

Plasmids for independently tunable, low-noise gene expression

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Abstract

Some microbiology experiments and biotechnology applications can be improved if it is possible to tune the expression of two different genes at the same time with cell-to-cell variation at or below the level of genes constitutively expressed from the chromosome (the “extrinsic noise limit”). This was recently achieved for a single gene by exploiting negative autoregulation by the tetracycline repressor (TetR), and bicistronic gene expression to reduce gene expression noise. We report new plasmids that use the same principles to achieve simultaneous, low-noise expression for two genes. The TetR system was moved to a compatible plasmid backbone, and a system based on the lactose repressor (LacI) was found to also exhibit gene expression noise below the extrinsic noise limit. We characterized gene expression mean and noise across the range of induction levels for these strains, applied the LacI system to tune expression for single-molecule mRNA detection in two different growth conditions, and showed that two plasmids can be co-transformed to independently tune expression of two different genes.

Introduction

We recently reported the development of a plasmid-based gene-expression system in which a gene of interest is expressed bicistronically with the tetracycline repressor (TetR) [1]. Using this gene expression system, cell-to-cell is below the “extrinsic noise limit” observed for genes expressed from the chromosome [2]. When TetR and GFP are expressed bicistronically, GFP induction and gene expression noise is similar to that observed for a TetR-GFP fusion protein with autoregulation [3]. Compared to induction of gene expression under the control of a constitutively expressed transcriptional repressor, the inducer dose-response is relatively linearized, and gene expression noise is much lower at intermediate induction levels.

Why this would it be useful to do this for two different genes at the same time? Example for stoichiometric production of a protein complex Expressing genes at their native levels ... e.g. for generating chromosome deletions

Figure 1: Regulatory constructs used in this study. (a) pZH509, (b) pJS102, (c) pJS103, (d) pJS23103

Methods

Strain construction

All plasmids were constructed using isothermal assembly [4] of fragments generated by PCR or double stranded DNA synthesis (IDT) and transformed Top10 E. coli cells (IBA #5-1600-020). Transformants were screened by colony PCR and verified by sequencing. Sequence maps are included as supplementary files and plasmids will be made available at AddGene after deposit and screening [5]. Purified plasmids were transformed into E. coli strain MG1655 by growing 2 mL of culture in SOB media at 37 C to OD600=0.4, washing twice with 1 mL ice-cold water, resuspending in 40 μ L water, electroporation of 1–10 ng plasmid with the EC1 setting of a Micropulser (BioRad), and recovering for 1 hour at 37 C in SOC media.

To generate pJS101 with a compatible backbone, plasmid pZH509 [1] was used as a template to amplify the bicistronic regulatory construct including the $P_{\text{TetO-1}}$ promoter [6], GFPmut2 [7], tn10 TetR [8] and rrnB T1 transcription terminator [9]. This was assembled by isothermal assembly with the backbone from pGB2 [10] with the pSC101 origin of replication and spectinomycin resistance to generate plasmid pJS101. Plasmids with the pSC101 and p15a origins of replication have been co-transformed in previous synthetic biology experiments [11].

Plasmids pJS102 and pJS103 were generated by 3-fragment isothermal assembly. Plasmid pZH509 was used as a template both for the vector backbone and for GFPmut2, with non-homologous extensions added to PCR primers to generate the P_{LlacO} and P_{LlacOsym} promoters.

Promoter $P_{LlacOsym}$ lacks the central G–C base pair in P_{LlacO} LacI binding sites, which are symmetrical [12] except that the second *lacOsym* sequence has one base changed to maintain the P_{LlacO} promoter –10 sequence. LacI was amplified from *E. coli* MG1655 by colony PCR. Plasmid pJS23103 was made by isothermal assembly of an inverse PCR product of pZH509, with non-homologous primer extensions adding the weak, constitutive BBa_J23103 promoter between GFPmut2 and TetR [13].

Plasmids were co-transformed into MG1655 by electroporation following the above protocol, except with 1 μ L each undiluted plasmid (~20–40 ng) and selecting on plates with both spectinomycin and carbenicillin.

Table 1: Plasmids used in this study.

Plasmid	Ori	Promoter	GOI	Repressor Expression	Reference
pZH501	p15a	CI-SNAP-tag	$P_{LtetO-1}$	bicistronic	[1]
pZH509	p15a	GFPmut2	$P_{LtetO-1}$	bicistronic	[1]
pZH520	p15a	GFPmut2	$P_{LtetO-1}$	constitutive	[1]
pJS101	pSC101	GFPmut2	$P_{LtetO-1}$	bicistronic	This work
pJS102	p15a	GFPmut2	P_{LlacO}	bicistronic	This work
pJS103	p15a	GFPmut2	$P_{LlacOsym}$	bicistronic	This work
pJS23103	p15a	GFPmut2	$P_{LtetO-1}$	bicistronic/ constitutive	This work
pZH742	pSC101	PP7cp- mNeonGreen	P_{LlacO}	bicistronic	This work
pZH740	p15a	mScarlet- I-24xPP7sl	$P_{LtetO-1}$	constitutive	This work

Cell Growth

Cells were grown in overnight cultures at 32 C in M9 minimal media supplemented with 1% SOB media, 50 μ g/mL carbenicillin or 50 μ g/mL spectinomycin. The next day, cells were diluted 1:100 or more (if multiple experiments were to be done sequentially over several hours) in the same media supplemented with anhydrotetracycline. In this growth condition, doubling time was approximately 30 minutes for MG1655 strains harboring these plasmids.

Microscopy

Microscopy methods and microscopy data analysis methods

Flow cytometry

Flow cytometry methods and data analysis methods

Results

Moving bicistronic autoregulatory construct to a compatible plasmid backbone

The first step in creating a low-noise system for tuning expression of two genes is to establish that a previously characterized bicistronic autoregulatory circuit functions well in a compatible plasmid backbone. In this expression system, GFP and TetR are expressed bicistronically from the TetR-repressible promoter $P_{\text{LtetO-1}}$ and expression is induced by the addition of ATc [14]. This system was shown to have low noise and a linearized dose response compared to a system in which TetR is constitutively expressed. We moved the system from a plasmid with a p15A replicon conferring ampicillin resistance to a lower-copy-number plasmid with a pSC101 replicon conferring spectinomycin resistance [10]. The p15A and pSC101 replicons have been used together in multiplasmid systems [15].

[14] my plosone paper [15] keasling biobricks

GFP expression mean and noise were characterized from low to high levels of induction by flow cytometry. Figure 1 shows that pJS101 induces at similar ATc concentrations as pZH509, with the change to the lower-copy pSC101 backbone resulting in a 58% drop in mean expression levels at a wide range of ATc concentrations. For a similar expression system in the absence of autoregulated TetR expression, moving the $P_{\text{LtetO-1}}$ promoter from a p15A to a pSC101 backbone resulted in an 87% drop in expression [6]. A smaller change is expected in our experiment since negative autoregulation will provide dosage compensation, just autoregulation can reduce noise in plasmid copy number [16,17,18].

[16] dublanche copy number variation [17] becskei serrano autoregulation noise [18] paulsson summing up the noise [6] lutz laco1 etc

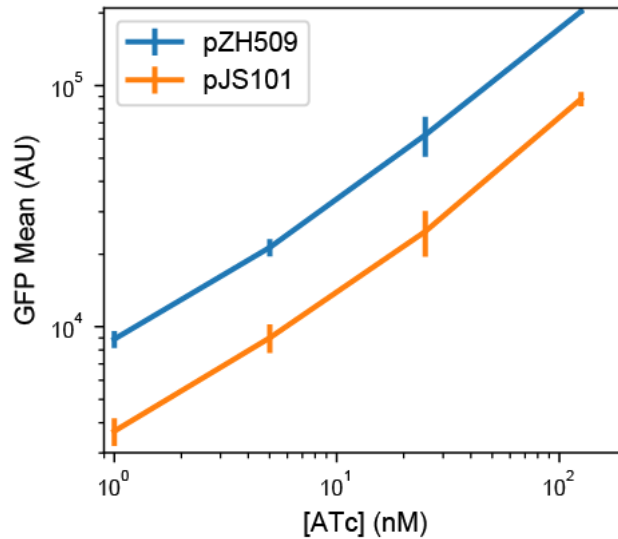


Figure 1: **Influence of plasmid backbone on induction of TetR expression system.** Mean single-cell GFP fluorescence for pZH509 (blue) and pJS101 (orange) plasmids as a function of ATc concentration. Error bars are 1 standard error.

Alternative regulatory constructs with LacI replacing TetR

We hypothesized that replacing $P_{\text{LtetO-1}}$ with the inducible promoter $P_{\text{LlacO-1}}$ with similar characteristics [6] and replacing TetR with LacI might result in a similarly useful expression system that could be tuned independently. However, regulatory parameters for TetR and LacI vary wildly, with ...

binding constants: tetR: LacI:

half induction: TetR ATc Tc LacI IPTG

Figure 2 shows

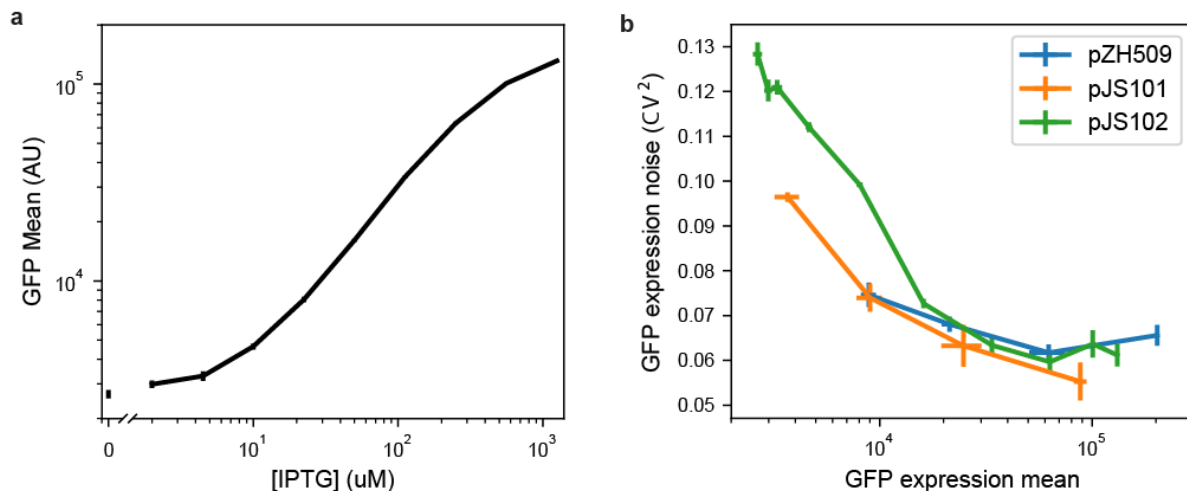


Figure 2: **Characterizing mean expression levels and noise for different gene expression systems.** (a) pJS102 mean induction (b) mean vs noise for all strains.

Using the new induction system for detection of single mRNA in living *E. coli*

blah blah

Figure 3 shows

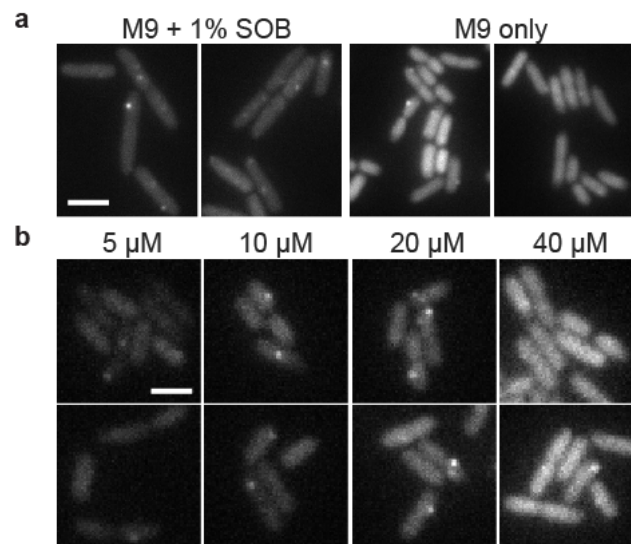


Figure 3: **Using IPTG to tune expression of a fluorescent RNA-binding protein for single mRNA detection in different growth conditions.** (a) 100 μ M IPTG works well in rich media conditions, but has too high an expression level to see single mRNA spots above background in minimal media conditions. (b) Using the pJS102 expression system, an optimal range of 10–20 μ M IPTG is quickly identified for single mRNA detection in minimal media.

Independent, tunable expression of two genes

Aggregation of mRNA-binding proteins from RNA bacteriophages makes it difficult to use fluorescent fusion proteins to detect single mRNAs [19]. We hypothesized that ...

[20] mNeonGreen [21] mScarlet-I [22] The Pf3 coat protein is a small membrane-spanning protein with a cytoplasm-facing C terminus.

Figure 4 shows

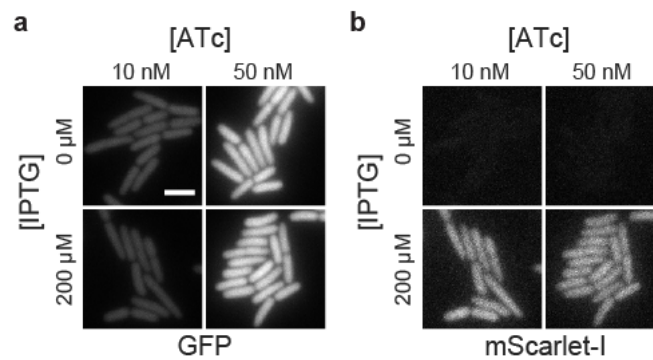


Figure 4: **Using IPTG to tune expression of a fluorescent RNA-binding protein for single mRNA detection in different growth conditions.** (a) 100 μ M IPTG works well in rich media conditions, but has too high an expression level to see single mRNA spots above background in minimal media conditions. (b) Using the pJS102 expression system, an optimal range of 10–20 μ M IPTG is quickly identified for single mRNA detection in minimal media.

Discussion

Possible to improve pJS23103 with somewhat stronger promoter?

Possible to extend to 3 genes? Possible using additional repressor, pMB1 origin of replication, and different antibiotic selection.

Author contributions

JS and ZH designed experiments, performed experiments, analyzed data, and wrote the paper. ZH supervised the project.

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References

1. A plasmid-based Escherichia coli gene expression system with cell-to-cell variation below the extrinsic noise limit

Zach Hensel

PLOS ONE (2017-10-30) <https://doi.org/10.1371/journal.pone.0187259>

2. Quantifying E. coli Proteome and Transcriptome with Single-Molecule Sensitivity in Single Cells

Y. Taniguchi, P. J. Choi, G.-W. Li, H. Chen, M. Babu, J. Hearn, A. Emili, X. S. Xie

Science (2010-07-29) <https://doi.org/10.1126/science.1188308>

3. Noise in transcription negative feedback loops: simulation and experimental analysis

Yann Dublanche, Konstantinos Michalodimitrakis, Nico Kümmerer, Mathilde Foglierini, Luis Serrano

Molecular Systems Biology (2006-08-01) <https://doi.org/10.1038/msb4100081>

4. Enzymatic assembly of DNA molecules up to several hundred kilobases

Daniel G Gibson, Lei Young, Ray-Yuan Chuang, J Craig Venter, Clyde A Hutchison, Hamilton O Smith

Nature Methods (2009-04-12) <https://doi.org/10.1038/nmeth.1318>

5. https://www.addgene.org/Zach_Hensel/

6. Independent and tight regulation of transcriptional units in Escherichia coli via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements.

R Lutz, H Bujard

Nucleic acids research (1997-03-15) <https://www.ncbi.nlm.nih.gov/pubmed/9092630>

7. FACS-optimized mutants of the green fluorescent protein (GFP).

BP Cormack, RH Valdivia, S Falkow

Gene (1996) <https://www.ncbi.nlm.nih.gov/pubmed/8707053>

8. Nucleotide sequence of the repressor gene of the TN10 tetracycline resistance determinant

Kathleen Postle, Toai T. Nguyen, Kevin P. Bertrand

Nucleic Acids Research (1984) <https://doi.org/10.1093/nar/12.12.4849>

9. Intrinsic termination of T7 RNA polymerase mediated by either RNA or DNA.

L Hartvig, J Christiansen

The EMBO journal (1996-09-02) <https://www.ncbi.nlm.nih.gov/pubmed/8887568>

10. A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors.

G Churchward, D Belin, Y Nagamine

Gene (1984-11) <https://www.ncbi.nlm.nih.gov/pubmed/6098521>

11. BglBrick vectors and datasheets: A synthetic biology platform for gene expression

Taek Lee, Rachel A Krupa, Fuzhong Zhang, Meghdad Hajimorad, William J Holtz, Nilu Prasad, Sung Lee, Jay D Keasling

Journal of Biological Engineering (2011) <https://doi.org/10.1186/1754-1611-5-12>

12. A perfectly symmetric lac operator binds the lac repressor very tightly.

JR Sadler, H Sasmor, JL Betz

Proceedings of the National Academy of Sciences of the United States of America (1983-11)
<https://www.ncbi.nlm.nih.gov/pubmed/6316325>

13. <http://parts.igem.org/Promoters/Catalog/Anderson>

14. A plasmid-based Escherichia coli gene expression system with cell-to-cell variation below the extrinsic noise limit.

Zach Hensel

PloS one (2017-10-30) <https://www.ncbi.nlm.nih.gov/pubmed/29084263>

15. BglBrick vectors and datasheets: A synthetic biology platform for gene expression.

Taek Soon Lee, Rachel A Krupa, Fuzhong Zhang, Meghdad Hajimorad, William J Holtz, Nilu Prasad, Sung Kuk Lee, Jay D Keasling

Journal of biological engineering (2011-09-20) <https://www.ncbi.nlm.nih.gov/pubmed/21933410>

16. Noise in transcription negative feedback loops: simulation and experimental analysis.

Yann Dublanche, Konstantinos Michalodimitrakis, Nico Kümmerer, Mathilde Foglierini, Luis Serrano

Molecular systems biology (2006-08-01) <https://www.ncbi.nlm.nih.gov/pubmed/16883354>

17. Engineering stability in gene networks by autoregulation.

A Becskei, L Serrano

Nature (2000-06-01) <https://www.ncbi.nlm.nih.gov/pubmed/10850721>

18. Summing up the noise in gene networks.

Johan Paulsson

Nature (2004-01-29) <https://www.ncbi.nlm.nih.gov/pubmed/14749823>

19. Spatial organization of the flow of genetic information in bacteria

Paula Montero Llopis, Audrey F. Jackson, Oleksii Sliusarenko, Ivan Surovtsev, Jennifer Heinritz,

Thierry Emonet, Christine Jacobs-Wagner

Nature (2010-06-20) <https://doi.org/10.1038/nature09152>

20. A bright monomeric green fluorescent protein derived from *Branchiostoma lanceolatum*

Nathan C Shaner, Gerard G Lambert, Andrew Chammas, Yuhui Ni, Paula J Cranfill, Michelle A Baird, Brittney R Sell, John R Allen, Richard N Day, Maria Israelsson, ... Jiwu Wang

Nature Methods (2013-03-24) <https://doi.org/10.1038/nmeth.2413>

21. mScarlet: a bright monomeric red fluorescent protein for cellular imaging

Daphne S Bindels, Lindsay Haarbosch, Laura van Weeren, Marten Postma, Katrin E Wiese, Marieke Mastop, Sylvain Aumonier, Guillaume Gotthard, Antoine Royant, Mark A Hink, Theodorus WJ Gadella

Nature Methods (2016-11-21) <https://doi.org/10.1038/nmeth.4074>

22. Negatively charged amino acid residues play an active role in orienting the Sec-independent Pf3 coat protein in the *Escherichia coli* inner membrane

Dorothee Kiefer, Xintong Hu, Ross Dalbey, Andreas Kuhn

The EMBO Journal (1997-05-01) <https://doi.org/10.1093/emboj/16.9.2197>