Synthesizing a novel genetic sequential logic circuit

Supplementary materials

Mathematical model of the genetic sequential logic circuit

Based on a previously defined model (Bintu *et al.* 2005), we employed a set of ordinary differential equations to describe the dynamic process of our genetic sequential logic circuit. The circuit's variables, such as the concentrations of mRNAs and proteins, were plot as functions of time in response to external stimulus. In order to reduce the control parameters in the equations, we assumed that different transcription factors could independently bind on their operators in the same promoter region. We also adopted a Hill function rather than partition function to describe the effect of each repressor or activator (de Jong 2002; Gardner *et al* 2000; Lou *et al* 2007). For the equation (1)-(11), the descriptions and values of parameters or variables are listed in Table S1.

$$\frac{d[mRNA_{CI}]}{dt} = \alpha_{PRM} \times \left(\frac{\alpha_{cI} \times \left(\frac{CI_{tot}}{K_{CI}}\right)^{n1} + 1}{1 + \left(\frac{CI_{tot}}{K_{CI}}\right)^{n1} + \left(\frac{CI434}{K_{CI434}}\right)^{n2} + \left(\frac{CI_{tot}}{K_{CI}}\right)^{n1}\left(\frac{CI434}{K_{CI434}}\right)^{n2}}\right) - r_{cImRNA} \times [mRNA_{CI}] \quad (1)$$

$$\frac{d[mRNA_{CI434}]}{dt} = \alpha_{PR} \times \left(\frac{1}{\left(\frac{CI_{tot}}{K_{CI}}\right)^{n1} + 1}\right) - r_{cI434mRNA} \times [mRNA_{CI434}] \quad (2)$$

$$\frac{d[mRNA_{LacI}]}{dt} = \alpha_{LacI} \times \left(\frac{\alpha_{cI} \times \left(\frac{CI_{tot}}{K_{CI}^{A}}\right)^{n1} + 1}{1 + \left(\frac{CI_{tot}}{K_{CI}^{A}}\right)^{n1} + \left(\frac{CI434}{K_{434}}\right)^{n2} + \left(\frac{CI_{tot}}{K_{CI}^{A}}\right)^{n1}\left(\frac{CI434}{K_{434}}\right)^{n2}}\right) - r_{lacImRNA} \times [mRNA_{LacI}] \quad (3)$$

$$\frac{d[mRNA_{CIind}]}{dt} = \alpha_{P_{NOR}} \times \left(\frac{1}{1 + \left(\frac{LacI}{K_{LacI}}\right)^{n3} + \left(\frac{LexA}{K_{LexA}}\right)^{n4} + \left(\frac{LacI}{K_{LacI}}\right)^{n3}\left(\frac{LexA}{K_{LexA}}\right)^{n4}}\right) - r_{CIindmRNA} \times [mRNA_{CIind}] \quad (4)$$

$$\frac{d[mRNA_{LexA}]}{dt} = \alpha_{LexA} \times \left(\frac{1}{\left(\frac{LexA}{K_{LexA}^{0}}\right)^{n5} + 1}\right) - r_{lexAmRNA} \times [mRNA_{LexA}] \quad (5)$$

$$\frac{d[CI]}{dt} = \beta_{SDA} \times mRNA_{CI} - (r_0 + d_{CI}) \times CI \quad (6)$$

$$\frac{d[CI434]}{dt} = \beta_{SDcro} \times mRNA_{CI434} - (r_0 + d_{CI434}) \times CI434 \quad (7)$$

$$\frac{d[LacI]}{dt} = \beta_{SDLacI} \times mRNA_{LacI} - r_0 \times LacI \quad (8)$$

$$\frac{d[CIind]}{dt} = \beta_{SDind} \times mRNA_{CIind} - r_0 \times CIind \quad (9)$$

$$\frac{d[LexA]}{dt} = \beta_{LexA} \times mRNA_{LexA} - (r_0 + d_{LexA}) \times LexA \quad (10)$$

$$[CI_{tot}] = [CI] + [CIind] \quad (11)$$

The effect of UV signal was described by the parameters d_{CI}, d_{CI434} or d_{LexA}. The values of these degradation rates listed in Table S1 represent the case in which the effect of UV irradiation is present; otherwise, they are zero. ODE15 solver in MATLAB was used to numerically solve the above equations. The time interval for integration was set to be 5500 minutes, and Figure 1E provides an example of the simulation.

The model of bistable switch

In order to investigate the robustness of the bistable switch circuits, we studied their behaviors with the corresponding ordinary differential equations. Three cases mentioned in the main text were considered here. (1) In the case I, the bistable switch circuit just consists of a double-negative feedback that is formed by two mutually suppressing dimerized repressors (CI and CI434). The corresponding ordinary different equations are Eqs. (12) and (13). Since there is no higher cooperation between the two repressor dimers, the values of Hill coefficients, n01 and n02, are fixed to 1.5. (2) In the case II, besides the double-negative feedback, the circuit also has a higher level of cooperation due to the interaction between the two CI dimers or CI434 dimers. The corresponding equations are the same as the case I, except that the values of parameters n01 and n02 were fixed to 3. (3) In the case III, the positive feedback of CI repressor was added into the circuit of the case II and the corresponding ordinary different equations are Eqs. (14) and (13). Here, the parameters μ_{CI}^0 and μ_{CI434}^0 in Eq. (13) and (14) integrated parameters that describe the transcriptional activity, the translational activity and the mRNA's decay of CI and CI434 respectively. All the parameters are listed in Table S1. With Eqs (12)-(14), we could calculate the bistable region for each of the above three cases. We found that the circuit that included all the three mechanisms had largest bistable region in the three cases (Figure S2).

$$\frac{d[CI]}{dt} = \mu_{CI}^0 \times \frac{1}{1 + (\frac{[CI434]}{K_{CI434}^0})^{n02}} - \gamma_{CI}[CI] \quad (12)$$

$$\frac{d[CI434]}{dt} = \mu_{CI434}^0 \times \frac{1}{1 + (\frac{[CI]}{K_{CI}^0})^{n01}} - \gamma_{CI434}[CI434] \quad (13)$$

$$\frac{d[CI]}{dt} = \mu_{CI}^{0} \times \frac{1 + \alpha_{cI} \times (\frac{[CI]}{K_{CI}})^{n01}}{1 + (\frac{[CI]}{K_{CI}^{0}})^{n01} + (\frac{[CI434]}{K_{CI434}^{0}})^{n02} + (\frac{[CI]}{K_{CI}^{0}})^{n01} (\frac{[CI434]}{K_{CI434}^{0}})^{n02}} - \gamma_{CI}[CI] \quad (14)$$

Bintu L, Buchler NE, Garcia HG, Gerland U, Hwa T, Kondev J, Kuhlman T, and Phillips R. 2005a. Transcriptional regulation by the numbers: applications. Current Opinion in Genetics & Development 15:125-135.

de Jong H. 2002. Modeling and simulation of genetic regulatory systems: a literature review. J Comput Biol 9:67-103.

Gardner TS, Cantor CR, and Collins JJ. 2000. Construction of a genetic toggle switch in Escherichia coli. Nature 403:339-342.

Lou CB, Yang XJ, Liu XL, He B, and Qi OY. 2007. A quantitative study of lambda-phage SWITCH and its components. Biophysical Journal 92:2685-2693.

Table S1

Variable/parameter	Description	Value
$[mRNA_{CI}]$	Mean number of cI's mRNA molecules per cell	
$[mRNA_{CI434}]$	Mean number of cI434's mRNA molecules per cell	
$[mRNA_{LacI}]$	Mean number of lacI's mRNA molecules per cell	
$[mRNA_{CIind}]$	Mean number of cIind-'s mRNA molecules per cell	
$[mRNA_{LexA}]$	Mean number of lexA's mRNA molecules per cell	
[CI]	Mean number of protein CI molecules per cell	
[CI434]	Mean number of protein CI434 molecules per cell	
[LacI]	Mean number of protein LacI molecules per cell	
[CIind]	Mean number of protein CIind- molecules per cell	
[LexA]	Mean number of protein LexA molecules per cell	
$[CI_{tot}]$	Total concentration of CI and CIind- molecules per cell	
α_{PRM}	Maximal rate of the transcription of cI	0.0187 min^{-1}
α_{CI}	the activation factor of CI on promoter P_{RM}	10 min^{-1}
α_{PR}	Maximal rate of the transcription of cI434 gene	4.375 min^{-1}
α_{LacI}	Maximal rate of the transcription of lacI gene	0.0019 min^{-1}
α_{PNOR}	Maximal rate of the transcription of cIind- gene	0.125 min^{-1}
α_{LexA}	Maximal rate of the transcription of lexA gene	0.4167 min^{-1}
β_{SDA}	Translation rate of CI	6.9 min^{-1}
β_{SDcro}	Translation rate of CI434	3.1 min^{-1}
β_{SDLacI}	Translation rate of LacI	0.93 min^{-1}
β_{SDind}	Translation rate of CIind-	6.8 min^{-1}
β_{LexA}	Translation rate of LexA	20 min^{-1}
$r_{cI\,mRNA}$	Decay rate constant of cI's mRNA	0.25 min^{-1}
$r_{cI\,434\,mRNA}$	Decay rate constant of cI434's mRNA	0.25 min^{-1}
$r_{lacI\,mRNA}$	Decay rate constant of lacI's mRNA	0.005 min^{-1}

$r_{cIind\mathrm{m}\,RNA}$	Decay rate constant of cIind-'s mRNA	0.25 min^{-1}
$r_{lexA\mathrm{m}\,RNA}$	Decay rate constant of lexA's mRNA	0.25 min^{-1}
r_0	Growth rate constant of E.coli	0.033 min^{-1}
d_{CI}	Decay rate constant of CI when the effect of UV is present	0.023 min^{-1}
d_{CI434}	Decay rate constant of CI434 when the effect of UV is present	0.091 min^{-1}
d_{LexA}	Decay rate constant of LexA when the effect of UV is present	0.91 min^{-1}
K_{CI}	Binding affinity of CI to its operator	**40** §
K_{CI434}	Binding affinity of CI434 to its operator	**50** §
K_{LacI}	Binding affinity of LacI to its operator	**30** §
K_{LexA}	Binding affinity of LexA to its consensus operator	**50** §
K^0_{LexA}	Binding affinity of LexA to its operator in the promoter region of itself	**50000** §
n_1	Hill coefficient to describe the cooperativity of CI effect	4
n_2	Hill coefficient to describe the cooperativity of CI434 effect	2
n_3	Hill coefficient to describe the cooperativity of LacI effect	4
n_4	Hill coefficient to describe the cooperativity of LexA effect on suppressing P_{NOR} promoter	2
n_5	Hill coefficient to describe the cooperativity of LexA effect on suspressing the itself promoter	1
n01	Hill coefficient to describe the cooperativity of CI	1.5 (3)
n02	Hill coefficient to describe the cooperativity of CI434	1.5 (3)
γ_{CI}	the degradation rate of CI	0.033 min^{-1}
γ_{CI434}	the degradation rate of CI434	0.033 min^{-1}
K^0_{CI}	the binding affinity of repressor CI to its binding site	$4 \sim 4\times10^6$ §
K^0_{CI434}	the binding affinity of repressor CI434 to its binding site	$4 \sim 4\times10^6$ §

μ_{CI}^{0}	The generation rate constant of CI	$2\times10^{-5}\sim2\times10$
μ_{CI434}^{0}	The generation rate constant of CI434	$3.1\times10^{-4}\sim3.1\times1$

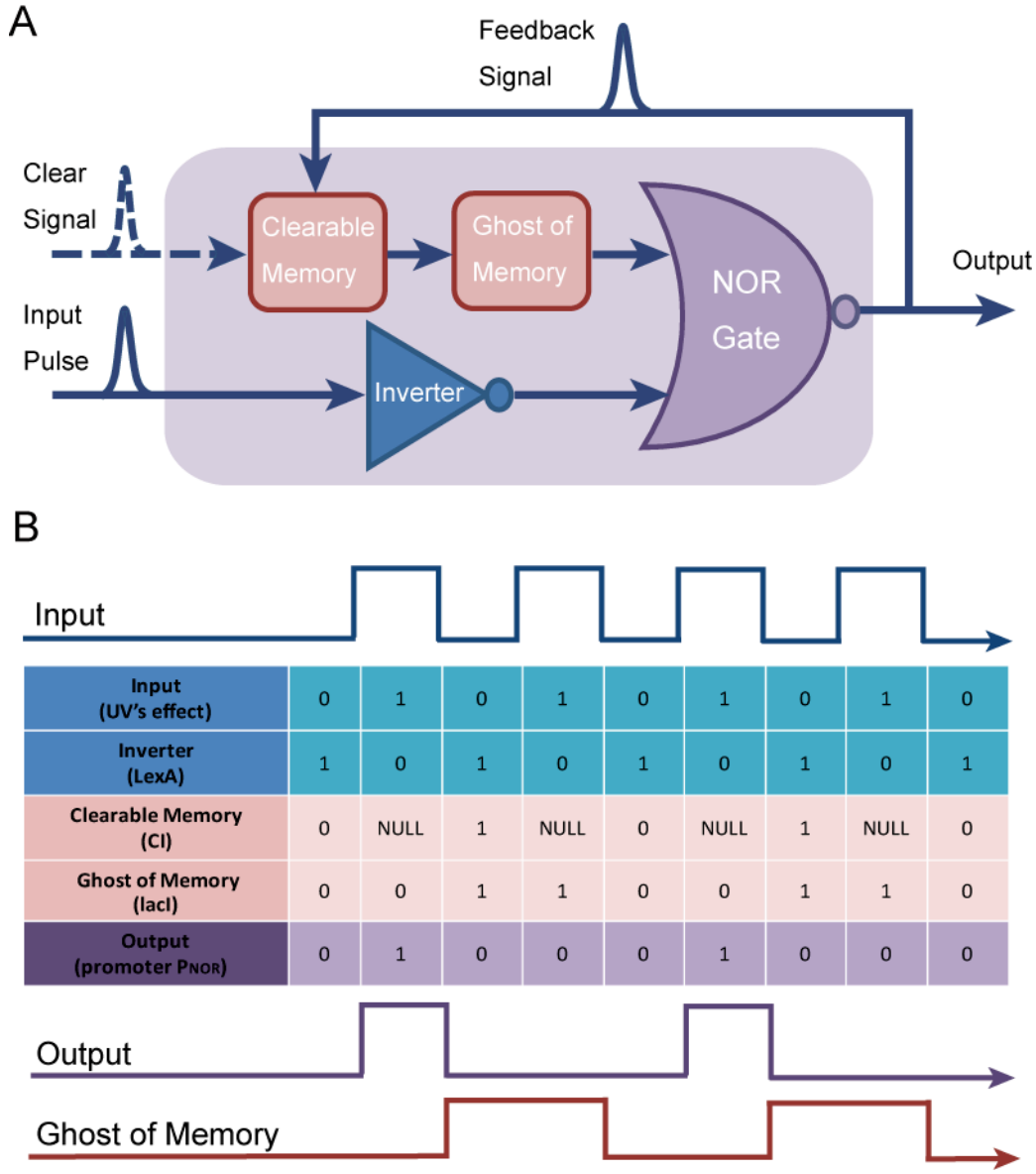
§ The unit of these dissociation constants has been converted from mol/L to number/cell. Here we assumed the concentration of one molecule in *E.coli* is 10^{-9}mol/L.

Table S2

Name	Relevant characteristic(s)	source
E. coli strains		
JM109	endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ Δ(lac-proAB) e14- [F'traD36 proAB+ lacIq lacZΔM15] hsdR17(rK -mK+)	Tiangen
JM110	rpsL thr leu thi lacY galK galT ara tonA tsx dam dcm glnV44 Δ(lac-proAB) e14- [F' traD36 proAB+ lacIq lacZΔM15] hsdR17(rK-mK+)	Yanhui Xiang CAS
AAEC072	MG1655 Δ*fim*	IC. Blomfield
AAEClac-	MG1655 Δ*fim* Δ*lacI*	This work
Plasmids		
P15Aswitch	Switch module constructed on pSB3K3	This work
pZS*Aswitch	Switch module subcloned into pZS*24MCS, Amp^R	This work
P15AcIindnor	cI_{ind} gene and P_{NOR} promoter constructed on pSB3K3	This work
pLXcmlacIOR	lacI gene and P_{RM} promoter constructed on pLXcm	This work
pSB3K3	p15A origin, Kan^R	iGEM biobrick
pZS*24MCS	pSC101* origin, Kan^R	Lutz and Bujard
pLXcm	pMB origin, Cm^R	Qi Ouyang's lab

Table S3 Survival Rate after UV irradiation

UV Dosage (J/m2)	0	15	20	25	30	35	40
Average of Survival Rate	1.00	0.59	0.15	0.12	0.04	0.01	0.00
Standard Deviated	0.00	0.26	0.07	0.06	0.02	0.01	0.00



Input (UV's effect)	0	1	0	1	0	1	0	1	0
Inverter (LexA)	1	0	1	0	1	0	1	0	1
Clearable Memory (CI)	0	NULL	1	NULL	0	NULL	1	NULL	0
Ghost of Memory (lacI)	0	0	1	1	0	0	1	1	0
Output (promoter P_{NOR})	0	1	0	0	0	1	0	0	0

Figure S1 Boolean logic state transition table of the genetic sequential logic circuit. (A) The analogous electronic circuit of the genetic sequential logic circuit. NOR gate represents the P_{NOR} promoter; output signal is the concentration of CI_{ind-}; clearable memory represents the bistable switch; ghost of memory represents LacI (it remembers the previous state of CI, when the state of the bistable switch is cleared by UV irradiation); Inverter represents RecA*'s effect on LexA; both input pulse and clear signal are the UV stimulus. (B) The corresponding state transition table of the circuit.

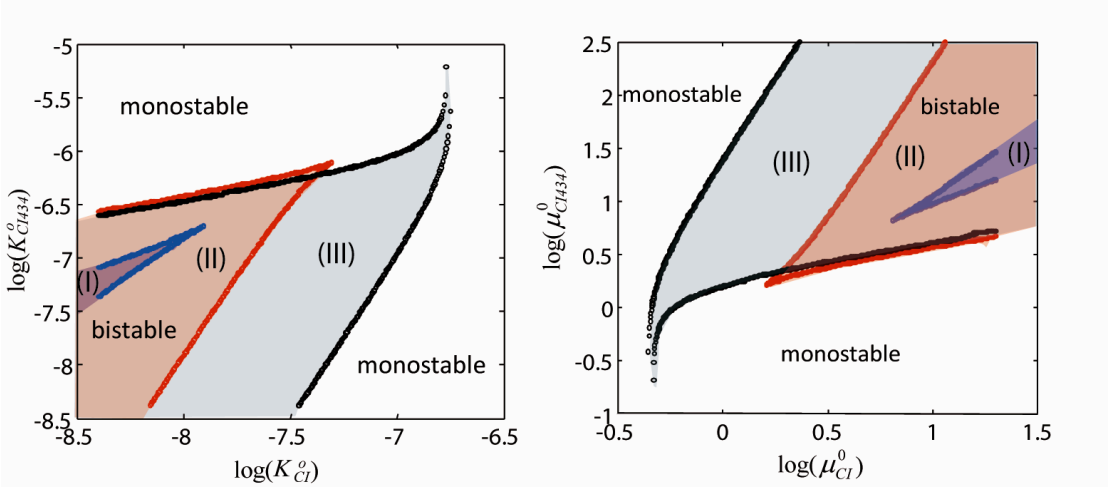


Figure S2 The phase diagram of the bistable switch. (A) bistable regions in the parameters space of the K_{CI}^{o} and K_{CI434}^{o}. (B) bistable regions in the parameters space of the μ_{CI}^{o} and μ_{CI434}^{o}. The blue shading indicates the bistable region for the case (I). The red shading indicates the bistable region for the case (II). The black shading region indicates the bistable region for the case (III).

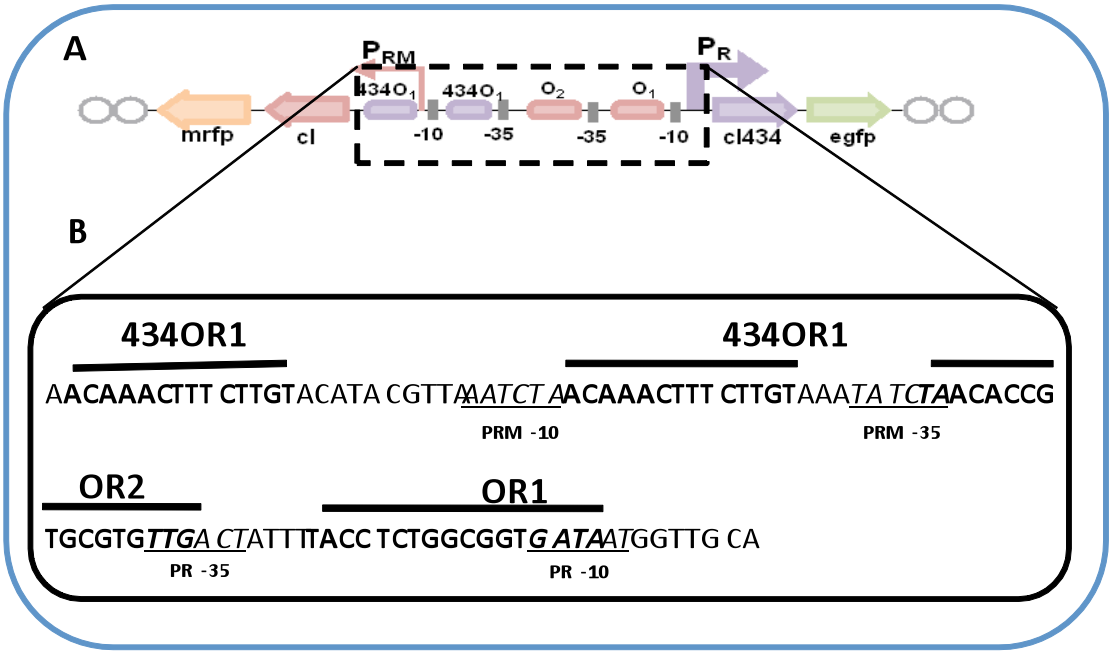


Figure S3 The sequence detail of the memory module's promoter region. (A) The schematics of the bistable module. Rectangles with arrow represent genes. Open ovals represent terminations. Lines with arrow represent transcriptional strength and direction of the promoters. Rectangles with colors represent repressors' binding sites. Gray squares represent the -10 and -35 region of the promoters. (B) The sequence detail of the promoter region. The bold sequences represent the binding sites of the repressors. The italic underlined letters represent the -35 and -10 consensus site of P_R and P_{RM} promoters.

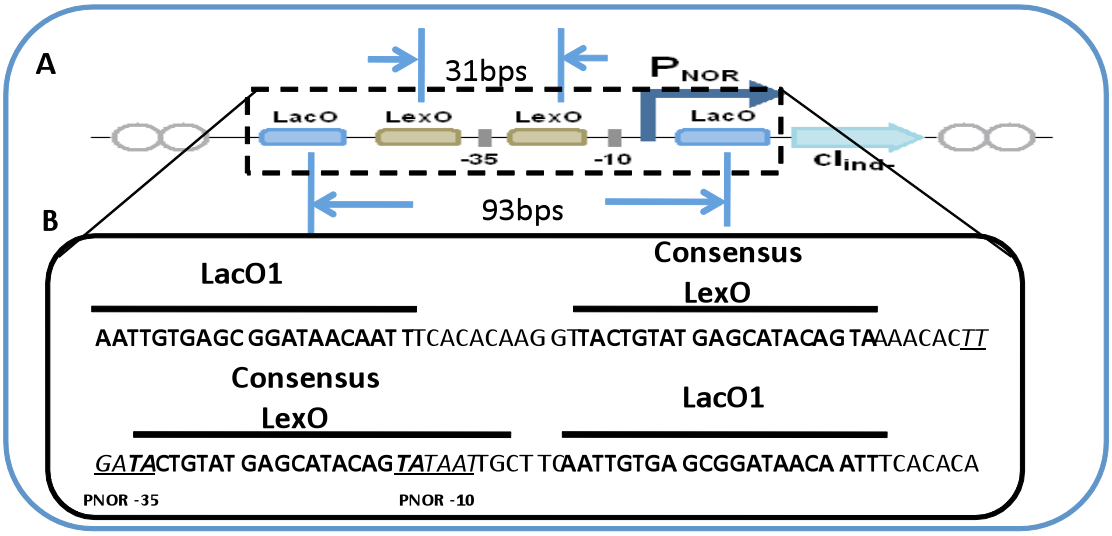


Figure S4 The sequence detail of the P_{NOR} promoter region. (A) The schematics of the NOR gate module. Rectangles with arrow represent genes. Open ovals represent terminations. Line with arrow represents transcriptional strength and direction of the promoters. Rectangles with colors represent repressors' binding sites. Gray squares represent the -10 and -35 region of the promoters. (B) The sequence of the P_{NOR} promoter region. The bold sequences represent the binding sites of LacI and LexA repressors. The italic underlined letters represent the -35 and -10 consensus site of P_{NOR} promoter.

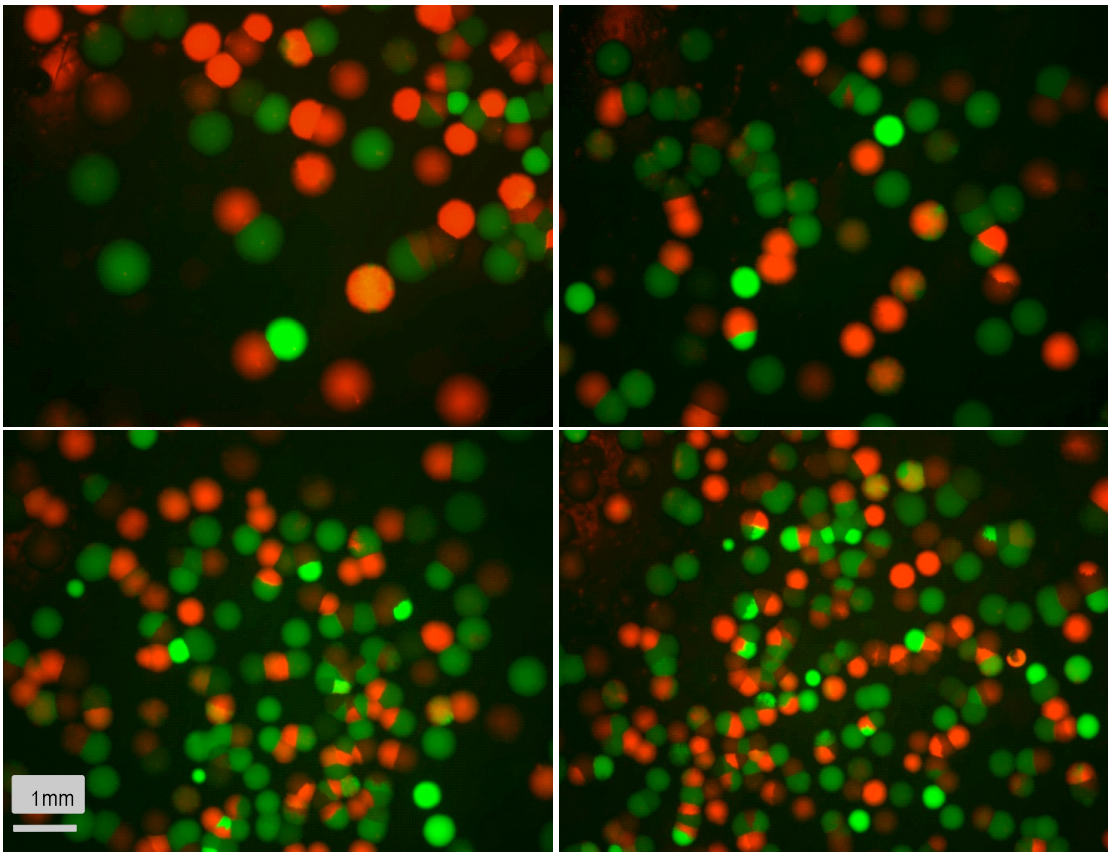
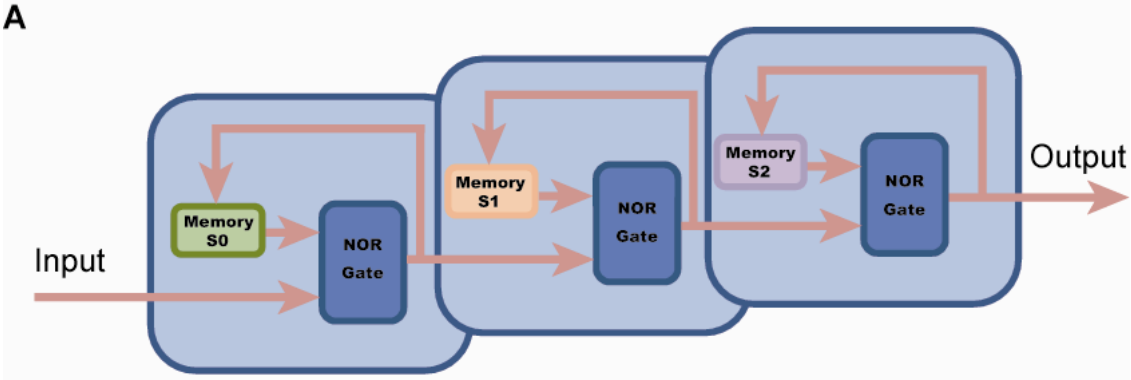


Figure S5 The mutation library yielded a diverse set of CI and CI434 expression levels.. The pictures indicate the colonies carrying two mutated plasmids. One of the plasmids mutated in cIind-'s RBS region; the other one mutated in lacI's RBS region. Each dot represents one colony. The pictures were directly merged from the gfp channel and the rfp channel. Four views were chosen to show the diversity of the fluorescent intensity.

A



B

State transitions	1	2	3	4	5	6	7	8
S0	1	0	1	0	1	0	1	0
S1	1	1	0	0	1	1	0	0
S2	1	1	1	1	0	0	0	0

Figure S6 A schematic and the state transition table of a 3-bit ripple-carry binary counter. (A) A 3-bit binary counter is composed of three coupling 1-bit ripple carry binary counter. (B) The state transition of the 3-bit counter is descending and the counter counts from 111 to 000.

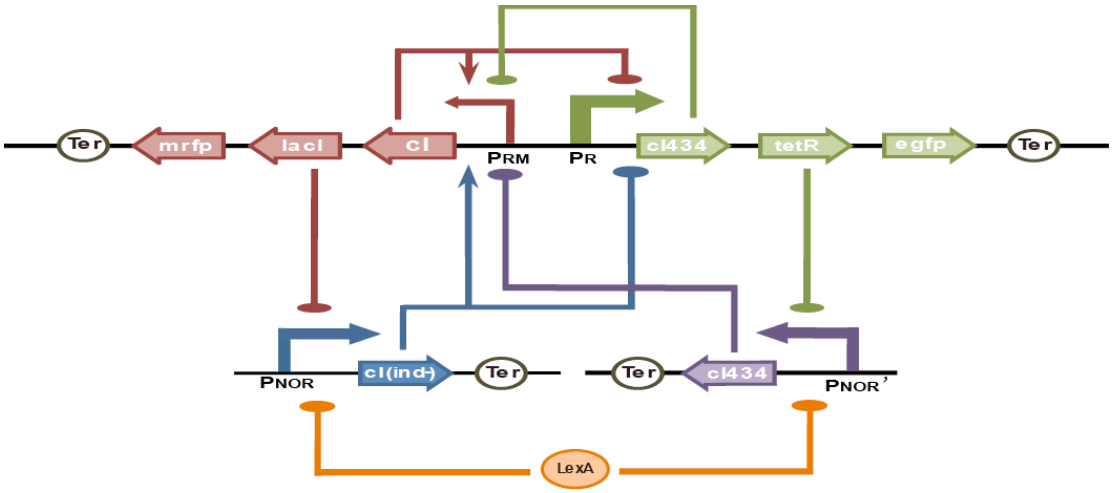


Figure S7 A more complex design for the push-on push-off switch. Another NOR gate is added into the circuit described in the main text. The additional NOR gate is connected into our GSLC circuit, taking TetR and LexA as two inputs and CI434 as the output. TetR is controlled by the P_R promoter, and cis-transcripted with CI434 in the memory module.

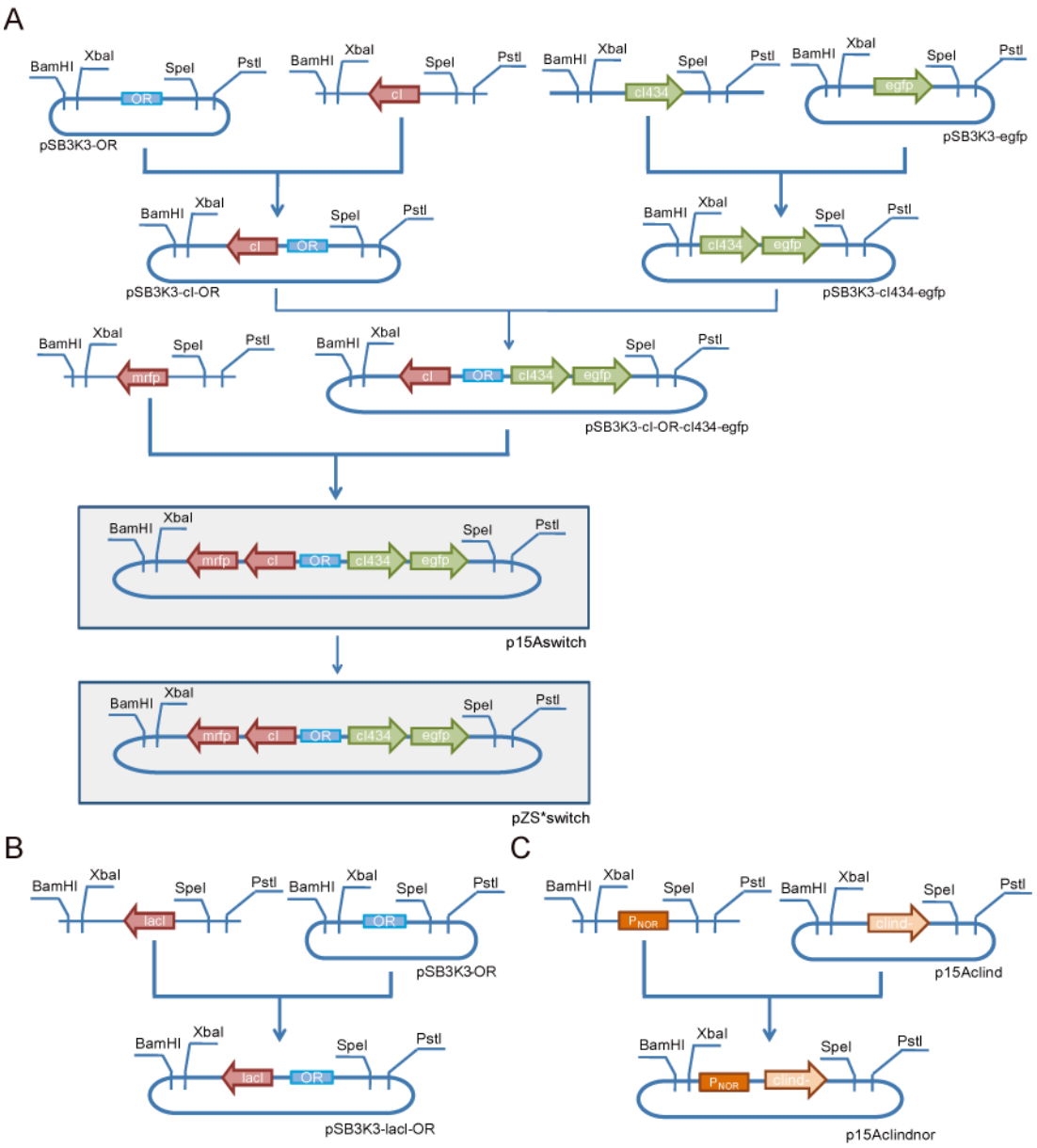


Figure S8 The flow of plasmid constructions. (A) The construction flow of the plasmid p15Aswitch and pZS*switch. (A) The construction flow of the plasmid pSB3K3-lacI-OR. (C) The construction flow of the plasmid p15AcIindnor. Rectangles represent the promoter regions; rectangles with arrow represent the genes; open ovals represent the backbone of the plasmids; polygonal lines branched from the open ovals represent the restriction sites.