New plasmids for low-noise gene expression in Escherichia coli

This manuscript (permalink) was automatically generated from zach-hensel/low-noise-manuscript@72dd71b on September 3, 2018.

Authors

- **Zach Hensel**
  0000-0002-4348-6229 · zach-hensel · alchemytoday
  Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal · Funded by Project LISBOA-01-0145-FEDER-007660 (Microbiologia Molecular, Estrutural e Celular)

- **João Silva**
  XXXX-XXXX-XXXX-XXXX
  Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

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, and bicistronic gene expression to reduce gene expression noise. We report several new plasmids that use the same principles to achieve simultaneous, low-noise expression for two genes. This system was moved to a compatible plasmid backbone, and a system based on the lactose repressor was found to also exhibit gene expression noise below the extrinsic noise limit. We further investigated the effects of increasing repressor binding strength and the addition of a weak constitutive promoter on protein induction and expression noise.

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 µL 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 $P_{LtetO-1}$ 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 µL 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	$_{LtetO-1}$	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 µg/mL carbenicillin or 50 µg/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

Info and testing for pJS101

Info for pJS102 / pJS103

Figure 2: pZH509 vs pJS101 (a) mean(aTc), (b) noise(mean) flow cytometry

Alternative regulatory constructs with LacI replacing TetR

We hypothesized that replacing $P_{LtetO-1}$ with a promoter with similar characteristics and replacing TetR with a …

Figure 3: pZH509 vs pJS102 vs pJS103 (a) mean(aTc), (b) noise(mean), (c) Microscope images at mid induction, (d) noise(mean) from microscope images

Increasing dynamic range with the addition of a weak constitutive promoter

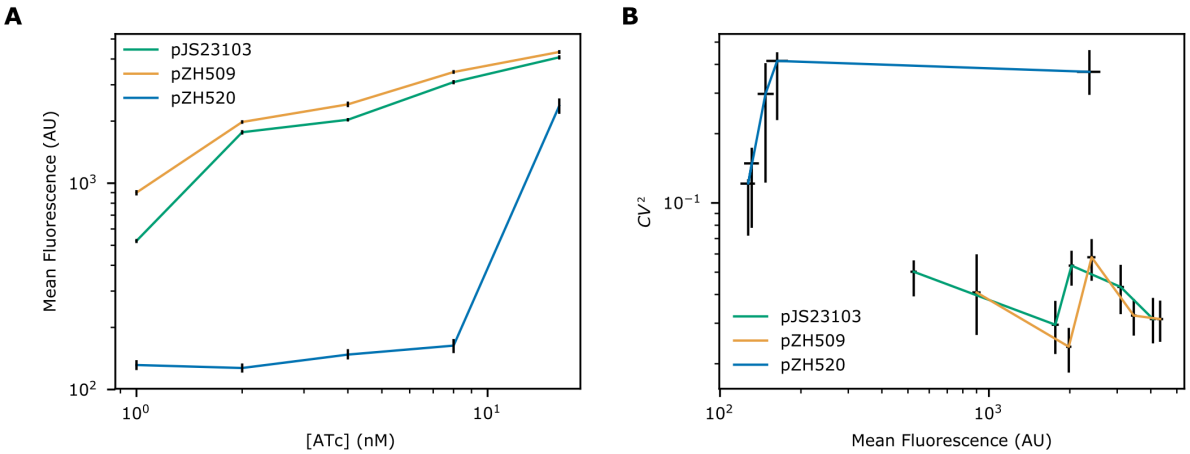

Figure 1: (**A**) Mean GFP fluorescence for pJS23103, pZH509 and pZH520 as a function of ATc concentration. The additional weak, constitutive promoter for TetR in pJS23103 results in lower GFP expression at low ATc concentrations. (**B**) Noise (CV^2) as a function of mean GFP expression for the same strains. pJS23103 retains the low gene expression noise characteristic of pZH509. Error bars in both plots are bootstrapped 95% confidence intervals from a single experiment. The mean and variance of the pZH501 strain which does not express GFP was subtracted from each sample.

Figure 4: ZH509 vs pZH520 vs pJS23103 (a) mean(aTc), (b) noise(mean), (c) Microscope images at mid induction, (d) noise(mean) from microscope images

Expression of two genes by remixing these components

Aggregation of mRNA-binding proteins from RNA bacteriophages makes it difficult to use fluorescent fusion proteins to detect single mRNAs [14]. We hypothesized that …

[15] mNeonGreen [16] mScarlet-I [17] The Pf3 coat protein is a small membrane-spanning protein with a cytoplasm-facing C terminus.

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.

Acknowledgments

This work was financially supported by: Project LISBOA-01-0145-FEDER-007660 (Microbiologia Molecular, Estrutural e Celular) funded by FEDER funds through COMPETE2020—Programa Operacional Competitividade e Internacionalização (POCI), by national funds through FCT—Fundação para a Ciência e a Tecnologia, and through a joint research agreement with the Okinawa Institute of Science and Technology (OIST).

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. **Addgene: Zach Hensel Lab Plasmids**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. **Promoters/Catalog/Anderson - parts.igem.org**http://parts.igem.org/Promoters/Catalog/Anderson

14. **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

15. **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

16. **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

17. **Negatively charged amino acid residues play an active role in orienting the Sec-independent Pf3 coat protein in theEscherichia coliinner membrane**
Dorothee Kiefer, Xintong Hu, Ross Dalbey, Andreas Kuhn
The EMBO Journal (1997-05-01) https://doi.org/10.1093/emboj/16.9.2197