Supplementary Material

1 Supplementary Text

1.1 Background subtraction in fluorescence assays

Raw absorbance and red fluorescence time series acquired from microplate experiments were background-subtracted as previously reported (Pasotti et al., 2017) to obtain OD$_{600}$ and RFP time series, proportional to the per-well cell density and fluorescent protein number. Sterile medium and a non-fluorescent TOP10 culture were used to measure absorbance and red fluorescence background, respectively. Since a significant cell density-dependent and growth rate-dependent autofluorescence was previously reported for GFP measurements in our experimental setup (Pasotti et al., 2017), green fluorescence was background-subtracted via a different procedure. We estimated the cell density- and growth rate-dependent autofluorescence as Eq.S1:

\[ GFP_{auto}(t) = e^{q + m \cdot OD_{600}(t)} \]  

in which OD$_{600}$ is the cell density of the culture, and $m$ and $q$ describe the linear relation between \( \ln(OD_{600}) \) and GFP autofluorescence level. The $m$ and $q$ coefficients are growth rate ($\mu$)-dependent parameters, with a function defined as Eqs.S2-S3:

\[ q(\mu) = \alpha_q + \beta_q \cdot \mu \]  
\[ m(\mu) = \alpha_m + \beta_m \cdot \mu \]

Therefore, the autofluorescence expression becomes (Eq.S4):

\[ GFP_{auto}(t) = e^{a_q + \beta_q \cdot \mu + (a_m + \beta_m \cdot \mu) \cdot OD_{600}(t)} \]  

Using Eq.S4, the four coefficients were fitted from autofluorescence data of different cultures exhibiting diverse growth rates. The Matlab \textit{regress} function was used for linear regression fitting in $GFP_{auto}$ calculation. This expression served as a calibration curve providing autofluorescence values that are then subtracted from raw green fluorescence values, given OD$_{600}$ and $\mu$.

1.2 Mathematical modelling of transcriptional cascade and NOR gate

Hill equation models were defined for both circuits and parametrized by fitting experimental data from their individual components to eventually compare model prediction and final circuit behaviour.

1.2.1 Transcriptional cascade model description

A previously adopted steady-state model (Pasotti et al., 2017) was used to describe the HSL-dependent RFP output of the X1TL circuit (Eq.S5).
\[ S_{tet,\text{max}} = \delta X_1 + \frac{\alpha x_1}{1 + (\frac{K x_1}{\text{HSL}}) \eta x_1} \]
\[ S_{lac,\text{max}} = \delta_T + \frac{\alpha_T}{1 + (\frac{T}{K_T}) \eta_T} \]
\[ S_{rfp,\text{max}} = \delta_L + \frac{\alpha_L}{1 + (\frac{L}{K_L}) \eta_L} \]
\[ D = 1 + \sum X_\lambda + J_{tet} \cdot S_{tet,\text{max}} \]
\[ T = \frac{1}{(\gamma_{tet} + \mu)} \cdot \frac{S_{tet,\text{max}}}{D} \]
\[ L = \frac{1}{(\gamma_{lac} + \mu)} \cdot \frac{S_{lac,\text{max}}}{D} \]
\[ S_{cell,X1TL} = \frac{a}{(a + \mu)} \cdot \frac{S_{rfp,\text{max}}}{D} \] (S5)

Parameters have the same meaning and units as in previous work (Pasotti et al., 2017), also summarized in Supplementary Table S2. Briefly, \( T \) and \( L \) represent the intracellular concentrations of TetR and LacI; \( S_{p,\text{max}} \) is the maximum synthesis rate of a generic regulated protein (\( p = \text{TetR}, \text{LacI}, \text{RFP} \)). Protein synthesis rate is modelled as a Hill function related to the activity of the upstream promoter (\( P_{\text{lux}}, P_{\text{LtetO1}} \) and \( P_{\text{LlacO1}} \), respectively) with parameters \( \delta, \alpha, K \) and \( \eta \), where \( \delta + \alpha \) is the maximum expression rate, \( K \) is the input level corresponding to 50\% of the expression rate range and \( \eta \) is the Hill coefficient; \( J_{tet} \) is the resource usage parameter of TetR; the cell load caused by LacI and RFP is assumed to be negligible at the expression levels spanned in the cascade circuit (Pasotti et al., 2017); \( \sum X_\lambda \) is the additional load given by the constitutive expression of LuxR. The term \( D \) is the scale factor between the maximum synthesis rate of a protein into the actual synthesis rate, obtained as the sum of cell load-related factors. The \( a, \mu, \gamma_{tet} \) and \( \gamma_{lac} \) represent the RFP maturation rate, cell growth rate, TetR and LacI protein degradation rates, respectively.

A model predicting the HSL-dependent output of the CRISPRi transcriptional cascade was derived from Eq.S5 by considering the \( gPtet_{\text{DEG9}}:dCas9 \) repressor (named \( \text{Cdeg9} \)) instead of TetR, under the following assumptions: the cell load caused by the expression of sgRNA is negligible; the intracellular \( gPtet_{\text{DEG9}}:dCas9 \) repressor complex was modeled, without describing the constitutive expression of dCas9 and its further binding to \( gPtet_{\text{DEG9}} \); the dilution rate of the CRISPRi repressor complex is equal to cell growth rate (Qi et al., 2013); an additional parameter (\( \Sigma C \)) is used in \( D \) to account for the GFP value that is slightly lower in the CRISPRi cascade compared with X1TL for HSL = 0 (Figure 7F), probably due to the presence of an additional plasmid, maintained at medium copy, that may represent a load for the cells (Pasotti et al., 2019). The resulting equations are reported in the Eq.S6 system.
\[ S_{\text{deg9},\text{max}} = \delta_{x_1} + \frac{\alpha_{x_1}}{1 + \left( \frac{K_{x_1}}{\text{HSL}} \right)^{\eta_{x_1}}} \]

\[ S_{\text{lac},\text{max}} = \delta_{\text{deg9}} + \frac{\alpha_{\text{deg9}}}{1 + \left( \frac{C_{\text{deg9}}}{K_{\text{deg9}}} \right)^{\eta_{\text{deg9}}}} \]

\[ S_{\text{rfp},\text{max}} = \delta_{L} + \frac{\alpha_{L}}{1 + \left( \frac{L}{K_{L}} \right)^{\eta_{L}}} \]

\[ D = 1 + \Sigma_{x_1} + \Sigma_{C} \]

\[ C_{\text{deg9}} = \frac{S_{\text{deg9},\text{max}}}{D \cdot \mu} \]

\[ L = \frac{S_{\text{lac},\text{max}}}{D \cdot (\gamma_{\text{lac}} + \mu)} \]

\[ S_{\text{cell,CRISPRi}} = \frac{a}{(a + \mu)} \cdot \frac{S_{\text{rfp},\text{max}}}{D} \]

1.2.2 NOR gate model description

A kinetic model of the main regulatory steps occurring in the NOR gate circuit was defined (Eq. S7).

\[ C_{\text{rep}} + D_{F} \xrightleftharpoons{K_{C}} D_{R1} \]

\[ \text{HSL} + R_{2} \xrightleftharpoons{K_{H}} Q \]

\[ Q + D_{F} \xrightleftharpoons{K_{R}} D_{R2} \]

\[ C_{\text{tot}} = 1 + \left( \delta_{I} + \frac{\alpha_{I}}{1 + \left( \frac{K_{I}}{\text{IPTG}} \right)^{\eta_{I}}} \right) \]

\[ D_{\text{tot}} = D_{F} + D_{R1} + D_{R2} \]

\[ C_{\text{tot}} = C_{\text{rep}} + D_{R1} \]

\[ R_{2T} = R_{2} + Q + D_{R2} \]

\[ D_{F} = \frac{D_{\text{tot}}}{1 + \frac{C_{\text{tot}}}{K_{C}} + \frac{R_{2T}/K_{R}}{1 + \frac{K_{H}}{\text{HSL}}}} \]

\[ S_{\text{cell,NOR}} = \frac{a}{(a + \mu)} \cdot \sigma \cdot D_{F} = \frac{a}{(a + \mu)} \cdot \frac{\beta}{1 + \frac{C_{\text{tot}}}{K_{C}} + \frac{U}{1 + \frac{K_{H}}{\text{HSL}}}} \]

The model describes the binding of the gPluxH:dCas9 repressor complex \((C_{\text{rep}})\) to the unoccupied \(P_{\text{luxRep}}\) promoter sequence \((D_{F})\) obtaining the promoter in a repressed state \((D_{R1})\), the binding of HSL to a LuxR dimer \((R_{2})\) obtaining the HSL-LuxR complex \((Q)\), the binding of \(Q\) to \(D_{F}\) obtaining the promoter in a second repressed state \((D_{R2})\), and the RFP synthesis rate per cell \((S_{\text{cell,NOR}})\) as proportional to \(D_{F}\) (with proportionality constant \(\sigma a/(a+\mu)\)). The symbols on the bidirectional arrows of the reactions indicate the resulting dissociation equilibrium constants. Conservation laws are also defined.
in Eq.S7 for total DNA, repressor and LuxR in cells. A steady-state solution for the free promoter $D_F$ was derived under the following assumptions: no significant cell load affects the recombinant strain; the total intracellular amount of repressors ($C_{tot}$ and $R_{2T}$) is much higher than the target DNA concentration; the binding events of $C_{rep}$ and $Q$ to the free promoter are mutually exclusive due to the short distance between the CRISPRi target site and the lux box; the activity of the $P_{luxRep}$ promoter in the repressed state is null; $C_{rep}$ is approximated by an IPTG-dependent Hill equation. In the final expression of $S_{cell,NOR}$, the lumped parameters $\beta = \sigma \cdot D_F$ and $U = R_{2T}/K_R$ are present. A summary of parameter values and units is reported in Supplementary Table S2.

### 1.2.3 Model implementation and fitting procedure

The models were implemented via Matlab R2017b (Mathworks, Natick, MA). Implicit equations (Eq.S5) were solved with a custom script implementing the fixed point method as it was carried out previously (Pasotti et al., 2017). The lsqnonlin routine, implementing the least squares algorithm, was used to fit experimental data (average values) to estimate the unknown parameters. For the transcriptional cascade, $\delta_{deg9}$, $\alpha_{deg9}$, $K_{deg9}$ and $\eta_{deg9}$ were estimated by fitting the RFP data of the gPtetDEG9 NOT gate (Figure 7B-C) using the model in Eq.S8.

\[
S_{deg9,max} = \delta_{X1} + \frac{\alpha_{X1}}{1 + \left(\frac{K_{X1}}{HSL}\right)^{\eta_{X1}}},
\]
\[
S_{rfp,max} = \delta_{deg9} + \frac{\alpha_{deg9}}{1 + \left(\frac{C_{deg9}}{K_{deg9}}\right)^{\eta_{deg9}}},
\]
\[
D = 1 + \Sigma_{X1} + \Sigma_C
\]
\[
C_{deg9} = \frac{S_{deg9,max}}{D} \cdot \frac{S_{rfp,max}}{D}
\]
\[
S_{cell} = \frac{a}{(a + \mu)} \cdot S_{rfp,max} / D
\]

The $\Sigma_C$ parameter was computed by solving $\frac{1 + \Sigma_{X1}}{1 + \Sigma_{X1} + \Sigma_C} = S_{cell,GFP}^{CRISPRi} / S_{cell,GFP}^{CRISPRi}$ using the GFP data in Figure 7F for HSL = 0.

For the NOR gate, the $\beta$ parameter was computed as $((a + \mu)/a) \cdot RFP_{control}$, where $RFP_{control}$ is the RFP output value of the control strain in Figure 5H for HSL=0; the RFP data of the No gPluxH condition (Figure 8B) were fitted with the $S_{cell,NOR}$ equation for $C_{tot} = 0$ to estimate the $U$ and $K_H$ parameters; the RFP data of the IPTG/P$_{LacO1}$-inducible system (Supplementary Figure S5) and of the IgLUX circuit (Figure 5H) were simultaneously fitted with Eq.S9 to estimate the $\delta_I$, $\alpha_I$, $K_I$, $\eta_I$ and $K_C$ parameters.
\[ S_{\text{cell,PLLacO1}} = \frac{a}{(a + \mu)} \cdot \left( \delta_l + \frac{\alpha_l}{1 + \left( \frac{K_l}{\eta} \right)^{\eta_l}} \right) \]

\[ C_{\text{tot}} = \frac{1}{\mu} \cdot \left( \delta_l + \frac{\alpha_l}{1 + \left( \frac{K_l}{\eta} \right)^{\eta_l}} \right) \]  

\[ S_{\text{cell,IGLUX}} = \frac{a}{(a + \mu)} \cdot \frac{\beta}{1 + \frac{C_{\text{tot}}}{K_C}} \]

A growth rate value was fixed for both fitting and simulations as the typical value measured in the two strains bearing the cascade and the NOR gate.

2 Supplementary Figures and Tables

2.1 Supplementary Figures

Supplementary Figure S1. Mathematical model simulations of circuit output as a function of dCas9, sgRNA and target DNA copy number. The output curves represent the intracellular concentration of free promoter DNA (D) normalized by the total concentration of available promoter DNA (D_{\text{tot}}). The independent variable of the simulations is the intracellular concentration of dCas9 (C_{\text{tot}}, expressed as nM concentration). Simulations are shown for different values of sgRNA (g_{\text{tot}}, expressed as nM concentration, in the columns) and DNA (D_{\text{tot}}, expressed as nM concentration, in the rows). Parameters: K_1 = 0.3 nM, K_2 = 2 nM.
Supplementary Figure S2. Mathematical model simulations of circuit output as a function of dCas9, sgRNA and target DNA copy number. The output curves represent the intracellular concentration of non-repressed promoter DNA (D) normalized by the total concentration of available promoter DNA (D_{tot}). The independent variable of the simulations is either the intracellular concentration of dCas9 (C_{tot}, expressed as nM concentration, panels in the left column) or the intracellular concentration of sgRNA (g_{tot}, expressed as nM concentration, panels in the right column). Simulations are shown for different values of sgRNA (left) or dCas9 (right), and DNA (D_{tot}, expressed as nM concentration) as different curves in each panel. Every panel reports six curves, corresponding to three different D_{tot} values, as indicated in the legend, and two different parameter sets (solid line: K_1 = 0.03 nM, K_2 = 0.2 nM; dashed line: K_1 = 3 nM, K_2 = 20 nM).
**Supplementary Figure S3.** Growth rate values of recombinant strains with HSL-inducible dCas9 and constitutive sgRNA. Data are reported as a function of HSL. In each panel, the copy number of the sgRNA constitutive cassette (low copy – LC, medium copy – MC) and the copy number of the target (medium copy – MC, high copy – HC) are reported. Two different targeting systems (Tet – panels A, C, E, and Lac – panels B, D, F) are reported: gPtet and gPlac, which repress the P_{LtetO1} and P_{LlacO1} promoters, respectively, that drive RFP. Each panel includes four curves: three of them correspond to circuits with the sgRNA under the control of three different constitutive promoters of diverse strengths (weak, medium and strong for J23116, J23100 and J23119, respectively), and one curve corresponds to a non-specific targeting control in which the medium-strength J23100 promoter constitutively transcribes a non-targeting sgRNA: gPlac and gPtet for the P_{LtetO1} and P_{LlacO1} promoters in the Tet and Lac systems, respectively. Data points represent the average value and error bars represent the standard errors of the mean of at least 3 independent experiments.
Supplementary Figure S4. GFP values of recombinant strains with HSL-inducible dCas9 and constitutive sgRNA. Data are reported as a function of HSL. In each panel, the copy number of the sgRNA constitutive cassette (low copy – LC, medium copy – MC) and the copy number of the target (medium copy – MC, high copy – HC) are reported. Two different targeting systems (Tet – panels A, C, E, and Lac – panels B, D, F) are reported: gPtet and gPlac, which repress the P_{LtetO1} and P_{LlacO1} promoters, respectively, that drive RFP. Each panel includes four curves: three of them correspond to circuits with the sgRNA under the control of three different constitutive promoters of diverse strengths (weak, medium and strong for J23116, J23100 and J23119, respectively), and one curve corresponds to a non-specific targeting control in which the medium-strength J23100 promoter constitutively transcribes a non-targeting sgRNA: gPlac and gPtet for the P_{LtetO1} and P_{LlacO1} promoters in the Tet and Lac systems, respectively. Data points represent the average value and error bars represent the standard errors of the mean of at least 3 independent experiments.
Supplementary Figure S5. Transfer function, with RFP as output, of the IPTG-inducible system including P_LlacO1. The Ir recombinant strain was used. Data are shown as the average RFP synthesis rate per cell, as a function of IPTG. Data points represent the average value and error bars represent the standard errors of the mean of at least 3 independent experiments.
Supplementary Figure S6. Copy number quantification in recombinant strains with two or more plasmids. All of them include a low-copy vector (highest molecular weight band). The medium- and high-copy vector amounts were quantified relative to the intensity of the low-copy vector. Electrophoresis gel (1% agarose with TBE) pictures, with ethidium bromide staining, are shown for strains H₃dgLAC₁₀₀,LC₉₄₅₆,₅₁₄₃P₄₄₃,₃₄₁₂,₅₆₇₈ and H₃dgLAC₁₀₀,MC₉₄₅₆,₅₁₄₃P₄₄₃,₃₄₁₂ (gel on the left) and H₃dgLAC₁₀₀,MC₉₄₅₆,₅₁₄₃P₄₄₃,₃₄₁₂ (gel on the right). Gel on the left: lanes 1, 5, 6 contain the H₃dgLAC₁₀₀,MC₉₄₅₆,₅₁₄₃P₄₄₃,₃₄₁₂ plasmids at different dilutions, digested with XbaI-SacII (XbaI is a single cutter in all the three plasmids and SacII is a single cutter only in the MC plasmid); lanes 2, 3, 4 contain the H₃dgLAC₁₀₀,LC₉₄₅₆,₅₁₄₃P₄₄₃,₃₄₁₂ plasmids at different dilutions, digested with XbaI-SacII (XbaI is a single cutter in all the three plasmids and SacII does not cut any of the plasmids). Gel on the right: lanes 1, 3, 5 contain the H₃dgLAC₁₀₀,MC₉₄₅₆,₅₁₄₃P₄₄₃,₃₄₁₂ plasmids at different dilutions, digested with XbaI (single cutter in both plasmids); lanes 2, 4, 6 are analogous to lanes 1, 3, 5, but they are relative to a biological replicate. The DNA ladder is GeneRuler 1 Kb (Thermo Scientific), with the lowest band reported in the pictures corresponding to the 1000-bp size.
Supplementary Figure S7. Growth rate values of recombinant strains with constitutive dCas9 and inducible sgRNA. Data are reported as a function of the inducer concentration driving sgRNA expression (HSL, in panels A-D, or IPTG, in panels E-H). In each panel, the CRISPRi targeting system is reported as gPtet, gPlac and gPluxH, which repress the P_{LtetO1}, P_{LlacO1} and P_{luxRep} target promoters, respectively, that drive RFP. Two different copy number contexts for the target are reported: medium copy (MC) and high copy (HC). Each panel includes two curves, corresponding to circuits with specific or non-specific targeting system. The latter is referred to as control and the used sgRNAs are gPlac (panels A-B), gPtet (panels C-D and G-H) and gPluxH (E-F). Data points represent the average value and error bars represent the standard errors of the mean of at least 3 independent experiments.
**Supplementary Figure S8.** GFP values of recombinant strains with constitutive dCas9 and inducible sgRNA. Data are reported as a function of the inducer concentration driving sgRNA expression (HSL, in panels A-D, or IPTG, in panels E-H). In each panel, the CRISPRi targeting system is reported as gPtet, gPlac and gPluxH, which repress the P_{LtetO1}, P_{LlacO1} and P_{luxRep} target promoters, respectively, that drive RFP. Two different copy number contexts for the target are reported: medium copy (MC) and high copy (HC). Each panel includes two curves, corresponding to circuits with specific or non-specific targeting system. The latter is referred to as control and the used sgRNAs are gPlac (panels A-B), gPtet (panels C-D and G-H) and gPluxH (E-F). Data points represent the average value and error bars represent the standard errors of the mean of at least 3 independent experiments.
Supplementary Figure S9. gPluxH variants not reported in the main text. (A) Description of the gPluxH variants. Blue nucleotides represent mismatches compared with the target sequence. (B) Transfer functions, with RFP as output, are reported as a function of IPTG concentration, driving the gPluxH variants expression in different circuits with constitutive dCas9, IPTG-inducible sgRNA, and P_{luxRep} as target driving RFP in a medium copy plasmid. The control represents an identical circuit but including the gPtet guide, which is not able to target P_{luxRep}. Data are shown as the average RFP synthesis rate per cell and data points represent the average value, with error bars representing the standard errors of the mean of at least 3 independent experiments.
Supplementary Figure S10. gPtet variants not reported in the main text. (A) sgRNAs with truncations and their effect on the HSL-inducible sgRNA circuit with constitutive dCas9 in MC and target in LC (HgTET<sub>d[n]</sub>P<sub>tet</sub> strains). All of them are transcribed with the three adenines present in the wild-type P<sub>lux</sub> promoter. The number of deleted nucleotides at the 5’ end of the guide is reported and the bars show the RFP output in the no induction and full induction conditions. The numbers above the bars indicate the percent repression. (B) Comparison between the gPtetDEG9 variant when expressed by the wild-type P<sub>lux</sub> and the modified P<sub>lux-3A</sub> promoters (HgTET<sub>DEG9P<sub>tet</sub></sub> and H<sub>3</sub>gTET<sub>DEG9P<sub>tet</sub></sub> strains, respectively). Data are shown as the average RFP synthesis rate per cell and data points and bars represent the average value, with error bars representing the standard errors of the mean of at least 3 independent experiments.
Supplementary Figure S11. NOR gate characterization. (A-C) Heatmaps and individual mean values of RFP, growth rate and GFP as a function of IPTG and HSL.
Supplementary Figure S12. Fitting of RFP data for the estimation of model parameters. Fitting of the (A) HSL-inducible gPtet<sub>DEG9</sub> NOT gate data (HgTET<sub>DEG9</sub>P<sub>tet</sub> strain), (B) IPTG-inducible P<sub>LlacO1</sub> data (Ir strain), (C) IPTG-inducible gPluxH NOT gate data with target promoter in high-copy plasmid (IgLUX<sub>d116PluxRep,HC</sub> strain), (D) HSL-repressible P<sub>luxRep</sub> promoter (NORcontrol strain). Data points represent the average data used for the fitting procedure and solid lines represent the fitted curves.
Supplementary Figure S13. Model simulations to predict the RFP output of the transcriptional cascades and the NOR gate. (A) Cascades. (B) NOR gate. The two graphs are consistent with the ones in Figure 7D and 8B, in which experimental data are reported.
### 2.2 Supplementary Tables

**Supplementary Table S1.** Description of the recombinant strains used in this work. All the plasmids are reported according with the nomenclature of the Registry of Standard Biological Parts, with the “BBa_” prefix omitted. Unless differently indicated, all the parts in the low-, medium- and high-copy plasmids are in the pSB4C5, pSB3K3 and pSB1A2 vector backbones.

| Strain | Description | Low-copy plasmid | Medium-copy plasmid | High-copy plasmid |
|--------|-------------|------------------|---------------------|-------------------|
| Hr     | HSL-inducible RFP in low-copy plasmid under the control of $P_{lux}$ | J107223 | - | - |
| H$_{3}$r | HSL-inducible RFP in low-copy plasmid under the control of $P_{lux-3A}$ | J107224 | - | - |
| Hd     | HSL-inducible dCas9 in low-copy plasmid under the control of $P_{lux}$ | J107225 | - | - |
| H$_{3}$d | HSL-inducible dCas9 in low-copy plasmid under the control of $P_{lux-3A}$ | J107216 | - | - |
| 116d   | Constitutive dCas9 cassette in medium-copy plasmid | - | J107202 | - |
| Ir     | IPTG-inducible RFP in low-copy plasmid under the control of $P_{LlacO1}$ | J107211 | - | - |
| J101R  | Reference strain for RFP fluorescence | J107029 | - | - |
| J101G  | Reference strain for GFP fluorescence | K173001 | - | - |

**Constructs with inducible dCas9 and constitutive sgRNA**

| H$_{3}$dgTET$_{116,LC}P_{tet,HC}$ | HSL-inducible dCas9 in low-copy plasmid with weak constitutive gPtet in low-copy and the target $P_{LtetO1}$ promoter driving RFP in high-copy | J107217 | - | I13521 |
| H$_{3}$dgTET$_{100,LC}P_{tet,HC}$ | HSL-inducible dCas9 in low-copy plasmid with medium-strength constitutive gPtet in low-copy and the target $P_{LtetO1}$ promoter driving RFP in high-copy | J107218 | - | I13521 |
| H$_{3}$dgTET$_{119,LC}P_{tet,HC}$ | HSL-inducible dCas9 in low-copy plasmid with strong constitutive gPtet in low-copy and the target $P_{LtetO1}$ promoter driving RFP in high-copy | J107219 | - | I13521 |
| H$_{3}$dgTET$_{116,MC}P_{tet,MC}$ | HSL-inducible dCas9 in low-copy plasmid with weak constitutive gPtet in medium-copy and the target $P_{LtetO1}$ promoter driving RFP in medium-copy | J107216 | J107237 | - |
| H$_{3}$dgTET$_{100,MC}P_{tet,MC}$ | HSL-inducible dCas9 in low-copy plasmid with medium-strength constitutive gPtet in medium-copy and the target $P_{LtetO1}$ promoter driving RFP in medium-copy | J107216 | J107238 | - |
| H$_{3}$dgTET$_{119,MC}P_{tet,MC}$ | HSL-inducible dCas9 in low-copy plasmid with strong constitutive gPtet in medium-copy and the target $P_{LtetO1}$ promoter driving RFP in medium-copy | J107216 | J107239 | - |
| Strain Description                                                                 | Promoter Details                                                                 | Constitutive Plasmid |
|-----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|---------------------|
| H₃dgTET116,MCP₅tet,HC                                                              | HSL-inducible dCas9 in low-copy plasmid with weak constitutive gPtet in medium-copy and the target P₅tetO₁ promoter driving RFP in high-copy | J107216  J107213   I13521 |
| H₃dgTET100,MCP₅tet,HC                                                              | HSL-inducible dCas9 in low-copy plasmid with medium-strength constitutive gPtet in medium-copy and the target P₅tetO₁ promoter driving RFP in high-copy | J107216  J107214   I13521 |
| H₃dgTET119,MCP₅tet,HC                                                              | HSL-inducible dCas9 in low-copy plasmid with strong constitutive gPtet in medium-copy and the target P₅tetO₁ promoter driving RFP in high-copy | J107216  J107215   I13521 |
| H₃dgLAC100,LCP₅tet,HC                                                               | Non-specific targeting control for the H₃dgTET116,LCP₅tet,HC strains              | J107221  -         I13521 |
| H₃dgLAC100,MCP₅tet,MC                                                                | Non-specific targeting control for the H₃dgTET119,MCPl₅tet,MC strains             | J107216  J107240   -   |
| H₃dgLAC116,LCPl₅lac,HC                                                               | HSL-inducible dCas9 in low-copy plasmid with weak constitutive gPlac in low-copy and the target Pl₅lacO₁ promoter driving RFP in high-copy | J107220  -         J107010 |
| H₃dgLAC100,LCPl₅lac,HC                                                               | HSL-inducible dCas9 in low-copy plasmid with medium-strength constitutive gPlac in low-copy and the target Pl₅lacO₁ promoter driving RFP in high-copy | J107221  -         J107010 |
| H₃dgLAC119,LCPl₅lac,HC                                                               | HSL-inducible dCas9 in low-copy plasmid with strong constitutive gPlac in low-copy and the target Pl₅lacO₁ promoter driving RFP in high-copy | J107222  -         J107010 |
| H₃dgLAC116,MCPl₅lac,MC                                                                | HSL-inducible dCas9 in low-copy plasmid with weak constitutive gPlac in medium-copy and the target Pl₅lacO₁ promoter driving RFP in medium-copy | J107216  J107231   -   |
| H₃dgLAC100,MCPl₅lac,MC                                                                | HSL-inducible dCas9 in low-copy plasmid with medium-strength constitutive gPlac in medium-copy and the target Pl₅lacO₁ promoter driving RFP in medium-copy | J107216  J107232   -   |
| H₃dgLAC119,MCPl₅lac,MC                                                                | HSL-inducible dCas9 in low-copy plasmid with strong constitutive gPlac in medium-copy and the target Pl₅lacO₁ promoter driving RFP in medium-copy | J107216  J107233   -   |
| H₃dgLAC116,MCPl₅lac,HC                                                                | HSL-inducible dCas9 in low-copy plasmid with weak constitutive gPlac in medium-copy and the target Pl₅lacO₁ promoter driving RFP in high-copy | J107216  J107207   J107010 |
| H₃dgLAC100,MCPl₅lac,HC                                                                | HSL-inducible dCas9 in low-copy plasmid with medium-strength constitutive gPlac in medium-copy and the target Pl₅lacO₁ promoter driving RFP in high-copy | J107216  J107208   J107010 |
### Constructs with inducible sgRNA and constitutive dCas9

| Construct Description                                                                 | Strain IDs                          |
|---------------------------------------------------------------------------------------|-------------------------------------|
| **H<sub>3</sub>dgLAC<sub>119</sub>,MC<sub>Plac,HC</sub>**                           | J107216, J107212, J107010            |
| HSL-inducible dCas9 in low-copy plasmid with strong constitutive gPlac in medium-copy and the target P<sub>LlacO1</sub> promoter driving RFP in high-copy |                                     |
| **H<sub>3</sub>dgTET<sub>100</sub>,LC<sub>Plac,HC</sub> Non-specific targeting control for the H<sub>3</sub>dgLAC<sub>1XX,LC</sub>,P<sub>lac,HC</sub> strains** | J107218 - J107010                    |
| **H<sub>3</sub>dgTET<sub>100</sub>,MC<sub>Plac,MC</sub> Non-specific targeting control for the H<sub>3</sub>dgLAC<sub>1XX,MC</sub>,P<sub>lac,MC</sub> strains** | J107216, J107241 - J107010           |
| **H<sub>3</sub>dgTET<sub>100</sub>,MC<sub>Plac,HC</sub> Non-specific targeting control for the H<sub>3</sub>dgLAC<sub>1XX,MC</sub>,P<sub>lac,HC</sub> strains** | J107216, J107214, J107010           |

**Constructs with inducible gPtet and constitutive dCas9**

| Construct Description                                                                 | Strain IDs                          |
|---------------------------------------------------------------------------------------|-------------------------------------|
| **H<sub>3</sub>gLAC<sub>d</sub><sub>116</sub>,MC<sub>Ptet,HC</sub>**               | J107206, J107242, J13521            |
| HSL-inducible gPtet in low-copy plasmid with constitutive dCas9 in medium-copy and the target P<sub>LtetO1</sub> promoter driving RFP in medium-copy |                                     |
| **H<sub>3</sub>gLAC<sub>d</sub><sub>116</sub>,P<sub>tet,MC</sub>**               | J107206, J107202, J13521            |
| Non-specific targeting control for the H<sub>3</sub>gLAC<sub>d</sub><sub>116</sub>,P<sub>tet,HC</sub> strain |                                     |
| **H<sub>3</sub>gLAC<sub>d</sub><sub>116</sub>,P<sub>tet,HC</sub>**               | J107226, J107242, J13521            |
| Non-specific targeting control for the H<sub>3</sub>gLAC<sub>d</sub><sub>116</sub>,P<sub>tet,HC</sub> strain |                                     |
| **H<sub>3</sub>gLAC<sub>d</sub><sub>116</sub>,P<sub>tet,MC</sub>**               | J107226, J107243, J13521            |
| HSL-inducible gPlac in low-copy plasmid with constitutive dCas9 in medium-copy and the target P<sub>LlacO1</sub> promoter driving RFP in medium-copy |                                     |
| **H<sub>3</sub>gLAC<sub>d</sub><sub>116</sub>,P<sub>tet,HC</sub>**               | J107226, J107202, J107010           |
| Non-specific targeting control for the H<sub>3</sub>gLAC<sub>d</sub><sub>116</sub>,P<sub>tet,HC</sub> strain |                                     |
| **H<sub>3</sub>gLAC<sub>d</sub><sub>116</sub>,P<sub>tet,MC</sub>**               | J107206, J107243, J13521            |
| Non-specific targeting control for the H<sub>3</sub>gLAC<sub>d</sub><sub>116</sub>,P<sub>tet,HC</sub> strain |                                     |
| **IgLUX<sub>d</sub><sub>116</sub>,P<sub>luxRep,MC</sub>**                       | J107206, J107244, J13521            |
| IPTG-inducible gPluxH in low-copy plasmid with constitutive dCas9 in medium-copy and the target P<sub>luxRep</sub> promoter driving RFP in medium-copy |                                     |
| IgLUXd116PluxRep,HC | IPTG-inducible gPluxH in low-copy plasmid with constitutive dCas9 in medium-copy and the target PluxRep promoter driving RFP in high-copy | J107229 | J107202 | J107100<sup>b</sup> |
|---------------------|-------------------------------------------------------------------------------------------------|--------|--------|------------------|
| IgTETd116PluxRep,MC | Non-specific targeting control for the IgLUXd116PluxRep,HC strain | J107230 | J107244<sup>a</sup> | - |
| IgTETd116PluxRep,HC | Non-specific targeting control for the IgLUXd116PluxRep,HC strain | J107230 | J107202 | J107100<sup>b</sup> |
| IgLUXd116P122,HC | IPTG-inducible gPluxH in low-copy plasmid with constitutive dCas9 in medium-copy and the target P122 promoter driving RFP in high-copy | J107229 | J107202 | J107111<sup>b</sup> |
| IgLUXd116P2,HC | IPTG-inducible gPluxH in low-copy plasmid with constitutive dCas9 in medium-copy and the target P2 promoter driving RFP in high-copy | J107229 | J107202 | J107101<sup>b</sup> |
| IgLUXd116P44,HC | IPTG-inducible gPluxH in low-copy plasmid with constitutive dCas9 in medium-copy and the target P44 promoter driving RFP in high-copy | J107229 | J107202 | J107105<sup>b</sup> |
| IgLUX<sub>δ</sub>[n]d116PluxRep,MC | IPTG-inducible gPluxH variants with [n]-nt truncations in low-copy plasmid with constitutive dCas9 in medium-copy and the target PluxRep promoter driving RFP in high-copy | J107229<sup>c</sup> | J107244<sup>a</sup> | - |
| IgLUX<sub>ε</sub>[n]d116PluxRep,MC | IPTG-inducible gPluxH variants with [n]-nt mismatching extension in low-copy plasmid with constitutive dCas9 in medium-copy and the target PluxRep promoter driving RFP in high-copy | J107229<sup>c</sup> | J107244<sup>a</sup> | - |
| IgLUX<sub>η</sub>[n]d116PluxRep,MC | IPTG-inducible gPluxH variants with mismatches (numbered and described in the manuscript) in low-copy plasmid with constitutive dCas9 in medium-copy and the target PluxRep promoter driving RFP in high-copy | J107229<sup>c</sup> | J107244<sup>a</sup> | - |
| **Transcriptional cascades** | | | | |
| X1T | HSL-inducible tetR-based NOT gate with RFP as output and constitutive GFP | J107245 | - | - |
| X1TL | lux-tet-lac cascade with RFP as output and constitutive GFP | J107246 | - | - |
| HgTET<sub>δ</sub>Tet | HSL-inducible gPtet in low-copy plasmid with constitutive dCas9 in medium-copy and the target P<sub>LtetO1</sub> promoter driving RFP in low-copy | J107249 | J107202 | - |
| HgTET<sub>ε</sub>[n]P<sub>Tet</sub> | HSL-inducible gPtet variants with [n]-nt truncations in low-copy plasmid with constitutive dCas9 in medium-copy and the target P<sub>LtetO1</sub> promoter driving RFP in low-copy | J107249<sup>c</sup> | J107202 | - |
|                     | HSL-inducible gPtet variants with degenerate nucleotides in low-copy plasmid with constitutive dCas9 in medium-copy and the target Ptet01 promoter driving RFP in low-copy | J107249c | J107202 | - |
|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|---------|---|
| HgTETDEG[P]tet      | HSL-inducible optimized gPtet variant (tgtcaatctctatcgcggat) in low-copy plasmid with constitutive dCas9 in medium-copy and the target Ptet01 promoter driving RFP in low-copy | J107252  | J107202 | - |
| HgTETDEG[P]tet      | HSL-inducible (Ptetlux3A) optimized gPtet variant (tgtcaatctctatcgcggat) in low-copy plasmid with constitutive dCas9 in medium-copy and the target Ptet01 promoter driving RFP in low-copy | J107254  | J107202 | - |
| HgTETDEG[P]tetLPlec | HSL-inducible (Ptetlux3A) low-burden transcriptional cascade including an optimized gPtet variant (tgtcaatctctatcgcggat) and a lacI-based logic inverter in low-copy plasmid with constitutive dCas9 in medium-copy and the target Ptet01 promoter driving RFP in low-copy | J107255  | J107202 | - |
| **NOR gate**        |                                                                                                                                         | J107256  | J107202 | J107100b |
| NORgPluxRep         | IPTG- and HSL-driven NOR gate                                                                                                          | J107202  | J107202 | J107100b |
| NORcontrol          | Non-specific targeting control for the NORgPluxRep strain                                                                                 | J107257  | J107202 | J107100b |

*a* Vector backbone is J107055, analogous to pSB3K3 but with a promoter-less RFP expression cassette between the SpeI and PstI sites.

*b* Vector backbone is J61002, analogous to pSB1A2 but with a promoter-less RFP expression cassette between the SpeI and PstI sites.

*c* Vector backbone is J107056, analogous to pSB4C5 but with a promoter-less RFP expression cassette between the SpeI and PstI sites.
## Supplementary Table S2. List of model parameters.

| Parameter | Units     | Value | Reference           |
|-----------|-----------|-------|---------------------|
| \(\delta X_1\) | AU/min\(^1\) | 0.2   | (Pasotti et al., 2017) |
| \(\alpha X_1\) | AU/min   | 24.33 | (Pasotti et al., 2017) |
| \(K_{X_1}\)    | nM       | 6.71  | (Pasotti et al., 2017) |
| \(\eta_{X_1}\) | -        | 1.19  | (Pasotti et al., 2017) |
| \(\delta T\)   | AU/min   | 0.21  | (Pasotti et al., 2017) |
| \(\alpha T\)   | AU/min   | 4.56  | (Pasotti et al., 2017) |
| \(K_{T}\)      | AU       | 6.92  | (Pasotti et al., 2017) |
| \(\eta_{T}\)   | -        | 2.57  | (Pasotti et al., 2017) |
| \(\delta I\)   | AU/min   | 0.22  | (Pasotti et al., 2017) |
| \(\alpha I\)   | AU/min   | 0.76  | (Pasotti et al., 2017) |
| \(K_{I}\)      | AU       | 34.92 | (Pasotti et al., 2017) |
| \(\eta_{I}\)   | -        | 1.93  | (Pasotti et al., 2017) |
| \(J_{tet}\)    | min/AU   | 0.31  | (Pasotti et al., 2017) |
| \(\Sigma_{X_1}\) | -       | 0.36  | (Pasotti et al., 2017) |
| \(\gamma_{tet}\) | min\(^{-1}\) | 0.0173 | (Pasotti et al., 2017) |
| \(\gamma_{loc}\) | min\(^{-1}\) | 0.0533 | (Pasotti et al., 2017) |
| \(\sigma\)    | min\(^{-1}\) | 0.0167 | (Pasotti et al., 2017) |
| \(\mu\)       | min\(^{-1}\) | 0.006 | This study           |
| \(\delta_{deg9}\) | AU/min | 0.48  | This study           |
| \(\alpha_{deg9}\) | AU/min | 6.5   | This study           |
| \(K_{deg9}\)   | AU       | 14.6  | This study           |
| \(\eta_{deg9}\) | -        | 1.11  | This study           |
| \(\Sigma_{C}\) | -        | 0.45  | This study           |
| \(\delta I\)   | AU/min   | 0.04  | This study           |
| \(\alpha I\)   | AU/min   | 4.77  | This study           |
| \(K_{I}\)      | \(\mu M\) | 2.94  | This study           |
| \(\eta_{I}\)   | -        | 2.46  | This study           |
| \(K_{C}\)      | AU       | 11.64 | This study           |
| \(U\)          | -        | 676.3 | This study           |
| \(K_{z}\)      | nM       | 2.2E+4| This study           |

\(^1\)AU: arbitrary units of RFP fluorescence.