTGAGGACAAAGCCCTACAATAATTGTTAAGAGCTATGGACTATGTTTCACAC AGGAAATACCAAGGATGGCACGTAACCGCAGCAGAGAACCCGTCAGCAGTATTGATGACGACTGGAAGTTTGTGAC TGGTAGTGTGCAACCCGATCAGATTGCACTGAAAGCCGGTTACCCGTTGCTGAGTTTACCGGATTTAATGTAACCTGGAAGTCTGCA GGCAGGTTCTGGCAACCCGTCAGCATCCGCTGGAACCTGATTACCCGGATCTGGTATTGAACTGAGCTGGGAAGCAGTTGTTGAGCTG GGATGAGTGTGATGAGTCAGTCAGACAGAACAGTTACCGGAAATTCTGATTATCAGGGCTGAGAAAGCCGCTGATTGATGATCTG GGCAAGCAGATGTTCTGCGATGATTATCATCAGGGCTGCGATCAGTTACCCAGGGTAACGCGGATAATCTGGATCTGAGACCA TGGTTTTAAAGGTTGATTAACCGGCTGCTGTTGAGGTTGCTGAGCAAAGTACAGCAGGACAGATTATCAAAGTTGCACTGGGTA TTGGGCACTGCTGCTGAGACCCGCTGTTCTGCTGTTGAGGTTGCTGAGAAAGACAGATTAAACAGGTGAAATCTCGAATAATTCA AGACCCGGTCTGTCACACTTCCAGTAAATGGGTGAGCAGGATGGGGTTTCTTCTCTCAA CTATGATTGTTGCTCAGATT ACCAATTGACAGCTAGCTCAGTCAGTCTAGGTATATACATACATGCTTGTGTTGAAAC</p>
V1-VanR	Gate	<p>AGTGGCTGCTGATGAAACTCGATCACCTGATGAGCTCAAGGCAAGCGAACACCCTACAATAATTGTTAAGAGCTATGGACTATGTT GAACATGGACATGCCTGATTAACCCGGTCAAGCGTAGCTGTTATGATGGCAGTCAGCTGGTAAATGATGCAAGCGGTAAATCAA AGGTTGAGCTGCTGCTGAGACCCGCTGTTCTGCTGTTGAGGTTGCTGAGAAAGACAGATTAAACAGGTGAAATCTCGAATAATTCA AGACCCGGTCTGTCACACTTCCAGTAAATGGGTGAGCAGGATGGGGTTTCTTCTCTCAA CTATGATTGTTGCTCAGATT ACCAATTGACAGCTAGCTCAGTCAGTCTAGGTATATACATACATGCTTGTGTTGAAAC</p>

TGGGTGCACGTGGTTATGCGAGCCGGTGGTGTAGCAGCATGAGATTCGTGATGCAATTGAAGGTTCTGGTGGTGTGGAAAGGTTTGCGACAGTC
 GTCTGGCAGAACGTGGATGACCGAAGAACCCATGCACCTTGTGTACTGATGAGAACGGACTTTGCGACAGTC
 GTGAAGATCTGGATCGTATGCCCATATACTAGGCATTCATGATACCCGTGTTAGCGCAGCAGGTAATGGTGCAGTTGAAAGGCCACTGGCAC
 GTAATGGTTGAACCGTTGCCAGCCGGTGCACTGGCCCTGGATCTGATGGACCTGTCCTGCCGAATATGAACATCTGCTGCCACACATGTC
 AGCATCAGGCAGTCTGGATGCAGTTAGCTGTGGTGTAGCCGAAGGGTGCAGAACGTATTATGCGTGTACATGCACCTGGCAGCAATTGTAATGCAA
 AAGTTTTGAAGCACCAACCGCACCGCTGGTGCAGCATGTCATTGCAAGGATTGATAAAACCCATGAGAAAGCCCCGAGA
 TCACCTCCGGGGCTTTTATTGCGCTGTATAAGTCGCCATTGGATCATTGACAGCTAGCTAGCTAGGTACATTGGATCCAAT

GCGGCGGCCATCGAATGGCGAAAACCTTCGGGTATGGCATGATAGGCCGAAGAGAGTCATTAGGGTTGAAT**ATGAAACAGTAAC**
 GTTATACGATGTCGAGAGTATGCCGTCTCTTATCACGCCCTCCGGTGTGAAACCAGGCCAGCCACGTTCTGC
 AGTGGAAAGCGCGATGGCGAGCTGAATACATTCCAAACCGCGTGGCACAACA
 CACTGGCGGCAAAACAGTC
 CAGTCTGGCCATGCCAGCCGCTGCAAAATTGCGCGGATTAATCTGCCGCGGCAACTGGGGTGCAGCGTGGTGTGCGATGTAACAG
 AAGCGCGTCAAGCGCTGCAAGCTTCTCGCAGGCGTGCACAATCTCTCGCAGCAGCGTGTGGCTGATCATTAACTGCCGCTGGATGACAGGAGTC
 CATTGCGTGGAAAGTGCCTGACTAATGTTCCGGCTTATTCTTGATGTCCTGACCCAGACACCCATCACAGTATTATTTCTCCATGAGGA
 CGGTAGCGACTGGCGTGGAGCATCTGCGTGGCATTGGGTGACCCAGAAATCCGCGTGTGTTAGCGGGCCATTAACTCTGCTCGCGCTCGC
 TCGTGGCTGGCTGCAAAATTCTCGCAGGCGGCTGCAACTGGGGTGCAGCTGCGGCACTGGGGTGCAGGTTTCCGACTGGA
 CATGCAATGTCGAATGGCCACTGTTCTGCGATGCGTGGGATACGACGATAACCGAAGATAGCTCATGTTATATCCGCCGTTAACACCCATCAACAGGATT
 GCTGCGCTGGTGTGGCGATATCTCGTAGTGGGATACGACGATAACCGAAGATAGCTCATGTTATATCCGCCGTTAACACCCATCAACAGGATT
 TCGCGCTGGGGAAACCCGGCTGCGGCTGCACTCTCAGGGGAGCCGTTGGCGGATTCATTAACTGAGCTGGCCAGTCTACAGTGGAA
 AAAGAAAACCCCTGGCGCCAATACCGCCACCCCTGGCGCTGCGGCGGCTGCGGCGGCTGCGGCGGCTGCGGCGGCTGCGGCGGCTGCGG
 AAGCGCGCAGTGAATCCAGGAGAAAAAAATGCGAGATTGATAAAAGTAAAGTAAACGGCATTAGAGCTGCTTAATGAGGTGGAAT
 CGAAGGTTAACACCCGTAACCTGCCAGAGCTGGGTGAGCATGGGTTGCGAAGACTCTCGCTTCGCAATTGAGCGACAGCTGGGCGTGGCGCTGCG
 TAGATGTCCTTACTAAGTCGCGATGGGAGCATGGGTTGCGAAGACTCTCGCTTCGCAATTGAGCGACAGCTGGGCGTGGCGCTGCG
 CTTTTATGCCAACAGGTTTCACTAGAAAGTCAATTATGCACTCAGGCCGTGGGCGATTACTTGTGTTGCGTATTGGAAGTCAGA
 GCATCAAGTCGCTAACAGAGAAAGGAAACACCTACTACTGATAGTATGCCCATATTACGACAAGTCATGCAATTATTTGATCACCAGGTG
 AGAGGAGCAGCCTCTTATTCGGCTGAAATTGATCATGGGATTAGAAAACAACCTAAATGTAAGGTTGGCTAA**TAATTGGTAACGAATCA**
 GACATTGACGGCTCGAGGGAGTAGCATAGGTTGCGAAGACTCTCTGGCGCTTCGCAATTGAGCGACAGCTGGGCGTGGCGCTTACAGGATGG
 ATGAGCAGATCTCTACCCGGGACATCGTGGCGCATCAGGCAAAATTCTGAGGTTTGTACAGCTAGCTCAGTCTAGGTTATGCGTGTAGAGGATA
 AAGAGGAGAAAATAGTGAACAAACATGCAATTGAGGAGGAGCATGGGACACATCAGGAAATTAAATTAAGGTTGAGAAGCAAAATGATGATTAAC
 ATAGCTTCTGATGACTAAATGTTGAGGAGGAGCATGGGACACATCAGGAAATTAAATTAAGGTTGAGAAGCAAAATGATGATTAAC
 CTTTTATGCCAACAGGTTTCACTAGAAAGTCAATTATGCACTCAGGCCGTGGGCGATTACTTGTGTTGCGTATTGGAAGTCAGA
 GCATCAAGTCGCTAACAGAGAAAGGAAACACCTACTACTGATAGTATGCCCATATTACGACAAGTCATGCAATTATTTGATCACCAGGTG
 AGAGGAGCAGCCTCTTATTCGGCTGAAATTGATCATGGGATTAGAAAACAACCTAAATGTAAGGTTGGCTAA**TAATTGGTAACGAATCA**
 GACATTGACGGCTCGAGGGAGTAGCATAGGTTGCGAAGACTCTCTGGCGCTTCGCAATTGAGCGACAGCTGGGCGTGGCGCTTACAGGATGG
 ATGAGCAGATCTCTACCCGGGACATCGTGGCGCATCAGGCAAAATTCTGAGGTTTGTACAGCTAGCTCAGTCTAGGTTATGCGTGTAGAGGATA
 AAGAGGAGAAAATAGTGAACAAACATGCAATTGAGGAGGAGCATGGGACACATCAGGAAATTAAATTAAGGTTGAGAAGCAAAATGATGATTAAC
 ATAGCTTCTGATGACTAAATGTTGAGGAGGAGCATGGGACACATCAGGAAATTAAATTAAGGTTGAGAAGCAAAATGATGATTAAC
 TAGATAATTACCCAAAATGAGGCAATTATGAGCGTCAATTAAATAAATGATCCTATAGTAGATTACTCAACTCCATCATTCA
 CAAATTGGAATATTTGAAACAAACTGTAATAAAAAAATCTCCAAATGTAATTAAAGAACGAAACATCAGGTTTACACTGGGTTA
 GTGGCTCTTCTGATGACTAAACATGGCTGGGAAATGCTTAGTTGCGATTACCTGCAAGAAAAGACAATATAGATGTTTACACTGGCT
 GTATGAGACATCAATTGTTCTCTGATGACTAAATGCAAAATTAAGGAAATAAATCAACACGATTAAACAAAAGAG
 AAAAGAATGTTAGCGTGGGCGATCGGAAGGGAAAGCTTGGGGATATTCAAAATATTAGGTTGAGCTGAGCGTACTGCACTTCCATTAA
 CCAATGCCAAATGAAACACTAACAAACCCCTGCCAAACTTCTAAAGCAATTAAACAGGAGCAATTGATGGCCCATACTTAA
 AACCCAAACAAGTACCCCTAGGAGGAGATA**ATGATTGAGAATACCTATAGCGAAAGGTTGAGTCCGGGTTGCAACAGATCAAAGCGCCGCAAC**
 GTGGATGCCGACATCGTATTCTCCAGGGGAATATACTCGATTCTCGCAGCTACCATCTGCCAGAACATGGGAGCAAGATCGATTCCGCC
 TTGCGTGCACCCACCATCCGGATGCGTGGGTTCCGTTACCTCTCAACTGCTATGTAAGGGTGCATCGATCATGCAAGCAGGGTCCGAACG
 CAGCTGCCCTCGACTGGAGGGCTGCAACCGACGCCGAGGGCTATGCCATGCTGGTGCAGCGCCAGAAAACAGGCATCGATGACAATGGCTAC
 TCCATCCCCGCGCCGACAAGGGCAGCGCCGCGCCCTGCTGCGTGAATGCCCATATGCCGAGGAGATGGACCGAGCTGCGCCGCTG
 CCCAATGAGTGGATGAGATGCCCATGATCACCCGCAAGGGCGTATATGAGCTGAGTGGCGCATTTGCGCCG
 GAGATCGAGTGGCTGCACTGGACGCCCTCGCAAGGATTACAAGGATATTGCGTACCTCTGGCATCAGAGCAACAGTCCGGCATTTGCGCC
 AACCGCCGCTTCAGGCTGGCTGCCACGATCTGCCGCCGCCGCTGCGTGAATGCCCATATGCCGAGGAGCTGCGCCGCTG
 ACAGCAGCTAATTGGTA**TAGCCAGGACATAAATAAACGAAAGGTCAGTCAAGGAAAGACTGGGCTTCTGTTATCTGTTGCGTGAACG**
 CTCTACTAGAGTCACACTGGCTCACCTCCGGTGGCTTCTGCGTGTATA

Regulator
operon for
backbone
JS_BB_4^d

Sensors

```

GGGGCGGCCATCGAATGGTCAAAACCTTCGGGTATGGCATGATAGCCCGGAAGAGAGTCATACTCAGGTGGTGAATATGAAAAACATAAA
TGCAGACACATACAGAATAATTAAATTAAAGCTGTAGAAGCAATAATGATATTAACTGTTATGACTAAATGGTACA
TTATGATGACGCTAATTAAATAAATGATCCTATAGTAGATTATCTAACCTCAATCCTAGATAATTACCTAAAAATGGAGGCAATA
TTATGATGACGCTAATTAAATAAATGATCCTATAGTAGATTATCTAACCTCAATCCTAGATAATTACCTAAAAATGGAGGCAATA
TGTAATAAAAATCTCCAATGTAATTAAAGAAGCGAAAACATCAGGTCTTATCAGGTCTTATCAGGTCTTATCAGGTCTAACAATGGCT
CGGAATGCTTAGTTGCACATTAGAAAAGACAATATAAGATAGTTTTACATCGGTGTATGACATACCATTAAATTGTCCTTCT
AGTTGATAATTATCGAAAATAATAGCAATAAAACACGATTAACCAAAGAGAAAAGAATTTAGCTGGGATCGCGAAGG
AAAAGCTTTGGGATATTCAAAAATTAAGGTTGAGCTGAGCTACTTCATTTAACTCCAAATGCAATCGGAAATGAAACCTCAATAACACAA
CCGCTCCAAGATTTAAAGCAATTAAACAGGAGATCTGATTGCCCATACTTAAATTAACATTCGAGGAGACGTACATGGCTGAAGC
GCAAAATGATCCCCCTGCGGGGATACCTGTTAACTGCCCCTGCGGGGTTAACCGGCAATGGCTTATCTGATTTTTAT
CGACCGACCGCTGGGATAGAAAGGTTATTTCTCAATCTCACCATCGCGTCAGGGGGTGTGAAAATCAGGGACGAGAATTGTTGCCACC
GGGTGATATTGCTGGTCCCGCAGGAGATTCTACACTACGGCTGTATCCGGGCTCAGGTATACCGAGTGGTTACTTCGTC
GGCGCCTACTGGCATGATGGCTTAACCTGGCGTATTGCGGATTAACCTGGGTTCTTCGGGGATGAGCGGACCCAGCGCATTTCAGCGA
CCTGTTGGCAAATCAACCGGGGCAAGGGGAAGGGCTATTGGAGCTGTGAGCAATTCTGTTAGCAATTGTTACTCGGGCAT
GGAAGCGATAACGAGTCGCTCATCCACCGATGGATAATCGGGTACGGAGGCTGTGAGTACATCAGCGATCACCTGGCAGACAGCAATTG
TATCCGGCAGCAGCTGGTCAACAGCTGTTGCTGGCGCTGTCACATCTGGGATTCAGGAGCTTAAACTGCTTAAACTGCTGGATGACC
GGACCAACGTATCAGCGCAGGCAAGCTTGGAGCACACCGGGATGCTTACCGGGCTCGCGTCAGTGGGTGACATGTTGGTTGACGATCAACTTA
TTCTCGGGTATTAAATAACCGGGGCAAGGGGAGCTGGGTAAAGAGAGCTTACGTTGAGCAATTCAGGGTGTGCGGTTAA
CAGTAACGTTACGATGTCGAGAGTATGGCGGTGTCCTTACGACCGTTCCCGCGTGGTGAACCAGGCCACGCTTCTCGAAAAGCG
GGGAAAAGTGGAAACGGCCGATGGGGAGCTGAATTACATCCCACACAAACATGGGGCAAACAGTCGTTGCTGATGGCGTTG
CCACCTCCAGCTGGCGCTGCAGCGCCGCGCAAAATGTCGCGGCGATTAAATCTCGCGGCGATCAACTGGTCCAGCGTGGTGTGCGATGG
TAGAACGAAAGCGCTGAAGCTGTAACAGCGGGTGCACAAATCTCTGGCAACCGCTAGTGGCTGATCATTAACATATCGCTGGATGACC
AGGATGCCATTGCTGGAGCTGCCACTAATGTCGGCTTATTCGATGTCCTGACCAAGCACCCATCAACAGTATTATTCGCCC
ATGAGGGACGGTACGGACTGGCGTGGGCTGATGGTCACTGGGCTGGGATGGCGATGGGCAATGGGGCCATTAGGTTCTGCGGCG
GTCTCGCGTCTGGCGCTGGCGATAAATATCTCACTCCGAATCAAACTCAGCGATAGCGGAACGGCAGCTGGAGTGCCTGCGGTTTC
AAACAAACCATGAAATGCTGAATGGGGCATGTCGCTCCACTGGGATGCTGGGCAAGGATGCTGAGTGGCGCATGGCCCAATTACCG
AGTCGGGCTGGCGTGGGCGATCTGGTGTGGGATACGCGATACCGGAAGGATAGCTCATGTTATATCCGGCGTTAACCCATCAAC
AGGATTTGCGCTGGGCGAACCGCGGCTGGCGTCAACTCTCGGGGCTGGGCGATGGCCGAGGGCAATCAGCTGGCGAGTGGTCTCAC
TGGTAAAGAAAACACCCCTGGGCCAATAGCGAAACCCGCTCTCCCGGGCTGGCGGATTCAATATGCACTGGCACAGGTTTCC
GACTGGAAAGCGGGCAGTGAATAATCCAAACCTACCCCGCGAGTAGCTCAGTCCAAATGTCAGATTAGATAAAAGTAAAGTATTAACAGCGC
ATTAGAGCTGCTTAATGAGGTGGAATGAGGTTAAACACCGTAAACTGCCAGAACGCTAGGTGAGAGCAGCTACATTGTTAGGCGATGT
AAAAAAATAAGCGGGCTTGTGCTGAGCCTTGGGATGGAGATGAGTGTAGATGGCACCATACTACCTTGGCCCTTAGAAGGGGAAAGCTGGCAAGA
TTTTTACGTAATAACGCTAAAGTTTACGATGTCCTTACGAGTGGCCTTACGAGTGGCACCATACTACCTTGGCCCTTACGAGTGGCCTTACGAGGAAAC
GTATGAAACTCTGAAATCAATTAGCTTTTATGCCAACAGGTTTACAGAGAATGCAATTATGCACTCAGCGCTGTGGGCATTTC
TTAGGTTGCGTATTGGAAGATCAAGAGCATCAAGTGGCTAAAGAAGAAAAGGAAACACCTACTACTGATAGTATGCGGCCATTATACGACAAGC
TATCGAATTATGATCACAAGGGTGCAGAGCGAGCCGCTTATTGCGCTTACGAGTGGGATGGCATAGGGTTGCGAGAATCCTGCTGCTGCGGTTGAG
TGGGTCTAAATTTGGAACGAATCAGACAATTGACGGCTCGAGGGAGTAGCATAGGGTTGCGAGAATCCTGCTGCTGCGGTTGAG
TTATGCGATGATAAGCTGTCACACATGAGCAGATCCTACGCGGCGACCATGTCGGCGCATCACCGGCCACAGGTGCGGTGCTGGC
CCTATATGCCGACATCACCGATGGGAAGATCGGGCTCCCATCGGGCTCATGAGCAAATTATTCG

```

a. DNA sequence colors: promoters (orange), ribozyme insulator (blue), RBS (green), open reading frames (red), terminators (black).

b. LacI and TetR expression cassette driven by constitutive P_{Laci} promoter. The plasmid map is shown in Appendix Fig S17A.

c. LacI and TetR expression cassette driven by constitutive P_{Laci} promoter and LuxR and CinR expression cassette driven by constitutive promoter J23104. The plasmid map is shown in Appendix Fig S17B.

d. LuxR, AraC*, LacI, and TetR expression cassette driven by constitutive P_{LaciQ} promoter. The plasmid map is shown in Appendix Fig S18.

Appendix Table S4: Genetic part sequence used in this work.

Part name	Type	DNA sequence
P _{AmeR2}	Promoter	TCGTCACTAGAGGGCGATAGGACAAACTTGACAACCATCCTACGTAGGCTGCTAGC
P _{AmtR2}	Promoter	CTTGTCCAACCAAATGATTGTTACCCCTTGACAGTTCTATCGATCTATAGATAATGCTAGC
P _{BetI2}	Promoter	AGCGCGGGTAGAGGGATTGTTACCAATAGACAATTGATTGGACGTTCAATATAATGCTAGC
P _{BM3R1}	Promoter	AATCCGCGTGTAGGCTGATTGTTACCAATTGACGGAATGACGTTATTCCGATAATGCTAGC
P _{CymR}	Promoter	TCGTGTAAGTAGCGTAACAAACAGACAATCTGGCTGTTGTATTGGAAAATTTCTGTATAATAGATTCAACAAACAGACAATCTGGCTGTTGTATTAT
P _{HlyIIR2}	Promoter	ACCAGGAATCTGAACGATTGTTACCAATTGCCATTAAATTCTGTTAAATGCTAGC
P _{LmrA2}	Promoter	CGCTCATTCACTAGGCTGATTGTTACCAATTGACAGCTGGTGGTCGAATCAAGATAATAGACCACTACTATATT
P _{PhIF}	Promoter	CGACGTACGGTGGAACTGATTGTTACCAATTGACATGATACGAAACGTACCGTATCGTTAAGGT
P _{SrpR}	Promoter	TCTATGATTGGTCAGATTGTTACCAATTGACAGCTAGCTCAGTCCTAGGTATATACATACATGCTTGTGTTGAAAC
P _{VanR}	Promoter	TGTATAAAGTCCGCCATTGGATCCAATTGACAGCTAGCTCAGTCCTAGGTACCATGGATCCAAT
P _{Tac}	Promoter	AACGATCGTTGGCTGTGTTGACAATTATCATCGGCTCGTATAATGTTGGAATTGTGAGCGCTCACAAATT
P _{Tet}	Promoter	TACTCCACCGTTGGCTTTTCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATAATGAGCAC
P _{Lux} *	Promoter	ATAGCTTCTTACCGGACCTGTAGGATCGTACAGGTTACGCAAGAAAATGGTTGTTACTTCAAA
P _{Lux2}	Promoter	ATAGCTTCTTACCGGACCTGTAGGATCGTACAGGTTACGCAAGAAAATGGTTGTTATTCGAAT
P _{Cin}	Promoter	CCCTTTGCGTCCAACCGAACGACGACGGCCTAAAGCGGTCGCGATCTTCAGATTGCGCTCCCGCTTTCAGCTTGTGTTG GCGCATGCGTTATCGAAAAACCGCTGCACACTTTGCGCGACATGCTGTGATCCCCCTCATCTGGGGGGCTATCTGAGGAAATT CCGATCCGGCTCGCTGAACCATTGCTTCCACGAACCTGAAAC
P _{Bad}	Promoter	ACTTTTACACTCCGCATTAGAGAAACCAATTGTCATATTGCGATCAGACATTGCGCTACTGGCTCTTACTGGCTCTTC TCGCTACCAAAACCGTAACCCCGCTTAAAGCATTCTGTAAACAAAGCGGACCAAAGCCATGACAAAAACGCGTAACAAAAGTG TCTATAATCACGGCAGAAAAGTCCACATTGATTATTGACCGGGCTCACACTTGTATGCCATAGCATTTTATCCATAAGATTAGC GGATCCTACCTGAGCCTTTATCGCAACTCTACTGTTCTCATACCGTTTTGGCTAGCCTGATGGCTTAGCGGGCTA
P _{LacI}	Promoter	GCGCGCGGCCATCGAATGGCGAAACCTTGCCTAGGTATTGCTAGC
J23104	Promoter	TTGACAGCTAGCTCAGTCCTAGGTATTGCTAGC
P _{LacIQ}	Promoter	GCGCGCGGCCATCGAATGGCGAAACCTTGCCTAGGTATTGCTAGC
J23101	Promoter	GATAAGTCCCTAATTACAGCTAGCTCAGTCCTAGGTATTGCTAGC
RiboJ54	Ribozyme	AGGGGTAGTTGATGTGCTTCAACTCTGATGAGTCAGTGACGAAACCCCTCTACAAATAATTGTTAA
BydvJ	Ribozyme	AGGGTGTCTCAAGGTGGTACCTTGACTGATGAGTCAGTGACGAAACCCCTCTACAAATAATTGTTAA
RiboJ57	Ribozyme	AGAAGTCAATTAATGTCCTTAACTCTGATGAGTCGGTGACGACGAAACTCCCTACAAATAATTGTTAA
SarJ	Ribozyme	AGACTGTCGCGGGATGTGATCCGACCTGACGATGGCCAAAAGGGCGAAACAGTCCTCTACAAATAATTGTTAA

LtsvJ	Ribozyme	AGTACGTCTGAGCGTGATACCCGCTCACTGAAGATGGCCCGTAGGCCGAAACGTACCTCTACAAATAATTGTTAA
RiboJ51	Ribozyme	AGTAGTCACCGCTGTGCTTGCCGTCTGATGAGCCTGTGAAGCGAAACTACCTCTACAAATAATTGTTAA
RiboJ64	Ribozyme	AGGAGTCATTAATGTGCTTTAATTCTGATGAGACGGTGACGTGCAAACCTCCCTACAATAATTGTTAA
RiboJ53	Ribozyme	AGCGGTCAACGCATGTGCTTGCGTTCTGATGAGACAGTGATGTCGAAACCGCCTCTACAAATAATTGTTAA
RiboJ10	Ribozyme	AGCGCTAACGGGTGTGCTTCCCCTCTGATGAGTCCGTGAGGACGAAAGCCCTCTACAAATAATTGTTAA
AraJ	Ribozyme	AGTGGTCGTGATCTGAAACTCGATCACCTGATGAGCTCAAGGCAGACGAAACCACCTCTACAAATAATTGTTAA
RiboJ	Ribozyme	AGCTGTACCGATGTGCTTCCGTCTGATGAGTCGTGAGGACGAAACAGCCTCTACAAATAATTGTTAA
F1	RBS	CTATGGACTATGTTTACATACGAGGGGATTAG
F2	RBS	TACGCTATGGACTATGTTCAACAGGAGCTAATAG
A1	RBS	AATGTTCCCTAATAATCAGCAAAGAGGTTACTAG
E1	RBS	CCCCCGAGGAGTAGCAC
B1	RBS	CTATGGACTATGTTTAACTACTAG
B2	RBS	CTATGGACTATGTTTCAAAGACGAAAAACTACTAG
B3	RBS	CCTAACGAGGCCGGAGG
C1	RBS	GACTTAAACGATTGGCCGACCGC
H1	RBS	ACCCCCGAG
N1	RBS	TACGCTATGGACTATGTTCTGCTATGGACTATGTTCACACACGAGATGCCTCG
P1	RBS	CTATGGACTATGTTAAAGGGAGAAACTACTAG
P2	RBS	GGAGCTATGGACTATGTTGAAAGGCTGAAACTACTAG
P3	RBS	CTTTACGAGGGCATCCT
S1	RBS	GAGTCTATGGACTATGTTTACAGAGGAGGTACAGG
S2	RBS	GAGTCTATGGACTATGTTTACATATGAGATAACAGG
S3	RBS	GAGTCTATGGACTATGTTTACAAAGGAAGTACAGG
S4	RBS	CTATGGACTATGTTTACACAGGAAATACAGG
V1	RBS	AAGACCATTATAAGGTTGAAC
B0064	RBS	TAATAGAGAAAGAGGGAAACTACTAG
ameR	Gene	ATGAAACAAACATTGATCAGGTGCTAAAGGTGATCGTAAAGCGATCTGCCGGTTCGTGTCGCGCTGTAGTGCCTCGAAAGAAA CCCGTCGTGATATTCTGCAAAAGCCGAAAGAAACTGTTCTGTAACGTGGTTAAATGCAAGTGCATTGCAAGATATTGCAAGCGCACT GAATATGAGTCGGCAATGTGTTAAACATTAGCAGCAAAACCGACTGGTGTGATGCAATTGGTTGGTCAGATTGGTTTT GAACGTCGAGATTGTCGCGTGGATAAAAGCCATGACCCGCTGGATCGTCTGCGTACATGGCACGTAATCTGATGGAACAGCATCATC AGGATCATTCAACACATCGGGTTTTATTCAAGATCCTGATGACCCCAAACAGGATATGAAATGTGCCGATTATTACAAAAGCGT GATTGCAAACACTGCTGGCGAAATTATCGTGTGTTGAAGCAGGTCTGTATATTGCAACCGATATTCCGGTTCTGGCGAAAC

		GTTCCTGCATGCACTGACCAGCGTATTCTACCGGTTCTGATTGACAAGAAGATATTGTAATCTGGCAACCGTTGTATCAGCTGG TTGATCTGATTGATGCAGGCTCGCTAACCGCTGGCAAATAAA
<i>amtR</i>	Gene	ATGGCAGCGCAGTGGCTGCCGCTCGTAGTGACCCGCTCGTAGCAGGTAAAAATCCCGTGAAGAAATTCTGGATGCAAGCGCAG AACTGTTACCCCTCAGGTTTGCACCCACAGTACCCATCAGATTCAGACTGGTATTCTGCAGGCAACCGTGTG TTTCCGAGAAACCGAAATCTTCTGACCCCTGCTGAAAAGCACCGTGAACCGACCGTCTGCGAGAAGATCTGAGCACCGT GATGCAGGCTCGGAAATCGCTCTGGCAATTGTCAGCGAAGTCAGGCTGAAGCGACTGACCAATGTTCTGATCTGCAAC ATCAGCTGCCGATTGGTAGCGGAAGAATATCATGAGCGCAGGTGAACCGACTGACCAATGTTCTGATCTGCAAC CGAGATTGGTAGCGCTGCCGAGACTGCCGATATTCTGCGAGGTTATGGAATGCGTGAAGCGTATTGAAATGCGTCA CCGAGCTCGTAGGCCAGATACCGCTGCCGAAACCGCAATTATCTGCGAGATGCAAGCGCTGGCAGTTCTGGTGCACCG CAGATCGTTGAAAAACCTGAACTGATTAACAGCGAGATGCAAAATAA
<i>betI</i>	Gene	ATGCCGAAACTGGGATGAGAGCATTCTCGTCGCTCAGCTGATTGATGCAACCCCTGGAAGCAATTAAATGAAGTTGGTATGCATGATG CAACCATTCGCACAGATTGACGTCGTCGGTTAGCACCGTATTATTAGCATTATTCCCGATAAAACCGTCTGCTGGAAGC AAACCTGCGTGAATTACCGACGCTGCTGATCAGCTGCTGATGAGCACGCTGCGCAGGGTAGCCAGAACACCGCTCG CAGGCAATTGTTGGTAGTGAATTCTGATGAAACCCGATGCGCAGAGCAATTGCGATGCGCAGGGTAGCCAGTGGCATT TGAACAGGCAAGAGGCAAGGGCAGGTTATGGCTGGCAGCACTGATTGATGTTCTGTTGCTGGCTGGTAGCAGCACTGAG AAACCCGTGCAAATAGCCTGACCCGCTACCTACCCGACATCTGCCGACCGATAA
<i>bm3R1</i>	Gene	ATGGAAAGCACCCGACCAACAGAAAGCAATTAGCGCAAGCCTGCTGTTGAGAAGCTGGTTTGATGCAACCACATGC CGATGATTGAGAAAATGCAAAGATGGTGCAGGACCATTTATCGTATTCTAAACAAAGAAAGCCTGGTAGCAAGAACCTGTTCA GCAGCATGTTATGAATTCTCGTAGTGGTATTGAAGCGCTGGCTGATCAGCTGCTGAGCTGGCAGGGTAGCCAGTGG GAAGGTATGGTAGCTTACAAAATCATCGCGTGCACGGTTTATCTAAACCCATAGCCAGGGCACCTTCTGACCGAAGAAA GCCGCTGGCATATCAGAAACTGGTTGAATTGTTGTCACCTTTTCTGTAAGGTCAAGGTCAGAACACGGGTGTATTGCA AAATGCACTGATTGCAATTCTGTTGAGCGTTATGGAAGTGTATGAAATGATCGAGAACGATTATCTGAGCCTGACCGAT CTGACCCGTTGTAAGAAACCGTGTGGCAGCACTGAGCCGTCAGAGCTAA
<i>cymR</i>	Gene	ATGAGCCC AACGTCGTACCCAGGAGAACGTGCAATGGAACCCAGGGTAAACTGATTGCAAGCAGCACTGGGTGTTCTGCGTAAA AAGGTTATGAGGTTCTGATGAGATGTTCCGGTGCAGCCGGTGTAGCGCTGTCACAGAGCCATATTCCGACCAA GGAACTGCTGCCGCAACCTTGAATGGCTGATGAGCAGATTACCGACGCTGCGCAGCTGCTGGCAA GATGTTATTCCAGAGATGCTGATGAGCAGATTCTCTGATGAGTATTGAGCTCAGCTGATGTTGCA ATCGTGAATCCGCACTGCGTAGAGTATTACCGTGTACCGTTGAGCTAATCGTTGTTGTAAGATATGTTGCTGG GAGCCGTTGCTGAGCCGTGATGAGCGAAGATCTGTTGCTGATTTAACACGCTTGTGCTGAGTTGCTAGCCGTG CAGAAAGATAAAGAACCTTTGAACCTGTGCCATTACCCATCCTGGTAAACGACCCCTGGAATTGCACTGAGCATT CCGATAAACCGAACATTAA
<i>hlyII R</i>	Gene	ATGAAATACTCTGTTGAGGTGCGAACGAACTGGTAAAGCCGTGAACAGACCATGGAAAATTCTGAAAGCAGC TCGGCAACGCTGTTATGAAGCACCCATTCAAGAAATTACCAAGAACGAAACTTAACGTTGCAATGCCAGCTATTACTTAA TGGCAAAGAACGACTGACTACGAGCTGTTCAAAAATACGGCTGCCAAATGAACCTGCCAACTTCTGAAAAAAAC CCGATTAATGCCCTCGCTGAATATCTGACCGTTTACCAACCATTAAGAAAATCCGAAATTGGCACCTGGCT TTATCAAAAGAACGACGCCGAAAGATACTGCGAGGTTATCTGCGAGCTGCCAGACGCTGAAAGAAATTCTG AAACACGGGTGTTTCAATTGACATCAACCATACCCATTGGTACCGATCTGTTCTGTTCCGAAATTCA ATCGATAGCTGGTCCGAATGAACCCATGATACCAATCATGAACTGATGCCGAAGATCTGTTAGCGCTT CCGATAAACCGAACATTAA
<i>lmrA</i>	Gene	ATGAGCTATGGTGTAGCCGTAAAAAATTCTGAGCGCAGCAACCCGCTGTTCAGCTGCAGGGTATTATGGCACCGGCTGAAATC AGATTATCAAAGAACGCGTGCACCGAACGGTAGCTGTTGATTATCATTTCCGGTGTAAAGAACAGCTGCCAATTGAAAGCAGTGA CGGAAATGAAAGAAATATCCGCAAGGAAATCCGGATTGATGAAAGCATGACCGATCCGGCAGAACGGTATTCTGAA GAACTGAGCTGCTAGTTAGCTGAGGATATTGAAAGGTCTGCTGGCTGCGAGCAGAACCGCTGAAAGCGAAC CGCTGCGTAGAACGATGTCAGGATATAAGAATACCCATTGGTACCGCTGAGCTGAGCCGGTTGAGCGAAC TGCAAAAGAACGACCGTTTAATGCAATGATTGAGGTGTTCTGCTGAGCCTGACCGCAAAATAGCACCGCTGCT CATATTAGCAGCTGATTCCGGATCTGCTGAAACCTTAA
<i>phlF</i>	Gene	ATGGCACGTACCCGAGCGTAGCAGCATTGGTAGCCTCGTAGTCGCATACCCATAAAGCAATTCTGACCGAC CTGAAAGAATGTTGTTATAGCGGCTGAGCATTGAAAGCGTTGCACGCTGCGCGTCAAGCAACCGAC CAATAAACGACACTGATTGCGCAAGTGTATGAAAGAACGAAACAGGTCGCTAAATTCCGGATCTGGTAGCTTAAAGCCGAT CTGGATTCTGCTGCCATTCTGCGAAGTTGGCTGAAACCCATTGTTGTTGAGACGCTTCTGTTATTGCA CAATGGTGAACCTGCCGAAAGAACCTACGACTGCTGCTGGATATGATTGTTGTTGTTGTTG CTGACCCGTTGAGCGATATTGAAAGAATTACCCATTGCTGATTAATGGTGTGTTGCTGACACCGCTTAA

<i>srpR</i>	Gene	ATGGCACGTAACCGCAGCAGAAGCAGAAAGAACCCGTCA CGTATTATGATGGCAGCACTGGAAAGTTTTGTCACAGGGTGT A GTGATGCAACCTGGATCAGATTGCACGTAAAGCCGGT TACCGGTGCACTGGTATTTGGCATTTAATGTAACACTGGAAAGTCT GCAGCAGTTCTGGCAAGCCGTCA CGTCACTGGTGA CAGTGGTACCTGGGATTCACCGGATCTGGGATTAAGCTGAAAGCAGTTGGT GTTCAATGCTGGATGCAGTTCACTAGTCGGCAGACCAAACAGTTAACCGAATTCGATTTATCAGGGTCTGGATGAAAGCCGTCTGA GATAATCTGGATCTGCAGCACGAGATTGGTTTAAAGGCTGATTACCGCTCGTGTATGAAGGCTGCTGAGAAAGATCAG CAGGCACAGATTCAAAGTGC ACTGGTAGCTTGACTGCTGCAACGCCCTGCTTCTGCTGTGTAAGAAGCACAGA TAAACAGGTGAAATCTCGAATAA
<i>vanR</i>	Gene	ATGGACATGCCCTGTATTAAACCGGGTCAGCGTGTATGATGGCACTCGTAAATGATTGCAAGCGGTGAAATCAAAGTGGTGAAC G TATGCAAGAAATCCGACCGCAGCAGCAGCACTGGGTGTTAGCGTATGCCGTTCTGATCGCACTCGCTTCACTGGAAACAAGAGTCT GGTGTCGTCCTGGGTCAGCTGGTTAACCGGGTGTGCACTGGCAGATCAGGATCAGGGTGTGCAATTTGCAAGTGAAGTTCGTTCTG GAAGGTTTCGACGACGCTGCTGGCAGAAGCGTGTGCAAGGCGAACCCATCGACGTCAGGCACTGGTGTGTTGTACTGATTGCAAGAGT CAGTGGTCACTGGGTCGCTGCTGCAATGGTGAAGATCTGCACTGGCCTATATACTAGGCAATTCTGATGATACCCCTGGATCTGATG AGCAGGTAATGGTCAGTTGAAAGCGCACTGGCACGTAATGGTTTGAAACCGGTTGCA CGCAGCCGGTGCACTGGCCCTGGATCTGATG GACCTGTCGCGGAATATGAAACATCTGCTGGCAGCACATCGTCAGGCACTGGCAGTTGCAAGTGAAGTCTGGATGCA GTTAGCTGTTGCAAGGCTATTATGCGTGTATGCACTGGCAGCAATTGTAATGCAAAGAAGCTTTGAAAGCAGCAGCAAGCGCAGGCACCGCT GGGTGCAGCATGGTCAATTGTCAGATTGATAA
<i>luxR</i>	Gene	ATGAAAAACATAATGCCGACGACACATACAGAATAATTAATAAAATTAAGCTGTAAGCAATAATGATATTAAATCAATGCTTAT CTGATATGACTAAAATGGTACATTGTAATATTATTAATCGCAATCATTTATCTCTTATATGGTAAATCCGATATTCAACTCCT AGATAATTACCTAAAAAATGGAGGAATATTATGACGCTAATTTAATAAAATATGCTTATAGTAGATTATTCTAACTCCAAT CATTCACTTAAATGGGAATATTGAAACATGCTGTAATAAAATATCCTTAAATGTTAAATGAGGAAACATCAGGTC TTATCAGGGTATGGTTAGCTCCCTATTCAGCTGCACTGGCTTGGGAATCTGTTAGCTTGGCAGATTCTAGGAAACATTTGCAATTTCTAGGAGCT ATAGAGTTTATTTCATCGGTGTGAAACATACCTTAAATTGTTCTCTAGTTGATAATTATGAAAAAATAAATATGCAAAT AATAAAATCAAACAAACGATTTAACAAAAGAGAAAAGATGTTAGCGTGGGATGCGAAGGAAAAGCTTGGGATATTCAA AAA TTTAGGTTGCACTGAGCGTACTGTCACTTCCATTAAACCAATGCGCAAATGAAACTCAATACAACAAACCCTGCCAAAGTATTTC TAAAGCAATTAAACAGGAGCAATTGCCCCATACTTAAATGAA
<i>araC*</i>	Gene	ATGGCTGAAGCGCAAATGATCCCTGTCGCGGGATACTCGTTAATGCCCATCTGGGGCGGTTAACCGCATTGGGCGATTGAGGCCAACG GTTATCTCGATTTTTATCGACCGACCGCTGGGAATGAAAGGTTATATTCTCAATCTCACCATTCCGGCTCAGGGGGTGGTGA AAAA TCAGGGACGAGAATTGTTGCGGACCGGGTGTATTTGCTGTTCCGGCAGGAGATTCACTACGGCTGTATCCGGAGCT CGCGAATGGTATCACCAGTGGGTTACTTCGTCGCGCGCCTACTGGCATGGCTTAACGGCTCAATTGGCAATACCGG TTGCTTCGCGGCGATGAAGCGCACCAGCCGCAATTCCAGCTGGGCAATTCTAGGGCAATTCAACGGCCGGAAAGGGGAAGGGCGCTA TTGGAGCTGCTGGCGATAAATCTGCTGGAGCAATTGTTACTGGCGCATGGAGCTTAACGAGTCGCTCCATCCACCGATGGT AATCGGCTACGGCGGCGCTGTAGCTACATCGCGATCACCTGGCAGACGAATTGGTATCTGGAGCTCCACAGCATTTGCT TGTCGCGCTGCGTGTGTCACATCTTCCGGCAGCAGTTAGGGATTAGCGCTTAAGCTGGCGGAGGACAGTATCAGCCAGG CAAAGCTGCTTTGAGCACCAACCCGGATGCTATCCCAACCGCTCGTGCAGTGGTGTGACGATCAACTTATCTCCGGGTA TTTAAAAAAATGACCGGGGCCACCGCGAGCGAGTTCTGGGGTTAA
<i>lacI</i>	Gene	ATGAAACCCAGTAACGTTACGATGTCGAGAGTATGCCGTGTTCTTACAGCCGTTTCCCGCTGGTAACCGGCAACCCACG TTTCTCGAAACACGGGGAAAAGTGGAGCGGCGATGGGGAGCTGAATTACATCCCAACCGCGTGGCACAAACAAACTGGGGCAA ACAGCTCGTCTGATTTGGCTTGGCCACCTCCAGTCTGGCCCTGACCGGGCGTGGCAATTGCGGGGATTAAATCTCGGGCGAT CAACTGGGTGCCAGCGTGGTGTGCGATGGTAGAACGAGCGCGTGAAGCGCTGTAAGCGGGTGTGACAATCTTCTCGCAAC GCGCTAGTGGGTGATCAATACCTGGCTGGATGACCGGAGGATGCGCTGTGCTGCACTAATGTTCCGGGTTATT TCTTGATGTTCTGACCAACACCATCAACAGTTATTTCTCCATGAGGAGCTGGCACTGGGGCTGGAGCATCTGGTGC GA TTGGGTCACCGAACATCCGGCTGTTAGGGGCCATTAAAGTCTGTCGCGGCTGCGCTGGCTGGCATAAATATCTCA CTCGCAATCAAATTCAGCGTAGCGGAACGGGAAGGGCAGTGGAGTGCCTGGCGTTTCAACAAACCATGCAATGCTGAATGA GGGCATCGTTCACACTGGCATGTTGGGATACGATGGCGCTGGCCCAATTACCGAGTCGGGCTCGGGTT GGTGCGGATATCTCGGTAGTGGGATACGCAATACCGAGAATAGCTCATGTTATATCCCGCTTAACCCACATCAAACAGATTTC GCGTCTGGGGCAACACCGCTGGCAGCGCTGCTCACTCTCAGGGCCAGGGCTGAAGGGCAATCAGCTGTCAGTCACT GTTAAAGAAAACACCCCTGGCGCCAATCGCAAACCGCTCTCCCGCGCTGGGCCATTCAATGAGCTGGCACAGACAG GTTCCCGACTGGAAAGCGGGCACTGATAA
<i>tetR</i>	Gene	ATGTCAGATTAGATAAAAGTAAAGTGAATACAGCGCATTAGAGCTGCTTAATGAGGTCGAATGAAAGTTAACACCGTAAAC TCGCCAGAAGCTAGGGTAGAGCAGCGCTACATTGTTAGCTGTA AAAAATAAAGCGGCTTGGCTCAGCCCTAGCCATTAGAT GTTAGATAGGCACCATACTTTGGGACTTAAAGGGAAAGTGGCAAGTGGCAATTAACGCTTAAAGGTTAGCTGTAATAGCTGGT GCTTACTAAGTCATCGCGATGGGAAAGTACATTAGGAGCTACGGCCATAGAAAACAGTATGAAACTCTCGAAATCAATTAG CCTTTTATGCCAACAGGTTTCACTAGAGAATGCTTATATGCACTCAGCGCTGTTGGGCTTACTTGTGCTATTGGA AGATCAAGAGCATCAAGTGCCTAAAGAAGAAAGGGAAACACCTACTAGTGTAGTATGCCGCTTATTACGACAAGCTATGAAATTA TTTGATCACCAAGGTGCAAGGCCACCGCTTATTGCGCTTGAATTGATCATATGCGGATTAGAAAACAACTTAAATGAAAGTG GGTCTCTA
<i>cinR</i>	Gene	ATGATTGAGAATACCTATAGCGAAAAGTCGAGTCGCGTCAACAGATCAAAGCGGGCCAACGTGGATGCCCATCGTATTCTC CAGGGGAATATAACCTCGATTCGTCACCTACCATCTGCCAGACAATCGCAGACAGATCGATTGCCCTTGTGCCACAC CTATCCGGATGGCTGGGTTCCGGTACCTCTCACTCGCTATGAGGTCAGTGGCATCATCAACGAGGCTTGCAGCGCACGT CCCTCGACTGGAGCAGGGTCAACCGACGCCGGCTATGCCATGCTGGTGCAGCCCAGAACACGGCATCGATGACAATGGCT

A	Scar	GGAG
B	Scar	TACG
B'	Scar	TACT
C	Scar	AATG
D	Scar	AGGT
E	Scar	GCTT
F	Scar	CGCT
U	Scar	GGGC
V	Scar	TCTG
X	Scar	TGTC
p15A	Origin	<p>GACCTCAGCGCTAGCGGAGTGATACTGGCTTACTATGTTGCACTGATGAGGGTGTCACTGAAGTGCTTCATGTGGCAGGAGAAAAAGGCTGCAC AGGCTGACCGGGTGCCTCACAGCAAATATGTGATAACAGGATATATTCCGCTTCCCTCGCTACTGACTCGCTACGCCCTGGTCTGTTGACT GCGGGCAGCGGAAATGGCTTACGAACGGGGCGGAGATTTCCTGGAAAGATGCCAGGAAGATACTTAACAGGGAAGTGAGAGGGCCGCG CAAAGCGTTTCCATAGGCTCCGCCCCCTGACAAGCATCACGAAATCTGACGCTCAAATCAGTGGTGGCAAACCGAACAGGACT ATAAAGATAACCAGCGTTTCCCCCTGGCGCTCCCTCGTGCCTCTCTGGTCTCTGGTTACCGGTTACCGGTTACCGGTTACCGGTT TGGCCGGTTGCTCATTCACGGCTGACACTCAGTCCGGTAGGCAGTTCGCTCCAAGCTGGACTGTATGCACGAACCCCCCGTT CAGTGGCAGCGCTGCCTTACCGTAAACTATCGCTTGAAGTCCACCGGAAAGACATGCAAAGCACCCTGGCAGCAGCCACTG GTAATTGATTAGGGAGTTAGCTTGAAGTCATGCCGGTTAACGCTAACATGAAAGGACAAGTTGGTACTGCCCTCTCC GCCAGTTACCTCGGTTCAAGAGTTGGTAGCTCAGAGAACCTCGAAAACCGCCCTGCAAGGGGTTTTCGTTTCAAGCAAGAAGA GATTACCGCAGACCAAAACGATCTCAAGAAGATCATTTAA</p>
p15A*	Origin	<p>GCTAGCGGAGTGATACTGGCTTACTATGTTGCACTGATGAGGGTGTCACTGAAGTGCTTCATGTGGCAGGAGAAAAAGGCTGCAC CGGTGCCTCAGCAGAAATATGTGATAACAGGATATATTCCGCTTCCCTCGCTACTGACTCGCTCGGTGACTGCCGAGC GGAAATGGCTTACGAACGGGGCGGAGATTTCCTGGAAAGATACCTTAACAGGGAAGTGAGAGGGCCCGCAGCCAAAGCG TTTCATAGGCTCCGGCCCTGACAAGCATCACGAAATCTGCGCTCAAATCAGTGGTGGCAAACCCGACAGGACTATAAAGATA CCAGCGTTTCCCCCTGGCGCTCCCTCGTGCCTCTCTGGTCTCTGGTTACCGGTTACCGGTTACCGCTGTATGCCGCGT TTGCTCATTCACCGCTGACACTCAGTCCGGTAGGCAGTTCGCTCCAAGCTGGACTGTATGCACGAACCCCCGGTTCACTGCCGAC CGCTGCGTTACCGGTTACCGTAAACTATGCTTGAAGTCCACCGGAAACGACATGCAAAGCACCCTGGCAGCAGCCACTGGTAAATTGAT TTAGAGGGATTAGCTTGAAGTCATGCCGGTTACCGCTAACATGAAAGGACAAGTTGGTACTGCCCTCTCAAAGCAGTTAC CTGGTTCAAAGAGTTGGTAGCTCAGAGAACCTCGAAAACCGCCCTGCAAGGGGTTTTCGTTTCAAGCAAGAGATTACCG CAGACCAAAACGATCTCAAGAAGATCATTTAA</p>
pSC101 var2	Origin	<p>AGTAAGACGGGTAAGCCTGTTGATGATAACCGCTGCCCTACTGGGTGCAATTAGCAGCTGTAATGACCTGTACGGGATAATCCGAAGT GGTCAAGACTGGAAAATCAGAGGGCAGGAACCTGCTGAACAGCAGAAAGTCAGATAGCACCACATAGCAGACCCGCAAAACGCCCTG AGAAGCCGTTGACGGCTTTCTGTTAGCTGTTCTGCTGATGAACTTACAGGAACTTACAGGGCTTAGCAGGCTATTCACCGGCTTAC CACTGCCAGAGCGTGAAGCCAGCAACTGAATGTCACGAAAAGACAGCAGCTCAGGGCTGATGGTGGAGACAAAAGGAATATT CAGCGATTGCCAGCTTGGAGGGTCTACTTAAGCTTCTAGGGTTAAAGTGTGTTGTAGAGGAGCAAACAGCGTTGGCAG ATCTTTTGTAACTCGGAACTGACTAAAGTGTGTTATACAGGCTGGATCTTCTTTTATCTTTTATCTTTTATCTTTCT TTATTCTATAAATATAACCTGTAATATAACAAAAAAACACAAACAAAGGCTAGCGGAAATTTCAGAGGGCTTAGCAGAATTTC AAGTTTCCAGCAAGGCTTAGCAGAAATTACAGATACCCACAACTAAAGGAAAGGACTAGTAAATTATCATGACTAGCCCATCTC AATTGGTATAGTGAATTAAATCACCTGACCAATTGAGATGTATGCTGTAATTAGTGTGTTCAAGCAAATGAACTAGCGATTAGTC GCTATGACTTAACGGAGCATGAACCAAAGCTAATTCTGCTGTTGCGACTCTAACCCCACGATTGAAAACCCCTACAAGGAAAGA ACGGACGGTACTTCACTTAAACCACTGCTGAGTAACTGAGTGGAAAAGCTTATGTTAGTAAAGCTGTTAGTAAAGCAGAAC AGAGAGCTGATGAGCAGAACTGGGAAATCAGGAATCTTGGTTAAAGCTTGGATTTCCAGTGACCAAACATGCAAAGTCT CAAGCGAAAATTTAGAATTAGTTAGTGAAGAGATATTGCTTATCTTCCAGTTAAAAAAATTCTAAAATAATCTGAAACA TGTTAAGTCTTGGAAACAAATCTCTATGAGGATTATGAGTGGTTAAAGGAAACTAACACAAAAGAAAACCTACAAGGCAAAT ATAGAGATTAGCTTGTGATGAAATTAGTCTGTTAGCTGAAATAACTACCATGAGTTAAAAGGCTTAAACCAAGTGGTTGA AACCAAATAAGTAAACATACAGCAATGAAATGAGTGGTTGATGAAAGGAGGGCCCGACTGATACGTTGATTTC AGTTGAACTAGATAGACAAATGGATCTGTAACCGAACATTGAGAACACCCAGATAAAATGAATGGTACAGAAAATACCAACAC ACATCAGATTCTACCTACATAACGGACTAAGAAAACACTACACGATGCTTAACTGCAAAATTCTAGCTCACAGTGGAGGCAA AATTGGTACTGAGCATGAAAGTAAAGTATGATCTCAATGGTCTGTTCTGATGGCTCACGCAAAAACACGAACCAACTAGAGAACAT ACTGGCTAAATACGGAAAGGATCTGAGTTCTATGGCTTATGCTATCATGTAACGACTAACAAACAAAGTAGAACAC TGTCACCGTTACATATAAGGGAAAATGTCCATGACAGATGAAACGGTGTAAAAAGATAGATACTACAGAGCTTAC GTTTGGTGCATTCAAAGCTGTCACCATGAAACAGATGACAATGTAACAGATGAAACAGCATGTAACACTAAAGAACAGGTGAAA CCAGTAAAACAAAGCAACTAGAACATGAAATGACACCTGAGACAACTTGTACAGCTCAAACAGTCACACATAGACAGCCTGAAACA GGCATGCTGCTTACGATCAAAGCTGCCGACACACGGAGGCCAGTGACGCCCTCCGGGGAAAAATCATGCCAATTCTGGAG</p>

AAATAGCGCTTCAGCCGGAAACCGGCTGAAGCCGGATCTGCGATTCTGATAACAAACTAGCAACACCAGAACAGCCCCTTGCGGG
CAGCAAAACCCGTAC

Appendix Table S5: Complete annotated sequences of the 7-segment circuits.

GGAGCCCCCTTGTGCGTCACAAAGCAGCACGGGCCCTCTAAAGCGGGTGCAGATCTTCACTGATTCCTCCGCTTCTCAGTTTGTGCGCATGTGCG
TTATCGCAAAACCGCTGCACACTTTGGCGACATGCTGTGATCCCCTCATCTGGGGGCTATCTGGGGAATTTCGACGCCGCTGCCGAACCAATT
CTCTGCTTCCACAGAACCTTGAAACGCTACCCGAACTTCGACAGATCTGGGCTTCAATTGCACTATTAAAATCTGTTTAAATCTGACAGCGCTCAACCC
TGTCGCTTCCGGTCTGATGAGACAGTGTGCAAAACGCCCTCTAAATAATTGTTTAATATGGACTATGTTGAAAGGGAAACATCTAGATGGCAC
GTACCCGGAGCGAGCAGCATGGTGGCTCGGTAGTCGCACATACCAAAAGCACTTGGGAGCAGCATTGAAACCTTGTGAAATGTTGTTATAGCG
GTCTGAGCATTTGAAACGGCTTCGACCTGCGGCCACAGCAACCCATTATCTGTTGGGACCAATAACAGCAGTACTGCGCAACTGTTGAA
ATGAAAGCAGAACAGGTGCTAAATTTCGGATCTGGGTAGCTTAAAGCGCATCTGGATTTCTGCTGGTAAATCTGTTGAAAGTCTGGCGTGAACCAATT
GTGGTGAGACCTTCGCTGGTATTGCGAGAGCAGCTGGACCCCTGCAACCCAGCTGGAAAGATCAGTTATGGAGCTGCTGTGAGATGGCA
AAAATCTGGTAAAGCCTACCATGTCGAACTGGCCAAGATACCATTCGTAACCTGCTGGATATGTTTGTGTTTGTGATCTGGCT
TGACCCGAACAGCTGACCGGTGAAAGGAGATATTGAAAGATTTCCTCTGCTGATTAATGTGTTTGTGCGGTACAGCGTTAAGGAAACACAGAAAAAA
GGCCGACCTGGACAGTGGGGCTTCTGGGACCAAAAGTAGCTGTGTAAGCTGGTAACAAACAGAACATCTGGTCTGGTTATATGAAATT
TTCTGTTAATAGTCAACACAGACAATCTGCTGTTGTTGTTATATGCTTCTACCGGACTTAGGATCTGAGCTTACAGGTTACCGAAGAAATGGT
TGTTATTTGAAATAAAAGGGTGTCTAACGGTGGCTACCTTGACTGATGAGTGGCTAACGGACAAACCCCTCTACAAAATATTGTTAAATGTTCC
TAATAATCAGCAAAAGGGTACTAGATGGCAGGGCAGTGGTGTGCTGGGCTAGTGGCAGCCTGGTCAACCCAGTACGATTTCTGCTGG
TCGAACCGAGACATGTTACCGCTCAGGGTTTCGACCCACGACTAACATGCTGGTATTCGAGGAAATCTCCGCTGAAGAAATTCTGG
TCGGACCCAAACCGAAATCTTCTGCTGACCCCTGCTGAAAGGACCGCTGGAACCGAGCACCCCTCTGGCAGAAAGATCTGACCCCTGGATGCG
GGCTGGCTGGGCAATTGTTGCGAGGAAGTCTGCTGCTGAGCACCAAATTGGATTTGTTGCTGCTGTGATCTGCGGAGATT
TCGAGATAATCATAGCGACCGTGAAGCAGTACCTGGGATCTGGTGTGAGCACCAAATTGGATTTGTTGCTGCTGTGATCTGCGGAGATT
TACCATGAGCGTTATTGAAATGCGTCGCAATGATGTTAAACCTGGAGTGGCTGAGCCAGAACATGGCTGGGAAACCCGAATTCTGGCGAGATGCG
CTCTGGCAGTCTGGGTGACCCGCTGCCGAGATCTGGTGTGAAAGAACCTGGACTGATTAACAGCGGAGCTGCAAAATAACTCGTACCAAAAGCAGAAC
ATAAGACGCTGAAAGCCTGTTTCTGTTGGTCTGGAGCTGGTGTGAGCTGGTGTGAGCTGGGAAACCCGCAATTCTGGCGAGATGCG
CTCTGGCGCTTCTGAGTTGGCTGGCAGTCTGGTGTGAGCTGGTGTGAGCTGGGAAACCCGCAATTCTGGCGAGTCTGGCGAGATGCG
GATGAGGAAACCCCTCTACAAATAATTGTTTAAATGCTGAGCTGTTACAGGCTAACACAGGCTAACATGAGCAACCCGCAATTCTGGCG
GATGAGGAAACCCCTCTACAAATAATTGTTTAAATGCTGAGCTGTTACAGGCTAACACAGGCTAACATGAGCAACCCGCAATTCTGGCG

Segment C Circuit

Segment D Circuit

Segment G Circuit

a. DNA sequence colors: AmeR (light blue), AmtR (cyan), BetI (blue), BM3R1 (red), CymR (dark blue), HlyII R (green), LmrA (orange red), PhIF (orange), SrpR (dark green), and VanR (light green).

References

Ackers GK, Johnson AD, Shea MA (1982) Quantitative model for gene regulation by lambda phage repressor. *Proceedings of the National Academy of Sciences* **79**: 1129

Brophy JAN, Voigt CA (2016) Antisense transcription as a tool to tune gene expression. *Molecular Systems Biology* **12**: 854

Dahirel V, Paillusson F, Jardat M, Barbi M, Victor J-M (2009) Nonspecific DNA-Protein Interaction: Why Proteins Can Diffuse along DNA. *Physical Review Letters* **102**: 228101

Roberts JW (2014) Molecular basis of transcription pausing. *Science* **344**: 1226

Nielsen AAK, Der BS, Shin J, Vaidyanathan P, Paralanov V, Strychalski EA, Ross D, Densmore D, Voigt CA (2016) Genetic circuit design automation. *Science* **352**: aac7341