Construction of a genetic toggle switch in *Escherichia coli* Supplementary Information

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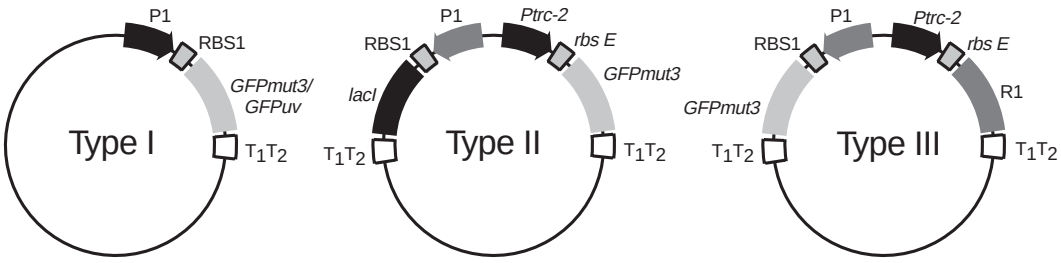

Figure 1: Promoters are marked by solid rectangles with arrowheads. Genes are denoted with solid rectangles. Ribosome binding sites and terminators ($T_1\,T_2$) are denoted by outlined boxes. The Ptrc-2 promoter with RBS-E and the lacI gene is used in all Type II and III plasmids.

Plasmids

The absolute strengths of the promoter/RBS pairs used to construct the toggle switches are listed in Table 1 in units of fluorescence. Measurements of promoter strengths were performed using Type I plasmids (Fig. 1) and assays were performed as described in the Methods. Leakage expression from the promoters under fully repressed conditions is also listed in Table 1. The efficacy of repression was tested using Type II plasmids (for Lac repression) or Type III plasmids (for λ or TetR repression). The extremely efficient λ repressor, expressed from Ptrc-2-E, achieves $\sim$6000 fold repression of the P_Ls1con-D promoter. On the other hand, the TetR repressor, also expressed from Ptrc-2-E, achieves only $\sim$100 fold repression of the $P_L$tetO-1-A promoter.

Table 1: Plasmids

Plasmid	Type	P1	RBS1	RBS2	GFP Expression
Bare Promoters					
pMKN7a*	I	Ptrc-2	E	—	732 ± 108
pBAG102	I	$P_L$tetO-1	C	—	5.5 ± 0.1
pBAG103	I	$P_L$tetO-1	A	—	660 ± 42
pBRT21.1*	I	P_Ls1con	D	—	9,390 ± 840
pBRT21.1*†	I	P_Ls1con	D	—	14,300 ± 400
pBRT123	I	P_Ls1con	G	—	387 ± 21
pBRT124	I	P_Ls1con	F	—	972 ± 43
pBRT125	I	P_Ls1con	H	—	15.9 ± 3.2
lacI Repression					
pTAK102	II	P_Ls1con	D	—	36.0 ± 3.8
pTAK103a	II	P_Ls1con	G	—	137 ± 8
cI Repression					
pTAK106	III	P_Ls1con	D	—	2.5 ± 0.3
pTAK107	III	P_Ls1con	G	—	2.0 ± 0.1
tetR Repression					
pIKE108	III	$P_L$tetO-1	A	—	5.8 ± 1.0
pIKE110	III	$P_L$tetO-1	C	—	2.3 ± 0.2
Toggles					
pTAK117	IV	P_Ls1con	D	B	bistable
pTAK130	IV	P_Ls1con	G	B	bistable
pTAK131	IV	P_Ls1con	F	B	bistable
pTAK132	IV	P_Ls1con	H	B	bistable
pIKE105	IV	$P_L$tetO-1	A	B	monostable
pIKE107	IV	$P_L$tetO-1	C	B	bistable

*Estimated from flow-cytometer assay of GFPuv-expressing promoters.

†Grown at 32°C.

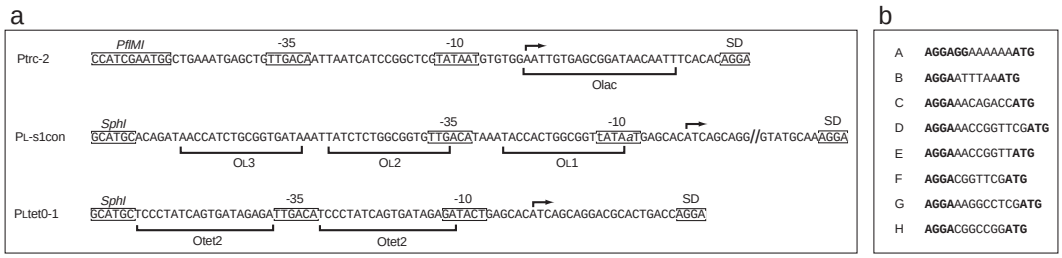

Figure 2: Promoters and ribosome binding sites used to construct the toggle plasmids. **a**, *Promoters. The upstream limit of each promoter is marked by the indicated restriction site. Operator sites are marked be a single underbracket. The initiation of transcription is denoted with arrows. SD denotes the Shine-Dalgarno sequence. Mutations in the -10 sequence of* P_L*s1con are indicated with lowercase letters.* **b**, *Ribosome binding sites. Shine-Dalgarno sequences and start codons are in boldface. Sequences are ranked in order of their translational efficiency (A = highest, G = lowest).*

Promoters

The structures of the three promoters used in the toggle are illustrated in Fig. 2. Bases -48 to +27 of the Ptrc promoter, where +1 is the initiation of transcription, are amplified by PCR from pTrc99a to form the Ptrc-2 promoter. Ptrc-2 is a highly efficient fusion of the Ptrp and Plac promoters and is nearly identical to the commonly used Ptac promoter. P_Ls1con is a shortened version of the wild-type P_L promoter with additional mutations conferring a consensus -10 sequence. P_Ls1con is amplified from bases -75 to the Shine-Dalgarno sequence of pXC46. Thus P_Ls1con eliminates the P_{L2} secondary promoter and the L1 and L2 integration host factor binding sites of the wild-type P_L promoter[1]. Elimination of P_{L2}, L1, L2 and introduction of the -10 mutations serve to weaken the native strength of the extremely strong P_L promoter while retaining all three operators for λ repressor binding. The $P_L$tetO-1 promoter, obtained through total synthesis according to the published sequence[2], contains two copies of the O2 operator of the Tn10 tetracycline resistance operon—one between the consensus -35 sequence and the -10 sequence of P_L, and one upstream of the -35 sequence. The $P_L$tetO-1 promoter is substantially less efficient than both Ptrc-2 and P_Ls1con, but it is effectively repressed by the wild-type TetR repressor.

References

[1] Giladi, H., Koby, S., Gottesman, M. E. & Oppenheim, A. B. Supercoiling, integration host factor, and a dual promoter system, participate in the control of the bacteriophage λ pL promoter. *J. Mol. Biol.* **224**, 937–948 (1992).

[2] Lutz, R. & Bujard, H. Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I_1-I_2 regualtory elements. *Nucleic Acids Res.* **25**, 1203–1210 (1997).