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

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Supporting Material

DNA sequence for bicistronic, autoregulatory sequence. The synthetic sequence incorporated into pZH509 is shown below. Transcription start and translation start/stop signals are capitalized and bolded and other features are marked as indicated:

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tccctatcagtgatagagattgacatccctatcagtgatagagatactgagcacAtcagcaggacgcactgaccg
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gctagaggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcgttttat
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TetO2 GFPmut2 TetR *rrnB* T1

41 *DNA sequences for "constitutive expression" (pZH514, pZH515, pZH516).* The sequence is
42 shown for pZH514 and annotated as above. The same mutated ribosome binding sites used
43 for pZH511 (weak RBS) and pZH512 (moderate RBS) are used in pZH515 and pZH516,
44 respectively.

45 tccctatcagtgatagagattgacatccctatcagtgatagagatactgagcac**A**tcagcaggacgcactgaccg
46 aattcattaaagaggagaaaggtaccgc**ATG**agtaaaggagaagaacttttcactggagttgtcccaattcttgt
47 tgaattagatggtgatgttaatgggcacaaattttctgtcagtggagagggtgaaggtgatgcaacatacggaaa
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55 ccacatggtccttcttgagtttgtaacagctgctgggattacacatggcatggatgaactatacaaa**TAATAA**tc
56 tagccatggtacgcgtgctagaggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcgttttat
57
58 TetO2 GFPmut2 *rrnB* T1
59

60

DNA sequences for "autoregulated repressor" (pZH517, pZH518, pZH519). The sequence is shown for pZH517 and annotated as above. The same mutated ribosome binding sites used for pZH511 (weak RBS) and pZH512 (moderate RBS) are used in pZH518 and pZH519, respectively.

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TetO2 GFPmut2 TetR *rrnB* T1

DNA sequences for "constitutive repressor" (pZH520, pZH521, pZH522). The sequence is shown for pZH520 and annotated as above. The same mutated ribosome binding sites used for pZH511 (weak RBS) and pZH512 (moderate RBS) are used in pZH521 and pZH522, respectively.

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agactgggcctttcgttttat

TetO2 GFPmut2 *rrnB* T1 proB TetR

122 # Supplemental Tables

123 **Table A.** Ribosome binding site strength estimates compared to GFP expression levels

124 (induction with 8 nM ATc) measured by flow cytometry (**Fig 3B**). GFP start codon and

125 mutations from pZH509 sequence are capitalized.

Plasmid	RBS sequence	RBS strength	GFP expression	Ratio
pZH509	aaagaggagaaaggtaccgcATG	12,905	1,175.5	10.98
pZH510	aaaTaggagaaaggtaccgcATG	7,189	998.2	7.20
pZH513	aaaCTCgagaaaggtaccgcATG	3,307	644.3	5.13
pZH512	aaagCCgagaaaggtaccgcATG	2,016	509.6	3.96
pZH511	aaaTTCgagaaaggtaccgcATG	1,471	179.2	8.21

126

127 **Table B.** Bootstrap fitting GFP expression distributions measured by fluorescence

128 microscopy. The convolution of the autofluorescence (pZH501) distribution and a log-

129 normal distribution is fit to 100 bootstrapped samples of sizes identical to the experimental

130 samples (samples of size N, sampled with replacement). This results in 100 estimates of the

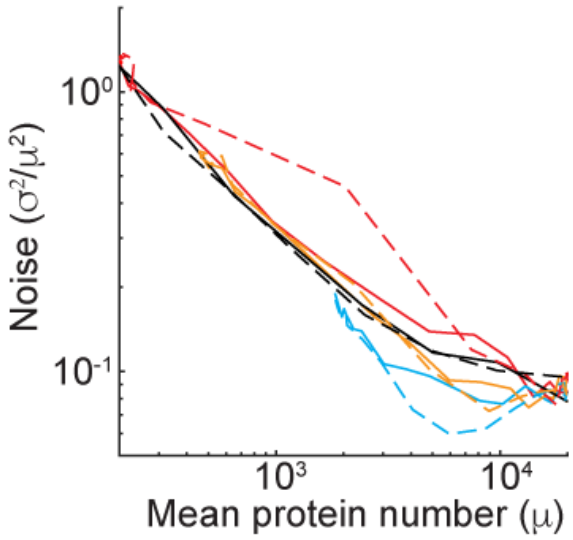
131 GFP expression mean (molecules/cell) and unitless noise (σ^2/μ^2). The mean and standard

132 deviation of these estimates is reported here.

Strain	[ATc] (nM)	Mean (molecules/cell)	Noise	N
pZH509	0	283.3 +/- 4.2	0.0440 +/- 0.0059	484
	0.5	1763.3 +/- 28.0	0.0792 +/- 0.0099	751
	8	4124.3 +/- 44.4	0.0563 +/- 0.0048	733
	32	7989.8 +/- 114.8	0.0821 +/- 0.0069	719
pZH511	0	54.7 +/- 1.2	0.0868 +/- 0.0191	893
	0.5	234.7 +/- 5.3	0.0586 +/- 0.0098	369
	8	322.9 +/- 4.4	0.0795 +/- 0.0088	764
	32	671.1 +/- 8.3	0.0468 +/- 0.0051	560
pZH512	0	120.3 +/- 3.7	0.0705 +/- 0.0143	424
	0.5	600.6 +/- 8.2	0.0846 +/- 0.0077	702
	8	870.2 +/- 11.1	0.0712 +/- 0.0056	800
	32	1871.2 +/- 24.5	0.0696 +/- 0.0047	809

133

134 ## Supplemental Figures



135

136 **Fig A**. Noise reduction for simulations in which free inducer numbers are held constant

137 (solid lines; identical to **Fig 4C**) or where inducer is removed when bound to repressor

138 (dashed lines) for constitutive expression (black), repressed expression (red), bicistronic

139 autoregulated repression (cyan), and hybrid regulation (orange).

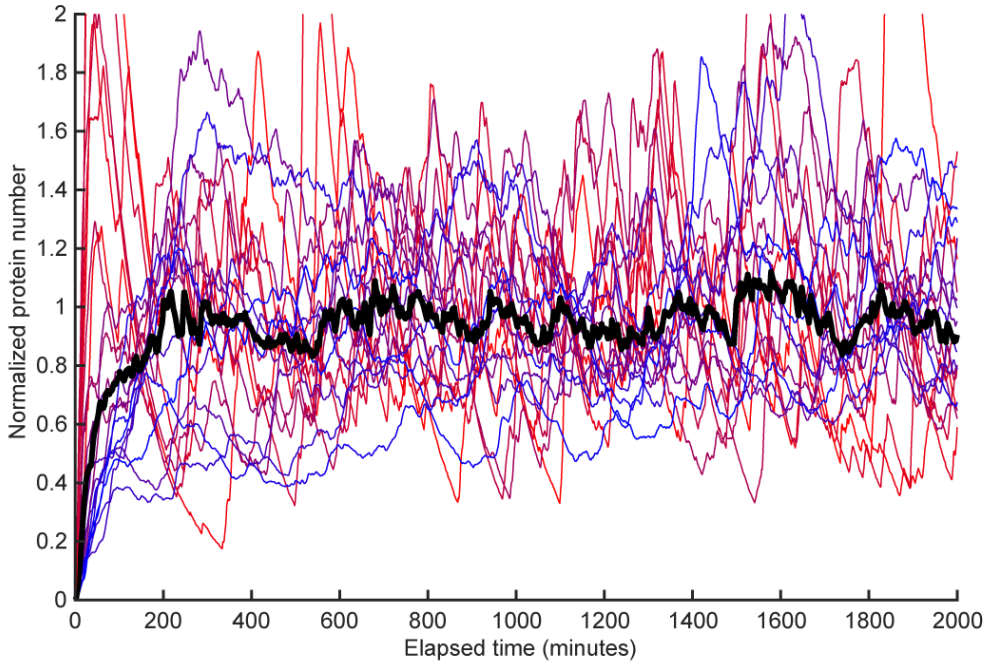


Fig B. First 2,000 minutes of simulated transgene expression for bicistronic, autoregulated construct at 21 different inducer levels ranging from 0 (red) to 524,288 molecules (blue), including extrinsic noise (data used for solid cyan lines in **Fig 1B** and **Fig 1C**). Protein levels are scaled by mean protein level calculated from time points from 1,000—101,000 minutes. The median of the 21 traces (thick black line) shows that equilibrium levels are typically reached after a few hundred minutes.

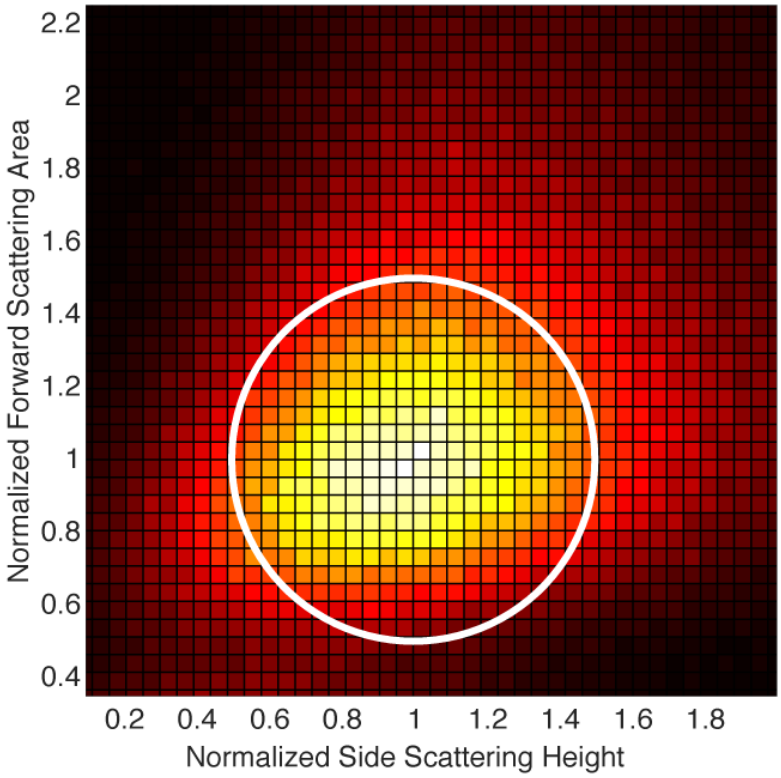
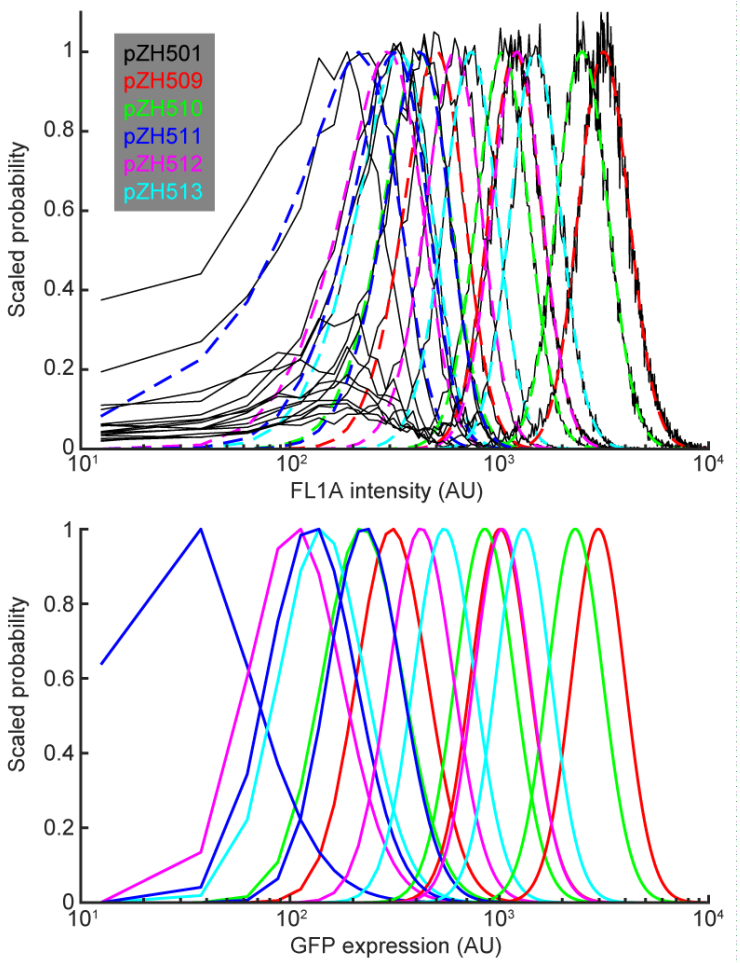


Fig C. Histogram of forward-scattering area and side-scattering height for all pZH509 data used for analysis underlying **Fig 2B**. The white circle indicates the gate used for selecting the >30% of cells in the neighborhood of the scattering peak.



153

154 **Fig D**. Top, raw GFP flow cytometry histograms (black lines) used for analysis underlying **Fig**

155 **3B** with fits (dashed, lines colored as indicated for different plasmids) to convolution of the

156 pZH501 distribution (no GFP, unfit black line) with log-normal distributions. Bottom,

157 resulting log-normal distributions after fit; lines are colored according to strain identity with

158 means monotonically increasing with ATc concentrations (2, 8, 32 nM).

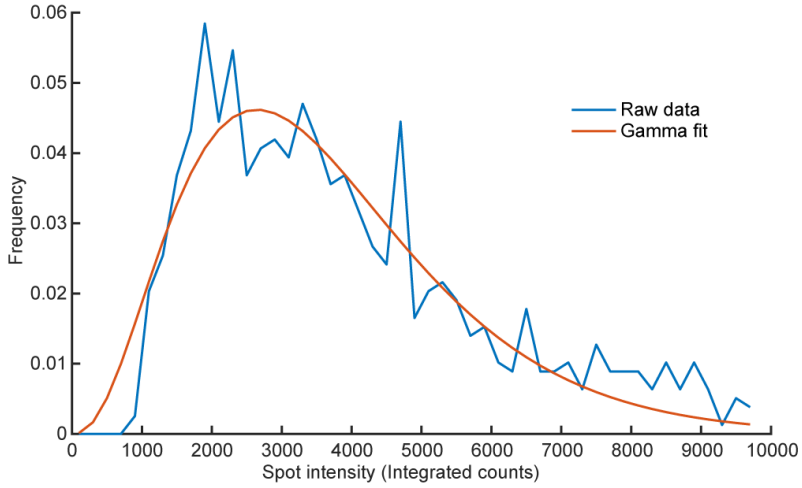


Fig E. Single-molecule spot intensity histogram (blue) fit to a gamma distribution (red) to estimate single-molecule intensities from GFP images at high laser power for pZH509 after photobleaching nearly all GFP molecules.

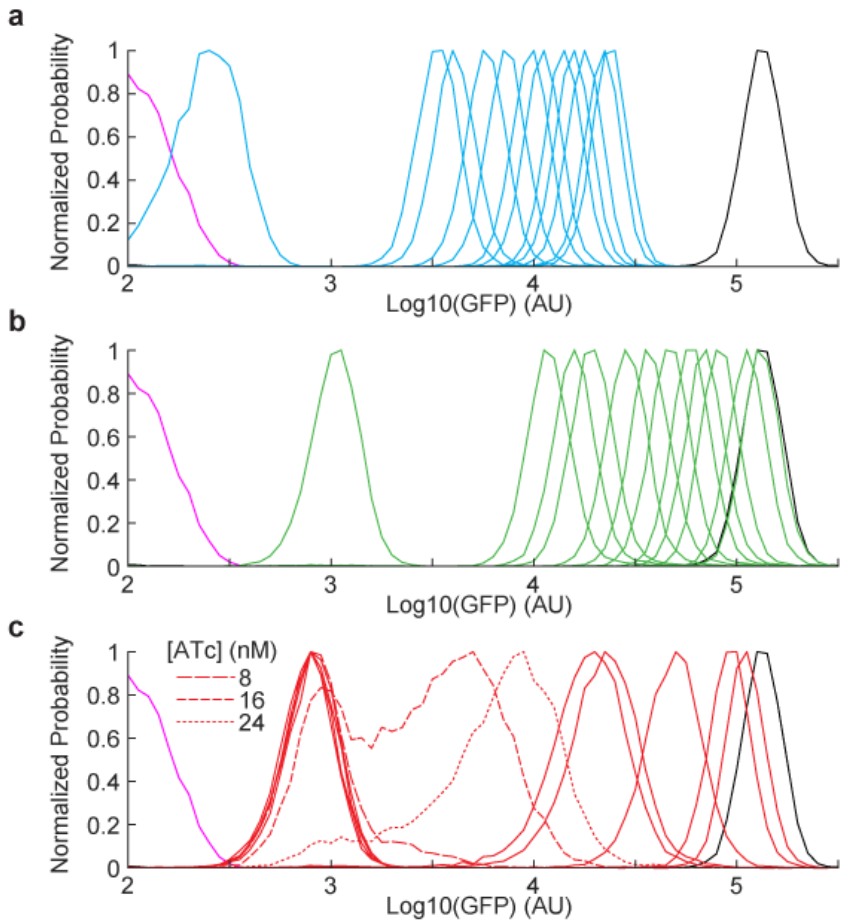


Fig F. Distribution of GFP fluorescence for alternative regulatory constructs measured by flow cytometry. Normalized probability of GFP fluorescence is shown for ATc concentrations of 0, 1, 2, 4, 8, 16, 24, 32, 40, 64, 128 and 256 nM (monotonic increase in expression with increasing ATc). (**a**) Bicistronic autoregulation plasmid pZH509. (**b**) Autoregulated repressor plasmid pZH517. (**c**) Constitutive repressor plasmid pZH520; three ATc concentrations are shown in differently dashed lines to show conditions giving high noise. For pZH520, [ATc] less than 8 nM gives very similar distributions.