Supporting Information for

"Amplification of small molecule-inducible gene expression via tuning of intracellular receptor densities"

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Supplementary Methods

Mathematical modelling and data fitting

Computational biochemical models were developed for individual transcription factor receptor modules to abstract their ligand-dependent signaling response behaviours. We focused on the average behaviour of the *E. coli* population to demonstrate the performance of the inducible gene expression systems at a steady state. The ordinary differential equation (ODE)-based deterministic model was used for accurately modelling the gene regulation and expression across the full input or output range of the system. The following describes the derivation of the transfer function (TF) for the small molecule-responsive gene expression module and the experimental data fitting to these models.

Deriving transfer function of the ligand-responsive inducible promoters

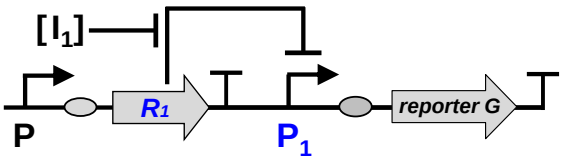


Figure S1: Schematics showing architecture of the inducible negatively regulated promoter P_1.

Figure S1 shows the exemplar architecture of the inducible promoter used in this study (*tetR*-P_{tet2}, *arsR*-P_{arsR} and *araC*-P_{BAD} promoters). The promoter P_1 is negatively regulated by its constitutively expressed repressor R_1 and is responsive to exogenous inducer I to activate transcription of downstream reporter gene *G*. The expression of the reporter can be modelled by[2-4]:

$$\frac{d[G]}{dt} = \alpha \cdot k_1 + \frac{k_1 \cdot [I]^{n_1}}{[I]^{n_1} + K_M{}^{n_1}} - d \cdot [G] \qquad \text{(S1)}$$

where $\alpha \cdot k_1$ is the basal constitutive activity of the promoter, $k_1 \cdot [I]^n / ([I]^n + K_M{}^n)$ is the activity due to cooperative transcription activation by assuming the concentration of the repressor is constant to allow modelling of the effect of varying the concentration of the inducer I, and $d \cdot [G]$ is the constitutive degradation activity of protein G. K_M and n are the Hill constant and coefficient relating to the promoter-regulator/inducer interaction, k_1 is the maximum expression rate due to induction and α is a constant relating to the promoter basal level due to leakage ($0 \leq \alpha < 1$), and d is the degradation rate of G.

The steady state solution of equation S1 is given by

$$f([I]) = [G]_{ss} = k \cdot (\alpha + [I]^n / (K_M{}^n + [I]^n)) \quad \text{(S2)}$$

where $k = k_1/d$ represents the maximum expression level due to induction. Equation S2 gives the reporter protein level at steady state for the inducible promoter P_1 and is also the TF of P_1. We used this TF to fit the characterisation data of the aTc (Fig. 2B), $3OC_6HSL$ (Fig. 3A) and $NaAsO_2$ (Fig. 4A) inducible promoters using the nonlinear least square fitting function (cftool) in Matlab (MathWorks R2010a). The bestfit parameters and coefficients (with 95% confidence bounds) are listed in Table S2 and the parameterised TFs are plotted in Fig. 2B and Fig. 3A respectively against their experimental data.

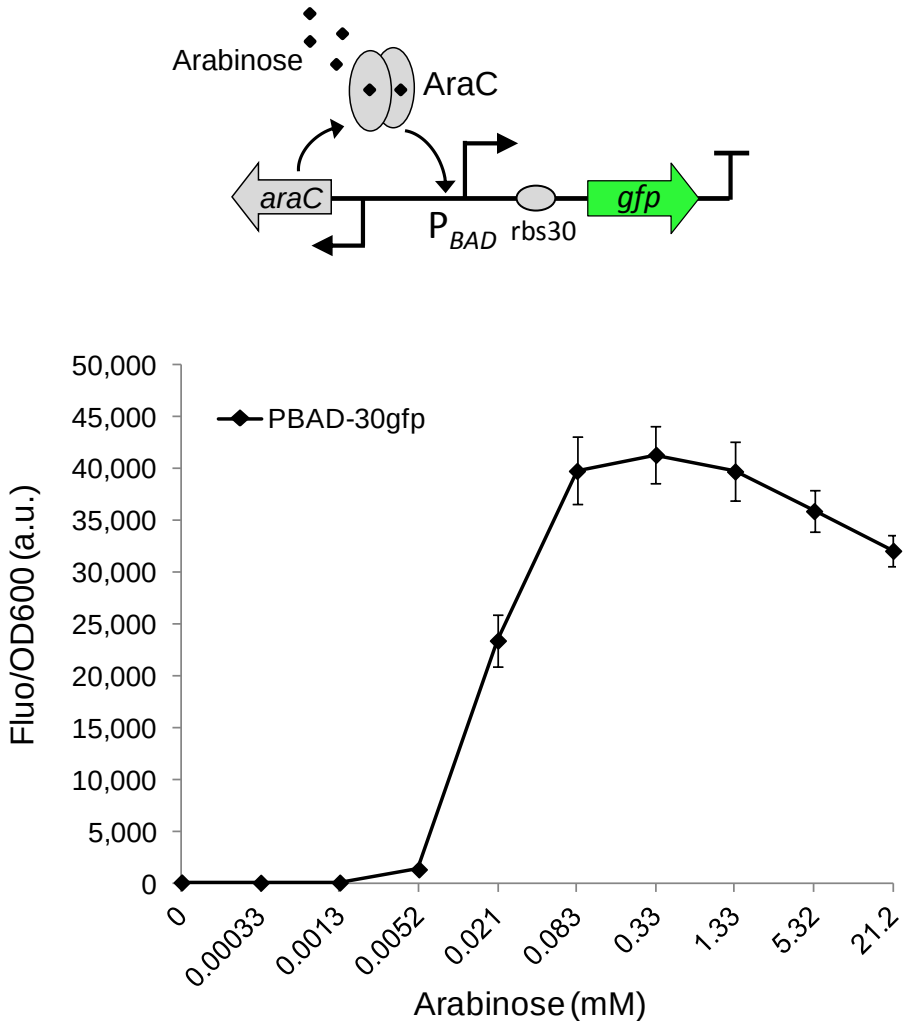
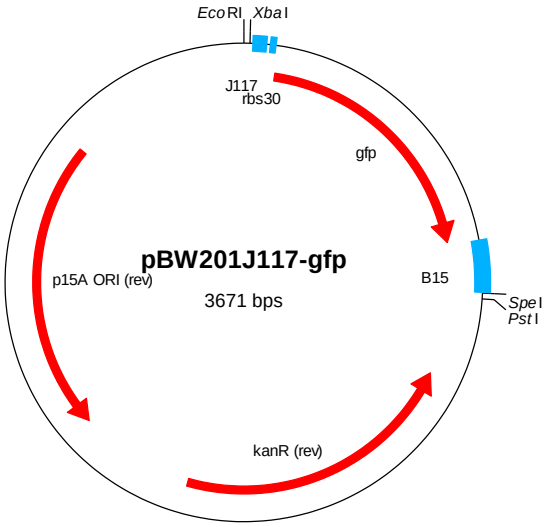


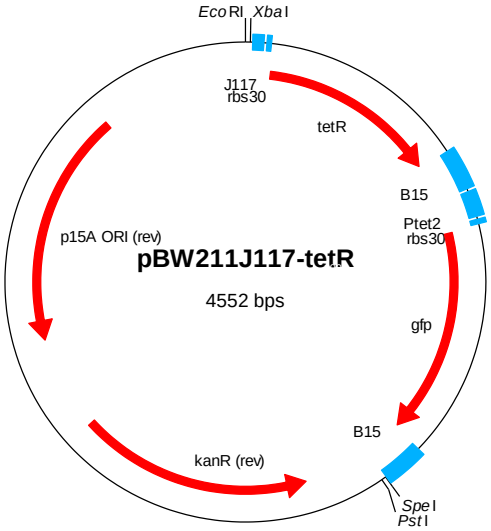
Figure S2: The characterised dose response of the arabinose inducible P_{BAD} promoter. The data were collected in *E. coli* TOP10 in LB media 6 hrs after induction by varying arabinose concentrations (0, 1.3×10^{-3}, 5.2×10^{-3}, 2.1×10^{-2}, 8.3×10^{-2}, 0.33, 1.33, 5.32 and 21.2 mM). Error bars, s.d. (n = 3).

A Pc-30gfp-B15 cassette (J117)



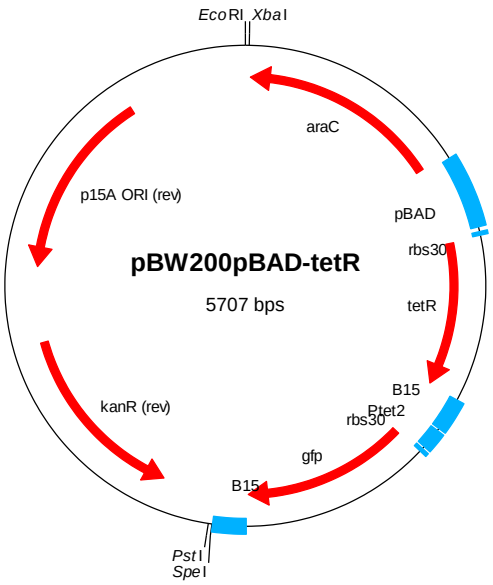
pSB3K3 carrying *J117*-30*gfp*-t

B Pc (J117) controlled TetR expression cassette



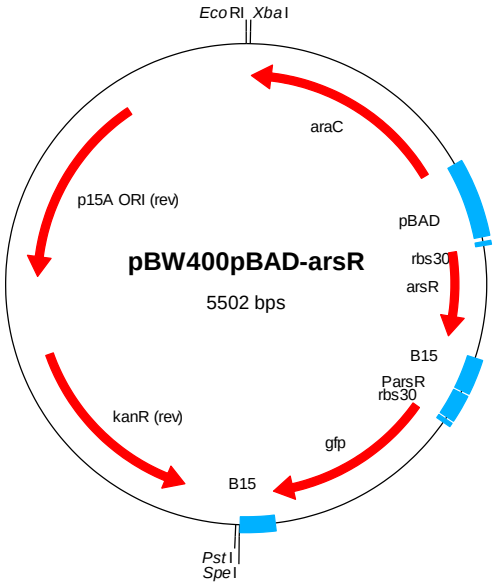
pSB3K3carrying *J117*-30*tetR*-t-P_{tet2}-30*gfp*-t

C pBAD controlled TetR expression cassette



pSB3K3 carrying *araC*-P_{BAD}-30*tetR*-t-P_{tet2}-30*gfp*-t

D pBAD controlled ArsR expression cassette



pSB3K3 carrying *araC*-P_{BAD}-30*arsR*-t-P_{arsR}-30*gfp*-t

Figure S3: Representative plasmid circuits used for sensor dose response characterisation. (**A**) Plasmid used for characterising constitutive promoter (e.g. J117, Fig. 2A). (**B-C**) Plasmids used for characterising aTc sensor response with the receptor TetR expressed from either a constitutive promoter (e.g. J117, Fig. 2B) or the arabinose inducible P_{BAD} promoter (Fig. 2C). (**D**) Plasmids used for characterising arsenic sensor response with the receptor ArsR expressed from the P_{BAD} promoter (Fig. 4C).

Table S1: List of plasmid circuit constructs used in this study

Plasmid	Description	Reference
pSB3K3	BioBrick vector, p15A ori, Kan^r	iGEM Registry[5]
pBW110pBAD	pSB3K3 carrying *araC*-P_{BAD}	This study
pBW111pBAD-gfp	pSB3K3 carrying *araC*-P_{BAD}-30*gfp*-t	This study
pBW201J117-gfp	pSB3K3 carrying *J117*-30*gfp*-t	This study
pBW202J114-gfp	pSB3K3 carrying *J114*-30*gfp*-t	This study
pBW204J115-gfp	pSB3K3 carrying *J115*-30*gfp*-t	This study
pBW205J105-gfp	pSB3K3 carrying *J105*-30*gfp*-t	This study
pBW206J106-gfp	pSB3K3 carrying *J106*-30*gfp*-t	This study
pBW206J101-gfp	pSB3K3 carrying *J101*-30*gfp*-t	This study
pBW211J117-tetR	pSB3K3 carrying *J117*-30*tetR*-t-P_{tet2}-30*gfp*-t	This study
pBW212J114-tetR	pSB3K3 carrying *J114*-30*tetR*-t-P_{tet2}-30*gfp*-t	This study
pBW213J115-tetR	pSB3K3 carrying *J115*-30*tetR*-t-P_{tet2}-30*gfp*-t	This study
pBW214J105-tetR	pSB3K3 carrying *J105*-30*tetR*-t-P_{tet2}-30*gfp*-t	This study
pBW215J106-tetR	pSB3K3 carrying *J106*-30*tetR*-t-P_{tet2}-30*gfp*-t	This study
pBW216J101-tetR	pSB3K3 carrying *J101*-30*tetR*-t-P_{tet2}-30*gfp*-t	This study
pBW200pBAD-tetR	pSB3K3 carrying *araC*-P_{BAD}-30*tetR*-t-P_{tet2}-30*gfp*-t	This study
pBW311J117-luxR	pSB3K3 carrying *J117*-30*luxR*-t-P_{lux2}-30*gfp*-t	This study
pBW312J114-luxR	pSB3K3 carrying *J114*-30*luxR*-t-P_{lux2}-30*gfp*-t	This study
pBW313J115-luxR	pSB3K3 carrying *J115*-30*luxR*-t-P_{lux2}-30*gfp*-t	This study
pBW314J105-luxR	pSB3K3 carrying *J105*-30*luxR*-t-P_{lux2}-30*gfp*-t	This study
pBW315J106-luxR	pSB3K3 carrying *J106*-30*luxR*-t-P_{lux2}-30*gfp*-t	This study
pBW316J101-luxR	pSB3K3 carrying *J101*-30*luxR*-t-P_{lux2}-30*gfp*-t	This study
pBW300pBAD-luxR	pSB3K3 carrying *araC*-P_{BAD}-30*luxR*-t-P_{lux2}-30*gfp*-t	This study
pBW413Pnull-arsR	pSB3K3 carrying P_{null}-30*arsR*-t-P_{arsR}-*gfp*-t	This study
pBW411J117-arsR	pSB3K3 carrying *J117*-30*arsR*-t-P_{arsR}-*gfp*-t	This study
pBW412J114-arsR	pSB3K3 carrying *J114*-30*arsR*-t-P_{arsR}-*gfp*-t	This study
pBW414J105-arsR	pSB3K3 carrying *J105*-30*arsR*-t-P_{arsR}-*gfp*-t	This study
pBW400pBAD-arsR	pSB3K3 carrying *araC*-P_{BAD}-30*arsR*-t-P_{arsR}-30*gfp*-t	This study

Table S2. List of genetic parts and sequences used in this study (promoters are in red, RBSs are in italic and bold, protein coding sequences are in brown and terminators are in bold)

Part name	Type and source	DNA sequence (5′ – 3′)
P_{J117}-*rbs30-tetR-B0015-*P_{tet2}	Inducible promoter with tetR receptor (de novo synthesized)	TTGACAGCTAGCTCAGTCCTAGGGATTGTGCTAGCTACTAGAG***ATTAAAGAGGAGAAA***TACCATATGTCCAGATTAGATAAAAGTAAAGTGATTAACAGCGCATTAGAGCTGCTTAATGAGGTCGGAATCGAAGGTTTAACAACCCGTAAACTCGCCCAGAAGCTAGGTGTAGAGCAGCCTACATTGTATTGGCATGTAAAAAATAAGCGGGCTTTGCTCGACGCCTTAGCCATTGAGATGTTAGATAGGCACCATACTCACTTTTGCCCTTTAGAAGGGGAAAGCTGGCAAGATTTTTTACGTAATAACGCTAAAAGTTTTAGATGTGCTTTACTAAGTCATCGCGATGGAGCAAAAGTACATTTAGGTACACGGCCTACAGAAAAACAGTATGAAACTCTCGAAAATCAATTAGCCTTTTTATGCCAACAAGGTTTTTCACTAGAGAATGCATTATATGCACTCAGCGCTGTGGGGCATTTTACTTTAGGTTGCGTATTGGAAGATCAAGAGCATCAAGTCGCTAAAGAAGAAAGGGAAACACCTACTACTGATAGTATGCCGCCATTATTACGACAAGCTATCGAATTATTTGATCACCAAGGTGCAGAGCCAGCCTTCTTATTCGGCCTTGAATTGATCATTTGCGGATTAGAAAAACAACTTAAATGTGAAAGTGGGTCCTAATAATACTAGAG**CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATA**TACTAGAGTTTTCAGCAGGACGCACTGACCTCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCACATAT
P_{J117}-*rbs30-luxR-B0015-*P_{lux2}	Inducible promoter with luxR receptor (de novo synthesized)	TTGACAGCTAGCTCAGTCCTAGGGATTGTGCTAGCTACTAGAG***ATTAAAGAGGAGAAA***TACTAGATGAAAAACATAAATGCCGACGACACATACAGAATAATTAATAAAATTAAAGCTTGTAGAAGCAATAATGATATTAATCAATGCTTATCTGATATGACTAAAATGGTACATTGTGAATATTATTTACTCGCGATCATTTATCCTCATTCTATGGTTAAATCTGATATTTCAATCCTAGATAATTACCCTAAAAAATGGAGGCAATATTATGATGACGCTAATTTAATAAAATATGATCCTATAGTAGATTTATTCTAACTCCAATCATTCACCAATTAATTGGAATATATTTGAAAACAATGCTGTAAATAAAAAATCTCCAAATGTAATTAAAGAAGCGAAAACATCAGGTCTTATCACTGGGTTTAGTTTCCCTATTCATACGGCTAACAATGGCTTCGGAATGCTTAGTTTTGCACATTCAGAAAAAGACAACTATATAGATAGTTTATTTTTACATGCGTGTATGAACATACCATTAATTGTTCCTTCTCTAGTTGATAATTATCGAAAAATAAATATAGCAAATAATAAATCAAACAACGATTTAACCAAAAGAGAAAAGAATGTTTAGCGTGGGCATGCGAAGGAAAAAGCTCTTGGGATATTTCAAAAATATTAGGTTGCAGTGAGCGTACTGTCACTTTCCATTTAACCAATGCGCAAATGAAACTCAATACAACAAACCGCTGCCAAAGTATTTCTAAAGCAATTTTAACAGGAGCAATTGATTGCCCATACTTTAAAAATTAATAACACTGATAGTGCTAGTGTAGATCACTACTAGAG**CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATA**TACTAGAGACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTTACTTTCGAATAAA
P_{J117}-*rbs30-arsR-B0015-*P_{arsR}	Inducible promoter with ArsR receptor[6] (de novo synthesized)	TTGACAGCTAGCTCAGTCCTAGGGATTGTGCTAGCTACTAGAG***ATTAAAGAGGAGAAA***TACTAGATGTCATTTCTGTTACCCATCCAATTGTTCAAAATTCTTGCTGATGAAACCCGTCTGGGCATCGTTTTACTGCTCAGCGAACTGGGAGAGTTATGCGTCTGCGATCTCTGCACTGCTCTCGACCAGTCGCAGCCCAAGATCTCCCGCCACCTGGCATTGCTGCGTGAAAGCGGGCTATTGCTGGACCGCAAGCAAGGTAAGTGGGTTCATTACCGCTTATCACCGCATATTCCAGCATGGGCGGCGAAAATTATTGATGAGGCCTGGCGATGTGAACAGGAAAAGGTTCAGGCGATTGTCCGCAACCTGGCTCGACAAAACTGTTCCGGGGACAGTAAGAACATTTGCAGTTAAAAATTTAGCTAAACACACATGAATTTTCAGATGTGTTTTATCCGGGTACTAGA**GCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATA**TACTAGAGCCAACTCAAAATTCACACCTATTACCTTCCTCTGCACTTACACATTCGTTAAGTCATATATGTTTTTGACTTATCCGCTTCGAAGAGAGACACTACCTGCAA

araC-P$_{BAD}$	Inducible promoter[7]	TTATGACAACTTGACGGCTACATCATTCACTTTTTCTTCACAACCGGCACGG AACTCGCTCGGGCTGGCCCCGGTGCATTTTTTAAATACCCGCGAGAAATAGA GTTGATCGTCAAAACCAACATTGCGACCGACGGTGGCGATAGGCATCCGGGT GGTGCTCAAAAGCAGCTTCGCCTGGCTGATACGTTGGTCCTCGCGCCAGCTT AAGACGCTAATCCCTAACTGCTGGCGGAAAAGATGTGACAGACGCGACGGCG ACAAGCAAACATGCTGTGCGACGCTGGCGATATCAAAATTGCTGTCTGCCAG GTGATCGCTGATGTACTGACAAGCCTCGCGTACCCGATTATCCATCGGTGGA TGGAGCGACTCGTTAATCGCTTCCATGCGCCGCAGTAACAATTGCTCAAGCA GATTTATCGCCAGCAGCTCCGAATAGCGCCCTTCCCCTTGCCCGGCGTTAAT GATTTGCCCAAACAGGTCGCTGAAATGCGGCTGGTGCGCTTCATCCGGGCGA AAGAACCCCGTATTGGCAAATATTGACGGCCAGTTAAGCCATTCATGCCAGT AGGCGCGCGGACGAAAGTAAACCCACTGGTGATACCATTCGCGAGCCTCCGG ATGACGACCGTAGTGATGAATCTCTCCTGGCGGGAACAGCAAAATATCACCC GGTCGGCAAACAAATTCTCGTCCCTGATTTTTCACCACCCCCTGACCGCGAA TGGTGAGATTGAGAATATAACCTTTCATTCCCAGCGGTCGGTCGATAAAAAA ATCGAGATAACCGTTGGCCTCAATCGGCGTTAAACCCGCCACCAGATGGGCA TTAAACGAGTATCCCGGCAGCAGGGGATCATTTTGCGCTTCAGCCATACTTT TCATACTCCCGCCATTCAGAGAAGAAACCAATTGTCCATATTGCATCAGACA TTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACC CCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAA CGCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCACATTGATTATTT GCACGGCGTCACACTTTGCTATGCCATAGCATTTTTATCCATAAGATTAGCG GATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT
rbs30	RBS[3]	TCTAGAG***ATTAAAGAGGAGAAA***TACTAG**ATG**
J117	Promoter[8]	TTGACAGCTAGCTCAGTCCTAGGGATTGTGCTAGC
J114	Promoter[8]	TTTATGGCTAGCTCAGTCCTAGGTACAATGCTAGC
J115	Promoter[8]	TTTATAGCTAGCTCAGCCCTTGGTACAATGCTAGC
J105	Promoter[8]	TTTACGGCTAGCTCAGTCCTAGGTACTATGCTAGC
J106	Promoter[8]	TTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGC
J101	Promoter[8]	TTTACAGCTAGCTCAGTCCTAGGTATTATGCTAGC
B0015 (B15)	Terminator[9]	**CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTT TATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCT TCGGGTGGGCCTTTCTGCGTTTATA**
gfp	Gene[3,10]	ATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAAT TAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGG TGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAA CTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAAT GCTTTGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGC CATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGG AACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATA GAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACA CAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAA CAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATG GAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGG CCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCG AAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAG CTGCTGGGATTACACATGGCATGGATGAACTATACAAATAATAA

pSB3K3	Plasmid backbone[1,5]	TACTAGTAGCGGCCGCTGCAGTCCGGCAAAAAAACGGGCAAGGTGTCACCACCCTGCC CTTTTTCTTTAAAACCGAAAAGATTACTTCGCGTTATGCAGGCTTCCTCGCTCACTGA CTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTA ATCTCGAGTCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGTTACAACCAATTAACCAA TTCTGATTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGA TTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGA GGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAAC ATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCA CCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGA CTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACC GTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGA CAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAA TATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGAT CGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGA AGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGG CAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAA TCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATAT AAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGAGCAAGACGTTTCCCGTTGAA TATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCA TGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACACAACGTGG CTTTGTTGAATAAATCGAACTTTTGCTGAGTTGAAGGATCAGATCACGCATCTTCCCG ACAACGCAGACCGTTCCGTGGCAAAGCAAAAGTTCAAAATCACCAACTGGTCCACCTA CAACAAAGCTCTCATCAACCGTGGCTCCCTCACTTTCTGGCTGGATGATGGGGCGATT CAGGCCTGGTATGAGTCAGCAACACCTTCTTCACGAGGCAGACCTCAGCGCTAGCGGA GTGTATACTGGCTTACTATGTTGGCACTGATGAGGGTGTCAGTGAAGTGCTTCATGTG GCAGGAGAAAAAAGGCTGCACCGGTGCGTCAGCAGAATATGTGATACAGGATATATTC CGCTTCCTCGCTCACTGACTCGCTACGCTCGGTCGTTCGACTGCGGCGAGCGGAAATG GCTTACGAACGGGGCGGAGATTTCCTGGAAGATGCCAGGAAGATACTTAACAGGGAAG TGAGAGGGCCGCGGCAAAGCCGTTTTTCCATAGGCTCCGCCCCCCTGACAAGCATCAC GAAATCTGACGCTCAAATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATACCAGG CGTTTCCCCTGGCGGCTCCCTCGTGCGCTCTCCTGTTCCTGCCTTTCGGTTTACCGGT GTCATTCCGCTGTTATGGCCGCGTTTGTCTCATTCCACGCCTGACACTCAGTTCCGGG TAGGCAGTTCGCTCCAAGCTGGACTGTATGCACGAACCCCCCGTTCAGTCCGACCGCT GCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGAAAGACATGCAAAAGCACC ACTGGCAGCAGCCACTGGTAATTGATTTAGAGGAGTTAGTCTTGAAGTCATGCGCCGG TTAAGGCTAAACTGAAAGGACAAGTTTTGGTGACTGCGCTCCTCCAAGCCAGTTACCT CGGTTCAAAGAGTTGGTAGCTCAGAGAACCTTCGAAAAACCGCCCTGCAAGGCGGTTT TTTCGTTTTCAGAGCAAGAGATTACGCGCAGACCAAAACGATCTCAAGAAGATCATCT TATTAAGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATG AGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAAT CAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGA GGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTC GTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATAC CGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAG GGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTCCATGG TGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCG TATCACGAGGCAGAATTTCAGATAAAAAAAATCCTTAGCTTTCGCTAAGGATGATTTC TGGAATTCGCGGCCGCTTCTAGAG

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