A biosensor strategy for *E. coli* based on ligand-dependent stabilization

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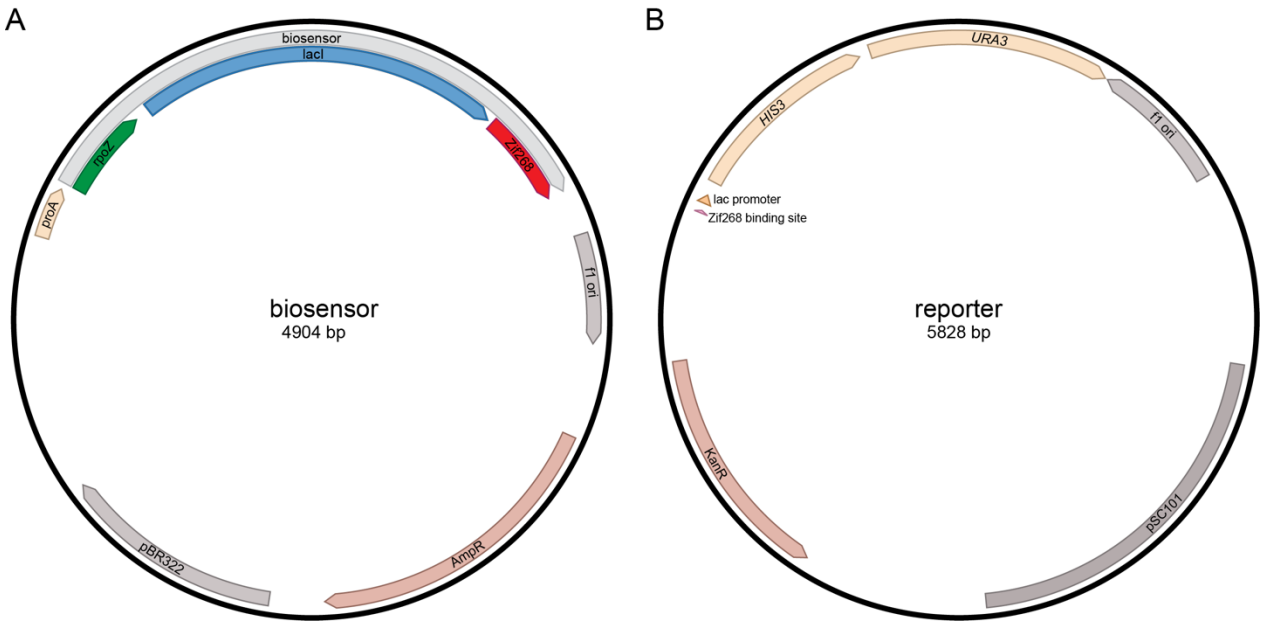

Figure S1. Plasmid maps of the biosensor and reporter plasmids. **A.** Map of plasmid encoding LacI-based biosensors and protein fusions. Biosensor expression is driven by the constitutively active *proA* promoter. The amino acid linkers between protein domains were chosen based on the original amino acid linker separating RpoZ and Zif268 in the bacterial one-hybrid assays.[1] The amino acid sequence between RpoZ and LacI is AAADYKDDDDKFRTG, and the amino acid sequence between LacI and Zif268 is SKTPPHGT. **B.** Map of plasmid encoding *HIS3 and URA3* reporter genes. *HIS3* expression is driven by a weak promoter derived from the native *lac* promoter. The Zif268 binding site is located 10 base pairs upstream of the -35 region of the promoter.

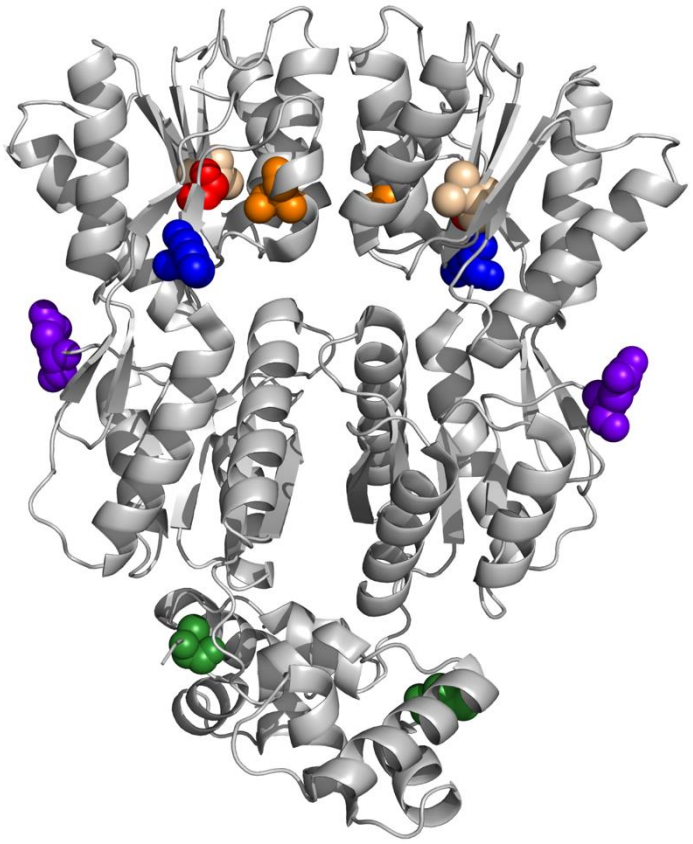

Figure S2. Structure of LacI dimer (taken from a co-crystal with DNA) showing amino acids 1-333 (PDB: 1EFA).[2] The location of the six amino acid mutations in L1.0 colored and shown as spheres (V4L: green; Q153L: violet; V244I: tan; G272R: red; S279R: orange; K290I: blue).

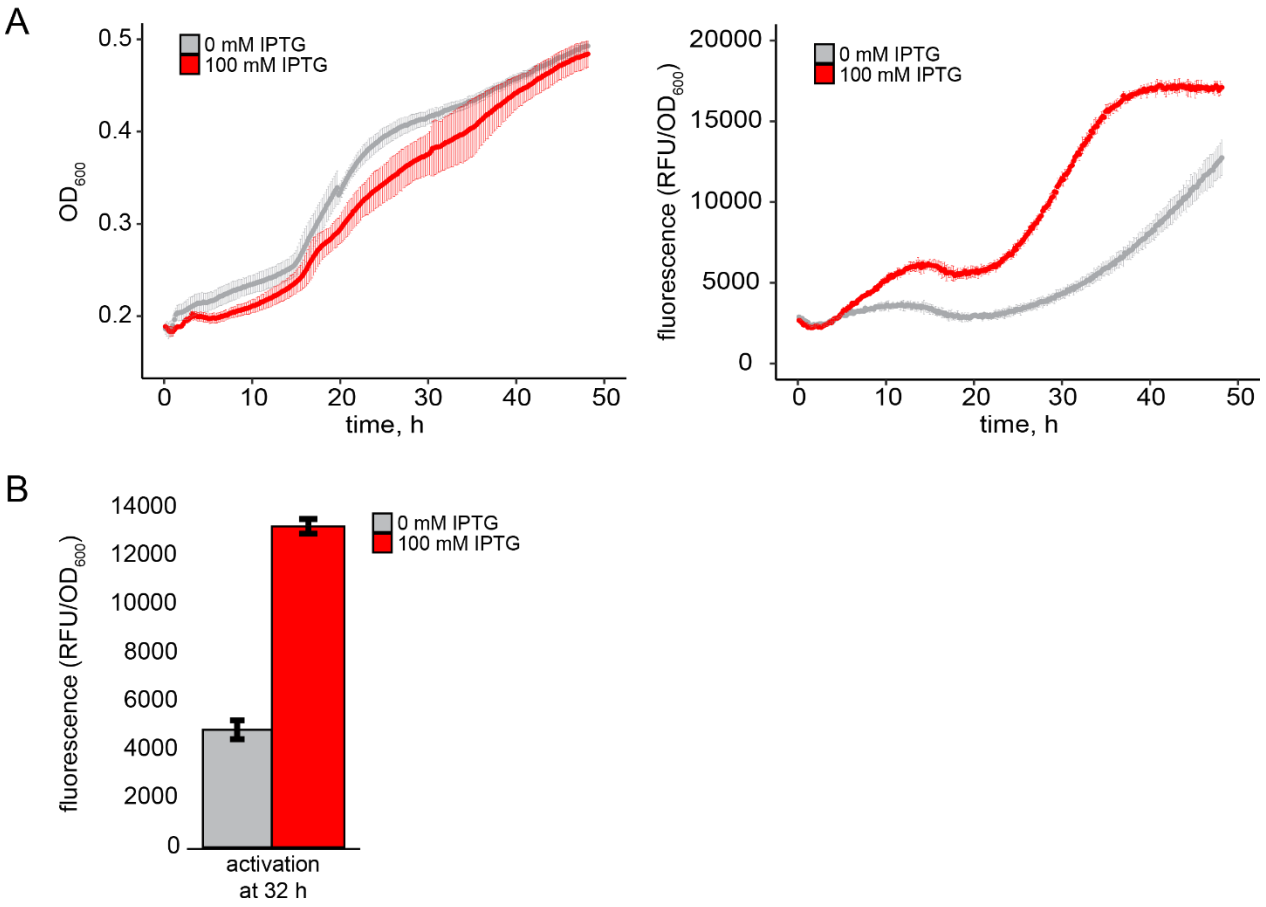

Figure S3. Biosensor L1.7-based activation of GFP. **A.** Plots showing OD_{600} and OD_{600}-normalized GFP with 0 mM and 100 mM IPTG. Each time point shows mean OD_{600} or mean fluorescence (n = 3 biological replicates; error bars represent ± one standard deviation). **B.** GFP expression at 32 h (from data in panel A). Bar plot shows mean fluorescence (n = 3 biological replicates; error bars represent ± one standard deviation).

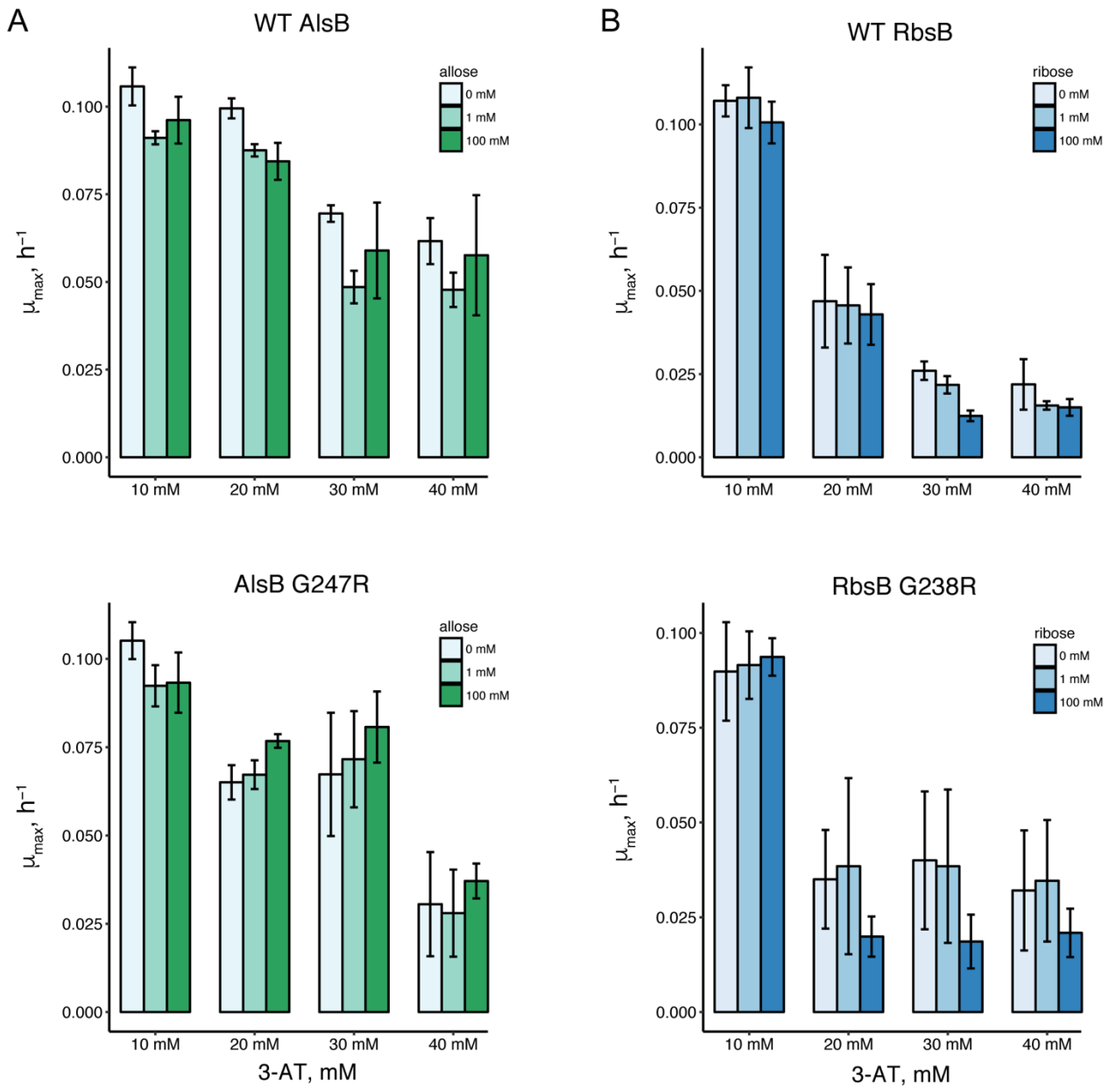

Figure S4. Response of protein fusions constructed from periplasmic binding proteins at 10-40 mM 3-AT. **A.** AlsB-based protein fusions induced with 0 mM, 1 mM, and 100 mM D-allose. **B.** RbsB-based protein fusions induced with 0 mM, 1 mM, and 100 mM D-ribose. Bar plots show mean μ_{max} (n = 3 biological replicates; error bars represent ± one standard deviation).

PCR and cloning procedures

General protocol for Gibson assembly

PCR product was assembled, together with linearized plasmid backbone, using New England Biolabs NEBuilder HiFi DNA Assembly Master Mix in a 10 μL reaction, incubating at 50 °C for 1 h. 2 μL of this product was transformed into chemically competent DH10B cells, and plasmid selection was performed on agar plates with LB supplemented with appropriate antibiotic. Plasmids were isolated using a Qiagen Qiaprep Spin Miniprep kit, and correct assembly of the desired biosensor construct was verified by Sanger sequencing.

Protocol for linearizing pB1H2ω2-Zif268: pB1H2ω2-Zif268 was linearized by inverse PCR with primers pB1H2ω2_Zif268 F and pB1H2ω2_Zif268 R using Kappa HiFi 2× Master Mix. The PCR product was desalted and concentrated using the Zymo Clean and Concentrate Kit, eluted into water, and used for cloning biosensors.

Protocol for cloning RpoZ-LacI-Zif268, RpoZ-AlsB-Zif268, and RpoZ-RbsB-Zif268: Each ligand binding domain was PCR amplified using primers that contain regions of homology for cloning by Gibson assembly. The PCR product was assembled with linearized pB1H2ω2-Zif268 using Gibson assembly.

Protocol for cloning proA constitutive promoter into L1.0: The *proA* promoter sequence containing 30 bp of homology to the adjacent regions of the transcriptional activator construct was synthesized as a gBlock by IDT. Together with L1.0 linearized by inverse PCR using primers BMB D133 and BMB D134 to remove the lac promoter and operator, this gBlock was assembled using Gibson assembly.

Protocol for reverting single amino acid mutations in L1.0 to generate L1.1-L1.6: L1.1 was generated by PCR amplification of the L1.0 ligand-binding domain with primers BMB D006 and BMB D007, and subsequently assembled with linearized pB1H2ω2-Zif268 using Gibson assembly. L1.2-L1.6 were generated using overlapping PCR primers, together with primers BMB D012 and BMB D013, to generate two overlapping PCR products of the L1.0 ligand-binding domain with the corresponding amino acid reverted back to its wild type identity. These two overlapping PCR products were assembled with linearized pB1H2ω2-Zif268 using Gibson assembly.

General protocol for inverse PCR

Two primers annealing adjacent to one another and oriented in opposite directions were used to introduce point mutations by PCR. Briefly, a 50 μL reaction containing 25 pmol of each primer, 1 ng of plasmid template, 250 μM dNTPs, 10 μL of 5× Phusion HF buffer, and 0.5 μL of Phusion polymerase was thermocycled for 25 cycles according to the following PCR protocol: 95 °C for 30 s, 24× (95 °C for 10 s, 65 °C for 10 s, 72 °C for 90 s), 72 °C for 4 min. The PCR product was assessed for correct size and lack of side products by running on a 1% agarose gel. A 20 μL reaction containing 4 μL of PCR product, 2 μL of 10x T4 DNA ligase buffer, and 1 μL of each DpnI, T4 PNK, and T4 DNA ligase was incubated at room temperature for 1 h. 2 μL of this product was transformed into chemically competent DH10B cells, and cells harboring plasmid were selected by plating on agar plates of LB supplemented with appropriate antibiotic. Plasmids were isolated using a Qiagen Qiaprep Spin Miniprep kit, and correct introduction of the desired mutation was verified by Sanger sequencing.

L1.7: L1.7 was generated by inverse PCR using primers BMB D104 and BMB D105 and RpoZ-LacI-Zif268 as template.

L1.7 + Q291T: L1.7 + Q291T was generated by inverse PCR using primers BMB D145 and BMB D146 and L1.7 as template.

Zif268 binding site mutant 1: Zif268 binding site mutant 1 was generated by inverse PCR using primers BMB D020 and BMB D019 and pH3U3-Zif268 as template.

Zif268 binding site mutant 2: Zif268 binding site mutant 2 was generated by inverse PCR using primers BMB D021 and BMB D019 and pH3U3-Zif268 as template.

Zif268 binding site mutant 3: Zif268 binding site mutant 3 was generated by inverse PCR using primers BMB D018 and BMB D019 and pH3U3-Zif268 as template.

RpoZ-AlsB G247R-Zif268: RpoZ-AlsB G247R-Zif268 was generated by inverse PCR using primers BMB D180 and BMB D181 and RpoZ-AlsB-Zif268 was template.

RpoZ-RbsB G238R-Zif268: RpoZ-RbsB G238R-Zif268 was generated by inverse PCR using primers BMB D186 and BMB D187 and RpoZ-RbsB-Zif268 was template.

Cloning of the library of MphR variants

Protocol for cloning erythromycin resistance gene into the biosensor backbone: The erythromycin resistance cassette *eryR* was PCR amplified from plasmid pJKR-H-mphR[3] using primers BMB D082 and BMB D083 and Kappa HiFi 2× Master Mix. Plasmid pB1H2ω2-Zif268 was linearized using EcoRI and desalted and concentrated using the Zymo Clean and Concentrate Kit. The PCR product and linearized vector were cloned using Gibson assembly.

Protocol for cloning mphR into the biosensor_eryR backbone: Plasmid pB1H2ω2-Zif268_eryR was linearized by inverse PCR with primers pB1H2ω2_Zif268 F and pB1H2ω2_Zif268 R using Kappa HiFi 2× Master Mix. The PCR product was desalted and concentrated using the Zymo Clean and Concentrate Kit. The *mphR* gene was PCR-amplified from plasmid plasmid pJKR-H-mphR using primers mphR_F and mphR_R, and cloned into linearized pB1H2ω2-Zif268_eryR using Gibson assembly.

Protocol for cloning the library of MphR variants: Error-prone PCR was performed using the MphR biosensor plasmid and primers BMB D012 and BMB D013 as described for the *lacI* gene. This PCR product was cloned into linearized pB1H2ω2-Zif268_eryR using Gibson assembly.

Transformation of the library of MphR variants: Gibson assembly product of MphR was desalted and concentrated using the Zymo Clean and Concentrate Kit. Invitrogen Electromax DH10B *E. coli* cells were transformed by electroporation with the Gibson assembly product and recovered in 1 mL of SOC shaking at 37 °C for 1 h. 1% and 0.1% of cells were plated on LB+Amp plates to estimate the number of unique transformants, and the remainder of the cells were grown overnight at 37 °C in 100 mL of LB supplemented with 100 μg/mL of carbenicillin. Plasmid was isolated from 5 mL of culture using the Qiagen miniprep kit.

200 ng of plasmid containing the error-prone PCR library plasmid was cotransformed with 200 ng of pH3U3-Zif268 (omega) into 200 μL of electrocompetent US0 *hisB*Δ *pyrF*Δ *rpoZ*Δ cells in four 50 μL transformations. The cells from each transformation were recovered by shaking in 1 mL of SOC media for 1 h at 37 °C. 1% of cells was plated on LB plates supplemented with ampicillin and kanamycin to estimate the number of unique colonies from the library transformation. The rest of the transformation was grown overnight at 37 °C in LB media supplemented with ampicillin and kanamycin, and frozen in 25% glycerol for use in subsequent selection experiments as described in the Materials and Methods section of the main text.

Plasmid	Antibiotic resistance marker	Description
pB1H2ω2-Zif268	Ampicillin	RpoZ-Zif268 direct fusion; mutated *lacUV5* promoter
pB1H2ω2-LacI-Zif268	Ampicillin	RpoZ-LacI-Zif268; mutated *lacUV5* promoter
pB1H2ω2-L1.0-Zif268	Ampicillin	RpoZ-L1.0-Zif268 biosensor; mutated *lacUV5* promoter
pB1H2ω2-LacI-Zif268_proA	Ampicillin	RpoZ-LacI-Zif268; *proA* constitutive promoter
L1.0 biosensor	Ampicillin	RpoZ-L1.0-Zif268 biosensor; *proA* constitutive promoter
L1.0 (rpoZ∆) biosensor	Ampicillin	RpoZ-L1.0-Zif268 biosensor; *proA* constitutive promoter
L1.1 biosensor	Ampicillin	RpoZ-L1.1-Zif268; V4L revert biosensor, *proA* constitutive promoter
L1.2 biosensor	Ampicillin	RpoZ- L1.2-Zif268 Q153L revert biosensor, *proA* constitutive promoter
L1.3 biosensor	Ampicillin	RpoZ- L1.3-Zif268 V244I revert biosensor, *proA* constitutive promoter
L1.4 biosensor	Ampicillin	RpoZ- L1.4-Zif268 G272R revert biosensor, *proA* constitutive promoter
L1.5 biosensor	Ampicillin	RpoZ-L1.5-Zif268 S279R revert biosensor, *proA* constitutive promoter
L1.6 biosensor	Ampicillin	RpoZ-L1.6-Zif268 K290I revert biosensor, *proA* constitutive promoter
L1.7 biosensor	Ampicillin	RpoZ-L1.7-Zif268; *proA* constitutive promoter
pH3U3-Zif268 (omega)	Kanamycin	Reporter with Zif268 DNA binding site upstream of *HIS3*
pH3U3-Zif268 (omega)_GFP	Chloramphenicol	Reporter with Zif268 DNA binding site upstream of sfGFP
pH3U3-Zif268 (omega)_binding site mutant 1	Kanamycin	Reporter with mutated Zif268 binding site upstream of *HIS3*; binding site = GCGTGGGaG
pH3U3-Zif268 (omega)_binding site mutant 2	Kanamycin	Reporter with mutated Zif268 binding site upstream of *HIS3*; binding site = GCGTGGGca
pH3U3-Zif268 (omega)_binding site mutant 3	Kanamycin	Reporter with mutated Zif268 binding site upstream of *HIS3*; binding site = GCGTGGGgG
L1.7 biosensor + Q291T	Ampicillin	RpoZ-L1.7 Q291T-Zif268; *proA* constitutive promoter
RpoZ-LacI Q291T-Zif268	Ampicillin	RpoZ-LacI Q291T-Zif268; *proA* constitutive promoter
RpoZ-AlsB-Zif268 fusion	Ampicillin	RpoZ-AlsB-Zif268; *proA* constitutive promoter
RpoZ-AlsB G247R-Zif268 fusion	Ampicillin	RpoZ-AlsB G247R-Zif268; *proA* constitutive promoter
RpoZ-RbsB-Zif268 fusion	Ampicillin	RpoZ-RbsB-Zif268; *proA* constitutive promoter
RpoZ-RbsB G238R-Zif268 fusion	Ampicillin	RpoZ-RbsB G238R-Zif268; *proA* constitutive promoter
RpoZ-MphR-Zif268	Ampicillin	RpoZ-MphR-Zif268; mutated *lacUV5* promoter
RpoZ-M1.0-Zif268	Ampicillin	RpoZ-M1.0-Zif268; mutated *lacUV5* promoter

Table S1. Plasmids used in this study.

Oligonucleotide	**Purpose**	DNA Sequence
pB1H2ω2_Zif268 F	Inverse PCR of pB1H2ω2_Zif268 to insert ligand-binding domain between RpoZ and Zif268	TCCAAGACACCCCCCCATGGTACC
pB1H2ω2_Zif268 R	Inverse PCR of pB1H2ω2_Zif268 to insert ligand-binding domain between RpoZ and Zif268	ACCGGTCCGGAACTTGTCGTCGTC
BMB D006	Amplification of LacI from pGEX-4T2 and cloning by Gibson assembly into linearized pB1H2ω2-Zif268	AAGGATGACGACGACAAGTTCCGGACCGGTgtgaaaccagtaacgttatacgatgtcgca
BMB D007	Amplification of LacI from pGEX-4T2 and cloning by Gibson assembly into linearized pB1H2ω2-Zif268	TGGGCGGGTACCATGGGGGGGGTGTCTTGGActgcccgctttccagtcgggaaac
BMB D012	Error-prone PCR of LacI in RpoZ-LacI-Zif268 fusion; reversion of single AA mutations	GACGACGACAAGTTCCGGACCGGT
BMB D013	Error-prone PCR of LacI in RpoZ-LacI-Zif268 fusion; reversion of single AA mutations	GGTACCATGGGGGGGGTGTCTTGGA
proA promoter	proA constitutive promoter for biosensor expression (synthesized as gBlock)	CACAGCTAACACCACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACTTTACGGGCATGCATAAGGCTCGtaggctATATTCAGGGAGACCACAACGGTTTCCCTCTACAAATAATTTTGTTTAACTTTTCACACAGGAAAGtactag
BMB D133	Linearization of biosensor plasmid to clone *proA* promoter	ATGGCACGCGTAACTGTTCAGGACG
BMB D134	Linearization of biosensor plasmid to clone *proA* promoter	CCGTTCCAGTAACCGGGCATGTTC
BMB D064	Reversion of Q153L mutation in L1.0	CTTGATGTCTCTGACCaGACACCCATCAACAGT
BMB D065	Reversion of Q153L mutation in L1.0	ACTGTTGATGGGTGTCtGGTCAGAGACATCAAG
BMB D066	Reversion of V244I mutation in L1.0	ACTGCGATGCTGgTTGCCAACGATCA
BMB D067	Reversion of V244I mutation in L1.0	TGATCGTTGGCAAcCAGCATCGCAGT

BMB D068	Reversion of G272R mutation in L1.0	CGGATATCTCGGTAGTGgGATACGACGATACCGA
BMB D069	Reversion of G272R mutation in L1.0	TCGGTATCGTCGTATCcCACTACCGAGATATCCG
BMB D070	Reversion of S279R mutation in L1.0	CGATACCGAAGACAGcTCATGTTATATCCCGC
BMB D071	Reversion of S279R mutation in L1.0	GCGGGATATAACATGAgCTGTCTTCGGTATCG
BMB D072	Reversion of K290I mutation in L1.0	CGTTAACCACCATCAaACAGGATTTTCGCCTG
BMB D073	Reversion of K290I mutation in L1.0	CAGGCGAAAATCCTGTtTGATGGTGGTTAACG
BMB D104	Introduction of G272R mutation in RpoZ-LacI-Zif268	CGGATATCTCGGTAGTGaGATACGACGATACCGA
BMB D105	Introduction of G272R mutation in RpoZ-LacI-Zif268	TCGGTATCGTCGTATCtCACTACCGAGATATCCG
BMB D020	Zif268 binding site mutant 1 in pH3U3-Zif268 (omega)	GCTGCGTGGGaGGGACGAATTC
BMB D021	Zif268 binding site mutant 2 in pH3U3-Zif268 (omega)	GCTGCGTGGGcaGGACGAATTCTTTAC
BMB D018	Zif268 binding site mutant 3 in pH3U3-Zif268 (omega)	GCTGCGTGGGgGGGACGAATTC
BMB D019	All mutated Zif268 binding sites in pH3U3-Zif268 (omega)	GGCCGCCCGGGTGTACAC
BMB D145	Introduction of Q291T mutation in L1.7	CACCATCAAAaccGATTTTCGCCTGCTG
BMB D146	Introduction of Q291T mutation in L1.7	GTTAACGGCGGGATATAAC
BMB D166	Amplification of AlsB from *E. coli* genome and cloning by Gibson assembly into linearized pB1H2ω2-Zif268	AAGGATGACGACGACAAGTTCCGGACCGGTAATA AATATCTGAAATATTTCAGCGGCACA
BMB D167	Amplification of AlsB from *E. coli* genome and cloning by Gibson assembly into linearized pB1H2ω2-Zif268	TGGGCGGGTACCATGGGGGGGTGTCTTGGATTG AGTGACCAGGATTGAATCGAC
BMB D180	Introduction of G247R mutation in RpoZ-AlsB-Zif268 fusion	GCTGGTCGTCaggACAGATGGCA

BMB D181	Introduction of G247R mutation in RpoZ-AlsB-Zif268 fusion	ACTTTTCCCGTTTTTCCGG
BMB D174	Amplification of RbsB from *E. coli* genome and cloning by Gibson assembly into linearized pB1H2ω2-Zif268	AAGGATGACGACGACAAGTTCCGGACCGGTAAC ATGAAAAAACTGGCTACCCTGG
BMB D175	Amplification of RbsB from *E. coli* genome and cloning by Gibson assembly into linearized pB1H2ω2-Zif268	TGGGCGGGTACCATGGGGGGGTGTCTTGGACTG CTTAACAACCAGTTTCAGATCAAC
BMB D186	Introduction of G238R mutation in RpoZ-RbsB-Zif268 fusion	GATGGTCGTCaggTTTGACGGTAC
BMB D187	Introduction of G238R mutation in RpoZ-RbsB-Zif268 fusion	ACATCCGATTTACCGGCA
BMB D082	Amplification of eryR from pJKR-H-mphR and cloning by Gibson assembly into pB1H2ω2-Zif268	TGAACATGCCCGGTTACTGGAACGGGAATTgcttgg attctcaccaataaaaaacgcc
BMB D083	Amplification of *eryR* from pJKR-H-mphR and cloning by Gibson assembly into pB1H2ω2-Zif268	CCGGAAGCATAAAGTGTAAAGCCCGGAATTcaaaa aacccctcaagacccgtttagag
mphR_F	Amplification of mphR from pJKR-H-mphR and cloning by Gibson assembly into pB1H2ω2-Zif268_eryR	AAGGATGACGACGACAAGTTCCGGACCGGTATG CCGCGTCCGAAACTGAAATCT
mphR_R	Amplification of mphR from pJKR-H-mphR and cloning by Gibson assembly into pB1H2ω2-Zif268_eryR	TGGGCGGGTACCATGGGGGGGTGTCTTGGACGC GTGCGGCTGCAGCAGCTGGAA

Table S2. Oligonucleotides used in this study.

References for Supporting Information

1. Noyes, M. B., Meng, X., Wakabayashi, A., Sinha, S., Brodsky, M. H., and Wolfe, S. A. (**2008**) A systematic characterization of factors that regulate Drosophila segmentation via a bacterial one-hybrid system, *Nucleic Acids Res. 36*, 2547-2560.
2. Bell, C. E., and Lewis, M. (**2000**) A closer view of the conformation of the Lac repressor bound to operator, *Nat. Struct. Biol. 7*, 209-214.
3. Rogers, J. K., Guzman, C. D., Taylor, N. D., Raman, S., Anderson, K., and Church, G. M. (**2015**) Synthetic biosensors for precise gene control and real-time monitoring of metabolites, *Nucleic Acids Res. 43*, 7648-7660.