Electronic Supplementary Material (ESI) for Chemical Communications

Artificial Repressors for Controlling Gene Expression in Bacteria

Electronic Supplementary Information

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Methods

Reagents:

All chemicals were purchased from Sigma Aldrich (St. Louis, Missouri) or Fisher Scientific (Waltham, Massachusetts). Enzymes used for plasmid construction were purchased from New England Biolabs (Ipswich, Massachusetts) and Promega (Madison, Wisconsin). Oligonucleotide primers were obtained from Integrated DNA Technologies (Coralville, Iowa). Cell culture medium was purchased from Fisher Scientific.

Bacterial Strains and Media:

All experiments were performed using the strain *E. coli* K12 MG1655 ΔaraBAD ΔlacI. This strain was constructed by P1 phage transduction from the appropriate KEIO collection mutant to *E. coli* K12 MG1655 ΔaraBAD, as previously described.[1-2,6] All experiments were conducted using LB medium (1% [wt/vol] tryptone, .5% [wt/vol] yeast extract, and 1% [wt/vol] sodium chloride). All cultures were supplemented with 100 μg/mL ampicillin sodium salt and 50 μg/mL kanamycin sulfate using 1000x stock solutions of each. Medium was supplemented as required with 1 mM IPTG from a 1 M stock and 1% (wt/vol) L-arabinose from a 20% (wt/vol) stock added prior to inoculation.

Gene Synthesis:

The custom TALE gene used in these studies, *TALElacO1*, was synthesized by GeneArt (Life Technologies, Grand Island, NY, USA). The gene was codon-optimized for expression in *E. coli* MG1655 and was sequence verified by GeneArt.

Plasmid Construction:

Plasmid pBT102 was created by restriction digest of pJ23102 (Registry of Standard Biological Parts, Cambridge, MA) with enzymes EcoRI and SpeI to release a 58-bp DNA fragment containing the constitutive promoter. The fragment was gel-purified and subcloned into pBT-2 using enzymes EcoRI and XbaI and T4 DNA ligase. The *TALElacO1* and *lacI* genes were inserted downstream of the promoter in pBT102 via Gibson assembly.[4] Briefly, primers ATW_pBT1xx_F and ATW_pBT1xx_R were used to amplify the pBT102 vector by PCR using *Pfu* polymerase. *TALElacO1* and *lacI* were amplified via PCR using primer pairs Gib_TALE_F / Gib_TALE_R and Gib_lacI_F / Gib_lacI_R, respectively. PCR products were DpnI digested, purified using a Qiagen PCR purification kit, and added to a Gibson master mix solution in a 3:1 (insert:vector) ratio. The composition of the Gibson master mix used has been previously described.[3] The Gibson assembly reaction was placed at 50°C for 1 hour before an aliquot was transformed into chemically competent DH5α cells. Transformants were plated on selective media (LB agar, 50 μg/ml kanamycin). Potential clones of pBT102-TALE and pBT102-lacI were screened via colony PCR using primers lacI_nested_R and pBT1xx_cPCR_F or TALE-lacO1_nested_R and pBT1xx_cPCR_F, respectively. Plasmids were further screened by restriction enzyme digest and confirmed by DNA sequencing (Functional Biosciences Inc., Madison, WI, USA).

Reporter plasmid pCherΔlacI was built by first inserting the *mCherry* gene downstream of the *trc* promoter of vector pMSB-6, followed by removal of the *lacI*^{q} gene. The *mCherry* gene was PCR amplified from the template plasmid pSW196 using primers mCherry_BamHI_F and mCherry_KpnI_R. The resulting PCR amplicon and plasmid pMSB-6 were each digested using restriction enzymes BamHI and KpnI and ligated together using T4 DNA ligase to yield plasmid pMSBmCherry. The *lacI*^{q} gene was removed from pMSBmCherry via Gibson assembly by amplifying the vector into two pieces using primer pairs Piece1_AmpF / Piece2_AmpF and Piece1_5'lacIq_R / Piece2_3'lacIq_F. PCR products were assembled as described above, transformed into chemically competent DH5α cells and selected on LB agar containing 100 μg/ml ampicillin. All reporter plasmid constructs were confirmed via DNA sequencing.

p70RG has been previously described.[5] The vector pMP1 was created by PCR amplification of p70RG using primers LacO_gibsonF and LacO_gibsonR, both of which contained the *lacO1* operator sequence or its complement in their 5' tails. The PCR product was then circularized using a one piece Gibson assembly reaction by adding 100 ng of PCR product to an appropriate amount of 1.33x Gibson master mix and nuclease free water. The transformation mixture was plated on LB agar with 100 μg/mL ampicillin to select for transformants. The resulting plasmid was sequence verified and electroporated into MG1655 ΔaraBAD ΔlacI using a 1 mm electroporation cuvette with the appropriate expression plasmids. Transformants were selected on LB agar with 50μg/mL kanamycin and 100μg/mL ampicillin.

Culturing of Bacteria:

All cultures were inoculated to an approximate OD_{600} of .01. Cultures were grown in tubes with 4x head space at 37°C with shaking (250 RPM). LB medium supplemented with the

aforementioned antibiotics and the appropriate additives (arabinose and/or IPTG) was used for all experiments.

Fluorescence and Optical Density Measurements:

All fluorescence and optical density measurements were taken using a Tecan Infinite M1000 plate reader. Fluorescence measurements were taken in black, flat, clear bottom 96-well plates (Corning Incorporated, Corning, New York). Optical density measurements were all taken at 600 nm using clear polystyrene 96-well plates (Corning Incorporated). Fluorescence measurements for cultures containing p70RG or pMP1 were taken at excitation/emission wavelengths of 558/583 nm and 400/510 nm for dsRed and GFPuv, respectively. Fluorescence measurements for cultures harboring pCherΔlacI were taken at excitation/emission wavelengths of 587/610 nm. All fluorescence and optical density measurements were taken from biological quadruplicates (n=4).

Supplementary Tables:

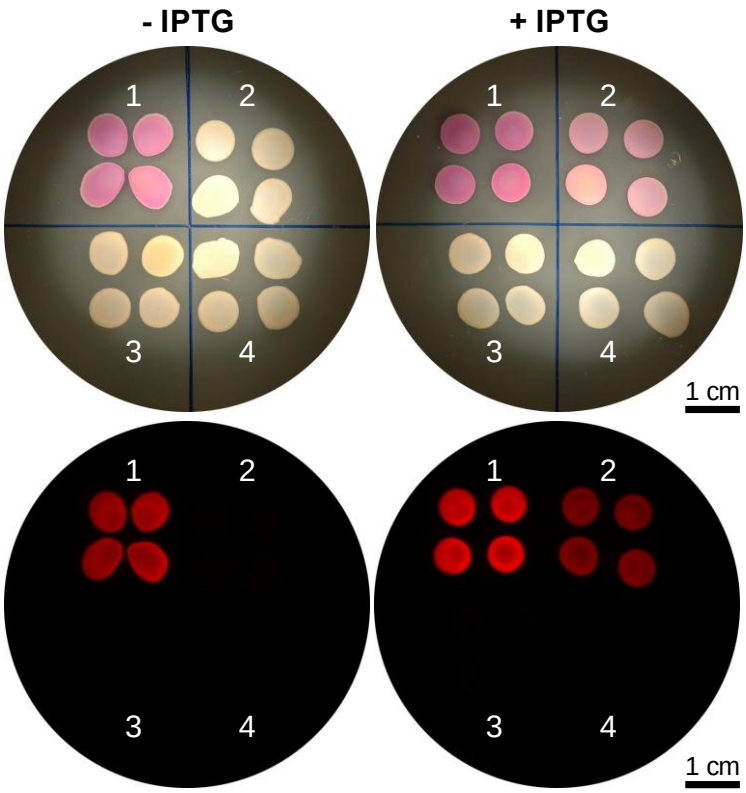
SI Table 1. Oligonucleotides used for this study.

Oligo Name	Sequence (5' to 3')
LacO_gibsonF	TGGAATTGTGAGCGGATAACAATTGTATTGATCTCCTAGGCGGGGTACCGTATTTT
LacO_gibsonR	AATTGTTATCCGCTCACAATTCCAAAATAATATTCTCGTATTTTCACCGCGGTCGC
ATW_pBT1xx_F	AATCATACCTGACCTCCATAGCAG
ATW_pBT1xx_R	TGGTCAGTATTGAGCGATATCTAG
Gib_TALE_F	CTAGATATCGCTCAATACTGACCAAGGAGGTATCTACA ATGGTTGATC
Gib_TALE_R	CTGCTATGGAGGTCAGGTATGATTAGGGTACCTTATTTATCATCATCATC
Gib_lacI_F	CTAGATATCGCTCAATACTGACCAAGGAGGTATCTACAGTGAAACCAGTAACGTTATACG
Gib_lacI_R	CTGCTATGGAGGTCAGGTATGATTACTCACATTAATTGCGTTGC
lacI_nested_R	ATTTGCTGGTGACCCAATGC
pBT1xx_cPCR_F	TCCATCAGCTTGTCCAGCAG
TALE-lacO1_nested_R	AATTGCAACAACCTGTTCCGG
mCherry_BamHI_F	TATGAAGGATCCAGGAGGTACAATCAATGGTGAGCAAGGGCGAGGAG
mCherry_KpnI_R	TCGATAGGTACCTTACTTGTACAGCTCGTCCATGCC
Piece1_AmpF	GTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGG
Piece2_AmpR	AAGTTGGCCGCAGTGTTATCAC
Piece1_5'lacIq_R	GCTGTCAAACCAGATCAATTCGCAACGTAAATGCATGCCGCTTC
Piece2_3'lacIq_F	GCGAATTGATCTGGTTTGACAGC

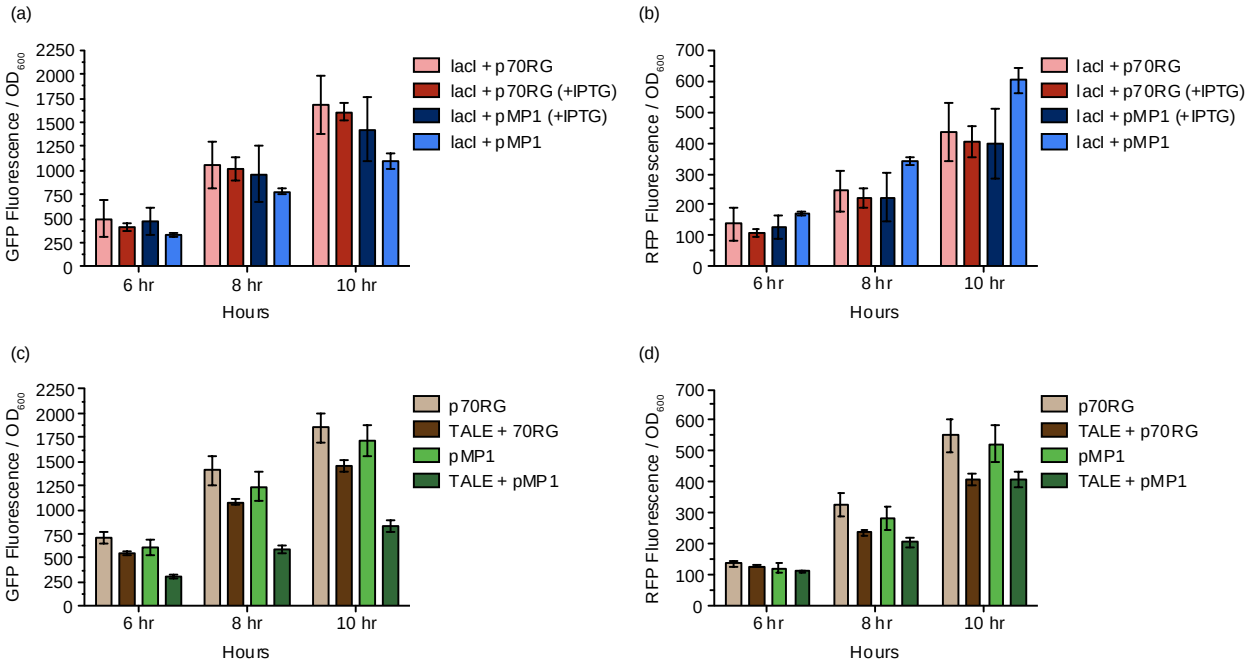
SI Table 2. List of plasmids used in the study.

Name	Description	Source
pMSB-6	Plasmid used to construct pMSBmCherry.	7
pSW196	Template used to amplify mCherry gene.	8
pMSBmCherry	pMSB-6 with the mCherry gene downstream of the *trc* promoter.	This work
pCherΔlacI	pMSBmCherry without the $lacI^q$ gene.	This work
pBT-2	Plasmid used to build *TALElacO1* and *lacI* expression vectors.	9
pJ23102	Source of constitutive promoter used in pBT102.	10
pBT102	pBT-2 with constitutive promoter from pJ23102.	This work
pBT102-lacI	pBT102 with *lacI* gene downstream of the constitutive promoter.	This work
pBT102-TALE	pBT102 with *TALElacO1* gene downstream of the constitutive promoter.	This work
pMK_RQ_2-TALE-lacO1	Template used to amplify *TALElacO1* gene.	This work
p70RG	Plasmid with GFPuv downstream of dsRed, under control of pBAD.	5
pMP1	p70RG with the *lacO1* operator inserted between dsRed and GFPuv.	This work

Supplementary Figures



SI Figure 1. Repression of *mCherry* expression via inhibition of transcription initiation by TALElacO1 and LacI. The top panel photographs are the same images presented in **Fig. 2**. The lower panel displays fluorescent images acquired by a Typhoon Imager (Excitation = 532 nm, Emission = LPR filter, PMT = 800) of the same agar plates shown in the top panel. The fluorescent colonies are false-colored red. All colonies are MG1655 Δ*lacI* Δ*araBAD* containing: **1** = pBT102 + pCherΔlacI, **2** = pBT102-lacI + pCherΔlacI, **3** = pBT102-TALE + pCherΔlacI, **4** = pBT102 + pMSB-6.



SI Figure 2. Fluorescence intensities of GFPuv and dsRed proteins over time for those cultures used to examine repression of transcription elongation. Plots (**a**) and (**b**) demonstrate that binding of LacI to the *lac* operator in pMP1 results in a reduction of GFP fluorescence, while levels of RFP fluorescence remain the same between strains or are slightly increased for those cells containing pBT102-lacI and pMP1. Plots (**c**) and (**d**) display similar results for cells expressing *TALElacO1*.

Supplementary References

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