New plasmids for low-noise gene expression in Escherichia coli

This manuscript (permalink) was automatically generated from zach-hensel/low-noise-manuscript@c0fd71b on August 31, 2018.

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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, 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.

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, GFPmut2 [6], tn10 TetR [7] and rrnB T1 transcription terminator [8]. This was assembled by isothermal assembly with the backbone from pGB2 [9] 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 [10].

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. 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 [11].

amplifying pZH509 by inverse PCR, adding

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

blah

Flow cytometry

ok

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