Development of a pigment-based whole-cell zinc biosensor for human serum

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Supporting Information

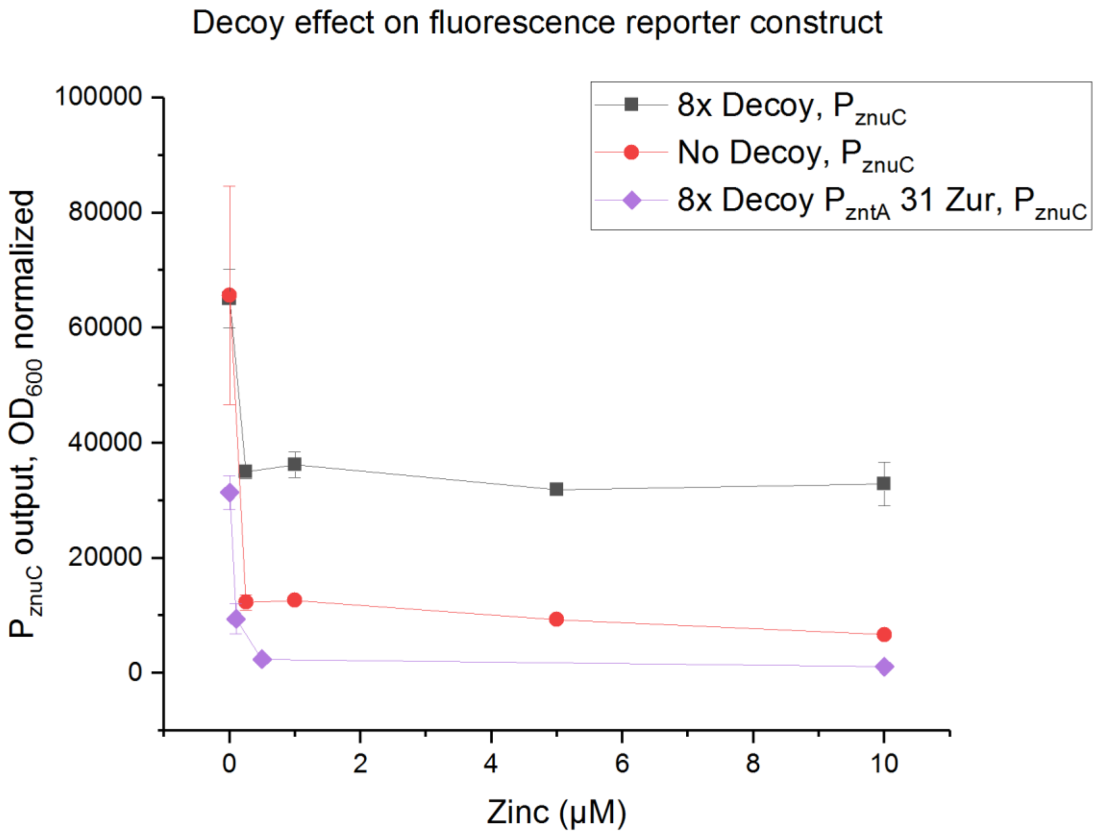

Figure S1: Effect of decoy Zur operators on the output of Pznuc. The unregulated reporter (red) has substantially lower expression in the repressed state than the construct with an 8x decoy array (grey). This is rectified upon zinc-dependent expression of Zur (purple). Error bars represent standard deviation.

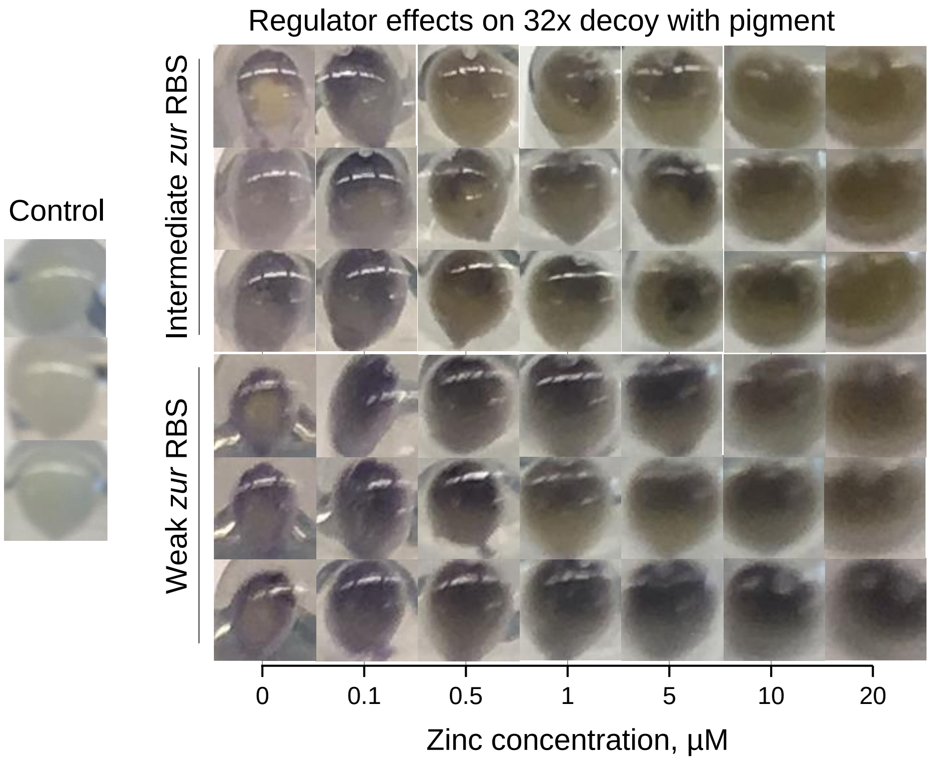

Figure S2: Cell pellets from the violacein extraction experiment described in Figure 3d. Note lower pigmentation at higher zinc with intermediate RBS

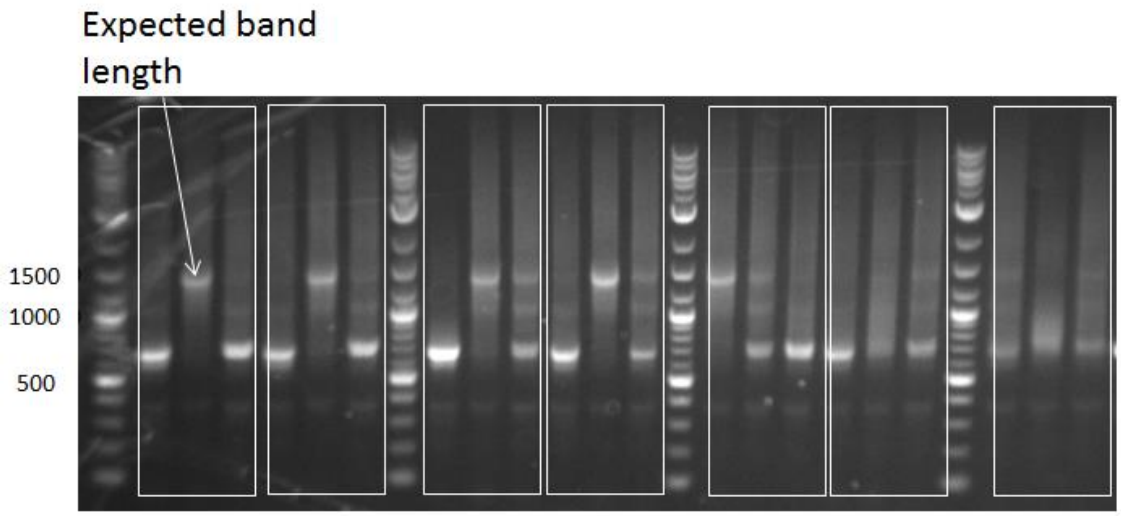

Figure S3: Characteristic banding pattern of CPCR of cells taken from a 32xdecoy pigment induction experiment. The expected banding pattern for an intact decoy array is 1.4 kb. Other bands indicate an inhomogeneous population with varying band length. Each box indicates biological replicates run in triplicate.

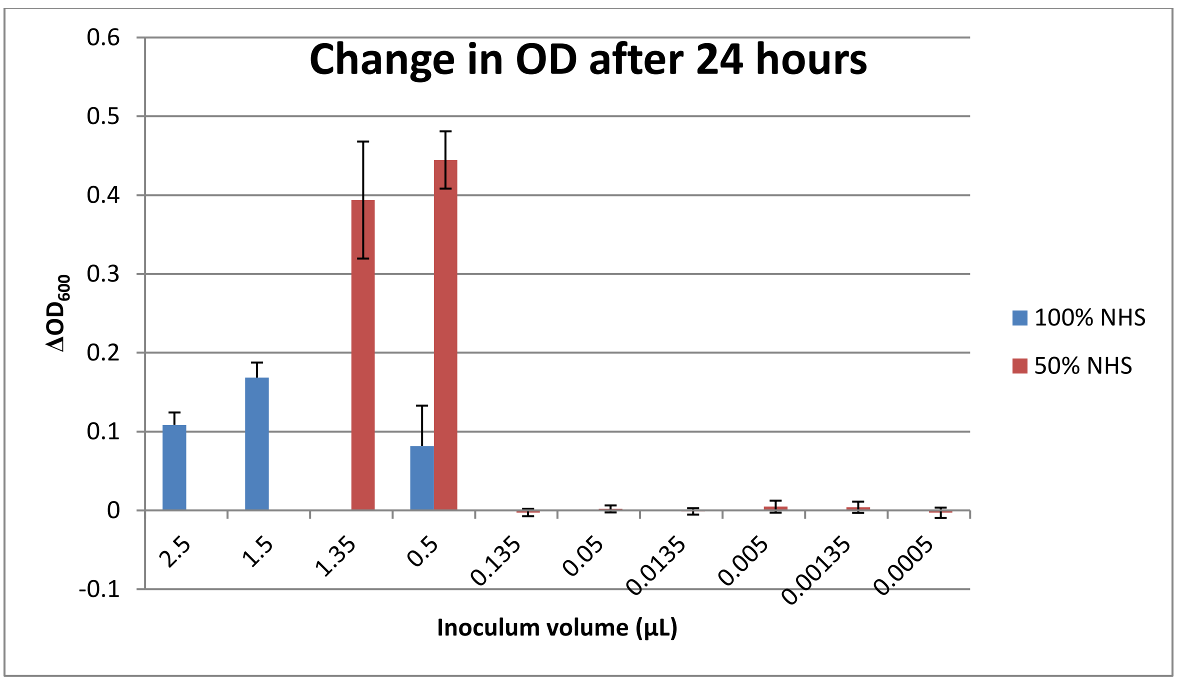

Figure S4: Overnight changes in OD in 100% and 50% serum in plates as a function of estimated initial OD, based on the fraction of OD_{600}:68 feeder culture. 100% NHS was only tested at 2.5, 1.5, and 0.5 μL. Error bars represent standard deviation.

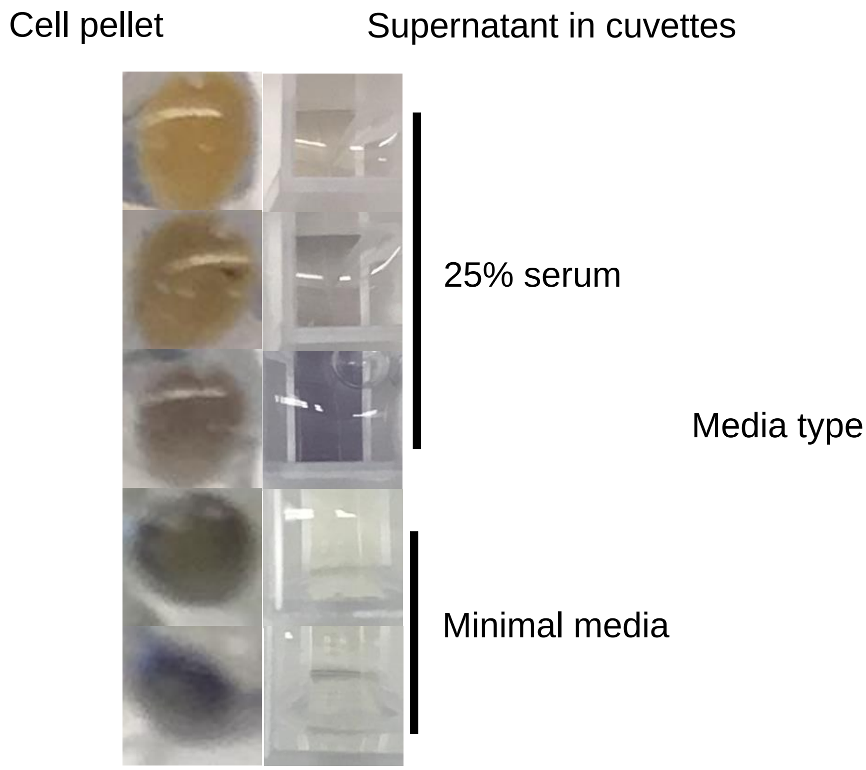

Figure S5: Comparison of images of cell pellets (left) and culture supernatants (right) from three color experiments in 25% serum versus M9 alone. Note the presence of substantial violacein in 25% serum supernatant, even as cell pellets from M9 alone demonstrate comparatively more violacein in cell pellets without visible presence in supernatant.

Rank order for ZntR			
P_{zntA} dynamic range	Regulator promoter/RBS	Zur predicted expression	ZntR predicted expression
252.3	7-4 7-1c (Pair 1)	480364	429402
168.3	7-4 7-1	480364	429402
147.7	3-4 3-4c (Pair 2)	334146	55663
142.9	2-4 7-1c	480364	2651
95.21	2-4 7-1	480364	2651
93.86	3-4 3-4	334146	55663

Table S1: Rank order list of top six fluorescent reporter dynamic ranges over 0-20 µM zinc for P_{zntA} with varying regulator pairs.

Rank order for Zur			
P_{znuC} Dynamic range	Regulator promoter /RBS	Zur predicted expression	ZntR predicted expression
235.1	7-4 7-1c (Pair 1)	480364	429402
153.4	3-4 3-4c (Pair 2)	334146	55663
146.5	2-4 7-1c	480364	2651
101.3	7-4 7-1	480364	429402
77.52	2-4 7-1	480364	2651
74.89	3-4 3-4	334146	55663

Table S2: Rank order list of top six fluorescent reporter dynamic ranges over 0-20 µM zinc for P_{znuC} with varying regulator pairs.

ID	RBS Calculator prediction (AU)	Promoter	relative transcriptional output
31zur	2965.21	112	1
32zur	5074.87	113	21
33zur	353.89	117	162
34zur	15911.72		
34zntr	2650.63		

Table S3: Output from RBScalc and relative transcriptional strength of promoters in Table 1.

The relative transcriptional strength of the constitutive reporters was taken from the registry of standard biological parts. The translation initiation rate (arbitrary units) was calculated with RBS calculator[1]. These two numbers were multiplied to approximate expected relative protein expression. While this metric is by no means expected to be quantitative, this prediction is used as a zeroth order approximation of protein expression. The naming convention here is promoter-ribosomal binding site for ZntR and then promoter-ribosomal binding site for Zur. For example, 7-4 7-1 denotes a construct with J23117 B0034 ZntR and J23117 B0031 Zur. 'c' denotes the results were from Chelex-treated media.

The rank order for dynamic range does not correspond with expected relative protein production. For example, in normal media for P_{zntA}, lower dynamic range over 20 μM was observed in 3-4 3-4 than in 2-4 7-1, even though the promoter controlling ZntR is exceptionally weak.

The behavior between media with and without treatment by Chelex between regulator pairs is also noteworthy. For Zur/P_{znuC}, the three arrangements exhibiting maximum dynamic range were all in Chelex-depleted media, and the next three were the same constructs in untreated media. The relative rank order of 3-4 3-4 and 2-4 7-1 is reversed in untreated vs treated media; however, the results in both cases are close enough that the differences are not significant. For P_{zntA}/ZntR, the top two performers were 7-4 7-1 in each media type. It is interesting to note that nearly all of the top P_{zntA}/ZntR candidates seem to correspond more with expected Zur expression than expected ZntR expression. This is consistent with increased levels of Zur outcompeting ZntR for zinc at low zinc concentrations, suppressing output from P_{ZntA}.

Description of supplementary files containing plasmid insert sequences

All files with the prefix 2c_ contain inserts for two-color pigment reporters controlling the production of lycopene and ß-carotene. The intermediate number represents the ribosomal binding site on crtY. An L following the RBS indicates the presence of an LAA degron tag. Finally, P1 or P2 indicate the regulator pair on the construct

The prefix 3c_ indicates the addition of P_{znuC} and *vioABCDE* to 2c_ constructs to make a three color reporter.

The prefix PaPc_ indicates a fluorescent reporter with eGFP as the readout for P_{zntA} and mRFP as the readout for P_{znuC}, followed by code for the promoter and ribosomal binding site controlling transcription factor expression. RPaPc_ is the same as above, but with the fluorescent reporter for each promoter swapped.

The prefix 32xd_ indicates the presence of a Zur operator decoy array.

Name	Type	Sequence	Description
vioABCDE	Operon Bba_k274002	ttaaggaggtaaaaaaaatgaaacattcttccgatatctgcattgttggtgctggtat ttctggtttgacgtgcgcaagccatctgctggacagcccggcatgccgtggtctgagc ctgcgtatctttgacatgcagcaagaagccggtggccgtatccgcagcaaaatgctg gatggtaaggcaagcattgaactgggcgcaggtcgctactcccctcagttgcacccg catttccaaagcgcaatgcagcactatagccaaaagagcgaagtctatccgttcacc cagttgaagttcaaatctcacgtgcagcaaaagctgaagcgcgccatgaatgaact gtccccgcgtctgaaagagcatggtaaagagagctttttgcagtttgtcagccgttat caaggtcacgatagcgcggttggtatgatccgctctatgggttacgacgcactgttcc tgccggatatcagcgcagaaatggcctacgacattgtgggtaagcacccggagatc cagagcgtgacggacaacgacgcgaaccaatggtttgcagcggaaacgggctttgc tggtctgattcagggcatcaaggctaaggttaaggcggcaggtgcgcgttttagcct gggttatcgtctgctgagcgtccgtaccgacggtgacggctacctgctgcaactggc aggtgacgacggctggaaactggagcaccgtacccgccatctgattctggcgattcc gccgagcgcgatggcgggtttgaatgttgattttccagaagcctggtccggtgcgcg ctatggcagcctgccgctgtttaagggctttctgacgtacggtgagccgtggtggttg gactacaaactggacgatcaggtgctgattgttgacaacccgctgcgcaaaatctat ttcaaaggcgataagtacctgttcttctatacCGATAGCGAGATGGCGAATT actggcgcggttgtgtcgcggagggcgaggacggttacctggagcaaattcgcacc catttggctagcgcactgggtatcgtccgtgaacgtatcccgcaaccgctggcacac gttcacaagtattgggcgcacggcgttgagttttgccgtgattctgatattgaccaccc gagcgcactgtctcatcgcgacagcggtatcatcgcgtgctccgatgcgtacacgga gcattgtggttggatggagggcggtctgctgagcgcccgtgaggcaagccgtctgct gttgcagcgtatcgccgcgtgattaaggaggtaaaaaaaatgagcattctggatttc ccgcgtatccacttccgtggctgggcccgtgtcaatgcgccgaccgcgaaccgcgat ccgcacggccacatcgatatggccagcaataccgtggcgatggcgggtgagccgtt cgacctggcacgccatcctacggagttccaccgtcacctgcgctccctgggtccgcgc ttcggcttggatggtcgtgctgacccggaaggcccgttcagcctggccgagggctac aacgctgccggtaacaaccacttttcgtgggagagcgcaaccgttagccacgtgcaa tgggatggcggtgaggcggatcgtggtgacggtctggtcggtgctcgtttggcactgt	Biosynthesis pathway for violacein

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crtY	Gene BBa_k118008	atgcaaccgcattatgatctgattctcgtgggggctggactcgcgaatggccttatcg ccctgcgtcttcagcagcagcaacctgatatgcgtattttgcttatcgacgccgcacc ccaggcgggcgggaatcatacgtggtcatttcaccacgatgatttgactgagagcca acatcgttggatagctccgctggtggttcatcactggcccgactatcaggtacgctttc ccacacgccgtcgtaagctgaacagcggctacttttgtattacttctcagcgtttcgct gaggttttacagcgacagtttggcccgcacttgtggatggataccgcggtcgcagag gttaatgcggaatctgttcggttgaaaaagggtcaggttatcggtgcccgcgcggtg attgacgggcggggttatgcggcaaattcagcactgagcgtgggcttccaggcgttt attggccaggaatggcgattgagccacccgcatggtttatcgtctcccattatcatgg atgccacggtcgatcagcaaaatggttatcgcttcgtgtacagcctgccgctctcgcc gaccagattgttaattgaagacacgcactatattgataatgcgacattagatcctga atgcgcgcggcaaaatatttgcgactatgccgcgcaacagggttggcagcttcagac actgctgcgagaagaacagggcgccttacccattactctgtcgggcaatgccgacgc attctggcagcagcgcccccctggcctgtagtggattacgtgccggtctgttccatccta ccaccggctattcactgccgctggcggttgccgtggccgaccgcctgagtgcacttga tgtctttacgtcggcctcaattcaccatgccattacgcattttgcccgcgagcgctggc agcagcagggctttttccgcatgctgaatcgcatgctgtttttagccggacccgccga ttcacgctggcgggttatgcagcgtttttatggtttacctgaagatttaattgcccgttt ttatgcgggaaaactcacgctgaccgatcggctacgtattctgagcggcaagccgcc tgttccggtattagcagcattgcaagccattatgacgactcatcgtTAA	Lycopene ß-cyclase to produce ß-carotene from lycopene
zur	gene	ATGGAAAAGACCACAACGCAGGAGTTATTAGCGCAGGCTGAAA AAATCTGCGCGCAGCGTAATGTGCGCCTGACCCCACAGCGCCTG GAAGTGTTGCGCCtgatgagtctccaagatggcGCTATCAGCGCTTATG ATCTGCTTGATTTACTGCGCGAAGCTGAACCGCAAGCCAAGCCG CCAACGGTTTATCGCGCGCTGGATTTTCTGCTTGAGCAAGGTTTT GTGCATAAGGTGGAATCCACCAACAGTTATGTGCTCTGTCATCT GTTCGATCAGCCCACCCATACGTCAGCCATGTTTATTTGCGATCG CTGCGGCGCAGTGAAAGAAGAGTGTGCAGAAGGCGTGGAAGA CATTATGCATACGCTGGCGGCAAAAATGGGGTTTGCCCTGCGGC ATAATGTGATTGAAGCACATGGGCTCTGTGCGGCATGTGTAGA AGTGGAAGCGTGTCGTCATCCTGAACAGTGCCAGCATGATCACT CTGTGCAGGTGAAAAAGAAACCGCGTTAA	Zinc-responsive transcriptional repressor
zntR	gene	ATGTATCGCATTGGTGAGCTGGCAAAAATGGCGGAAGTAACAC	Zinc-

		CCGACACGATTCGTTATTACGAAAAACAGCAGATGATGGAGCATGAAGTGCGTACTGAAGGTGGGTTTCGCCTATATACCGAAAGCGATCTCCAGCGATTGAAATTTATCCGCCATGCCAGACAACTAGGTTTCAGTCTGGAGTCGATCCGCGAGTTGCTGTCGATCCGCATCGATCCTGAACACCATACCTGTCAGGAGTCAAAAGGCATTGTGCAGGAAAGATTGCAGGAAGTCGAAGCACGGATAGCCGAGTTGCAGAGTATGCAGCGTTCCTTGCAACGCCTTAACGATGCCTGTTGTGGGACTGCTCATAGCAGTGTTTATTGTTCGATTCTTGAAGCTCTTGAACAAGGGGCGAGTGGCGTTAAGAGTGGTTGTTGA	responsive transcriptional activator
Pznuc	promoter	AACATAATGCGACCAATAATCGTAATGAATATGAGAAGTGTGATATTATAACATTT	
Pznta	promoter	CTGTATCTCTGATAAAACTTGACTCTGGAGTCGACTCCAGAGTGTATCCTTCGGTTAAT	
BBa B0031	RBS	tcacacaggaaacc	
BBa B0032	RBS	tcacacaggaaag	
BBa B0033	RBS	tcacacaggac	
BBa BBa B0034	RBS	AAAGAGGAGAAA	
LAA	Signal tag	GCTGCTAACGACGAAAACTACGCTCTGGCTGCT	
Bba J23112	Promoter	ctgatagctagctcagtcctagggattatgctagc	
Bba J23113	Promoter	ctgatggctagctcagtcctagggattatgctagc	
Bba J23117	Promoter	ttgacagctagctcagtcctagggattgtgctagc	
32xd	Decoy Zur operator array	TGTTATGTTATAACATAACATACTAGATGTTACAATATAACATTACATACTAGATGTTATGTTATAACATAACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTATGTTATAACATAACATACTAGATGTTACAATATAACATTACATACTAGATGTTATGTTATAACATAACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTATGTTATAACATAACATACTAGATGTTACAATATAACATTACATACTAGATGTTATGTTATAACATAACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTATGTTATAACATAACATACTAGATGTTACAATATAACATTACATACTAGATGTTATGTTATAACATAACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACATACTAGATGTTACAATATAACATTACA	A combination of Zur operator palindromes and Zur operator sites from Pzint

mRFP	gene	atggcttcctccgaagacgttatcaaagagttcatgcgtttcaaagttcgtatggaag gttccgttaacggtcacgagttcgaaatcgaaggtgaaggtgaaggtcgtccgtacg aaggtacccagaccgctaaactgaaagttaccaaaggtggtccgctgccgttcgctt gggacatcctgtccccgcagttccagtacggttccaaagcttacgttaaacacccgg ctgacatcccggactacctgaaactgtccttcccggaaggtttcaaatgggaacgtgt tatgaacttcgaagacggtggtgttgttaccgttacccaggactcctccctgcaagac ggtgagttcatctacaaagttaaactgcgtggtaccaacttcccgtccgacggtccgg ttatgcagaaaaaaaccatgggttgggaagcttccaccgaacgtatgtacccggaa gacggtgctctgaaaggtgaaatcaaaatgcgtctgaaactgaaagacggtggtca ctacgacgctgaagttaaaaccacctacatggctaaaaaaccggttcagctgccgg gtgcttacaaaaccgacatcaaactggacatcacctcccacaacgaagactacacc atcgttgaacagtacgaacgtgctgaaggtcgtcactccaccggtgcttaataa	
eGFP	gene	ATGGCTAGCAAAGGAGAAGAACTCTTCACTGGAGTTGTCCCAAT TCTTGTTGAATTAGATGGTGATGTTAACGGCCACAAGTTCTCTGT CAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACC CTGAAGTTCATCTGCACTACTGGCAAACTGCCTGTTCCATGGCca accctggtcactactctgTGCTATGGTGTTCAATGCTTTTCAAGATACC CGGATCATATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCC GAAGGTTATGTACAGGAAAGGACCATCTTCTTCAAAGATGACG GCAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATAC CCTTGTTAATAGAATCGAGTTAAAAGGTATTGACTTCAAGGAAG ATGGCAACATTCTGGGACACAAATTGGAATACAACTATAACTCA CACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCA AAGTGAACTTCAAGACCCGCCACAACATTGAAGATGGAAGCGT TCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATG GCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTG CCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTT CTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGA ACTGTACAATTAA	
B0015	Transcript-ional terminator	ccaggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcgttttatct gttgtttgtcggtgaacgctctctactagagtcacactggctcaccttcgggtgggcct ttctgcgtttata	

Table S4: A list of genetic components used to construct plasmids used in this study.

VF2	tgccacctgacgtctaagaa
VR	attaccgcctttgagtgagc
crtY5'r	ATC CAA CGA TGT TGG CTC TC
crtY3'f	TCC TAC CAC CGG CTA TTC AC
crtE5'r	CCA CAT GCT CTC CGT AAT GA
crtI3'F	CCA AAA CTA CGC GAC CGT AT
vioA	ATG CTT GCC TTA CCA TCC AG

5’R	
vioE 3’F	AAA AGT CGC CTA TGG TCG TG
Pal.F	CTA GGA ATT CGC GGC CGC TTC TAGATG TTA TGT TAT AAC ATA ACA
Pal.r	CAG TCT GCA GCG GCC GCT ACTAGT ATG TTA TGT TAT AAC ATA ACATCT A GA AGC GG
Zint. R	CAG TCT GCA GCG GCC GCT ACTAGT ATG TAA TGT TAT ATT GTA ACATCT A GA AGC GG
Zint.F	CTA GGA ATT CGC GGC CGC TTC TAGATG TTA CAA TAT AAC ATT ACA

Table S5: List of primers used in this study

Psb3c5	tactagtagcggccgctgcaggagtcactaagggttagttagttagattagcagaaagtcaaaagcctccgaccggaggcttttg actaaaacttcccttggggttatcattggggctcactcaaaggcggtaatcagataaaaaaaatccttagctttcgctaaggatga tttctgctagagatggaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggttt attgctgataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgt agttatctacacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagca ttggtaactgtcagaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatcc tttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccTTAATAAGATGATCTTCT TGAGATCGTTTTGGTCTGCGCGTAATCTCTTGCTCTGAAAACGAAAAAACCGCCTTGCAGGGCGGT TTTTCGAAGGTTCTCTGAGCTACCAACTCTTTGAACCGAGGTAACTGGCTTGGAGGAGCGCAGTCA CCAAAACTTGTCCTTTCAGTTTAGCCTTAACCGGCGCATGACTTCAAGACTAACTCCTCTAAATCAA TTACCAGTGGCTGCTGCCAGTGGTGCTTTTGCATGTCTTTCCGGGTTGGACTCAAGACGATAGTTA CCGGATAAGGCGCAGCGGTCGGACTGAACGGGGGGTTCGTGCATACAGTCCAGCTTGGAGCGAA CTGCCTACCCGGAACTGAGTGTCAGGCGTGGAATGAGACAAACGCGGCCATAACAGCGGAATGA CACCGGTAAACCGAAAGGCAGGAACAGGAGAGCGCACGAGGGAGccgccaggggaaacGCCTGGTA TCTTTATAGTCCTGTCGGGTTTCGCCACCACTGATTTGAGCGTCAGATTTCGTGATGCTTGTCAGGG GGGCGGAGCCTATGGAAAAACGGCTTTGCCGCGGCCCTCTCACTTCCCTGTTAAGTATCTTCCTGG CATCTTCCAGGAAATCTCCGCCCCGTTCGTAAGCCATTTCCGCTCGCCGCAGTCGAACGACCGAGC GTAGCGAGTCAGTGAGCGAGGAAGCGGAATATATCCtgtatcacatattctgctgacgcaccggtgcagcctttt ttctcctgccacatgaagcacttcactgacaccctcatcagtgccaacatagtaagccagtatacactccgctagcgctgaggtct gcctcgtgaagaaggtgttgctgactcataccaggcctgaatcgccccatcatccagccagaaagtgagggagccacggttgatg agagctttgttgtaggtggaccagttggtgattttgaacttttgctttgccacggaacggtctgcgttgtcgggaagatgcgtgatct gatccttcaactcagcaaaagttcgatttattcaacaaagccacgttgtgtctcaaaatctctgatgttacattgcacaagataaaa atatatcatcatgaacaataaaactgtctgcttacataaacagtaatacaaggggtgtttactagaggttgatcgggcacgtaag aggttccaactttcaccataatgaaataagatcactaccgggcgtatttttgagttatcgagattttcaggagctaaggaagctaa aatggagaaaaaaatcacgggatataccaccgttgatatatcccaatggcatcgtaaagaacattttgaggcatttcagtcagtt gctcaatgtacctataaccagaccgttcagctggatattacggcctttttaaagaccgtaaagaaaaataagcacaagttttatcc ggcctttattcacattcttgcccgcctgatgaacgctcacccggagtttcgtatggccatgaaagacggtgagctggtgatctggga tagtgttcacccttgttacaccgttttccatgagcaaactgaaacgttttcgtccctctggagtgaataccacgacgatttccggcag tttctccacatatattcgcaagatgtggcgtgttacggtgaaaacctggcctatttccctaaagggtttattgagaatatgtttttgt ctcagccaatccctgggtgagtttcaccagttttgatttaaacgtggccaatatggacaacttcttcgcccccgttttcacgatgggc aaatattatacgcaaggcgacaaggtgctgatgccgctggcgatccaggttcatcatgccgtttgtgatggcttccatgtcggccg

	catgcttaatgaattacaacagtactgtgatgagtggcagggcggggcgtaataatactagctccggcaaaaaaacgggcaagg tgtcaccaccctgccctttttctttaaaaccgaaaagattacttcgcgtttgccacctgacgtctaagaaaaggaatattcagcaat ttgcccgtgccgaagaaaggcccacccgtgaaggtgagccagtgagttgattgctacgtaattagttagttagcccttagtgactc gaattcgcggccgcttctagag
Psb3t5	tactagtagcggccgctgcaggagtcactaagggttagttagttagattagcagaaagtcaaaagcctccgaccggaggcttttg actaaaacttcccttggggttatcattggggctcactcaaaggcggtaatcagataaaaaaaatccttagctttcgctaaggatga tttctgctagagatggaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggttt attgctgataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgt agttatctacacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagca ttggtaactgtcagaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatcc tttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccttaataagatgatcttcttgagat cgttttggtctgcgcgtaatctcttgctctgaaaacgaaaaaaccgccttgcagggcggtttttcgaaggttctctgagctaccaac tctttgaaccgaggtaactggcttggaggagcgcagtcaccaaaacttgtcctttcagtttagccttaaccggcgcatgacttcaag actaactcctctaaatcaattaccagtggctgctgccagtggtgcttttgcatgtctttccgggttggactcaagacgatagttaccg gataaggcgcagcggtcggactgaacggggggttcgtgcatacagtccagcttggagcgaactgcctacccggaactgagtgtc aggcgtggaatgagacaaacgcggccataacagcggaatgacaccggtaaaccgaaaggcaggaacaggagagcgcacgag ggagccgccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccaccactgatttgagcgtcagatttcgtgatgcttg tcaggggggcggagcctatggaaaaacggctttgccgcggccctctcacttccctgttaagtatcttcctggcatcttccaggaaat ctccgccccgttcgtaagccatttccgctcgccgcagtcgaacgaccgagcgtagcgagtcagtgagcgaggaagcggaatatat cctgtatcacatattctgctgacgcaccggtgcagcctttttttctcctgccacatgaagcacttcactgacaccctcatcagtgccaa catagtaagccagtatacactccgctagcgctgaggtctgcctcgtgaagaaggtgttgctgactcataccaggcctgaatcgccc catcatccagccagaaagtgagggagccacggttgatgagagctttgttgtaggtggaccagttggtgattttgaacttttgctttg ccacggaacggtctgcgttgtcgggaagatgcgtgatctgatccttcaactcagcaaaagttcgatttattcaacaaagccacgtt gtgtctcaaaatctctgatgttacattgcacaagataaaaatatatcatcatgaacaataaaactgtctgcttacataaacagtaa tacaaggggtgtttactagaggagattctcatgtttgacagcttatcatcgataagctttaatgcggtagtttatcacagttaaattg ctaacgcagtcaggcaccgtgtatgaaatctaacaatgcgctcatcgtcattctcggcaccgtcaccctggacgctgtaggcatag gcttggttatgccggtactgccgggcctcttgcgggatatcgtccattccgacagtattgccagtcactatggcgtgctgcttgcgct ctatgcgttgatgcaatttctttgcgcacccgttctcggagccctgtccgaccgctttggccgccgtccagtcctgctcgcttcgctcc ttggagccactatcgactacgcgatcatggcgaccacacccgtcctgtggattctctacgccggacgcatcgtggcgggcatcacg ggtgccacaggtgcggttgctggtgcctatatcgccgacatcaccgatggggaagatcgggctcgccacttcgggctcatgagcg cttgtttcggcgtgggtatggtggcaggccccgtggccgggggactgttgggtgccatctccttgcatgcaccattccttgcggcgg cggtgctcaacggcctcaacctcctcctgggctgcttccttatgcaggaatcgcataagggagagcgccgtccgatgcccttgcgt gccttcaatccagtcagctccttccggtgggcgcggggcatgactatcgtcgccgcacttatgactgttttctttatcatgcaactcg taggacaggttccggcagcgctctgggtcattttcggcgaggaccgctttcgctggagcgcgacgatgatcggcctgtcgcttgcg gtattcggaatcttgcacgccctcgctcaagccttcgtcacgggccccgccaccaaacgtttcggcgagaagcaggccattatcgc gggcatggcggccgacgcgctgggctacgtcttgctggcgttcgcgacgcgcggctggatggccttccccattatgattcttctcgc ttccggcggcatcggtatgcccgcgttgcaggccatgctgtcccgccaagtagatgacgaccatcagggacagcttcaagggtcg ctcgcggctcttaccagcctcacttcgatcattggaccgctgatcgtcacggcgatttatgccgcctcggcgagcacatggaacgg gttggcatggattgtaggtgccgccctttaccttgtctgcctccccgcgttgcgtcgcggtgcatggagccgggccacctcgaccta ataatactagctccggcaaaaaaacgggcaaggtgtcaccaccctgccctttttctttaaaaccgaaaagattacttcgcgtttgc cacctgacgtctaagaaaaggaatattcagcaatttgcccgtgccgaagaaaggcccacccgtgaaggtgagccagtgagttga ttgctacgtaattagttagttagcccttagtgactcgaattcgcggccgcttctagag

Table S6: Sequence of vectors used in this study.

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