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***Escherichia coli* “Marionette” strains with 12 highly optimized small-molecule sensors**

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Supplementary Information for:

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Supplementary Table 1: Promoters

Regulator	Promoter	DNA sequence ^a	Source ^b
AcuR	P _{Acu}	CGCTAGCAAGTAAGCCGACGCTTCACAACCGCACTTGATT TAATAGACCATACCGTCTATTAT TTCTGGCCAT	1-2
AraCE	P _{BAD}	AGAAACCAATTGTCATATTGCATCAGACATTGCCGTCAGTGGCTCTTTTACTGGCTCTTCTCGCTAACCAACCGGTA ACCCCGCTTATTAAAGCATTCTGTAAACAAAGCGGACCAAGCCATGACAAAAACGG TAACAAAAGTGTCTATAATC ACGGCAGAAAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATT TTTTATCCATAAGATTAGC GGATCCTAC CTGACGCTTTTATCGCAACTCTCTACTGTTTCTCCATACCCG	3
AraCE	P _{BADΔ30}	ATTGCCGTCAGTGGCTCTTTTACTGGCTCTTCTCGTAACCAACCGGTAACCCCGCTTATTAAAGCATTCTGTAAACA AAGCGGGACCAAGCCATGACAAAAACGG TAACAAAAGTGTCTATAATC ACGGCAGAAAAGTCCACATTGATTATTTG CACGGCGTCACACTTTGCTATGCCATAGCATT TTTTATCCATAAGATTAGCGGATCCTAC CTGACGCTTTTATCGCAAC TCTCTACTGTTTCTCCATACCCG	Rational
AraCE	P _{BADΔ135}	ACGT TAACAAAAGTGTCTATAATC ACGGCAGAAAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCAT AGCATT TTTTATCCATAAGATTAGCGGATCCTACCTGACGCTTTTATCGCAACTCTCTACTGTTTCTCCATACCCG	Rational
AraCE	P _{BADnoCRP}	AGAAACCAATTGTCATATTGCATCAGACATTGCCGTCAGTGGCTCTTTTACTGGCTCTTCTCGCTAACCAACCGGTA ACCCCGCTTATTAAAGCATTCTGTAAACAAAGCGGACCAAGCCATGACAAAAACGG TAACAAAAGTGTCTATAATC ACGGCAGAAAAGTCCACATTGTTGCGTTTCCCTCGATCTTCTAACCGCACCCCATAGCATT TTTTATCCATAAGATTAGC GGATCCTAC CTGACGCTTTTATCGCAACTCTCTACTGTTTCTCCATACCCG	Rational
AraCE	P _{BADmin}	TAGCATT TTTTATCCATAAGATTAGCGGATCCTACCTGACGCTTTTATCGCAACTCTCTACTGTTTCTCCATACCCG	Rational
BetI	P _{BetI}	AGCGCGGGTGAGAGGGATTGTTACCAATAGACA ATTGATTGGACGTTCAATATAA TGCTAGC	4-5
CinR	P _{Cin}	CCCTTTGTGCGTCCAAACGGACGACGCGGCTCTAAAGCGGGTGGCGATCTTTCAGATTTCGCTCCTCGGCTTTTCAGTC TTGTTTTGGCGCATGTCGTTATCGCAAAACGCTGCACACTTTTGGCGGACATGCTCTGATCCCCCTCATCTGGGGGG GCCTATCTGAGGGAATTC CGATCCGGCTCGCCTGAACCATCTGCTTTCCACGAACCTGAAAACGCT	iGEM R0078
CymR	P _{CymR}	GAAATCATAAAAATTTATTTGCTTTTCAGGAAAATTTTCTGTATAATAGATT CAACAAACAGACAATCTGGTCTGTTT GTATTAT	Rational ⁶
	P _{CymRA}	GAAATCATCAGAACTTTATTTCTTTTCAGGAAAATTTTCTGTATAATAGATT CAACAAACAGACAATCTGGTCTGTTT GTATTAT	Rational
	P _{CymRB}	AACAAACAGACAATCTGGTCTGTTTGTAT TATTTCTTTTCAGGAAAATTTTCTGTATAATAGATT CAACAAACAGACA ATCTGGTCTGTTTGTATTAT	Rational
	P _{CymRC}	AACAAACAGACAATCTGGTCTGTTTGTAT TATGGAATTTTCTGTATAATAGATT CAACAAACAGACAATCTGGTCT GTTTGTATTAT	Rational
LacI	P _{Tac}	TGTTGACAATTAATCATCGGCTCGTATAATGTGTGCA ATTGTGAGCGCTCACAATT	4, 7
	P _{T5LacO}	AATCATAAAAATTTATTTGCTTT TGTGAGCGGATAACAATT TATAATAGATT CAATTGTGAGCGGATAACAATT	pQE (c)
	P _{B4}	AATTGTGAGCGGATAACAATTGACTTGTGAGCGGATAACAATGATACT TCGTGC	8
LuxR	P _{Lux}	ACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTATAGTCGAATAAA	9
	P _{LuxA}	ACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTAT TGTGCGAATAAA	Rational
	P _{LuxB}	ACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTAT AGTCGAATAAA	Rational
	P _{Lux*}	ACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTAT CTTCGAATAAA	9
MphR	P _{Mph}	GGATTGAATATAACC GACGTGACTGTT TACATTAGG TGGCTAAACCCGCTCAA	1, 10
NahR	P _{Sal}	GGGGCTCGCTTGGGTTATTGCTGGTGCCCGCCGGCG CGCAATATTCA TGTTGATGATT TATTATATATC GAGTGGTGT ATT TATCA TATTGTTGCTCCGT TATCGT TATTAAAC	11
	P _{SalTTC}	GGGGCTCGCTTGGGTTATTGCTGGTGCCCGCCGGCG CGCAATATTCA TGTTGATGATT TATTATATATC GAGTGGTGT ATT TAT TATTATTGTTGCTCCGT TAC CGTTATTAAAC	Evolved
	P _{Nah}	ACATGACTACTTCAGACGATT CGCAGTATTAC GCTGGTGAT TAACAAATCAACT ATGCTTTAT TGACA ATAAAAGC ACGCTCACCATCATCGCGAATAC	11-12
PcaU	P _{3B5}	TTTTGTTTCGATTATCGAACA AAATTATT GAAATATCGAACA AAACCTCTAACTACTGTGGCACTGAATCAAAAAATTAT AAACAATGATCAGA	13
	P _{3B5A}	TTTTGTTTCGATTATCGAACA AAATTATT GAAATATCGAACA AAACCTCTAACTACTGTGGCACTGAATCAAAAAATTAT AAAC TAT GATCAGA	Rational
	P _{3B5B}	TTTTGTTTCGATTATCGAACA AAATTATT GAAATATCGAACA AAACCTCTAACTACTGTGGCACTGAATCAAAAAATTAT AAAC TAT GATCAGA	Rational
	P _{3B5C}	TTTTGTTTCGATTATCGAACA AAATTATT GAAATATCGAACA AAACCTCTAACTACTGTGGCACTGAATCAAAAAATTAT AAAC CT TGATCAGA	Rational
PhIF	P _{PhIF}	CGACGTACGGTGAATCTGATTCGTTACCAATTGAC ATGATACGAAACGTACCGTATCGTTAAGGT	5
TetR	P _{Tet*}	TTTTCAGCAGGACGCACTGACCT CCCTATCAGT GATAGAG ATTGACA CTCCCTATCAGT GATAGAGTACT GAGCAC	9
	P _{D18}	TACAACGTCGTGTAGCTGCCCTTCGTCCTCAATAATCT TTGACA CTCCCTATCAGT GATAGAGTACT TGAATA	8

	P _{D79}	TACAACGTCGTGTTAGCTAGCCTTTTCGTCTTCAATAATCTTAGACATCCCTATCAGTGATAGAGATACTTGAATA	8
TetR	P _{TetR}	TCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGATATAATGAGCAC	5
TtgR	P _{Ttg}	CACCCAGCAGTATTACAAACAACCATGAATGTAAGTATATTCCTTAGCAA	1, 14
VanR	P _{Van}	ATTGGATCCAAATTGACAGCTAGCTCAGTCCTAGGTATAATTGGATCCAAT	15
	P _{VanCC}	ATTGGATCCAAATTGACAGCTAGCTCAGTCCTAGGTACCATTTGGATCCAAT	Evolved
SrpR	P _{SrpR}	GATTCGTTACCAATTGACAGCTAGCTCAGTCCTAGGTATATACATACATGCTTGTGTTTGTAAAC	5
σ70	P _{LacI}	GCGGCGGCCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGATAGCGCCC	<i>E. coli</i> genome
	P _{LacIQ}	GCGGCGGCCATCGAATGGTGCAAACCTTTCGCGGTATGGCATGATAGCGCCC	Rational ¹⁶
	P _{LacIR}	GCGGCGGCCATCGAATGGTGAACCTTTCGCGGTATGGCATGATAGCGCCC	Rational
	P _{J23101}	TTTACAGCTAGCTCAGTCCTAGGTATTATGCTAGC	iGEM J23101
	P _{J23100}	TTGACGGCTAGCTCAGTCCTAGGTACAGTGTAGC	iGEM J23100
	P _{J23119}	TTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGC	iGEM J23119

a) -35 box, -10 box, and +1 are underlined. Operators are shown in bold. Evolved or rational point mutations are shown in blue.
b) Rational mutations were made by altering the -10 box, adding or eliminating operators, or (where noted) inspired by previous work. Evolved mutations arose during selection.
c) Qiagen (Hilden, Germany)

Supplementary Table 2: Insulators

Insulator	DNA sequence	Source
RiboJ	AGCTGTACCGGATGTGCTTTCGGTCTGATGAGTCCGTGAGACGAAACAGCCTCTACAAATAATTTGTTTAA	17
RiboJ10	AGCGCTCAACGGGTGTGCTTCCGTTCTGATGAGTCCGTGAGGACGAAAGCGCCTCTACAAATAATTTGTTTAA	4
RiboJ51	AGTAGTCACCGGCTGTGCTTCCGTTCTGATGAGCCTGTGAAGGCGAAACTACCTCTACAAATAATTTGTTTAA	4
RiboJ53	AGCGGTCAACGCATGTGCTTTCGTTCTGATGAGACAGTGTGTCGAAACCGCCTCTACAAATAATTTGTTTAA	4
BydvJ	AGGGTGTCTCAAGGTGCGTACCTTGACTGATGAGTCCGAAAGGACGAAACACCCCTCTACAAATAATTTGTTTAA	4
ElvJ	AGCCCATAGGGTGGTGTGTACCACCCCTGATGAGTCCAAAGGACGAAATGGGCGCTCTACAAATAATTTGTTTAA	4

Supplementary Table 3: RBSs

Protein	RBS	DNA sequence ^a	Source ^b
AcuR	acu1	GGAAGAGAGTCAATT CAGGGTGGT GAAT	<i>E. coli</i> genome
	acu2	GGAAGAGAGTCAATT CAGGAGGT GAAT	Evolved
AraC	ara1	GGAAGAGAGTCAATT CAGGGTGGT GAAT	<i>E. coli</i> genome
	ara2	GGATCCCAATGATGTGGGTCCCCTTT CAAGGAGGT AAAT	RBS calculator ^{12, 18}
AraE	e1	TAAGATCCTATTCCAGCGGGATTAA AGAGGAGCG ATTAAAGC	Evolved
BetI	bet1	GGAAGAGAGTCAATT CAGGGTGGT GAAT	<i>E. coli</i> genome
	bet2	GGAAGAGAGTCAATT CACGGGGT GAAT	Evolved
	bet3	GGAAGAGAGTCATT CA TGGAGGTGAAT	rational
	bet4	GGAAGAGAGTCAATT CACGGAGGT GAAT	rational
CinR	cin1	GGAAGAGAGTCAATT CAGGGTGGT GAAT	<i>E. coli</i> genome
	cin2	AGGTCCGAGACGCCGCTC AACGGAGAA CGGCGA	RBS calculator ^{12, 18}
CymR	cym1	GGAAGAGAGTCAATT CAGGGTGGT GAAT	<i>E. coli</i> genome
CymR	cym2	AATGGAATATTTCATAGTAAATACCA AACGGAGAT TCTT	rational

	cym3	AATGGAATATTTCATAGTAAATACCA AACGGATAT TCTT	RBS calculator ^{12, 18}
LacI	lac1	GGAAGAGAGTCAATT CAGGGTGGT GAA	<i>E. coli</i> genome
	lac2	GGAAGAGAGTCAATT CATGGAGGT GAA	Rational
	lac3	GGAAGAGAGTCAATT CAGGGTAGAGAA C	Rational
	lac4	GGAAGAGAGTCAATT CAGGGTAGAGAA T	Rational
	lac5	GGAAGAGAGTCAATT CAGGGCGGT GAA	Rational
LuxR	lux1	GGAAGAGAGTCAATT CAGGGTGGT GAA	<i>E. coli</i> genome
	lux2	AGGTACTACGCGAGGACTTAAGCCT TTCGGAGGG GCAGA	RBS calculator ^{12, 18}
MphR	mpr1	GGAAGAGAGTCAATT CAGGGTGGT GAA	<i>E. coli</i> genome
	mph2	GGAAGAGAGTCAATT TTAGGAGGT GAA	evolved
	mph3	GGAAGAGAGTCAATT CAGGGAGGT GAA	Rational
NahR	nah1	GGAAGAGAGTCAATT CAGGGTGGT GAA	<i>E. coli</i> genome
	nah2	GGAAGAGAGTCAATT CATGGGGT TGAA	Evolved
	nah3	ACCCCTATAAGAAA AAAGACTTAA CTATCC	RBS calculator ^{12, 18}
PcaU	pca1	GGAAGAGAGTCAATT CAGGGTGGT GAA	<i>E. coli</i> genome
	pca2	GGAAGAGAGTCAATT CAGGGAGGT GAA	Evolved
	pca3	CGCTTACAATAGACGAACAAT AAAGGAGAA TTAACCG	Rational
	pca4	CGCTTACAATAGACGAACAAT TAAGGAGAA TTAACCG	RBS calculator ^{12, 18}
PhlF	phl1	GGAAGAGAGTCAATT CAGGGTGGT GAA	<i>E. coli</i> genome
	phl2	GGAAGAGAGTCAATT CATGGGGT GAA	Evolved
	phl3	CTATGGACTATGTTTGA AAAGGAGAA ATACTAG	4
	phl4	GGAAGAGAGTCAATT CAGGGGGT GAA	Rational
	phl5	GGAAGAGAGTCAATT CAGGGTGGT GAA	Rational
	phl6	CTATGGACTATGTTTGA AAAGGATAC ATACTAG	Rational
TetR	tet1	GGAAGAGAGTCAATT CAGGGTGGT GAA	<i>E. coli</i> genome
	tet2	GTAATAAT CAAGGTGG CAAAAA	Rational
	tet3	GTAATAAT CCAGGAGG AAAAA	RBS calculator ^{12, 18}
	tet4	GTAATAAT CCGGAGG CAAAAA	Rational
TtgR	ttg1	GGAAGAGAGTCAATT CAGGGTGGT GAA	<i>E. coli</i> genome
	ttg2	GGAAGAGAGTCAATT CAGGGAGGT GAA	Rational
	ttg3	TTACGAATTAGAT TAAGTAGGT AAAAAC	Rational
	ttg4	TTACGAATTAGAT TAAGAAGGT AAAAAC	RBS calculator ^{12, 18}
VanR	van1	GGAAGAGAGTCAATT CAGGGTGGT GAA	<i>E. coli</i> genome
	van2	GGAAGAGAGTCAATT CAGGGGGT GAATA	Evolved
	van3	GCTTAAACTAACGAACGTAAAT TAAGGAGGA TAGAC	RBS calculator ^{12, 18}
	van4	GCTT CAGGGGGT GAATA	Rational
	van5	GCTT TAAGGAGGT GAATA	Rational
Ery ^R	ery1	TAATATTGAAAAAGGAAGAT	1
YFP	BBa_B0064	TACTAGAGAAAGAGGGGAAATACTAG	iGEM BBa_B0064
PheS	BBa_B0064	TACTAGAGAAAGAGGGGAAATACTAG	iGEM BBa_B0064
KOD	BBa_B0064	TACTAGAGAAAGAGGGGAAATACTAG	iGEM BBa_B0064

PK6	BBa_B0064	TACTAGAGAAAGAGGGGAAATACTAG	iGEM BBa_B0064
SrpR	srp1	GAGTCTATGGACTATGTTTTCACAGAGGAGGTACCAGG	4
CrtI	crt1	ATAACAAACTTTGACTGTAGGCGGTATTTCTACGGAAGGAGGTAGAC	19
CrtB	crt2	AAATTTAATGATGAGCGAAAAACCCCTTAACCTCTACTAAGGAGGAACGAC	19
CrtE	crt3	TCACACTATACTACATGAGAAGGAGGTTAAGGAAGGGATAA	19
Idi	idi1	ATTCTCGTAGTTTATACTCGTTTAGTTACAATAAGTAGGAGGTTTTTA	RBS calculator ^{12, 18}
Dxs	dxs1	CTAAATATTTCATTAGACACATAGGAGGTTAACA	RBS calculator ^{12, 18}

a) Evolved or rational point mutations are shown in blue.

b) Rational mutations were made by altering the Shine-Dalgarno sequence (bold). See Methods. Evolved mutations arose during selection. The RBS Calculator was used to design RBSs *de novo*.

Supplementary Table 4: Proteins

Protein	Amino acid sequence ^a	Source ^b
AcuR	MPLTDTPPSPVQKPRRGRPRGAPDASLAHQSLIRAGLEHLTEKGYSSVGVEILKAARVPKGSFYHYFRNKADFGGLALIEAYDTYFARLLDQA FLDGS LAPLARLR LRTMAEEG MARHGFRRCGLVGNLQGMALPDDFRAALIGVLETWQRRTAQLFREAQACGELSADHDPALAEAFWIGW EGAILRAKLELRPDLHSFTRTFGRHFVTRTQE	Addgene 62569 ¹
AcuR ^{AM}	MPLTDTPPSPVQKPRRGRPRGAPDASLAHQSLIRAGLEHLTEKGYSSVGVEILKAARVPKGSFYHYFRNKADFGGLALIKAYDTYFARLLDQA FLDGS LAPLARLR LRTMAEEG MARHGFRRCGLVGNLQGMALPDDFRAALIGVLETWQHRTAQLFREAQACGELSADHDPALAEAFWIGW EGAILRAKLELRPDLHSFTRTFGRHFVTRTQE	Evolved
AraC	MAEAQNDPLLPYGSFNAHLVAGLTPIEANGYLDFFIDRPLGMKGYILNLTIRGQGVVKNQGREFVCRPGDILLFPFGEIHYYGRHPEAREWYH QWVYFRPRAYWHEWLNWPSIFANTGFFRPDEAHQPHFSDFFGQIINAGQGEGRYSELLAINLLEQLLLRRMLAINSLHPPMDNRVREACQYI SDHLADSNFDIASVAQHVCLSPSRSLSHLFRQQLGISVLSWREDQRISQAKLLSSTRMPATVGRNVGFDQQLYFSRVFKKCTGASPSSEFRAG CEEKVNDVAVKLS	3
AraC ^{AM}	MAEAQNDPLLPYGSFNAHLVAGLTPIEANGYLDFFIDRPLGMKGYILNLTIRGQGVVKNQGREFVCRPGDILLFPFGEIHYYGRHPEAREWYH QWVYFRPRAYWHEWLNWPSIFANTGFFRPDEAHQPHFSDFFGQIINAGQGEGRYSELLAINLLEQLLLRRMLAINSLHPPMDNRVREACQYI SDHLADSNFDIASVAQHVCLSPSRSLSHLFRQQLGISVLSWREDQRISQAKLLSSTRMPATVGRNVGFDQQLYFSRVFKKCTGASPSSEFRAG LEEKVNDVAVKLS	Evolved
AraE	MVTINTESALTPRSRLDTRRMNMFVSAAVAGLLFGLDIGIAGALFFITDHFVLTSRLQEWVSSMMLGAAIGALFNGWLSFRLGRKYSIM AGAILFVLGSGISAFATSVEMLIAARVVLGIAVGIASYTAPLYLSEMASENVRGKMISMYQLMVTLGIVLAFSLDTAFSYSGNWRAMLGVLLAL PAVLLIILVVFLENPSRWLAEGKRHIEAEVLRMLRDTSEKAREELNEIRESKLGQGWALFKINRNVRAVFLGMLLQAMQQTGMNIIIMY YAPRIFKMGAGTTTTEQQMIATLVVGLTFMFATPIAVFTVKDAGRKPALKIGFSVMALGTVLVGCLMQFDNGTASSGLSWLSVGMTMCIAGY AMSAAPVWVILCEISQPLKCRDFGITCSTTTNNVSNMIGATFTLLDSIGAAGTFWLYTALNIAFVGITFWLIPETKNVTLEHIERKLMAGE KLRNIGV	Addgene 18987 ³
BetI	MPKLGMSIRRRQLIDATLEAINEVGMHDATIAQIARRAGVSTGIIISHYFRDKNGLLEATMRDITSQLRDAVLNRLHALPQGSAEQRLQAIIVG GNFDETVQSSAAMKAWLAFWASSMHQPMYRLQVQSSRRLLSNLVSEFRRELPREQAQAEAGYGLAALIDGLWLRALSGKPLDKTRANSLTRH FITQHLPTD	5
BetI ^{AM}	MPKLGMSIRRRQLIDATLEAINEVGMHDATIAQIARRAGVSTGIIISHYFRDKNGLLEATMRDITSQLRDAVLNRLHALPQGSAEQRLQAIIVG GNFDETVQSSAAMKAWLAFWAISMHQPMLYRLQVQSSRRLLSNLVSEFRRELPREQAQAEAGYGLAALIDGLWLRALSGKPLDKTRANSLTRH FITQHLPTD	Evolved
CinR	MIENTYSEKFESAFEQIKAAANVDAAIRILQAEYNLDFVTYHLAQTIAISKIDSPFVRTTYPDAWVSRYLLNSYVKVDPPIVKQGFERQLPFWDWS EVEPTPEAYAMLVDQAKHGIDNGYSIPVADKAQRALLSLNAHIPADEWTELVRRRCNEWIEIAHLHRKAVYELHGENDVPALSPREIEC LHWTALGDKYKDISVILGISEHTTRDYLKTARFKLGCATTISAAASRAVQLRIINP	iGEM C0077 ²⁰⁻²¹
CinR ^{AM}	MIENTYSEKFESAFEQIKAAANVDAAIRILQAEYNLDFVTYHLAQTIAISKIDSPFVRTTYPDAWVSRYLLNCYVKVDPPIIKQGFERQLPFWDWS EVEPTPEAYAMLVDQAKHGIDNGYSIPVADKAQRALLSLNAHIPADEWTELVRRRCNEWIEIAHLHRKAVYELHGENDVPALSPREIEC LHWTALGDKYKDISVILGISEHTTRDYLKTARFKLGCATTISAAASRAVQLRIINPYRIMTRRNW	Evolved
CymR	MSPKRRTQAERAMETQGKLIAAALGVLEKGYAGFRIADVPGAAGVSRGAQSHHFPTKLELLLATFEWLYEQITERSRARLAKLKPEDDVIQQ MLDDAAEFFLDDDFSIGLDLIVAADRPALREGIQTVERNRFVVEDMWLGVLVSRGLSRDDAEDILWLFNSVRGLAVRSLWQDKKERFERV RNSTLEIARERYAKFKR	5-6
CymR ^{AM}	MSPKRRTQAERAMETQGKLIAAALGVLEKGYAGFRIADVPGAAGVSRGAQSHHFPTKLELLLATFEWLYEQITERSRARLAKLKPEDDVIQQ MLDDAAEFFLDDDFSIGLDLIVAADRPALREGIQTVERNRFVVEDMWLGVLVSRGLSRDDAEDILWLFNSVRGLVVRSLWQDKKERFERV RNSTLEIARERYAKFKR	Evolved
LacI	MKPVTLYDVAEYAGVSYQTVSRVVNQASHVSAKTREKVEAAMELNYIPNRVAQQLAGKQSLIGVATSSALHAPSQIVAAIKSRADQLGAS VVVSMVERSVEACKAAVHNLLAQRVSGLIINYPLDDQDAIAVEAACTNVPALFLDVSQDTPINSIIFSHEDGTRLGVHEHLVALGHQQIALLA GPLSSVSARLRLAGWHKYLTRNQIQPIAEREGDWSAMSGFQQTQMQLNEGIVPTAMLVANDQMALGAMRAITESGLRVGADISVVGYYDDTEDS SCYIIPPLTTIKQDFRLLGQTSVDRLLQLSQGQAVKGNQLLPVSLVKRKTTLAPNTQTASPRALADSLMQLARQVSRLESQ	9
LacI ^{AM}	MKPVTLYDVAEYAGVSYTVSRVVNQASHVSAKTREKVEAAMVELNYIPNRVAQQLAGKQSLIGVATSSALHAPSQIVAAIKSRADQLGAS VVVSMVERSVEACKAAVHNLLAQRVSGLIINYPLDDQDAIAVEAACTNVPALFLDVSQDTPINSIITYSHEDGTRLGVHEHLVALGHQQIALLA GPLSSVSARLRLAGWHKYLTRNQIQPIAEREGDWSAMSGFQQTQMQLNEGIVPTAMLVANDQMALGAMRAITESGLRVGADISVVGYYDDTEDS SCYIIPPLTTIKQDFRLLGQTSVDRLLQLSQGQAVKGNQLLPVSLVKRKTTLAPNTQTASPRALADSLMQLARQVSRLESQ	Evolved
LuxR	MKNINADDTYRIINKIKACRSNNNDINQCLSDMTKMVHCEYYLLAIYPHSMVKSISILDNYPKKWRQYYDDANLIKYDPIVDYSNSNHSPIN WNIFENNAVNKSPNVKEAKTSGLITGFSFPIHTANNFGMLSFHSEKDNIDSLFLHACMNIPLVPSLVDNYRKNIANNNKSNNDLTRK EKECLAWACEGKSSWDISKILGCSERTVTPHLTNAQMKLNTNRQCSISKAILTGAIDCPYFKN	9
MphR	MRPKLLKSDDEVLEAATVVLKRCGPIETFLSGVAKEVGLSRAALIQRFTNRDTLVRRMERMGEVQRHYLNAIPIGAGPQGLWEFLQVLVRSR NTRNDFSNNYLSISWYELQVPELRTLAIQRNRVVEGIRKRLPPGAPAAAEALLHSHVIAGATMQWAVDPDGEADHVLAIQIAAILCLMPFHEHD FQLLQAH	Addgene 62570 ¹

MphR ^{AM}	MMPRKLSKSDDEVLEAATVVLKRCGPIEFRTLGSVAKEVGLSRAALIQRFTNRDITLLVRMMERGVEQVRHYLNAIPIGAGPQGLWEFLQVLVSRM DTRNDFSVNYLISWYELQVPELRTLATIQRNRAVVEGIRKRLPPGAPAAAEALLHSHVIAAGATMQWAVDPDGLADHVLQAQTAAILCLMFPHEHDD FQLLQAHA	Evolved
NahR	MELRDLNLNLVVFNQQLLVDRRVSITAENLGLTQPAVSNALKRLRLTSLQDPLFVRTHQGMEPTPYAAHLAEPVTSAMHALRNALQHESFDPL TSERTFTLAMTDIGEIYFMPRLMDVLAHQAPNCVISTVRDSSMSLMQALQNGTVDLAVGLLPNLQTGFQRRLLQNHVYVCLCRKDHVPVTRERPL TLERFCSYGHVRVIAAGTGHGEVDTYMTRVGIRRDIRLEVPHFAAVGHILQRTDLLATVPICLADCCVEPFGLSALPHFPVVLPEIAINMFWHA KYHKDLANIWLRLQMLFDLFTD	12
NahR ^{AM}	MELRDLNLNLVVFNQQLLVDRRVSITAENLGLTQPAVSNALKRLRLTSLQDPLFVRTHQGMEPTPYAAHLAEHVTSAMHALRNALQHESFDPL TSERTFTLAMTDIGEIYFMPRLMDLAHQAPNCVISTVRDSSMSLMQALQNGTVDLAVGLLPNLQTGFQRRLLQNHVYVCLCRKDHVPVTRERPL TLERFCSYGHVRVIAAGTGHGEVDTYMTRVGIRRDIRLEVPHFAAVGHILQRTDLLATVPICLADCCVEPFGLSALPHFPVVLPEIAINMFWHA KYHKDLANIWLRLQMLFDLFTD	Evolved
PcaU	MWSNMDDKKVKEEKILHNSTNKKIRHEDFVAGISKGMAILDSFGTDRHRLNITMAAEKTMTRAAARRHLLTLEYLGYLESDDGHYFYLTTPKI LKFSGSYLGAQLPKISQPLNLLTTQTSLIYSVMVLDGYEAITIARSAHQQTDRVNPYGLHGNRLPAHATSAGKILLAYLDDHAQQEWLN QYPLQRLTKYTYTNNIDFLRLLSEIKEQGWCYSSEEHGLGVHALAVPIYQQSRVVAALNIVSPMTMRTTKEYLIQHILPLLQETARELRNIL	13, 22
PcaU ^{AM}	MWSNMDDKKVKEENILHNSTNKKIRHEDFVAGISKGMAILDSFGTDRHRLNITMAAEKTMTRAAARRHLLTLEYLGYLESDDGHYFYLTTPKI LKFSGSYLGAQLPKISQPLNLLTTQTSLIYSVMVLDGYEAITIARSAHQQTDRVNPYGLHGNRLPAHTTSAGKILLAYLDDHAQQEWLN QYPLQRLTKYTYTNNIDFLRLLSEIKEQGWCYSSEEHGLGVHALAVPIYQQSRVVAALNIVSPMTMRTTKEYLIQHILPLLQETARELRNIL	Evolved
PhlF	MARTPSRSSIGSLRSPHTHKAILTSTIEILKECGYSGLSIESVARRAGASKPTIYRWWTNKAALIAEVYENESEQVRKFPDLGSFKADLDFLL RNLWKVWRETI CGEAFRCVIAEAQLDPATLTQLKQDFMERRRMPKKLVENAINSGELPKDNTRELLDLMIFGFCWYRLLTEQLTVEQDIEEF TFLLINGVCPGTQR	5
PhlF ^{AM}	MARTPSRSSIGSLRSPHTHKAILTSTIEILKECGYSGLSIESVARRAGAKPTIYRWWTNKAALIAEVYENEIEQVRKFPDLGSFKADLDFLL HNLWKVWRETI CGEAFRCVIAEAQLDPVTLTQLKQDFMERRREIPKKLIVEDAINSGELPKDINRELLDLMIFGFCWYRLLTEQLTVEQDIEEF TFLLINGVCPGTQC	Evolved
TetR	MSRLDKSKVINSALBLNEVGIEGLTTRKLAQKLGEVQPTLYWHVKNKRALLDALAIEMLDHRHHTFCPLEGESWQDFLRNNAKSFRCALLSH RDGAKVHLGTRPTEKQYETLENQLAFLCQQGFSLENALYALSAVGHFTLGCVLEDQEHQVAKEERETPTTDSMPPLLRQAIELFDHQGAEPAP LFGLELIICGLEKQKCESGS	9
TtgR	MVVRTKEEAQETRAQIEAAERAFYKRGVARTTLADIAELAGVTRGAIYWHFNNKAELVQALLDSLHETHDHLARASESEDEVDP LGC MRKLL LQVFNEVLVDARTTRINEIILHKKCEFTDDMCEIRQQRSVAVLDCHKGITLALANAVRRGQLPGE LDAERA AVAMFAYVDGLIRRWLLLPDSVD LLGDVEKWVDTG LDM LRLSPALRK	1, 5
TtgR ^{AM}	MVVRTKEEAQETRAQIEAAERAFYKRGVARTTLADIAELAGVTRGAIYWHFNNKAELVQALLDSLHETHDHLARASESEDEVDP LGC MRKLL LQVFNEVLVDARTTRINEIILHKKCEFTDDMCEIRQQHQS AVLDCHKGITLTLANVVRGQLPGE LDAERA AVAMFAYVDGLIRRWLLLPDSVD LLGDVEKWVDTG LDM LRLSPALRK	Evolved
VanR	MDMPRIKPGQVRMMALRKMIASGEIKSGERIAEIPATAALGVSRMPVRTALRSLEQEGVLVRLGARGYAARGVSSDQIRDAIEVRGVLEGFAA RRLAERGMTAETHARFVALIAEGEALFAAGRLNGEDLDRYAAYNQAFHDTLVSAAGNGAVESALARNGFEPPAAAGALALDMLDPAEYEHLL AAHRHQAVLDVAVSCGDAEGAERIIMRDHALAAIRNAKVFAAASAGAPLGAAWSIRAD	15, 23
VanR ^{AM}	MDMPRIKPGQVRMMALRKMIASGEIKSGERIAEIPATAALGVSRMPVRTALRSLEQEGVLVRLGARGYAARGVSSDQIRDAIEVRGVLEGFAA RRLAERGMTAETHARFVLLIAEGEALFAAGRLNGEDLDRYAAYNQAFHDTLVSAAGNGAVESALARNGFEPPAAAGALALDMLDPAEYEHLL AAHRHQAVLDVAVSCGDAEGAERIIMRDHALAAIRNAKVFAAASAGAPLGAAWSIRAD	Evolved
Ery ^R	MNEKNIKHSQNFITSKHNDIKIMTNIRLNEHDNIFEIGSGKGHFTLELVKRCNFVTAIEIDHKLCKTTENKLVHDHNFQVLNKKDILQFKFPKN QSKYIGNIYNINSTDIIRKIVFDSIANEYILIVEYGFARLLNTRKSLALLLMAEVDISILSMVPREYHPKPKVNSSLIRLSKKSKSRISHK DKQKYNFYVMKWVNKEYKKTFTKNQFNNSLKHAGIDDLNNISFEQFLSLFNSYKLFNK	Addgene 62570 ¹
YFP	MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGDATYKGLTLKFICTTGKLPVPWPTLVTTTFGYGLQCFARYPDHMKLHDFFKSAMPEGY VQERTIFFKDDGNYKTRAEVKFEQDITLVNRIELKIDFKEDGNIILGHKLEYNYNSHNVIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQN TPIGDGVPVLLPDNHYLSYQSALS KDPNEKRDH MVLEFVTAAGITLGMDELYK	4
PheS	MSHLAELVASAKAAISQASDVAALDNVRVEYLKGGKHLTQMOTTLRELPPPEERPAAGAVINEAKEQVQQAALNARKAELESAAALNARLAETID VSLPGRRRIENGLHVPVTRTIDRIESFFGELGFTVATGEIEDDYHNFDAIIPGHHPARADHDTFWFDTTLLRRTQTSQGVQIRTKMAQFPPIR IAPGRVVRNDYDQTHTFMFHQMEGLIVDTNISFTNLKGTLHDFLRNFFEDDLQIRFRPSYFFPTPEPSAEVDVMGKNKGWLEVLCGMVHPNV LRNVGIDPEVYSGFGFGMGMERLTMRLRYGVTDLRSFFENDLRLFKQFK	24-25
KOD	MILDTDYITEDGKPVIRIFKKENGFEKIEYDRTFEPYFYALLKDDSAIEEVKKITAERHGTVVTVKRVEKVQKKFLGRPVEVWKLYFTHPQDV PAIRDKIREHPAVIDIYEYDIPFAKRYLIDKGLVPMEGDEELKMLAFDIETLYHEGEEFAEGPIMISYADEEGARVITWKNVDLPYVVDVST EREMIKRFLRVVKEKDPDVLITYNGDNDFAYLKKRCEKLGINFALGRDGSSEPKIQRMGDRFAVEVKGRIFDLYPVIRRTINLPTYTLEAVY EAVFGQPKKVVYAEIITAWETGENLERVARYSMEDAKVTYELGKEFLPMEAQLSRLIGQSLWDVRSSTGNLVEWFLRLKAYERNELAPNKP DEKELARRRQSYEGGYVKEPERGLWENIVYLDFRSLYPSIIITHNVSPDTLNRGCKEYDVAPOVGHFRCKDFPGFIPSLLDLLEERQKIKK KMKATIDPIERKLLDYRQRAIKILLANSFYGGYARARWYCKECAESVTAWGREYITMTIKEIEKYGFKVIYSDTDGFFATIPGADAETVKK KAMEFLKYINAKLPGALELEYEYFKRGFFVTKKYAVIDEEGKITTRGLEIVRRDWEIAKETQARVLEALLKGDGVEKAVRIKVEVTEKLS KYEVPPEKLVIEHQITRDLDKYKATGPHVAVAKRLAARGVKIRPGTVISYIVLKGSGRIGDRAIPFDEFDPTKHKYDAEYIENQVLPFAVERI LRAFGYRKEDLRYQKTRQVGLSAWLKPKGTLE	26
PK6	MILDTDYITEDGKPVIRIFKKENGFEKIEYDRTFEPYFYALLKDDSAIEEVKKITAERHGTVVTVKRVEKVQKKFLGRPVEVWKLYFTHPQDV PAIRDKIREHPAVIDIYEYDIPFAKRYLIDKGLVPMEGDEELKMLAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKNIDLPYVVEVSS EREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLTIGRDGSEPKMQRIGDMTAVEVKGRIFDLYPVIRRTINLPTYTLEAVY EAVFGQPKKVVYAEIITAWETGENLERVARYSMEDAKVTYELGKEFLPMEAQLSRLIGQSLWDVRSSTGNLVEWFLRLKAYERNELAPNKP DEKELARRRQSYEGGYVKEPERGLWENIVYLDFRSLYPSIIITHNVSPDTLNRGCKEYDVAPOVGHFRCKDFPGFIPSLLDLLEERQKIKK KMKETQDPIDKILLDYRQRAIKILLANSFYGGYARARWYCKECAESVTAWGREYITMTIKEIEKYGFKVIYSDTDGFFATIPGADAETVKK KAMEFLKYINAKLPGALELEYEYFKRGFFVTKKYAVIDEEGKITTRGLEIVRRDWEIAKETQARVLEALLKGDGVEKAVRIKVEVTEKLS KYEVPPEKLVIEHQITRDLDKYKATGPHVAVAKRLAARGVKIRPGTVISYIVLKGSGRIGDRAIPFDEFDPTKHKYDAEYIENQVLPFAVLR LEGFGYRKEDLRYQKTRQVGLSAWLKPKGTLE	27
SrpR	MARKTAAEAETQRQIRIDAALEVFAQGVSDATLDQIARKAGVTRGAVYWHFNGKLEVLQAVLASRQHPLELDFTPD LGIERSWEAVVVA ML D AVHSPQSKQFSEILTYQGLDESGLIHNRMVQASDRFLQYIHQVLRHAVTGELPINLDLQTSIGVFGLITGLLYEGLRSKQQAQIIKVALG SFWALLREPRFRLCEEAIKQVKSFE	5
CrtI	MKKTVVIGAGFGGLALAIRLQAAGIPTVLEQRDKPGGRAYVWHQDGFTFDAGPTVITDPTALEALFTLAGRRMEDYVRLLPVKPFYRLCWES GKTLDYANDSAELEAQITQFNPRDVEGYRRFLAYSQAVFQEGYLRLGSPVFLSRDMLRAGPQLLKQAWQSVYQSVSRFIDEHLRQAFS SLLVCGNFPTSSITYLIIHALEREWGVNFPCEGTGALVNGMVKLFTDLGGEIELNARVEELVVDNRVSVQVRLADGRILDTDAVASADNVUNT YKKLLGHHFVGQKRAAALERKSMSNSFLVLYFGLNQPHSLAHHTICFGPRYRELIIDEIFTSALADDFSLYLHSPCVTDP LSPAPPGCASFYV LAPVPHLGNAPLDWAQEGPKLRDRIIDYLEERYMPLGRSLQVTQRIFTPADFHDTLDAHLGSAFSEIPLLTQSAWFRPHNRSDIANLYLVGA GTHPGAGIPGVVASAKATASLMIEDLQ	19
CrtB	MSQPELLDHATQTMALNGSKSFATAAKLFDPATRRSVMLMYTWCRCDDVIDDQTHGFASAAAAEEATQRLARLRLTLAAFEAGEMAQDPFA AFQEVLELDTGIRTRMAGLDHGFAMDVAQRYRYFTFDTLCYCHVAGVGLMMARVMGVDRDVRDLDRACDLGLAFQLTNIAARDIIDAAIDRC	19

	YLP AEWLQDAGLTPENYAARENRAALARVAERLIDAAEPYIISQAGLHDLPPRCAWAIATARSVYREIGIKVKAAGGSAWDRRQHTSKGEKI AMLM AAFPQVIRAKTTRVTPRPAGLWQRPV	
CrtE	MVSGSKAGVSPHREIEVMRQSIDDHLAGLLPETDSQDIVSLAMREGVMAPGKRIRPLMLLAARDLRYQGSMP TLLDLACAVELTHTASLM LD DMPCMDNAELRRGPTTHKKFGESVAILASVGLLSKAFGLIAATGDLPGERRAQAVNELSTAVGVQGLVLGQFRDLNDAALDRTPDAILSTNH LKTGILFSAMLQIVAIASASSPSTRETLHAFALDFGQAFQLLDDLRDDHPETGKDRNKDAGKSTLVNRLGADAA RQKLREHIDSADKHLTFAC PQGGAIRQFMHLWFGHLADWSFVMKIA	19
Idi	MQTEHVILLNAQGVPTGTLEKYAAHTADTRLHLAFSSWL FNAKGQLLVTRRLSKKAWPGVWTVNSVCGHPQLGESNEDAVIRRCRYELGVEIT PPESIYPDFRYRATDP SGIVENEVCVFFAARTTSALQINDDEVMDYQWCDLADVLHGIDATPWAFSPWMVMQATNREARKRLSAFTQLK	19
Dxs	MSFDIAKYPTLALVDSTQELRLLPKESLPKLCDELRRYLLDSVSRSSGHFASGLGTVELTVALHYVYNTPF DQLIWDVGHQAYPHKILTGRRD KIGTIRQKGG LHPFPWRGESEYDVL SVGHSSTSISAGIGIAVAAEKEGKNRRTVCVIGDGAITAGMAFEAMNHAGDIRPDMLVILNDNEMSIS ENVGALNNH LAQLLSGKLYSSLREGGKKVFSGVPP IKELLKRTEEHIKGMVVP GTLFEELGFNYIGPVDGHDV LGLITTLKNMRDLKGPQFLH IMTKKGRGYEPAEKDPI THAVPKFDPSSGCLPKSSGGLPSYSKIFGDWLCETA AKDNKLMAITPAMREGSGMVEFSRKFPDRYFDVAIAEQH AVTFAAGLAIGYKPIVAIYSTFLQRAYDQVLHDVAIQKLPVLFAIDRAGIVGADGQTHQGAFDLSYLRCIPEMVIMTPSDENECRQMLYTG HYNDGPSAVRYP RGNVAVGVELTPLEKLP IKGKIVKRRGEKLA I LNF GTLMPEAAKVAESLNATLVDMRFVKPLDEALILEMAASHEALVTVEE NAIMGAGSGVNEVLMHRKVPVVLNIGLPDFFIPQGTQEEMRAELGLDAGMEAKIKAWLA	19

- a) Evolved mutations are shown in blue, bold, and underlined.
b) Evolved mutations arose during selection.

Supplementary Table 5: Terminators

Terminator	Sequence	Source
B0053	TCCGGCAAAAAACGGGCAAGGTGTACCACCC TGCCTTTTCTTTAAACCGAAAAGATTACTTCGCGTT	iGEM B0053
ECK120017009	GATCTAACTAAAAAGCCGCTCTCGCGCCTTTTCTTTTCACT	28
ECK120033736	AACGCATGAGAAAGCCCCGGAAGATCACCTTCCGGGGCTTTTTTATTGCGC	28
ECK120033737	GGAAACACAGAAAAAGCCCGCACCTGACAGTGGGGCTTTTTTTCGACCAAAGG	28
IOT	TAATTGGTAACGAATCAGACAATTGACGGCTCGAGGGAGTAGCATAGGGTTTGAGAATCCCTGCTCGTCCATTTGAC AGGCACATTATGCATCGATGATAAGCTGTCAAACATGAGCAGATCCTCTACGCCGACGCATCGTGGCCGGCATCACCG GGCCACAGGTGCGGTTGCTGGCGCTATATCGCCGACATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCAT GAGCAATATTTTATCTG	5
L3S2P21	CTCGGTACCAAATTCAGAAAAAGAGCCCTCCCGAAAGGGGGCCTTTTTTCGTTTTGGTCC	28
L3S3P21	CCAATTATTGAAGGCCTCCCTAACGGGGGCCTTTTTTGTTTCTGGTCTCCC	28
L3S2P56	CTCGGTACCAAATTTTCGAAAAAGACGCTGAAAGCGCTTTTTTCGTTTTGGTCC	28
L3S3P41	AAAAAAAAAAACACCCTAACGGGTGTTTTTTTTTTTTTTGGTCTCCC	28
L3S1P52	TCTAACTAAAAAGGCCTCCCAAATCGGGGGCCTTTTTTATTGATAACAAAA	28
L3S2P41	CTCGGTACCAAAAAAAAAAAAAAGACGCTGAAAGCGCTTTTTTTTTTTGGTCC	28

Supplementary Table 6: Plasmid-encoded regulators response function parameters^a

Inducer	Plasmid	Regulator	Promoter	y_{max} (RPU)	y_{min} (RPU $\times 10^{-3}$)	K (μ M)	n	Dynamic range
DAPG	pAJM.847	PhIF ^{AM}	P _{PhIF}	2.5	2.5	1.7	2.1	1000
Cuma	pAJM.657	CymR ^{AM}	P _{CymRC}	3.7	4.3	8.9	2.4	870
OC6	pAJM.474	LuxR	P _{LuxB}	1.3	2.4	0.12	1.8	540
Van	pAJM.773	VanR ^{AM}	P _{VanCC}	3.0	2.4	26	2.3	1200
IPTG	pAJM.336	LacI ^{AM}	P _{Tac}	3.3	4.8	140	1.8	690
aTc	pAJM.011	TetR	P _{Tet*}	2.4	4.9	0.013	3.8	500
Ara	pAJM.677	AraC ^{AM}	P _{BAD}	1.2	2.4	37	1.5	500
Cho	pAJM.683	BetI ^{AM}	P _{BetI}	2.6	8.5	4100	2.7	310
Nar	pAJM.661	TtgR ^{AM}	P _{Ttg}	0.46	3.4	95	1.9	140
DHBA	pAJM.690	PcaU ^{AM}	P _{3BSB}	1.6	4.5	370	1.8	360
Sal	pAJM.771	NahR ^{AM}	P _{SalTTC}	2.8	4.7	43	1.8	600
OHC14	pAJM.1642	CinR ^{AM}	P _{Cin}	1.5	3.0	0.43	2.3	490
Acr	pAJM.884	AcuR ^{AM}	P _{Acu}	3.1	37	130	2.5	83
Ery	pAJM.969	MphR ^{AM}	P _{Mph}	0.29	8.0	65	1.5	36

a) Response data from three days were averaged and fit to Equation 1 (Methods). Full response functions are provided in Supplementary Note 1. Definitions of parameters are provided in the main text. All data are from experiments using plasmid based-regulators in *E. coli* DH10B cells in mid-log growth in LB media.

Cluster Sequence^{a,b}

Marionette

TAACCAATCAGGCTTCTTACTTTACAGAATTGAGAAAGAGGATGTGGAAACGCTTACTAGCTCCGGCAAAAAACGGGCAAGGTGTACCAACCCCTGCCCTTTTCTTTAAACCGGA
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GAAATCGAACAGGTAGCTAAATTTCCGGAATTTGGGTAGCTTTTAAACCGCGATTCGTGTAATTTCTCGCTGCAATAATCTGTGAAAGTTTGGCGTGAAACCATTTTGGTGGAAGCATTTT
GTGTGTTATTGCGAAGCAGATTGGACCGCTGTAACTTACCGCACTGAAAGATTCAGTTATTGGAACAGCTGCTGTGAGATACCGCAAAACCTGGTTGGAAGTAGGCTATTGAAAT
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ACCTTCTCGCTGTTTAATGGTGTGTTCCGGGTACACAGTTGTGATAAATGGAATTTACATAGTAATACCAACCGAGATTCCTTATGCGCCGAACCTGCTACCCAGCGAGA
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GGTGACAGAGCCCATCTTTCCGCAACCTGGAACCTGCTGTGGCAACCTTTGATAGGCTGTATGACAGATTTACCGAACCTAGCCGTGCACGCTGTGCCAAACCTGAAACCGG
AAGATGATGTTATTACAGAGCATCGGTGATGTCAGCAGAAATTTTCTGGAATGATTTTAGCATCGGCTGGAATCTGATTTGCGAGCAGCATGCTGATCGGCACCTGCGTGA
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TTTAAACAGCGTTCGTGGTGTGCTGGATTGCTGAGCTGTGGCAGAAAGATAAAGACGTTTGAACCGTGTGGGATATAGACCACTGGAATTTGCACTGAGCTGATGAAATTTT
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TGACATCTTCTGCTGGCAGCATGCTGACATCAGGCATTTCTGATGAGCTTACTGTGATGCGCAAGGTTGCAAGCATTAATGCTGATGATGACCTGGCAGCAATTCGT
AATGCAAAAGTTTTTGAAGCAGCAGCAAGCGCAGGCGCACCGCTGGGTGACGATGGTCAATTCGTGCAAGTTGAATAGTAGAATTTGGTAAACGAATCAGACAATTCAGCGCTCGAGG
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GCAATCAAAATTCAGCGGATAGCGGAACGGAAGCGACTGGAGTGCATGTCGCGTTCCTCAACAAACCATGCAAAATGCTGAATGAGGCGCATCTGTTCCCACTGCGATGCTGGTGTG
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CCAAATTTCTCAGTGGTTGTCGAGGCGGTGGAAGCACCTTTACGCCA

- a) DNA sequence colors correspond to promoters (orange), RBSs (green), protein coding sequences (red), terminators (purple), spacers (black), Chloramphenicol resistance cassette (pink), and *g/lc* homology regions (blue).
b) Sequence was verified by PCR and sequencing from the genome of Marionette.

Supplementary Table 8: Marionette genome sequences

Strain number	Strain name	Reference	Mutation ^a	locus	Mutation also found in parental strain
sAJM.1506	Marionette-Wild	NC_00913	Synonymous substitution A547,694G	<i>ylbE</i>	Yes
			1 bp insertion at 547,836	<i>ylbE</i> ^b	Yes
			1 bp insertion at 1,211,312	Intergenic	Yes
			776 bp deletion (IS1) from 1,976,520 to 1,977,296	Intergenic	Yes
			Non-synonymous substitution G2,017,485A	<i>fliK</i> (V359I)	Yes
			Marionette cluster insertion at 3,860,010	<i>glvC</i> (replaced)	No ^c
			1199 bp insertion (IS5A) at 3,888,103	<i>tnaA</i> (disrupted)	Yes
			Substitution C3,957,958T	Intergenic	Yes
			2 bp insertion at 4,294,405	Intergenic	Yes
sAJM.1504	Marionette-Clo	NC_010473 ^d	1331 bp insertion (IS2A) at 142,870	<i>fhuA</i> (disrupted)	Yes
			1199 bp insertion (IS5A) at 399,877	Intergenic	Yes
			776 bp insertion (IS1) at 1,154,509	Intergenic	Yes
			777 bp deletion (IS1) at 1,339,315	Intergenic	Yes
			Non-synonymous substitution G1,917,065A	<i>astD</i> (A422V)	Yes
			16,510 bp deletion from 2,455,019 to 2,471,529	Δ <i>arnB-elaA</i>	Yes
			1336 bp insertion (IS2A) at 3,062,665	<i>ygdQ</i> (disrupted)	Yes
			1338 bp insertion (IS10) at 3,217,235	Intergenic	Yes
			1336 bp insertion (IS2A) at 3,462,690	Intergenic	Yes
			1446 bp insertion (IS150) at 3,630,429	<i>envZ</i> (disrupted)	Yes
			66 kb transduced element replacing 3,957,000 to 4,010,000	<i>glvG</i> to <i>glmU</i>	No ^e
			1 bp insertion at 4,272,973	Intergenic	Yes
			9,934 bp deletion from 4,377,242 to 4,387,176	Δ <i>yjcD-nrfA</i>	Yes
sAJM.1505	Marionette-Pro	CP010816 ^f	55 kb transduced element replacing 151,000 to 204,000	<i>panC</i> to <i>ipxD</i>	Yes
			70 kb transduced element replacing 3,706,000 to 3,764,000	<i>uhpB</i> to <i>bglG</i>	No ^e
			7 kb transduced element replacing 3,768,000 to 3,775,000	<i>pstC</i> to <i>atpD</i>	No ^e
			Synonymous substitution A4,366,616T	<i>pepA</i>	Yes

- a) Numbering based on reference sequence (*E. coli* MG1655 NCBI accession number NC_000913, *E. coli* DH10B NC_010473, and *E. coli* BL21 CP010816)
- b) Undoes a frameshift that is likely an error in the reference sequence.
- c) Introduced by recombineering. See Methods.
- d) Marionette-Clo derives from *E. coli* NEB10beta from New England Biolabs. NEB confirms their *E. coli* NEB10beta cells contain the noted mutations other than the transduction event from Marionette-Wild.
- e) Transduction from Marionette-Wild; introduces Marionette-cluster. See Methods.
- f) Marionette-Pro derives from *E. coli* BL21 from New England Biolabs. NEB confirms their *E. coli* BL21 cells contain the noted mutations other than the transduction event from Marionette-Wild.

Supplementary Table 9: Genome-encoded regulators response function parameters^a

Inducer	Reporter Plasmid	Regulator	Promoter	y_{max} (RPU)	y_{min} (RPU $\times 10^{-3}$)	K (μ M)	n	Dynamic range
DAPG	pAJM.711	PhlF ^{AM}	P _{PhlF}	3.4	6.5	2.1	2.3	530
Cuma	pAJM.712	CymR ^{AM}	P _{CymRC}	7.6	3.6	22	2.3	2100
OC6	pAJM.713	LuxR	P _{LuxB}	4.1	8.6	0.012	1.7	480
Van	pAJM.714	VanR ^{AM}	P _{VanCC}	4.5	4.0	14	2.1	1100
IPTG	pAJM.715	LacI ^{AM}	P _{Tac}	2.6	8.3	190	1.7	320
aTc	pAJM.717	TetR	P _{Tet*}	3.2	3.6	0.012	4.4	890
Ara	pAJM.716	AraC ^{AM}	P _{BAD}	1.8	3.6	43	1.7	500
Cho	pAJM.718	BetI ^{AM}	P _{BetI}	3.7	4.0	1900	2.0	930
Nar	pAJM.719	TtgR ^{AM}	P _{Ttg}	0.50	4.0	280	2.3	130
DHBA	pAJM.1459	PcaU ^{AM}	P _{3BSC}	0.81	8.0	240	1.5	100
Sal	pAJM.721	NahR ^{AM}	P _{SalTTC}	2.9	3.8	29	2.1	760
OHC14	pAJM.944	CinR ^{AM}	P _{Cin}	1.2	3.6	0.25	3.0	340

a) Response data from three days were averaged and fit to Equation 1 (Methods). Full response functions are provided in Supplementary Note 1. Definitions of parameters are provided in the main text. All data are from experiments using genome based-regulators in Marionette-Wild cells in mid-log growth in LB media.

Supplementary Table 10: Extracellular and intracellular concentrations of each inducer

Inducer	[Added to culture] (μM)	[Detected in supernatant ^a] (μM)		[Detected in pellet ^a] (μM)	
		Average	s.d.	Average	s.d.
DAPG	25	15	14	71	45
Cuma	100	55	36	130	50
OC6	10	7.5	2.3	14	11
Van	100	97	16	230	97
IPTG	1000	1500	830	6500	3700
aTc	0.20	0.088	0.075	0.81	0.43
Cho	10000	5800	4100	6700	1200
Nar	1000	550	310	2700	1500
DHBA	1000	560	200	2500	320
Sal	100	88	9.4	290	150
OHC14	10	2.9	2.8	84	59

a) See Methods for details. All data are from experiments using Marionette-Wild cells in mid-log growth in LB media. The average and standard deviation were calculated based on three replicates performed on different days. No measurements for Ara are above the limit of detection.

Supplementary Table 11: mRNA levels in *E. coli* MG1655 and Marionette-Wild

Gene	mRNA levels ^a		log(fold change) ^b	RefSeq annotation
	<i>E. coli</i> MG1655	Marionette-Wild		
glvG	34.2	0.2	2.2	pseudogene
chiA	3.1	0.2	1.2	periplasmic endochitinase
ydiH	1.3	0.1	1.1	uncharacterized protein
casD	1.5	0.1	1.0	CRISP RNA (crRNA) containing Cascade antiviral complex protein
gatD	1.7	0.3	0.8	galactitol-1-phosphate dehydrogenase, Zn-dependent and NAD(P)-binding
yhaI	0.7	0.1	0.8	DUF805 family inner membrane protein
cysN	3.1	0.5	0.8	sulfate adenyltransferase, subunit 1
mglB	1.1	0.2	0.8	methyl-galactoside transporter subunit
malE	1.2	0.2	0.8	chromate reductase, quinone reductase, FMN-linked; N-Ethylmaleimide reductase; old yellow enzyme
ykgF	0.8	0.1	0.7	ferridoxin-like LutB family protein; putative electron transport chain YkgEFG component
malK	0.5	0.1	0.7	maltose ABC transportor ATPase
gltF	2.0	0.4	0.7	periplasmic protein
cysJ	3.2	0.7	0.7	sulfite reductase, alpha subunit, flavoprotein
ytjA	1.0	0.2	0.6	uncharacterized protein
srlB	0.4	0.1	0.6	glucitol/sorbitol-specific enzyme IIA component of PTS
ygjH	0.5	0.1	0.6	putative tRNA binding protein; putative tRNA corner chaperone
yodC	1.8	0.4	0.6	uncharacterized protein
cysC	1.9	0.4	0.6	adenosine 5'-phosphosulfate kinase
ykfF	0.7	0.2	0.6	CP4-6 prophage; uncharacterized protein
fxsA	2.6	0.6	0.6	suppressor of F exclusion of phage T7
ilvC	2.7	0.6	0.6	transcriptional activator of ilvC; autorepressor
yedF	0.7	0.2	0.6	putative TusA family sulfurtransferase
pspC	0.5	0.1	0.6	psp operon transcription co-activator
ydiZ	0.9	0.2	0.6	uncharacterized protein
ygbT	1.0	0.2	0.6	multifunctional endonuclease Cas1, CRISPR adaptation protein; DNA repair enzyme
ychQ	0.8	0.2	0.6	SIRB family inner membrane protein
yehR	0.5	0.1	0.6	lipoprotein, DUF1307 family
yhcO	0.6	0.1	0.6	putative barnase inhibitor
yicT	0.6	0.1	0.6	pseudogene
zraP	1.0	0.2	0.6	Zn-dependent periplasmic chaperone
yedJ	0.4	1.5	-0.6	putative HD superfamily phosphohydrolase
acrA	1.4	5.7	-0.6	multidrug efflux system
cnu	0.4	1.6	-0.6	nucleoid-associated oriC-binding protein; H-NS and StpA stabilizing factor
arfB	0.2	0.8	-0.6	alternative stalled-ribosome rescue factor B; peptidyl-tRNA hydrolase, ribosome-attached
dld	0.2	1.0	-0.6	D-lactate dehydrogenase, FAD-binding, NADH independent
yhdN	0.2	0.9	-0.6	DUF1992 family protein
elaB	0.3	1.2	-0.6	putative membrane-anchored DUF883 family ribosome-binding protein
ycbC	0.1	0.5	-0.6	
yfiR	0.2	0.7	-0.6	putative periplasmic inhibitor of YfiN activity
uidR	0.3	1.2	-0.6	transcriptional repressor
yciK	0.2	1.0	-0.6	putative EmrKY-TolC system oxoacyl-(acyl carrier protein) reductase
mobB	0.2	0.7	-0.7	molybdopterin-guanine dinucleotide biosynthesis protein B
ybaA	0.2	1.1	-0.7	DUF1428 family protein
fdnI	0.1	0.6	-0.7	formate dehydrogenase-N, cytochrome B556 (gamma) subunit, nitrate-inducible
ydhO	0.1	0.5	-0.7	
cof	0.1	0.5	-0.7	thiamine pyrimidine pyrophosphate hydrolase; HMP-PP phosphatase
cueR	0.2	1.0	-0.7	copper-responsive regulon transcriptional regulator
ytfH	0.2	1.2	-0.7	DUF24 family HxIR-type putative transcriptional regulator
gadW	0.1	0.7	-0.8	transcriptional activator of gadA and gadBC; repressor of gadX
blc	0.2	1.0	-0.8	outer membrane lipoprotein cell division and growth lipocalin

yhhQ	0.1	0.9	-0.8	DUF165 family inner membrane protein
yebB	0.1	1.1	-0.9	DUF830 family protein
yhcN	1.3	12.9	-1.0	periplasmic protein, function unknown, YhcN family
gnsB	0.5	6.5	-1.1	Qin prophage; multicopy suppressor of secG(Cs) and fabA6(Ts)
creA	0.2	2.7	-1.2	putative periplasmic protein

a) FPKM in arbitrary units. See Methods for full description.

b) Positive log(fold change) denotes higher mRNA levels in Marionette-Wild. Negative log(fold change) denotes higher mRNA levels in *E. coli* MG1655. Only genes with a |fold change| greater than four and a FPKM higher than 0.1 a.u. in either strain are shown.

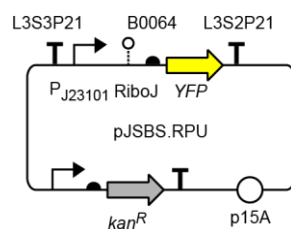
Supplementary Table 12: Protein synthesis rates in *E. coli* MG1655 and Marionette-Wild

Gene	Protein synthesis rate ^a		log(fold change) ^b	RefSeq annotation
	<i>E. coli</i> MG1655	Marionette-Wild		
casA	0.4	70.2	2.2	casA; CRISP RNA (crRNA) containing Cascade antiviral complex protein
glvG	1.8	75.8	1.6	pseudogene
casC	2.8	118.2	1.6	casC; CRISP RNA (crRNA) containing Cascade antiviral complex protein
casB	1.8	65.3	1.5	casB; CRISP RNA (crRNA) containing Cascade antiviral complex protein
malK	0.5	15.2	1.5	malK; maltose ABC transporter ATPase
casE	1.9	40.4	1.3	CRISPR RNA precursor cleavage enzyme; CRISP RNA (crRNA) containing Cascade antiviral complex protein
casD	1.7	33.3	1.3	casD; CRISP RNA (crRNA) containing Cascade antiviral complex protein
gspG	0.1	2.1	1.3	pseudopilin, cryptic, general secretion pathway
chiA	5.1	98.2	1.3	putative bifunctional chitinase/lysozyme precursor
yghS	0.9	15.4	1.3	putative ATP-binding protein
mglC	0.2	2.2	1.1	methyl-galactoside transporter subunit
lamB	2.3	30.1	1.1	maltose outer membrane porin (maltoporin)
ygbT	0.6	7.3	1.1	multifunctional endonuclease Cas1, CRISPR adaptation protein; DNA repair enzyme
uidA	0.8	9.8	1.1	beta-D-glucuronidase
yghR	0.2	1.9	1.1	putative ATP-binding protein
yiiG	2.6	28.2	1.0	DUF3829 family lipoprotein
gspD	0.5	4.9	1.0	general secretory pathway component, cryptic
gspC	0.8	7.3	1.0	general secretory pathway component, cryptic
gspE	0.3	2.4	0.9	general secretory pathway component, cryptic
galS	0.3	2.3	0.9	galactose- and fucose-inducible galactose regulon transcriptional isorepressor; mgl operon transcriptional repressor; autorepressor
gspF	0.2	1.4	0.9	general secretory pathway component, cryptic
ykgE	2.0	14.5	0.9	cysteine-rich LutA family protein; putative electron transport chain YkgEFG component
envR	0.3	2.0	0.9	DNA-binding transcriptional regulator
torD	0.1	0.7	0.8	TorA-maturation chaperone
yjhl	0.2	1.0	0.8	putative DNA-binding transcriptional regulator
rem	0.2	1.5	0.8	Qin prophage; uncharacterized protein
yfgH	2.2	14.8	0.8	outer membrane integrity lipoprotein
yghT	0.5	3.4	0.8	putative ATP-binding protein
srlA	1.7	10.4	0.8	glucitol/sorbitol-specific enzyme IIC component of PTS
malE	12.4	74.4	0.8	malE; maltose transporter subunit
pitB	1.4	8.4	0.8	hypothetical protein
ybeQ	0.3	1.6	0.7	Sel1 family TPR-like repeat protein
bssR	0.1	0.8	0.7	repressor of biofilm formation by indole transport regulation
rrrD	0.2	1.3	0.7	rrrD; DLP12 prophage; putative lysozyme
ykgF	3.8	19.9	0.7	ferridoxin-like LutB family protein; putative electron transport chain YkgEFG component
paaG	0.2	0.9	0.7	1,2-epoxyphenylacetyl-CoA isomerase, oxepin-CoA-forming
malM	3.3	16.8	0.7	maltose regulon periplasmic protein
yfeN	0.2	1.0	0.7	putative outer membrane protein
yghQ	0.4	2.2	0.7	putative inner membrane polysaccharide flippase
ynfO	0.3	1.3	0.7	uncharacterized protein, Qin prophage
araB	0.3	1.2	0.7	enzyme, degradation of small molecules: carbon compounds
ydaV	0.1	0.6	0.7	Rac prophage; putative DNA replication protein
yahl	0.1	0.5	0.7	carbamate kinase-like protein
lldP	1.8	8.1	0.7	L-lactate permease
gspA	0.1	0.7	0.7	general secretory pathway component, cryptic
cysD	21.6	98.0	0.7	sulfate adenylyltransferase
mglB	6.8	31.0	0.7	mglB; methyl-galactoside transporter subunit
yfgI	1.4	6.2	0.7	nalidixic acid resistance protein, putative periplasmic protein
yjeJ	4.8	21.3	0.6	uncharacterized protein
malF	0.8	3.6	0.6	malF; maltose transporter subunit

tnaA	1.0	4.1	0.6	tryptophanase/L-cysteine desulfhydrase, PLP-dependent
hscC	0.5	2.1	0.6	Hsp70 family chaperone Hsc62
yccM	0.2	0.9	0.6	putative 4Fe-4S membrane protein
cysC	8.1	33.8	0.6	adenosine 5'-phosphosulfate kinase
psuG	1.4	5.8	0.6	pseudouridine 5'-phosphate glycosidase
yjfl	0.8	3.3	0.6	DUF2170 family protein
napH	0.8	0.2	-0.6	ferredoxin-type protein
yadK	0.5	0.1	-0.6	putative fimbrial-like adhesin protein
yhcN	597.7	142.3	-0.6	cadmium and peroxide resistance protein, stress-induced
ulaC	1.3	0.3	-0.6	L-ascorbate-specific enzyme IIA component of PTS
feoC	8.8	2.0	-0.6	putative DNA-binding transcriptional regulator
ycaP	0.7	0.2	-0.6	UPF0702 family putative inner membrane protein
uidR	33.0	7.5	-0.6	transcriptional repressor
yeeO	1.0	0.2	-0.7	putative multidrug exporter, MATE family
dld	41.1	8.7	-0.7	LDHD, DLD; lactate dehydrogenase D
ybhG	5.1	1.0	-0.7	putative membrane fusion protein (MFP) component of efflux pump, membrane anchor
yraN	2.1	0.4	-0.7	UPF0102 family protein
yhjY	0.7	0.1	-0.8	autotransporter beta-domain protein
ybhF	4.2	0.6	-0.8	putative ABC transporter ATPase
ybiH	5.9	0.9	-0.8	ybiH; DUF1956 domain-containing tetR family putative transcriptional regulator
yohJ	4.5	0.6	-0.9	UPF0299 family inner membrane protein
ybhR	1.5	0.2	-0.9	putative ABC transporter permease
ykgH	1.3	0.1	-1.1	putative inner membrane protein
betA	32.3	0.8	-1.6	choline dehydrogenase, a flavoprotein
acrR	37.7	0.8	-1.7	transcriptional repressor
betB	46.4	0.8	-1.8	betB; betaine aldehyde dehydrogenase, NAD-dependent

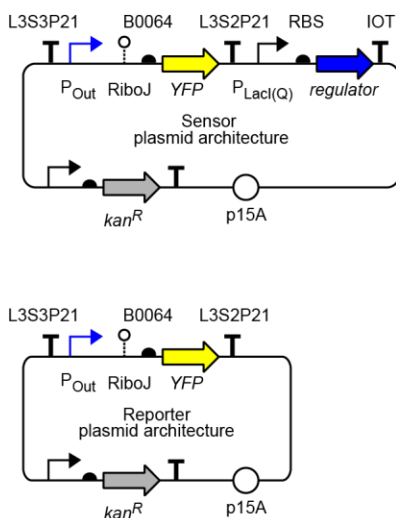
a) Ribosome density in arbitrary units. See Methods for full description.

b) Positive log(fold change) denotes higher translation in Marionette-Wild. Negative log(fold change) denotes higher translation in *E. coli* MG1655. Only genes with a |fold change| greater than four and a ribosome density higher than 0.1 a.u. in either strain are shown.



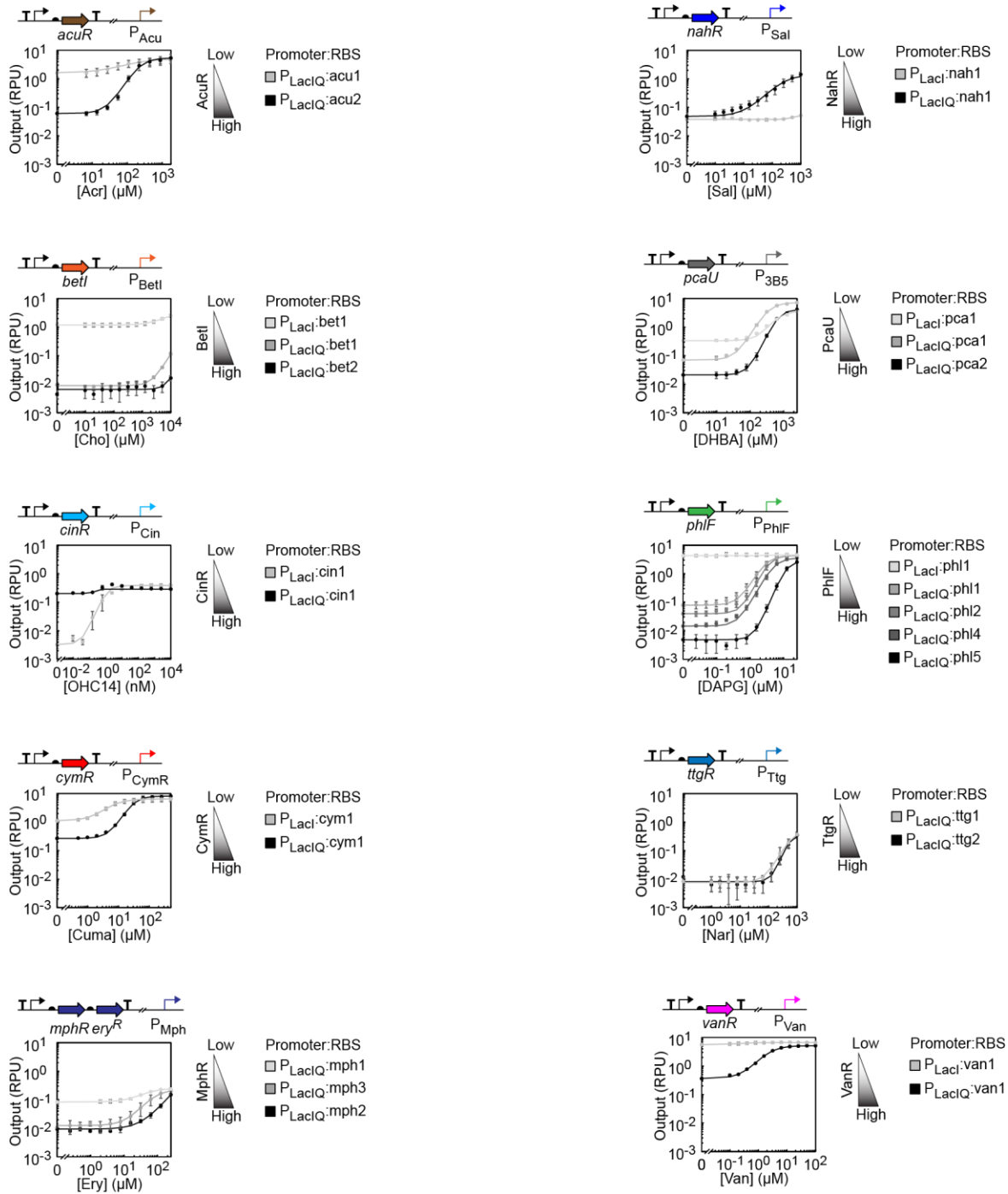
Supplementary Figure 1: RPU Standard plasmid

The RPU standard plasmid is used to normalize fluorescence (Methods). The promoter (P_{J23101}) sequence is provided in Supplementary Table 1. The insulator (RiboJ) sequence is provided in Supplementary Table 2. The RBS (BBa_B0064) sequence is provided in Supplementary Table 3. The protein (YFP) sequence is provided in Supplementary Table 4. The terminator (L3S3P21, L3S2P21) sequences are provided in Supplementary Table 5. The full plasmid sequence is provided in Supplementary Table 14.



Supplementary Figure 2: General plasmid architectures

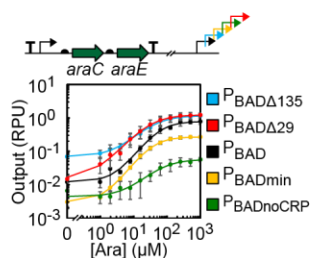
All sensors are assembled into the same sensor plasmid architecture for characterization. Examples include pAJM.011, pAJM.336, pAJM.474, pAJM.657, pAJM.661, pAJM.677, pAJM.683, pAJM.690, pAJM.771, pAJM.773, pAJM.847, pAJM.884, pAJM.969, and pAJM.1642. All output promoters are assembled into the same reporter plasmid architecture for use in Marionette strains. Examples include pAJM.711, pAJM.712, pAJM.713, pAJM.714, pAJM.715, pAJM.716, pAJM.717, pAJM.718, pAJM.719, pAJM.721, pAJM.944, and pAJM.1459. The promoter (*P_{LacI}*, *P_{LacIQ}*) sequences are provided in Supplementary Table 1. The insulator (*RiboJ*) sequence is provided in Supplementary Table 2. The RBS (BBa_B0064) sequence is provided in Supplementary Table 3. The protein (*YFP*) sequence is provided in Supplementary Table 4. The terminator (*L3S3P21*, *L3S2P21*, *IOT*) sequences are provided in Supplementary Table 5. The full plasmid sequences are provided in Supplementary Note 4.



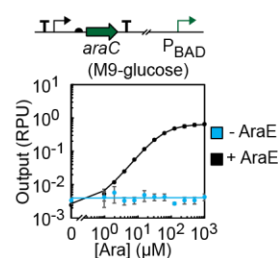
Supplementary Figure 3: Initial sensor tuning

The promoter and/or RBS driving regulator expression was tuned, and the response function for each construct is shown. Darker color denotes higher expression of the regulator, as determined by the relative promoter and RBS strengths. All data are from experiments using *E. coli* DH10B cells in mid-log growth in LB media. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. The promoter and RBSs are listed and their sequences are provided in Supplementary Tables 1 and 3.

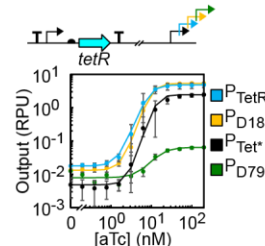
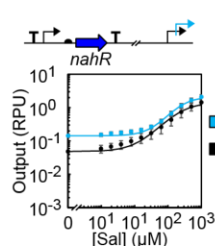
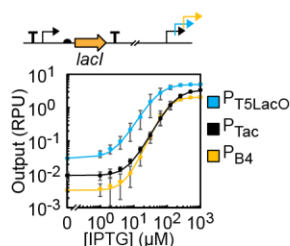
a. Operator placement



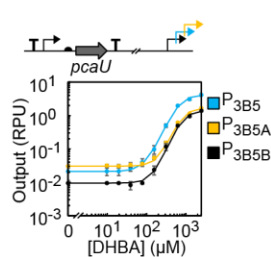
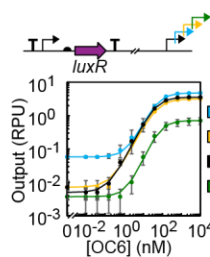
b. Transport



c. Literature mining

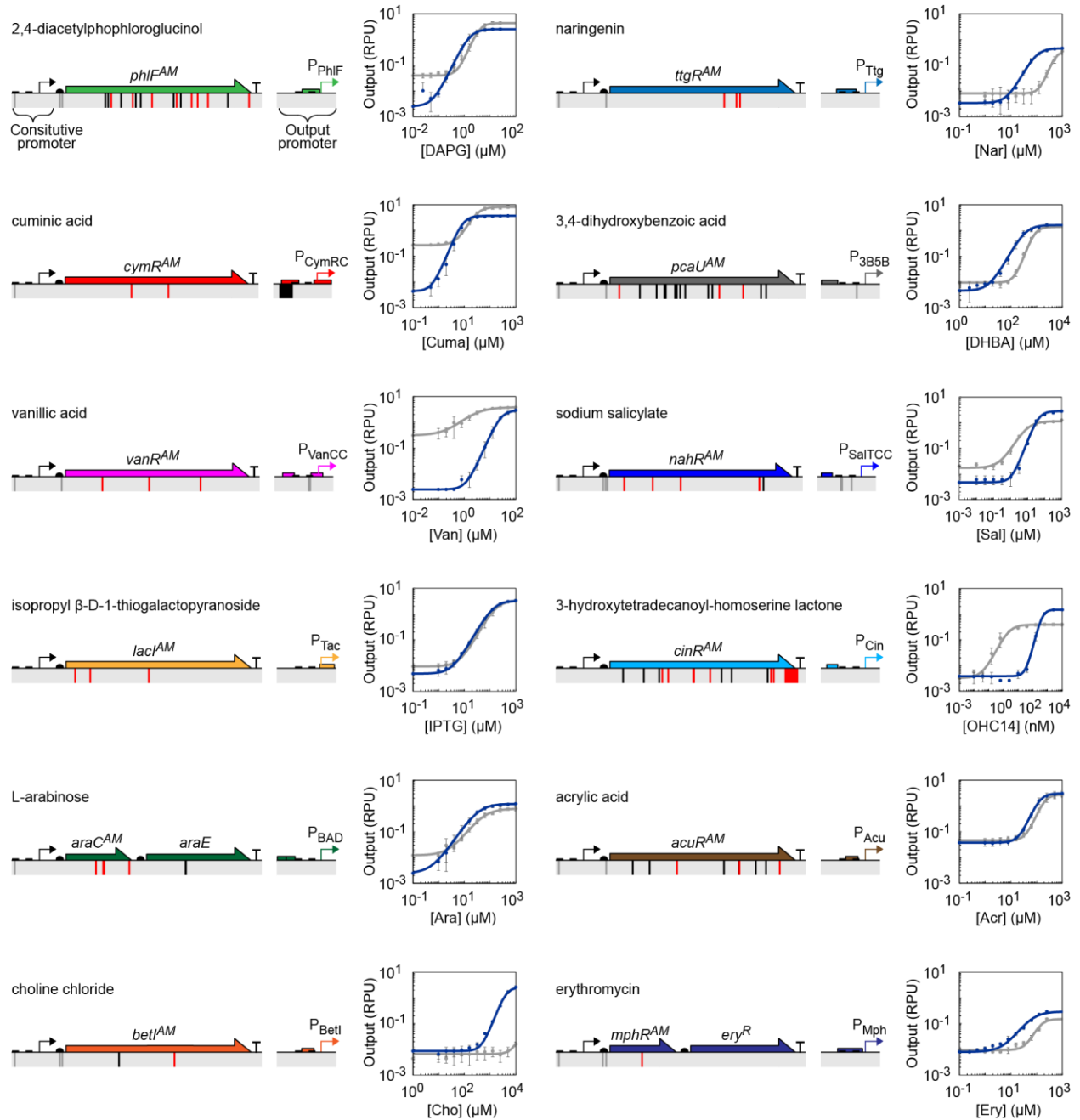


d. -10 box mutations



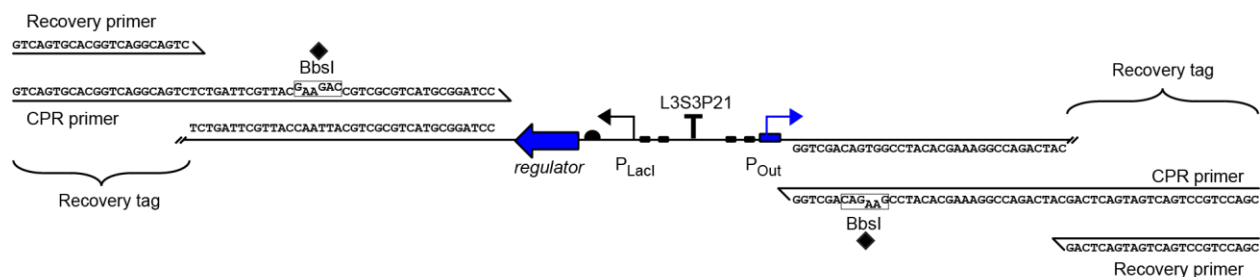
Supplementary Figure 4: Initial sensor engineering

Mutations were made based on either rational design or inspired by the literature. **a)** Output promoters with operators added, removed, or moved were tested. **b)** The Ara sensor was tested with and without the constitutive expression of the AraE transporter. **c)** Multiple promoters described in the literature were tested. **d)** Output promoters with mutations made to the -10 box were tested. Multiple promoters in the diagram denote that multiple promoter variants were tested. All data are from experiments using *E. coli* DH10B cells in mid-log growth in LB media (except M9-glucose was used in part b.) The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Promoter variants are listed and their sequences are provided in Supplementary Table 1.

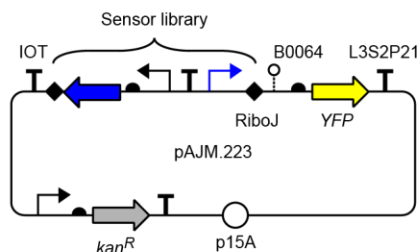


Supplementary Figure 5: Effect of sensor evolution on response functions

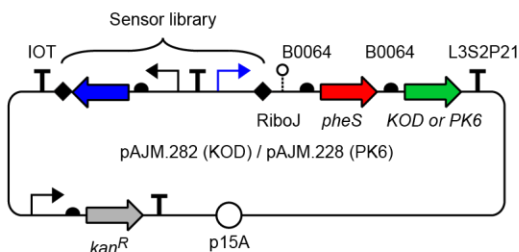
The genetic design of each sensor is shown, with non-synonymous (red) and non-coding or synonymous (grey/black) mutations noted. Mutations in grey were also applied to the parental sensor. The corresponding response functions comparing the evolved (blue) and parental (grey) sensors. All data are from experiments using *E. coli* DH10B cells in mid-log growth in LB media. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Response functions were obtained by fitting the experimental data to Equation 1 and are shown in Figure 2a. Response function parameters for evolved sensors are provided in Table 1. Sequences of promoters and regulators are provided in Supplementary Table 1 and 4.



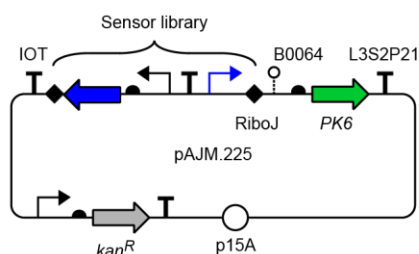
YFP screening plasmid



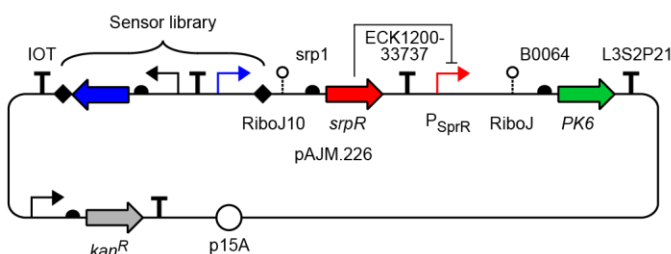
Dual selection plasmid



Positive selection plasmid

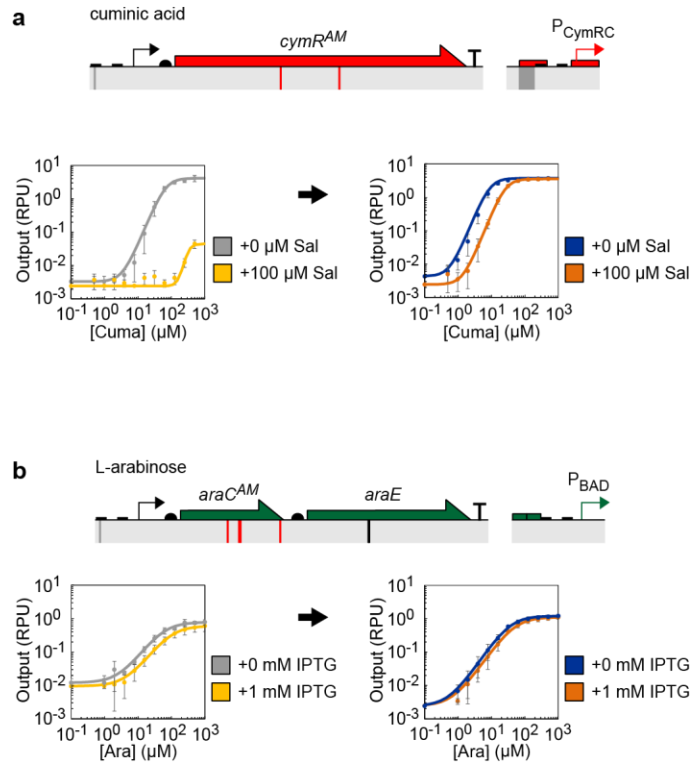


Negative selection plasmid



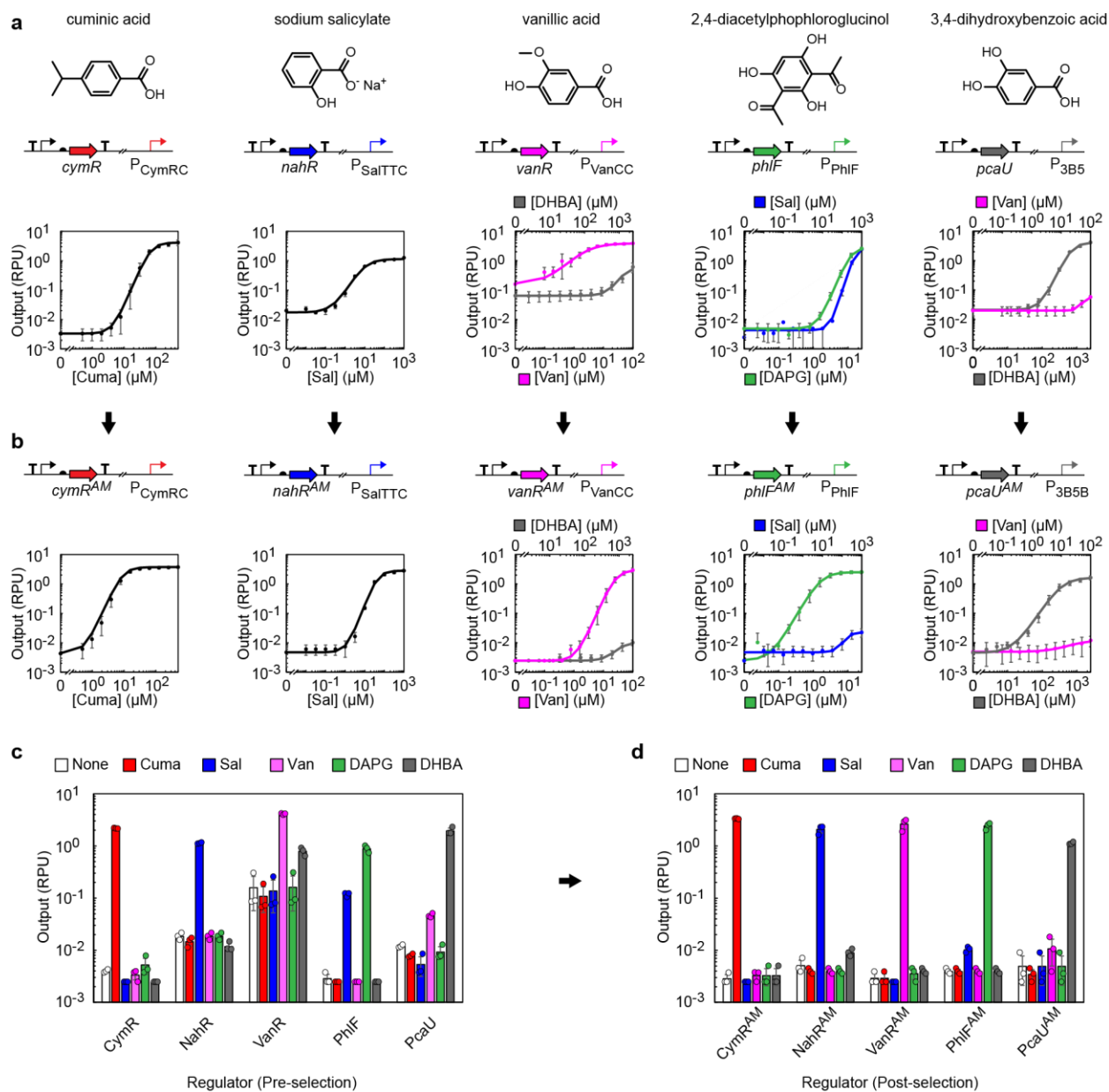
Supplementary Figure 6: Directed evolution selection plasmids

Sensor libraries were constructed as divergent operons flanked by BbsI sites (black diamonds). Most libraries were Golden Gate assembled into a dual selection plasmids for selection and into the YFP screening plasmid for on/off screening. The first two rounds of the LacI selection utilized the positive and negative selection plasmids, respectively. The negative selection plasmid contains *srpR* and P_{SrpR} in order to invert the selection from positive to negative. CPR primers are used in the emulsion PCR. CPR primers re-introduce BbsI sites and append recovery tags to the library. Recovery primers are used in the recovery PCR. Recovery primers anneal to the recovery tags. The promoter (P_{SrpR}) sequence is provided in Supplementary Table 1. The insulator (RiboJ, RiboJ10) sequences are provided in Supplementary Table 2. The RBS (BBa_B0064, *srp1*) sequences are provided in Supplementary Table 3. The protein (YFP, PheS, PK6, KOD, *SrpR*) sequences are provided in Supplementary Table 4. The terminator (L3S2P21, L3S2P21, IOT, ECK120033737) sequences are provided in Supplementary Table 5.



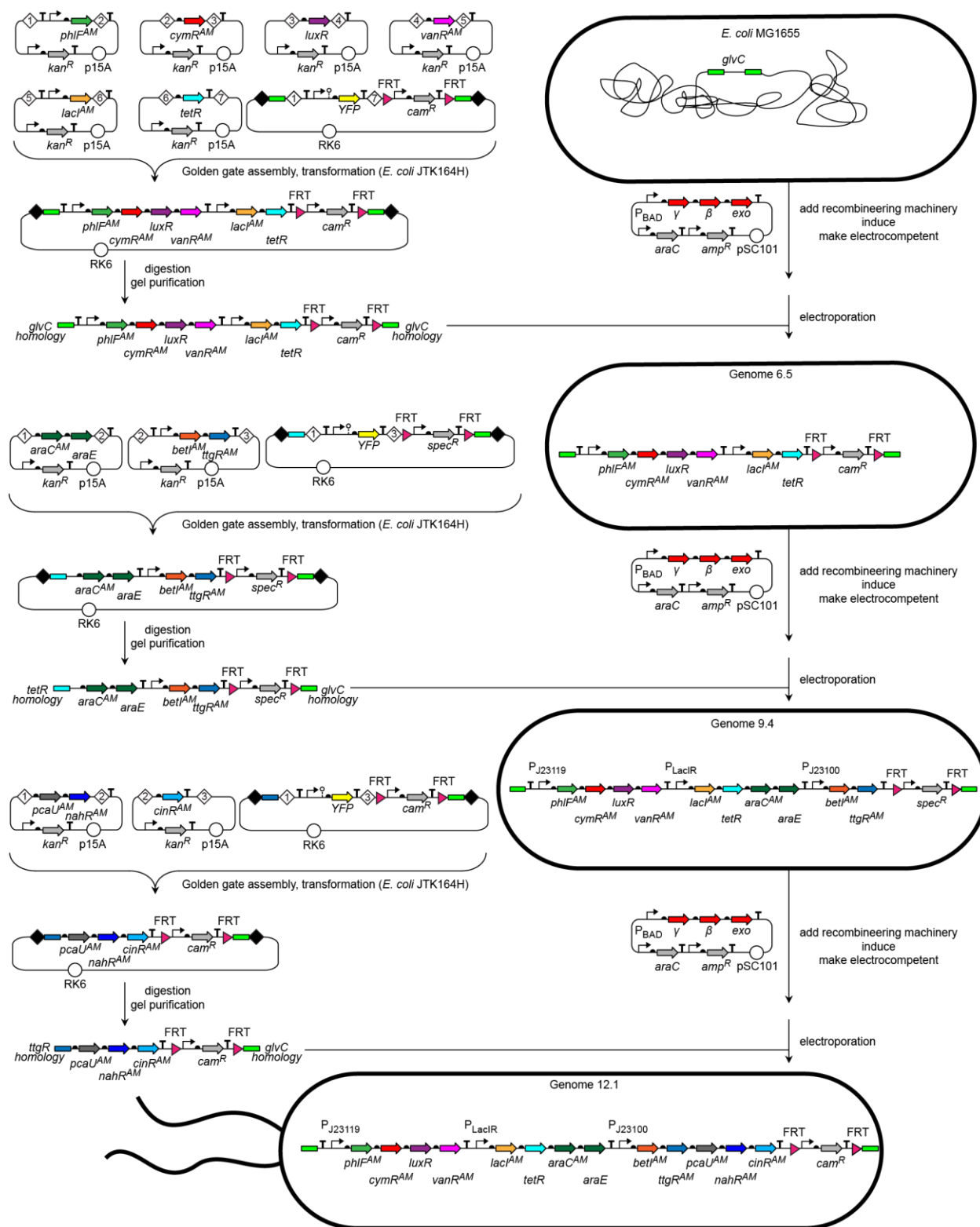
Supplementary Figure 7: Chemical antagonism

a) Response functions with Sal (light/dark orange) and without Sal (grey/blue) for parental CymR (left) and evolved CymR^{AM} (right) are shown. **b)** Response functions with IPTG (light/dark orange) and without (grey/blue) IPTG for parental AraC (left) and evolved AraC^{AM} (right) are shown. All data are from experiments using *E. coli* DH10B cells in mid-log growth in LB media. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Response functions were obtained by fitting the experimental data to Equation 1. Sequences of promoters and regulators are provided in Supplementary Table 1 and 4.



Supplementary Figure 8: Chemical cross-reactivity

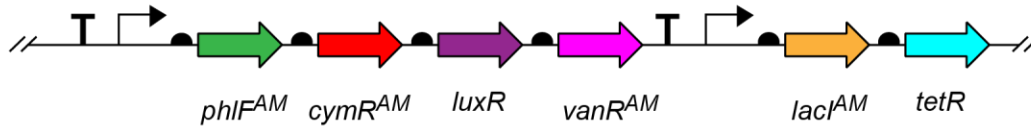
CymR, NahR, VanR, PhlF, and PcaU respond to similar chemical inducers. **a)** Response functions for the parental regulators with cognate inducers and cross-reactive inducers (where appropriate) are shown. **b)** Response functions for the evolved sensors with cognate inducers and cross-reactive inducers (where appropriate) are shown. Response functions were obtained by fitting the experimental data to Equation 1. **c)** Cross-reactivity responses of parental regulators are shown. **d)** Cross-reactivity responses of evolved regulators are shown. Cross-reactivity heat maps are provided in Figure 2b. Inducer concentrations are 100 μ M Cuma, 100 μ M Sal, 100 μ M Van, 10 μ M DAPG, 1 mM DHBA. All data are from experiments using *E. coli* DH10B cells in mid-log growth in LB media. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Sequences of promoters and regulators are provided in Supplementary Table 1 and 4.



Supplementary Figure 9: Genomic integration of the Marionette cluster

Regulators were assembled into RK6 suicide vectors. Regulators were serially integrated into *glvC* locus of the *E. coli* MG1655 genome using λ Red recombineering (Methods). All part sequences are provided in Supplementary Tables 1-5. The sequence of the full cluster is provided in Supplementary Table 7.

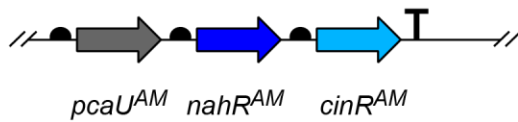
Construct	RBS	Score	Note	RBS	Score	Note	RBS	Score	Note	RBS	Score	Note	RBS	Score	Note	RBS	Score	Note
Plasmid	phl2	6066		cym1	5300		lux1	23403		van2	10916		lac1	3379		tet1	25607	
Genome 6.1	phl6	620	low	cym3	391	low	lux2	4056	good	van4	13069	low	lac3	328	low	tet3	62294	high
Genome 6.2	phl3	1999	good	cym2	4631	good	lux2	4056	good	van5	26850	low	lac4	973	low	tet4	52502	high
Genome 6.3	phl3	1999	good	cym2	4631	good	lux2	4056	good	van3	42112	good	lac5	3868	low	tet2	17908	good
Genome 6.4	phl3	1999	good	cym2	4631	good	lux2	4056	good	van3	42112	good	lac5	3868	low	tet2	17908	good
Genome 6.5	phl3	1999	good	cym2	4631	good	lux2	4056	good	van3	42112	good	lac2	10889	good	tet2	17908	good



Construct	RBS	Score	Note	RBS	Score	Note	RBS	Score	Note	RBS	Score	Note
Plasmid	ara1	2953		e1	930		bet2	1147		ttg2	17865	
Genome 9.1	ara2	6374	good	e1	930	good	bet2	1147	low	ttg4	19594	high
Genome 9.2	ara2	6374	good	e1	930	good	bet2	1147	low	ttg3	17908	good
Genome 9.3	ara2	6374	good	e1	930	good	bet4	1720	low	ttg3	17908	good
Genome 9.4	ara2	6374	good	e1	930	good	bet3	8155	good	ttg3	17908	good

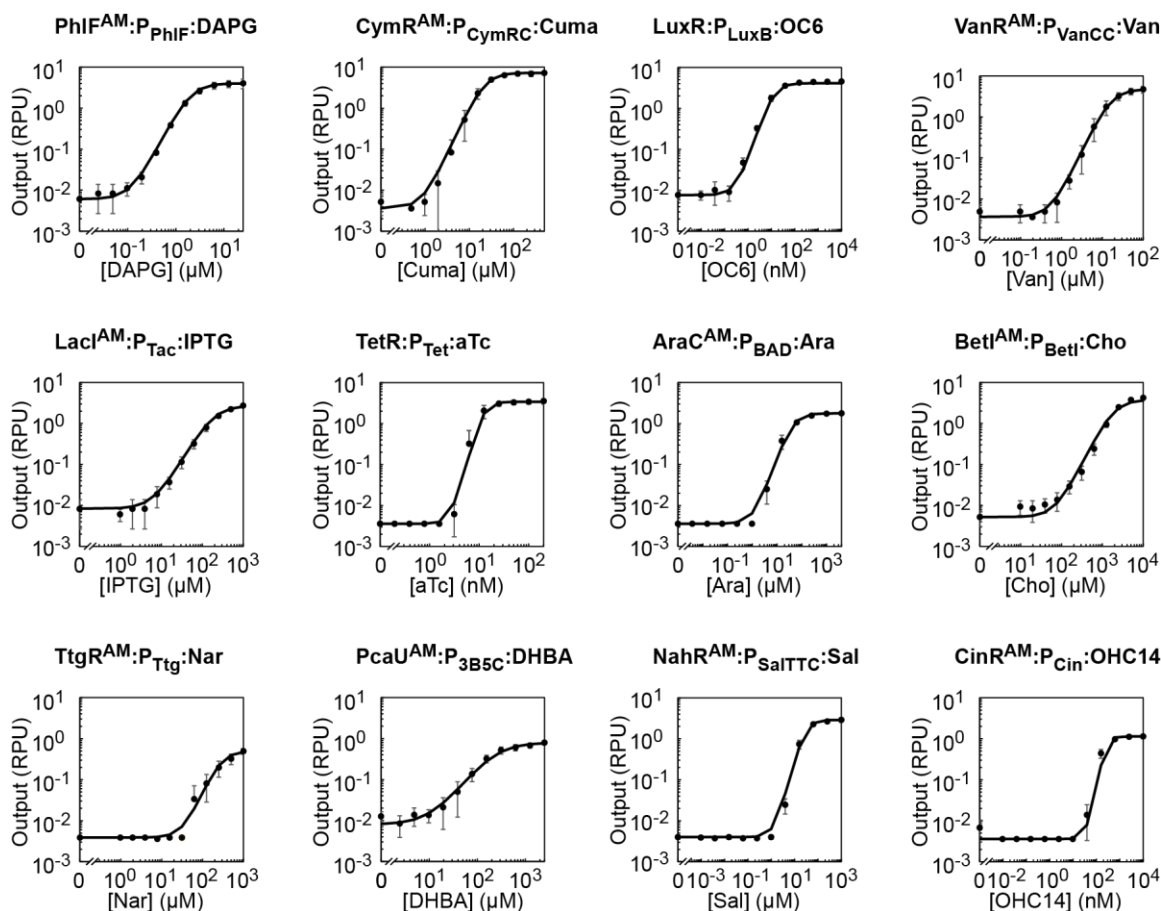


Construct	RBS	Score	Note	RBS	Score	Note	RBS	Score	Note
Plasmid	pca2	17865		nah2	2450		cin1	22373	
Genome 11.1	pca4	43031	low	nah3	3135	good			
Genome 11.2	pca3	105849	good	nah3	3135	good			
Genome 12.1	pca3	105849	good	nah3	3135	good	cin2	2076	good



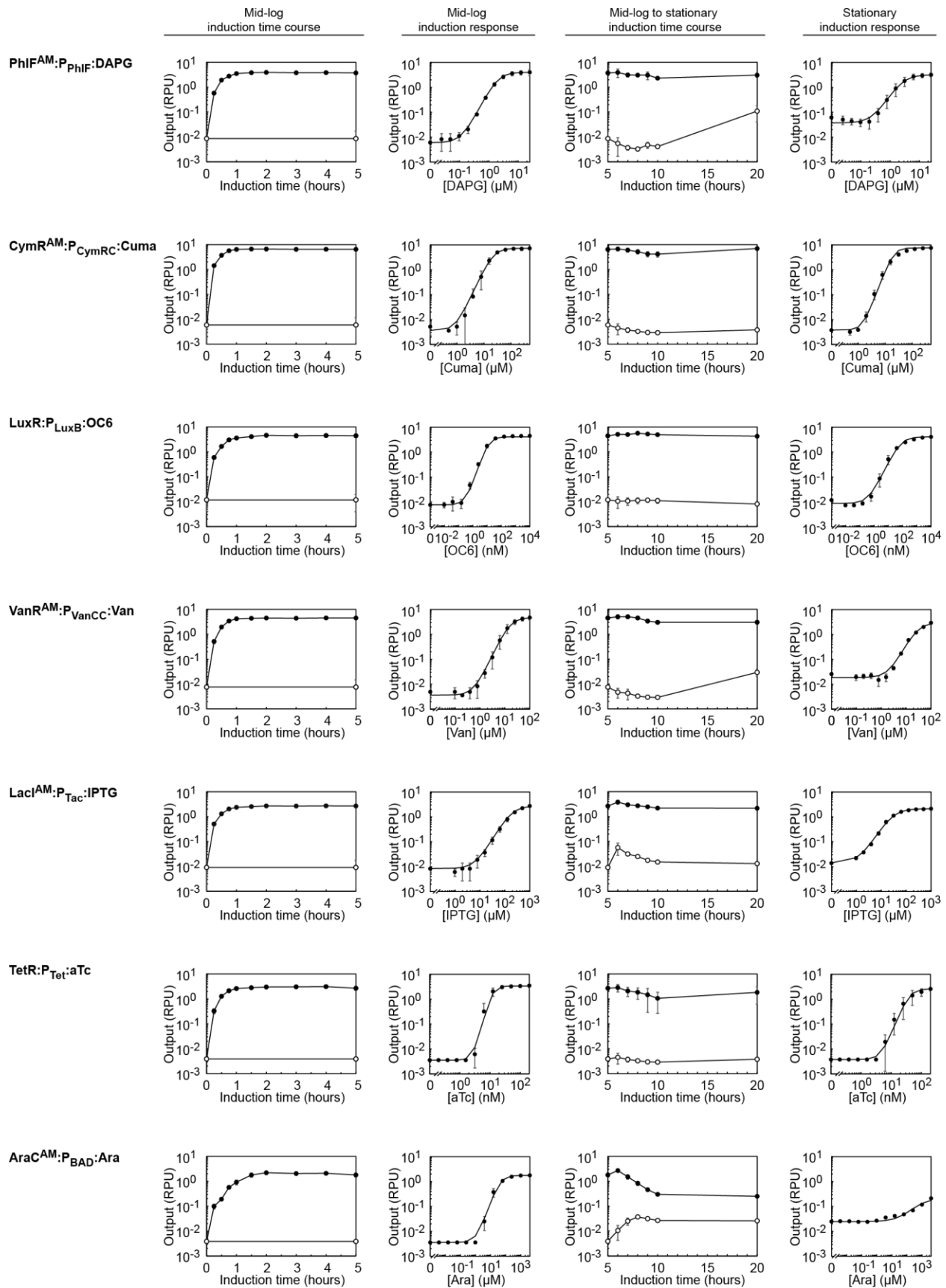
Supplementary Figure 10: Tuning the RBSs in the Marionette cluster

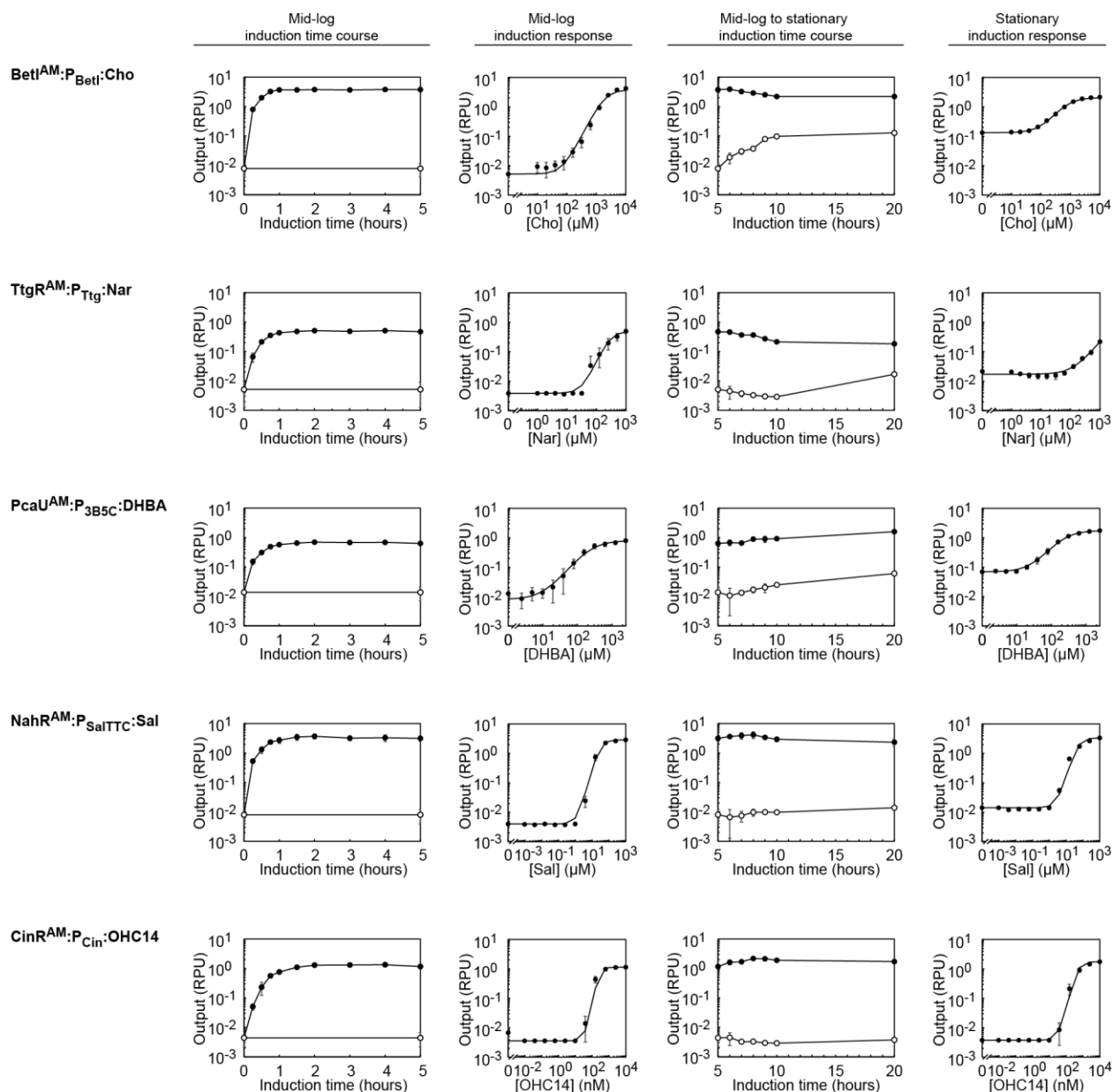
The Marionette cluster was integrated in three sections (Supplementary Figure 9). The RBS strength of each regulator was tuned by iteratively designing, building, and testing multiple versions of each section. The RBS name, score (Translation Initiation Rate from the RBS Calculator in arbitrary units), and a qualitative assessment of the RBS strength are provided for each regulator in each construct. See Methods for full description. All part sequences are provided in Supplementary Tables 1-5.



Supplementary Figure 11: Marionette-Wild response functions

Response functions for each of the 12 output promoters with their cognate inducers are shown. All data are from experiments using cells in mid-log growth in LB media. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Response functions were obtained by fitting the experimental data to Equation 1 and are shown in Figure 3b. Response function parameters for genomic sensors are provided in Table 1. Sequences of promoters are provided in Supplementary Table 1. Sequences of reporter plasmids are provided in Supplementary Note 4.

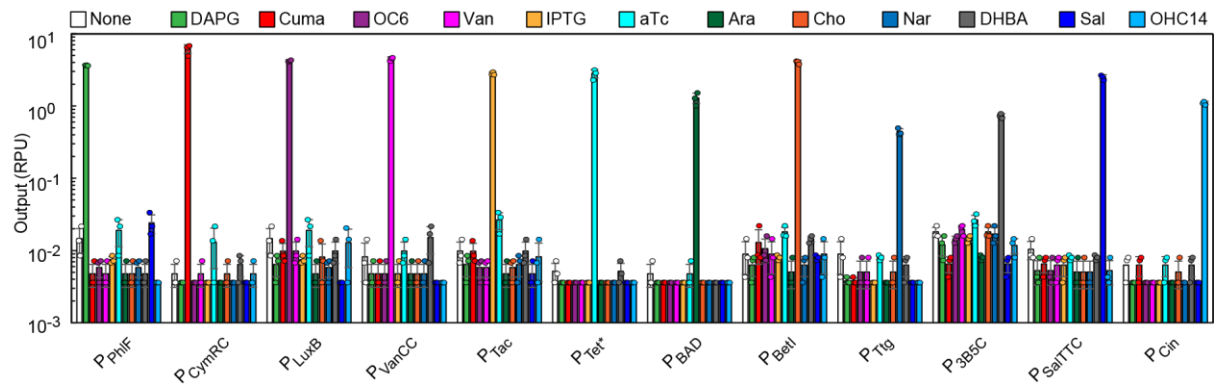




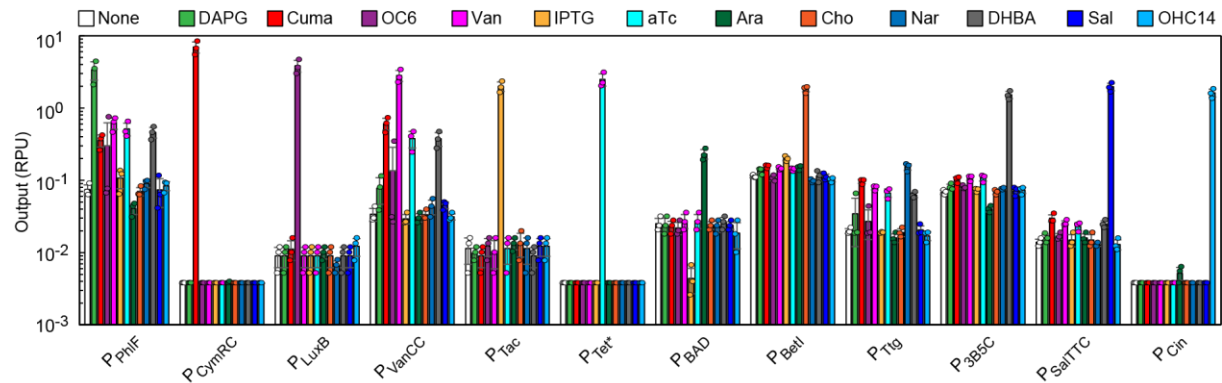
Supplementary Figure 12: Marionette-Wild induction time course

First column) Time course data from mid-log phase are shown. Inducer was added at different times (black) or not at all (white). **Second column)** Inducer response functions after five hours of induction are shown. **Third column)** Time course data from mid-log to stationary phase are shown. Inducer was added at different times (black) or not at all (white). **Fourth column)** Inducer response functions after 20 hours of induction are shown. See Methods for growth and induction details. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Inducer concentrations were: 25 μ M DAPG, 100 μ M Cuma, 10 μ M OC6, 100 μ M Van, 1 mM IPTG, 200 nM aTc, 4 mM Ara, 10 mM Cho, 1 mM Nar, 1 mM DHBA, 100 μ M Sal, and 10 μ M OHC14. LuxR, AraC, NahR, and CinR are activators, while the other regulators are repressors.

Marionette-Wild mid-log

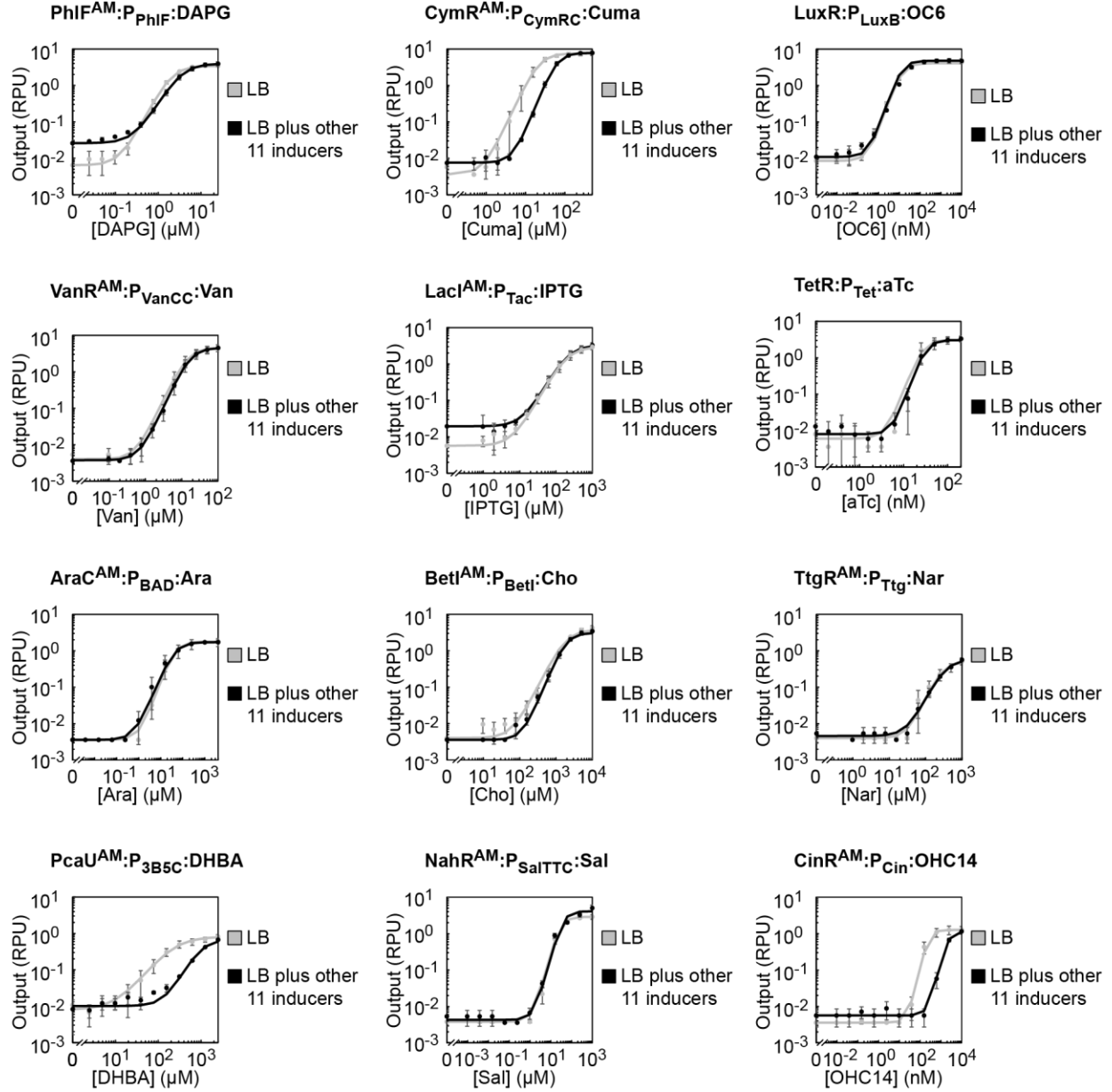


Marionette-Wild stationary



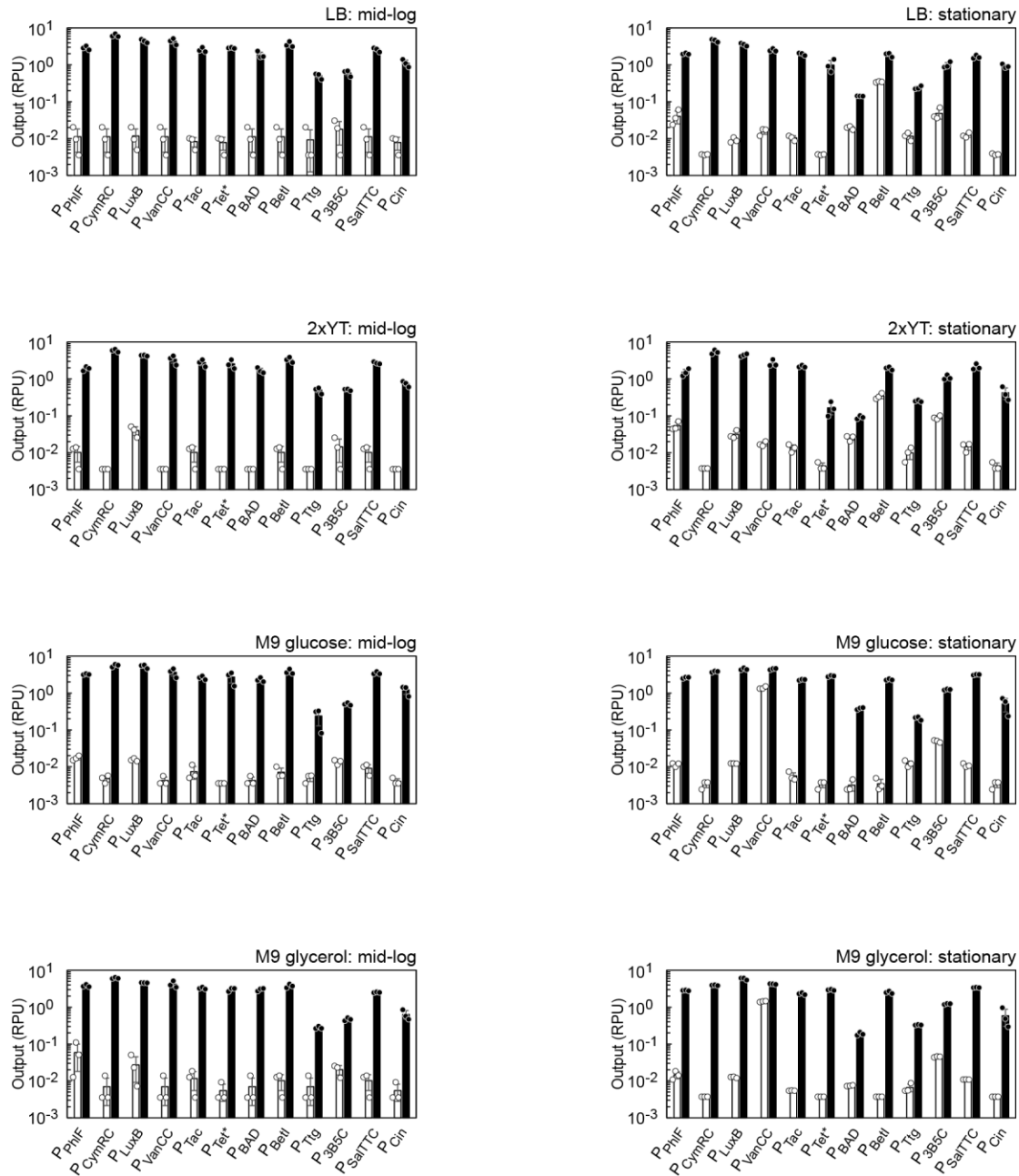
Supplementary Figure 13: Inducer cross reactivity in Marionette-Wild in mid-log and stationary phase

Cross-reactivity responses in mid-log and stationary phase (Methods) are shown. A cross-reactivity heat map of the mid-log data is provided in Figure 3c. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Inducer concentrations were: 25 μ M DAPG, 500 μ M Cuma, 10 μ M OC6, 100 μ M Van, 1 mM IPTG, 200 nM aTc, 4 mM Ara, 10 mM Cho, 1 mM Nar, 2.5 mM DHBA, 250 μ M Sal, and 10 μ M OHC14.



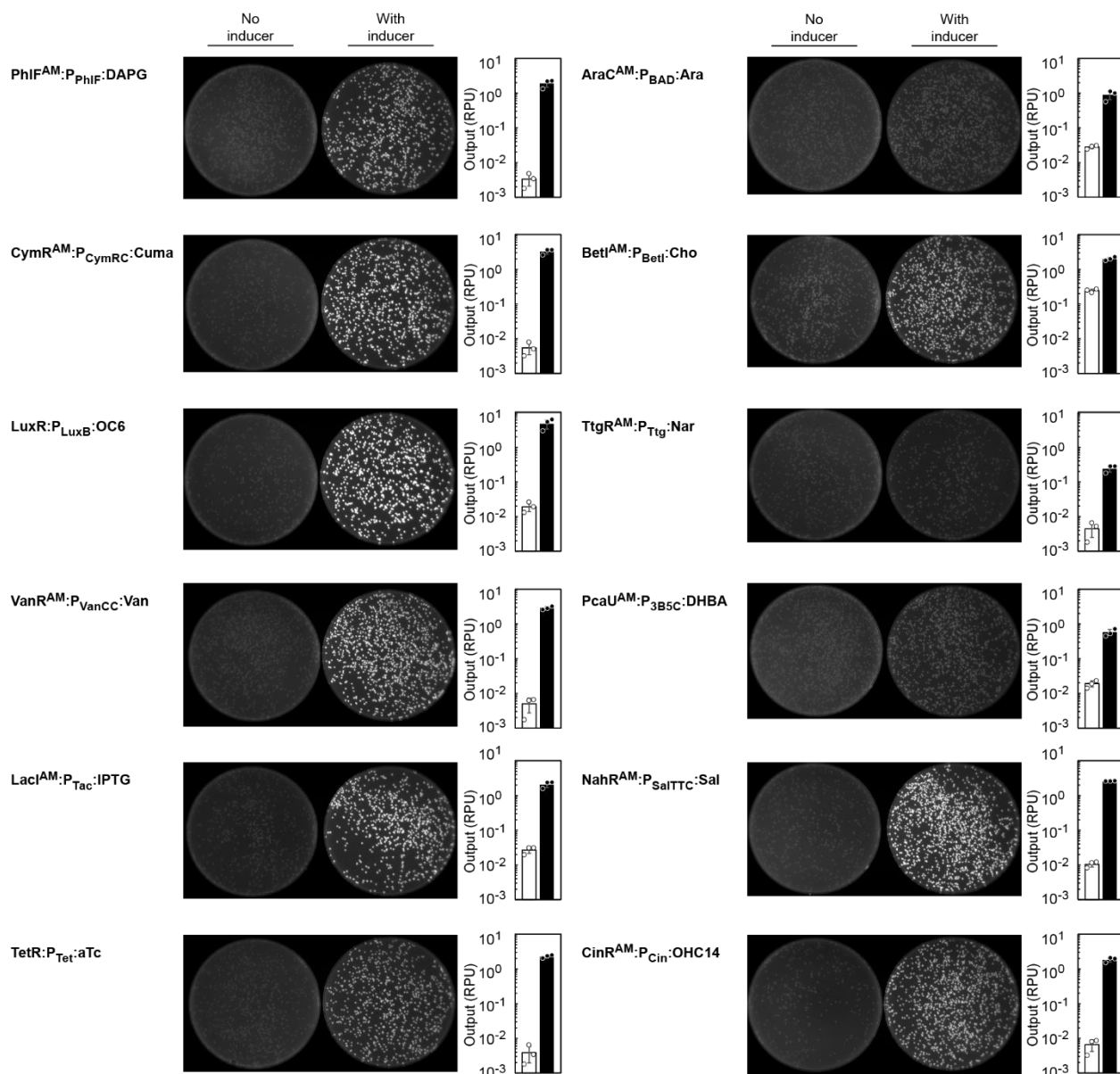
Supplementary Figure 14: Impact of simultaneous presence of all inducers on response functions

Marionette-Wild was transformed with each reporter plasmid. Response functions for each output reporter with the appropriate inducer were determined in LB (grey) or in LB containing the other 11 inducers (black). Measurements were taken of cells from mid-log phase. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Inducer concentrations were: 6.25 μM DAPG, 100 μM Cuma, 1 μM OC6, 100 μM Van, 1 mM IPTG, 50 nM aTc, 1 mM Ara, 5 mM Cho, 1 mM Nar, 625 μM DHBA, 100 μM Sal, and 1 μM OHC14.



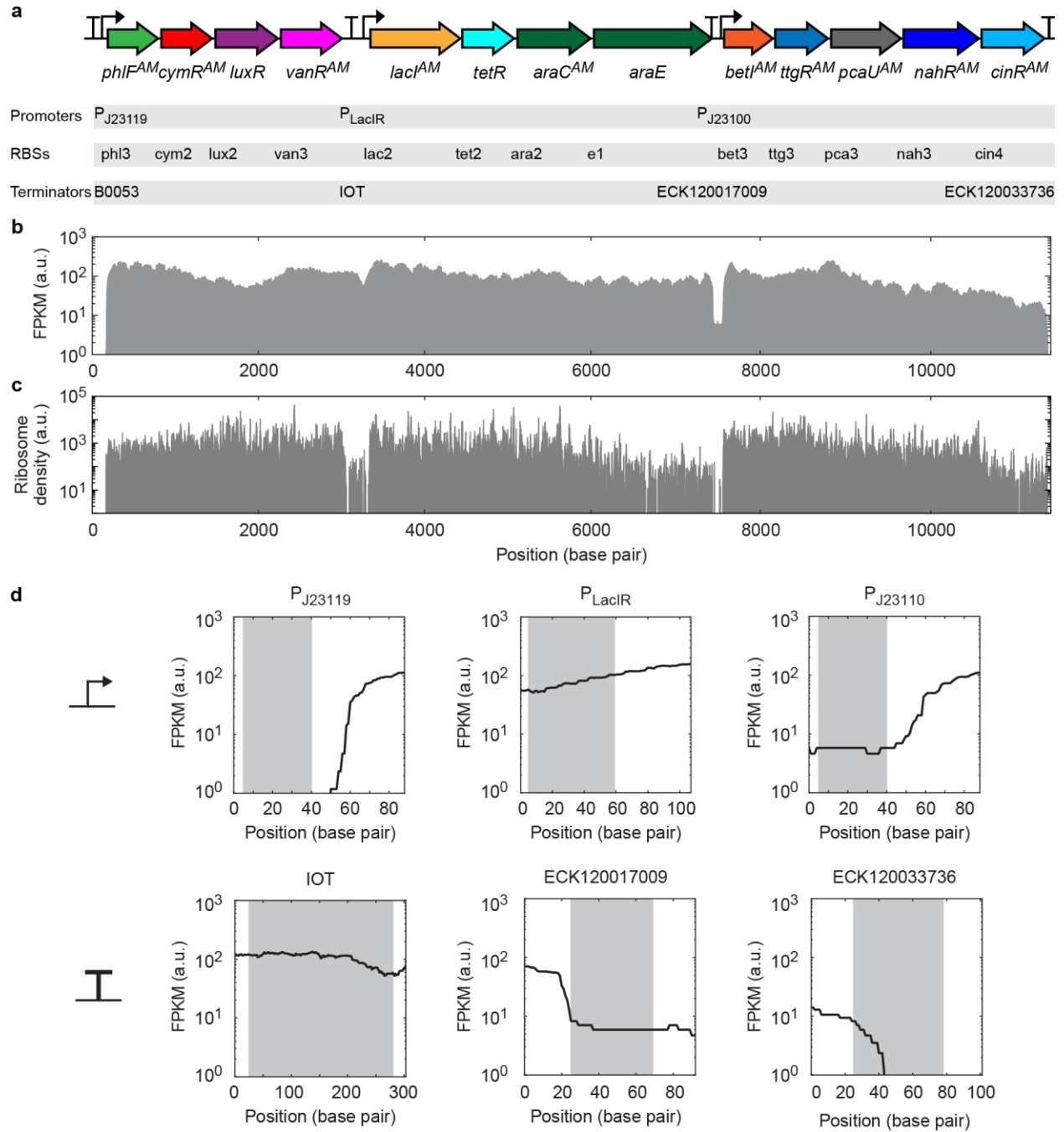
Supplementary Figure 15: Impact of growth media on sensor performance

Marionette-Wild was transformed with each reporter plasmid and grown with (black) and without (white) the cognate inducer in a variety of media compositions (Methods). Measurements were taken of cells from mid-log and stationary phase. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Inducer concentrations were: 6.25 μ M DAPG, 100 μ M Cuma, 1 μ M OC6, 100 μ M Van, 1 mM IPTG, 50 nM aTc, 1 mM Ara, 5 mM Cho, 1 mM Nar, 625 μ M DHBA, 100 μ M Sal, and 1 μ M OHC14.



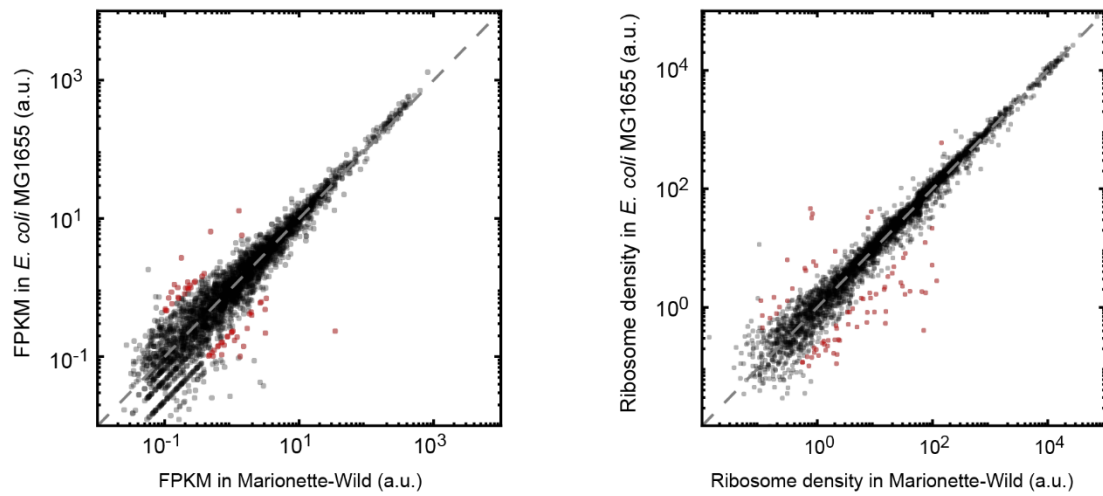
Supplementary Figure 16: Induction of sensors on plates

Marionette-Wild was transformed with each of the 12 reporter plasmids and plated on LB-agar with and without the appropriate inducer. After overnight growth at 37 °C, plates were incubated at 4 °C for one hour and imaged (Methods). Individual colonies from plates with (black) and without (white) inducer were picked, resuspended in PBS, and assayed by cytometry. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Inducer concentrations were: 25 μ M DAPG, 100 μ M Cuma, 10 μ M OC6, 100 μ M Van, 1 mM IPTG, 200 nM aTc, 4 mM Ara, 10 mM Cho, 1 mM Nar, 1 mM DHBA, 100 μ M Sal, and 10 μ M OHC14.

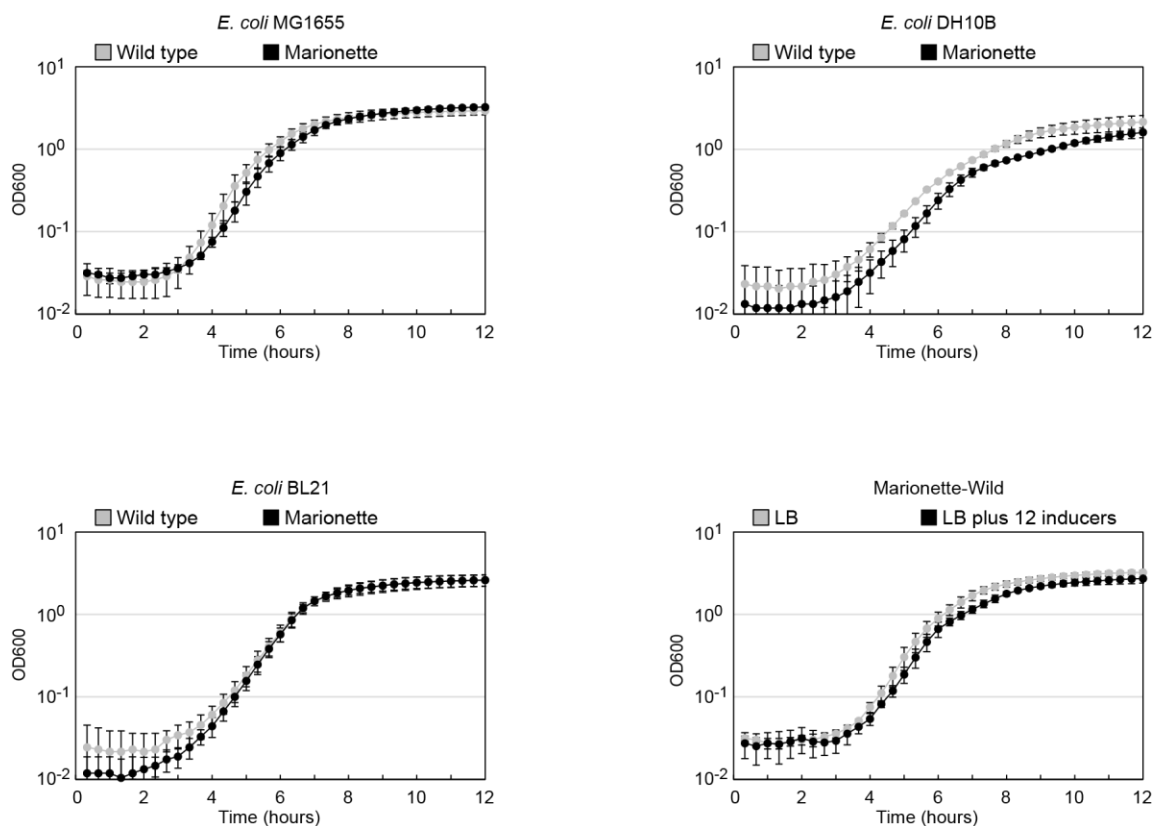


Supplementary Figure 17: Transcription and translation of the Marionette Cluster

a) The genetic schematic of the Marionette cluster is provided for reference. **b)** The mRNA profile of the sensor array in Marionette-Wild is shown. **c)** The translation profile of the sensor array in Marionette-Wild is shown. **d)** The mRNA profiles of promoters and terminators are shown. Shaded regions denote the boundaries of the part. Read count at each nucleotide position was normalized by the total sequencing reads. These data are the result of a single experiment. See Methods for details. All part sequences are provided in Supplementary Tables 1-5.

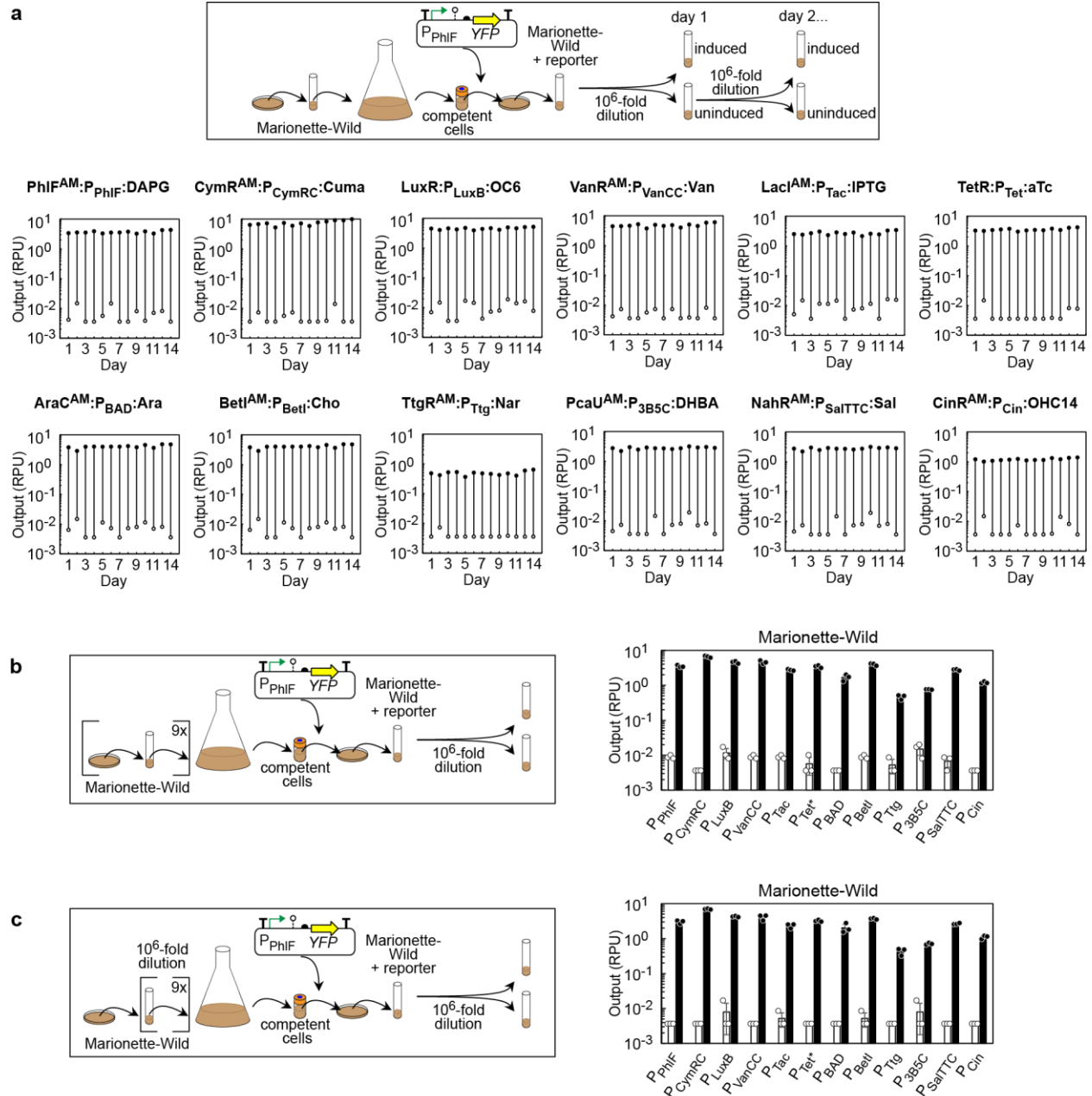


Supplementary Figure 18: mRNA levels and protein synthesis rates in *E. coli* MG1655 and Marionette-Wild
Left) A comparison of the levels of endogenous mRNAs in Marionette-Wild strain and in *E. coli* MG1655 is shown. Genes that are differentially abundant ($|\text{fold change}|$ greater than four between the two strains), with a FPKM higher than 0.1 a.u. in either strain are highlighted (red) and listed in Supplementary Table 11. These data are the result of a single experiment. **Right)** A comparison of the synthesis of endogenous proteins in Marionette-Wild strain and in *E. coli* MG1655 is shown. Genes that are differentially expressed ($|\text{fold change}|$ greater than four between the two strains), with a ribosome density higher than 0.1 a.u. in either strain are highlighted (red) and listed in Supplementary Table 12. These data are the result of a single experiment.



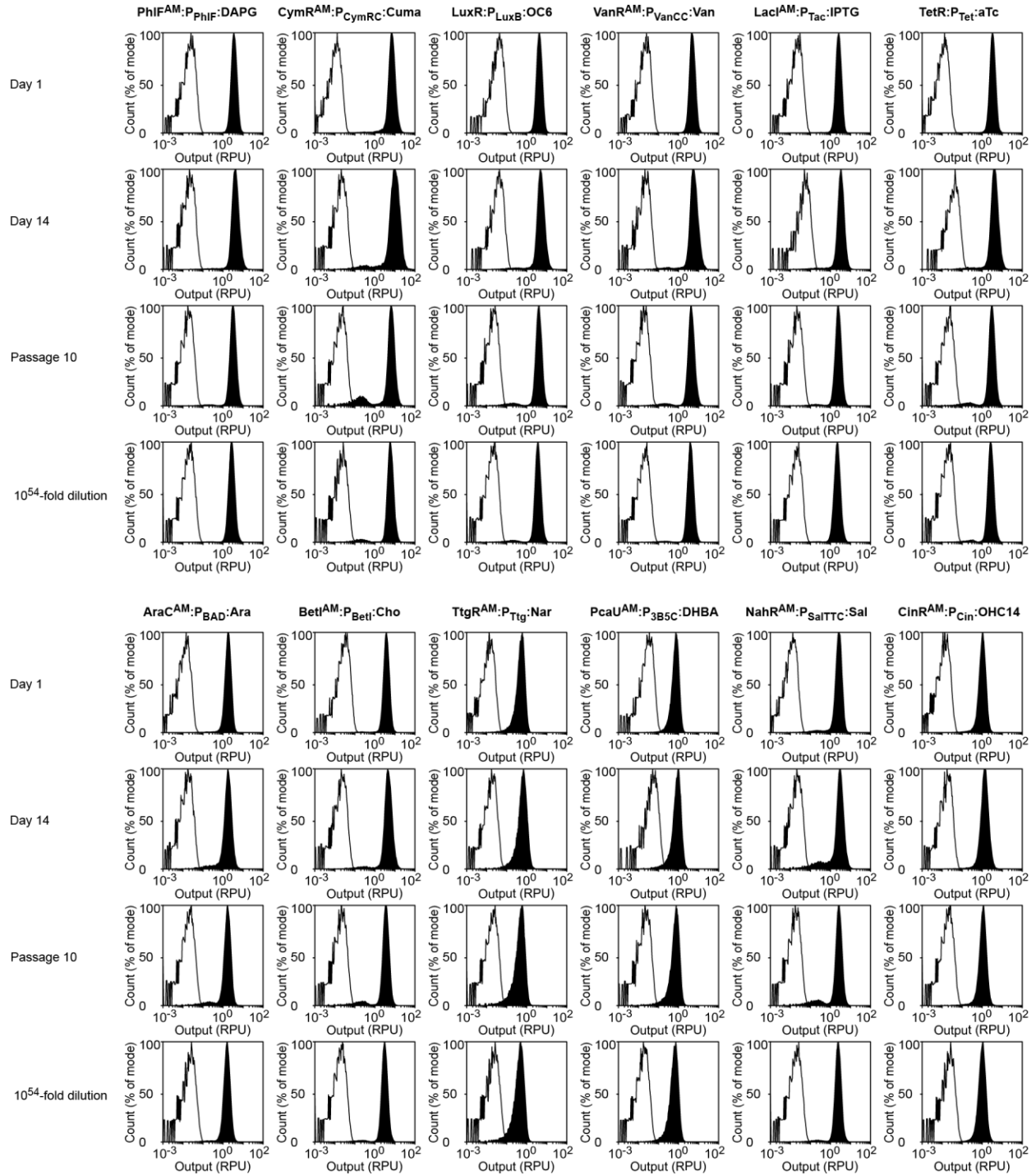
Supplementary Figure 19: Growth impact of maintaining genomically encoded Marionette cluster

Marionette-Wild, -Clo, and -Pro (black) as well as their parental strains, *E. coli* MG1655, DH10B, and BL21 respectively (grey) were grown in LB. Marionette-Wild was also grown in LB plus all 12 inducers. OD600 measurements were taken every 20 minutes (Methods). The Marionette-Wild strain grows with a doubling time of 29.0 ± 1.9 minutes as compared 27.1 ± 1.2 for wild-type *E. coli* MG1655. The Marionette-Clo strain grows with a doubling time of 42.3 ± 3.8 minutes as compared 45.1 ± 3.8 for wild-type *E. coli* DH10B. The Marionette-Pro strain grows with a doubling time of 31.1 ± 1.1 minutes as compared 34.5 ± 1.6 for wild-type *E. coli* BL21. The Marionette-Wild strain grows with a doubling time of 29.0 ± 1.9 minutes in LB as compared 31.4 ± 2.3 for LB plus all 12 inducers. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Inducer concentrations were: 25 μ M DAPG, 100 μ M Cuma, 10 μ M OC6, 100 μ M Van, 1 mM IPTG, 200 nM aTc, 4 mM Ara, 10 mM Cho, 1 mM Nar, 1 mM DHBA, 100 μ M Sal, and 10 μ M OHC14.



Supplementary Figure 20: Evolutionary stability of Marionette-Wild

a) Marionette-Wild was transformed with each of the 12 reporter plasmids and grown for 14 days. Each day, cells were assayed with (black) and without (white) the corresponding inducer (Methods). These data are the result of a single experiment. Data from days 1, 7, 11, and 14 are provided in Figure 3d. **b)** Marionette-Wild was passaged between plates and liquid culture nine times and was then transformed with each of the 12 reporter plasmids and assayed with (black) and without (white) the corresponding inducer (Methods). **c)** Marionette-Wild was diluted 10^6 -fold in liquid culture nine times and was then transformed with each of the 12 reporter plasmids and assayed with (black) and without (white) the corresponding inducer (Methods). The mean of three replicates performed on different days is shown and error bars represent the standard deviation. All data are from experiments using cells in mid-log growth in LB media. Inducer concentrations were: 25 μ M DAPG, 500 μ M Cuma, 10 μ M OC6, 100 μ M Van, 1 mM IPTG, 200 nM aTc, 4 mM Ara, 10 mM Cho, 1 mM Nar, 2.5 mM DHBA, 250 μ M Sal, and 10 μ M OHC14.



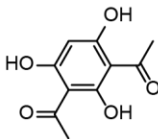
Supplementary Figure 21: Fluorescence distributions of Marionette-Wild after passaging

Cytometry histograms with (black) and without (white) the corresponding inducer are shown. Raw values are white cell corrected and RPU normalized. Data are from Day 1, Day 14, Passage 10, and 10^{54} -fold dilution populations of the evolutionary stability assays (Methods, Supplementary Figure 20). Inducer concentrations were: 25 μ M DAPG, 500 μ M Cuma, 10 μ M OC6, 100 μ M Van, 1 mM IPTG, 200 nM aTc, 4 mM Ara, 10 mM Cho, 1 mM Nar, 2.5 mM DHBA, 250 μ M Sal, and 10 μ M OHC14. These data are the result of a single experiment.

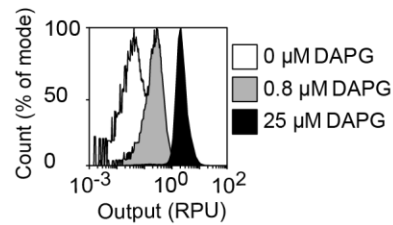
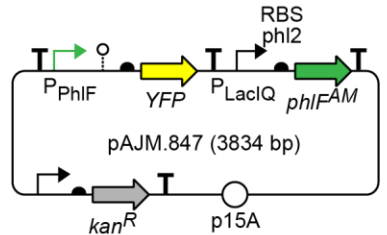
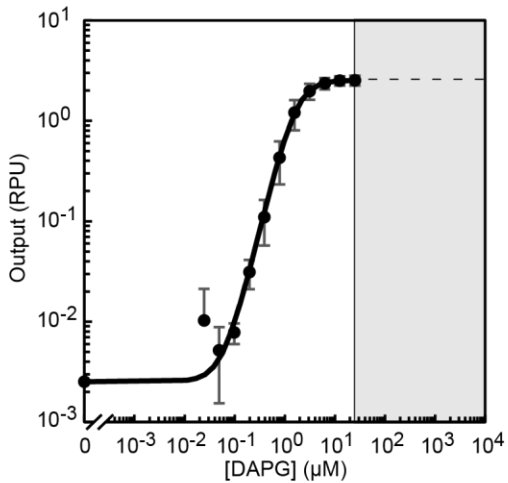
Supplementary Note 1: Sensor specification sheets.

Each page details the performance specifications of 14 sensors.

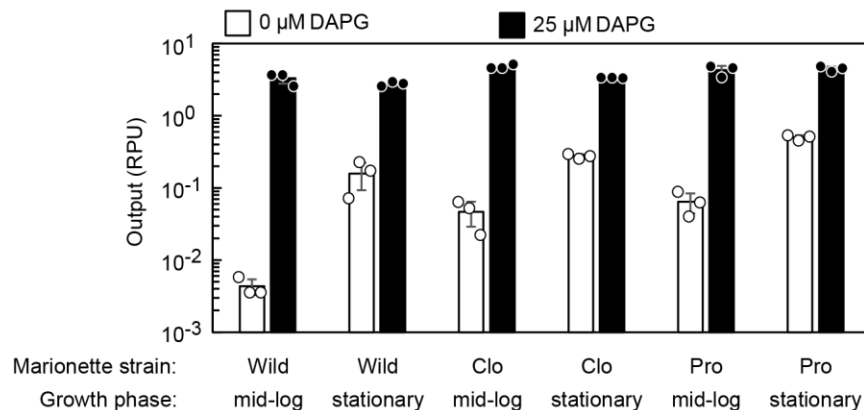
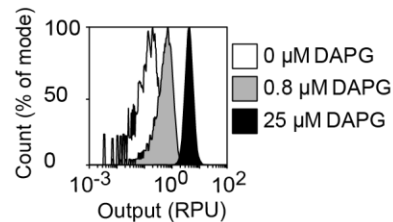
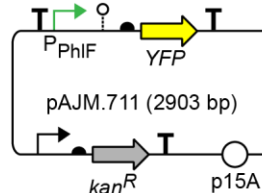
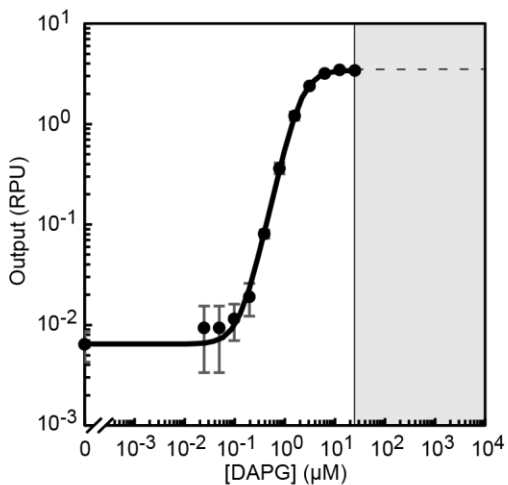
Regulator:P_{Out}:Inducer Information about the inducer including recommendations for preparation and storage of stocks is provided. Recommended maximum induction concentrations are based on induction saturation, solubility, cellular toxicity, cross-reactivity, and antagonism.	Notes Notes that may prove useful to the user are provided. Known instances of cross reactivity are reported; values are based on fold-induction. Known instances of antagonism are reported; values are based on the change in fold-induction at the midpoint of the response function.
Plasmid-encoded regulator Information about the performance of the plasmid-encoded regulators in <i>E. coli</i> DH10B is provided. Response of the output promoter (encoded on the plasmid shown) as a function of inducer concentration in <i>E. coli</i> DH10B in mid-log phase is plotted. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Dashed lines indicate the extension of the response function beyond inducer concentrations for which data have been collected. The grey boxed region denotes inducer concentrations above the recommended maximum induction concentration. Recommended maximum induction concentrations are based on induction saturation, solubility, cellular toxicity, cross-reactivity, and antagonism. Each response function was obtained by fitting the experimental data to Equation 1 (Methods) the curves for which are shown in Figure 2a. The response function parameters are provided in Table 1 and Supplementary Table 6. The plasmid map used to generate these data is shown, and their sequences are provided in Supplementary Note 4. Cytometry histograms for no induction (white), moderate induction (grey), and maximum induction (black) are shown.	
Genome-encoded regulator Information about the performance of the genome-encoded regulators in Marionette strains is provided. Response of the output promoter (encoded on the plasmid shown) as a function of inducer concentration in Marionette-Wild in mid-log phase is plotted. The mean of three replicates performed on different days is shown and error bars represent the standard deviation. Dashed lines indicate the extension of the response function beyond inducer concentrations for which data have been collected. The grey boxed region denotes inducer concentrations above the recommended maximum induction concentration. Recommended maximum induction concentrations are based on induction saturation, solubility, cellular toxicity, cross-reactivity, and antagonism. Each response function was obtained by fitting the experimental data to Equation 1 (Methods) the curves for which are shown in Figure 3b. The response function parameters are provided in Table 1 and Supplementary Table 9. The plasmid map used to generate these data is shown, and their sequences are provided in Supplementary Note 4. Cytometry histograms for no induction (white), moderate induction (grey), and maximum induction (black) are shown. Outputs in the presence (black bars) and absence (white bars) of the appropriate inducer in Marionette-Wild, -Clo, and -Pro in mid-log and stationary phase are shown. The mean of three replicates performed on different days is shown and error bars represent the standard deviation.	

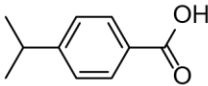
PhlFAM:P_{PhlF}:DAPG 	Inducer	2,4-Diacetylphloroglucinol	Notes -PhlF is a TetR family repressor from <i>Pseudomonas fluorescens</i> -PhlFAM:P _{PhlF} is 2.6-fold induced by 100 μ M Sal
	Source	Santa Cruz sc-206518	
	Stock	25 mM	
	Solvent	DMF	
	Max induction	25 μ M	
	Storage	-20 $^{\circ}$ C	

Plasmid-encoded regulator

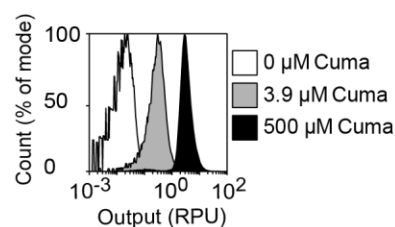
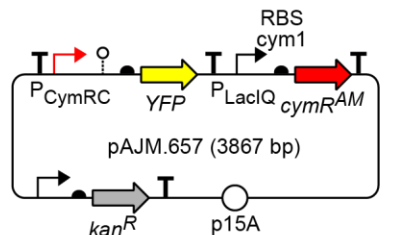
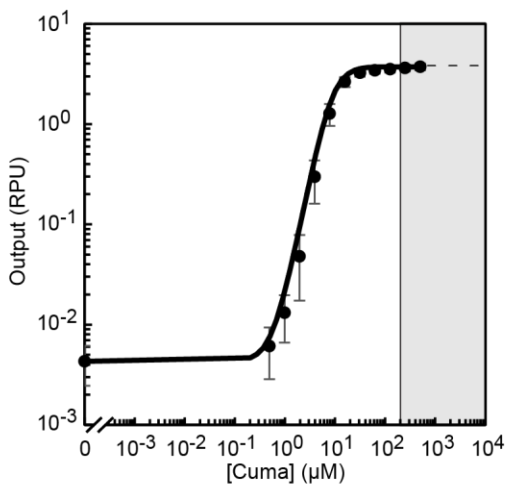


Genome-encoded regulator

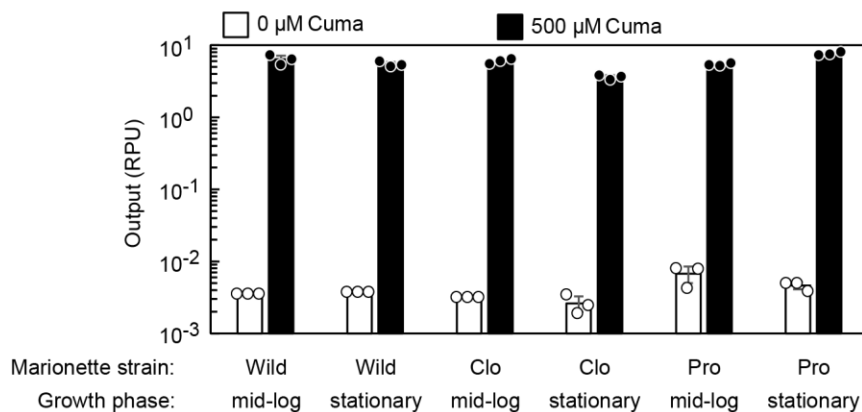
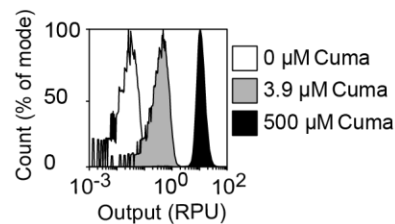
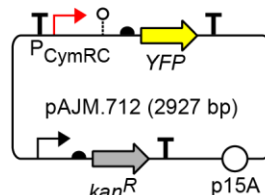
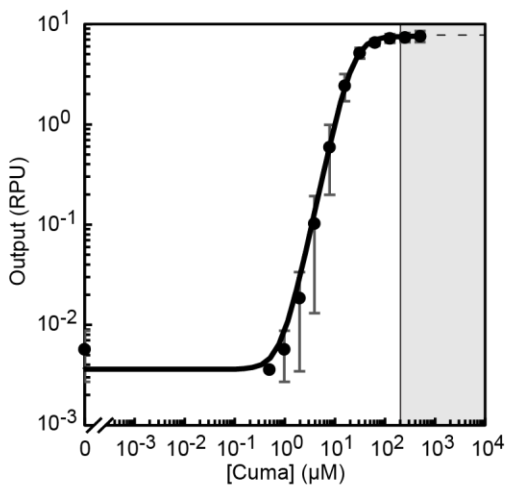


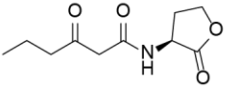
CymRAM:P_{CymRC}:Cuma		Inducer	Cuminic acid	Notes -CymR is a TetR family repressor from <i>Pseudomonas putida</i> -CymRAM:P _{CymRC} :Cuma induction is 13-fold antagonized by 100 μ M Sal
		Source	Sigma 268402	
		Stock	100 mM	
		Solvent	EtOH	
		Max induction	100 μ M	
		Storage	-20 °C	

Plasmid-encoded regulator

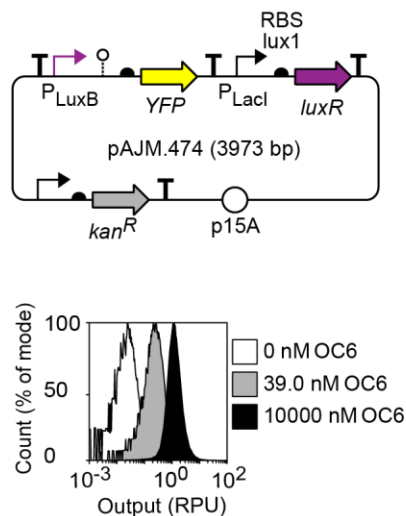
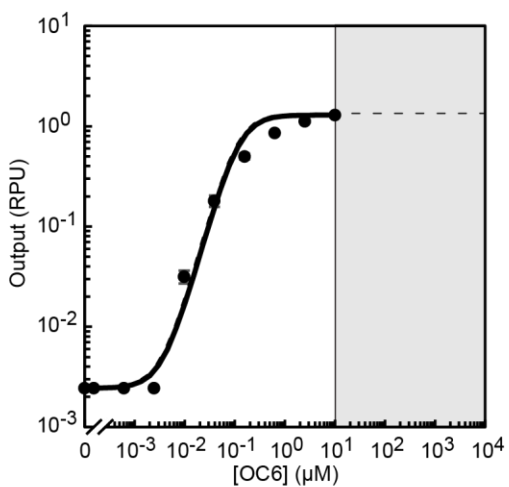


Genome-encoded regulator

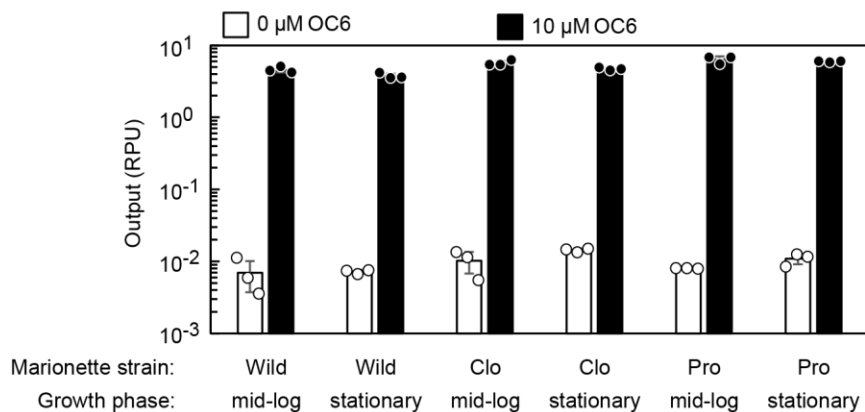
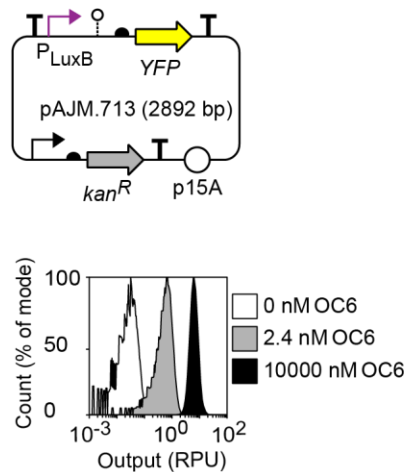
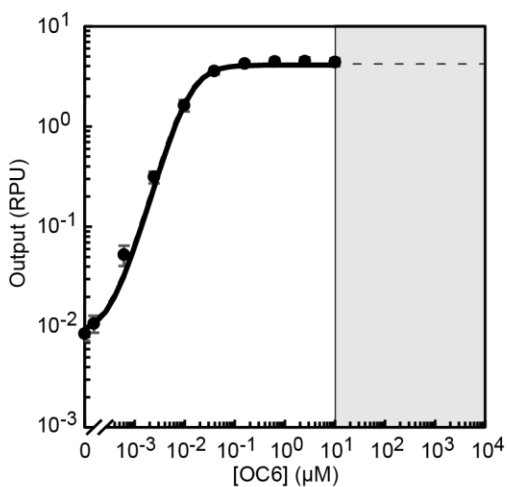


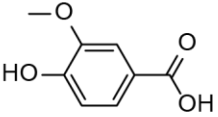
LuxR:P_{LuxB}:OC6 	Inducer	3OC6-HSL	Notes -LuxR is an activator from <i>Vibrio fischeri</i>
	Source	Sigma K3007	
	Stock	10 mM	
	Solvent	DMF	
	Max induction	10 μ M	
	Storage	-20 $^{\circ}$ C	

Plasmid-encoded regulator

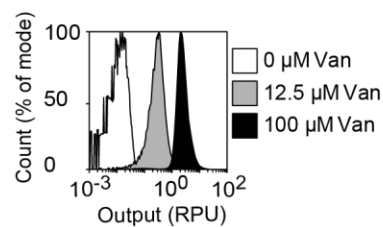
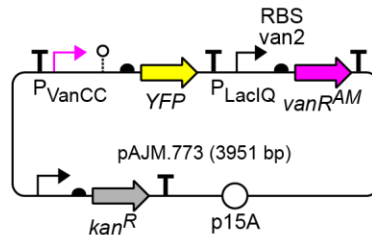
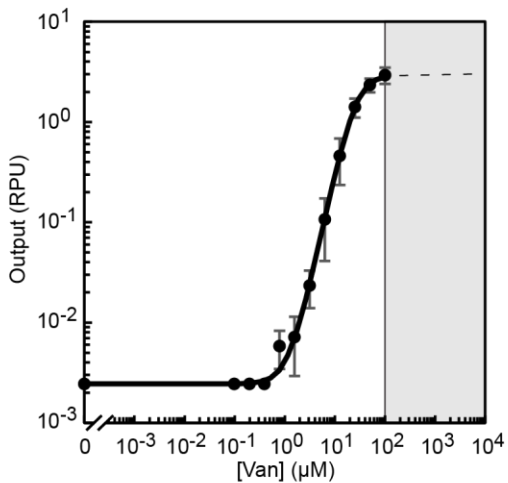


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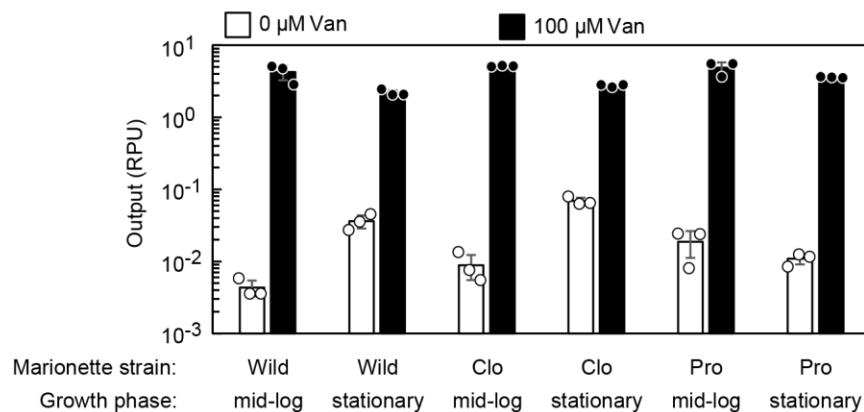
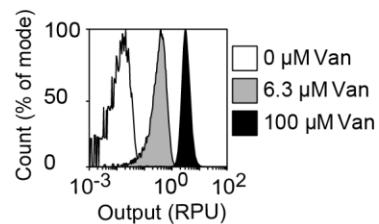
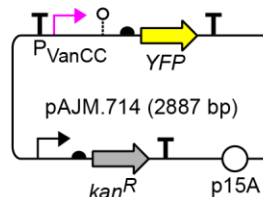
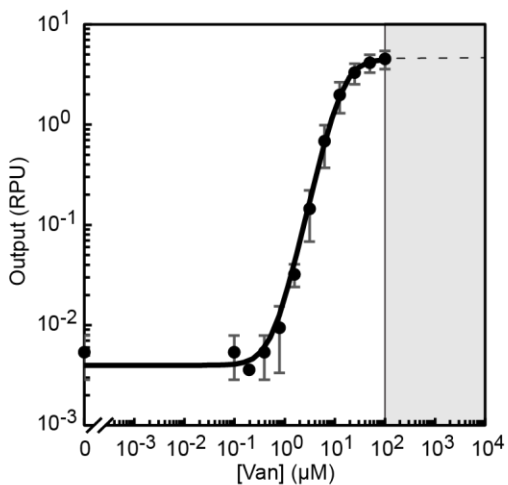


VanRAM:P_{VanCC}:Van 	Inducer	Vanillic acid	Notes -VanR is a GntR family repressor from <i>Caulobacter crescentus</i> -PcaU ^{AM} :P _{3B5} is 2.2-fold induced by 100 μ M Van -VanRAM:P _{VanCC} is 1.3-fold induced by 1 mM DHBA
	Source	Sigma 94770	
	Stock	100 mM	
	Solvent	EtOH	
	Max induction	100 μ M	
	Storage	-20 $^{\circ}$ C	

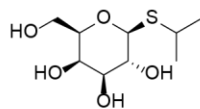
Plasmid-encoded regulator



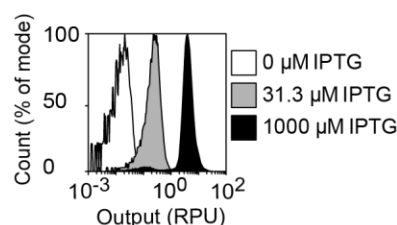
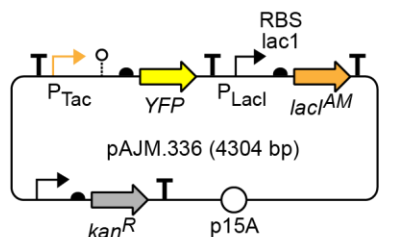
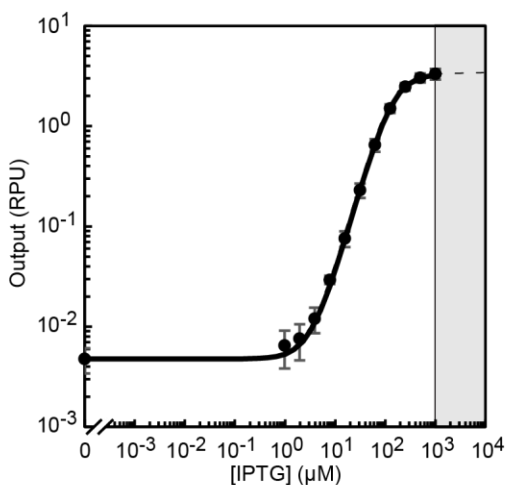
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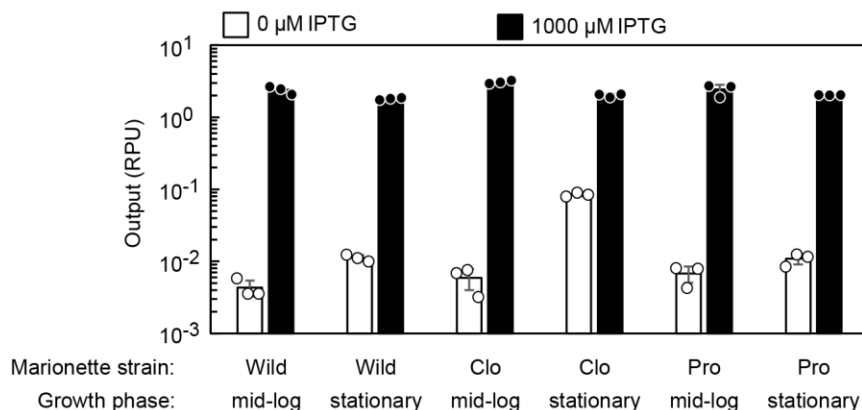
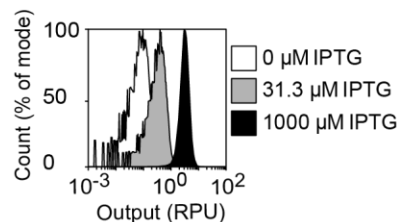
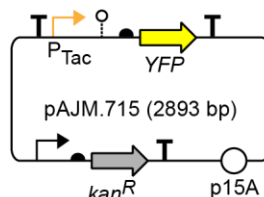
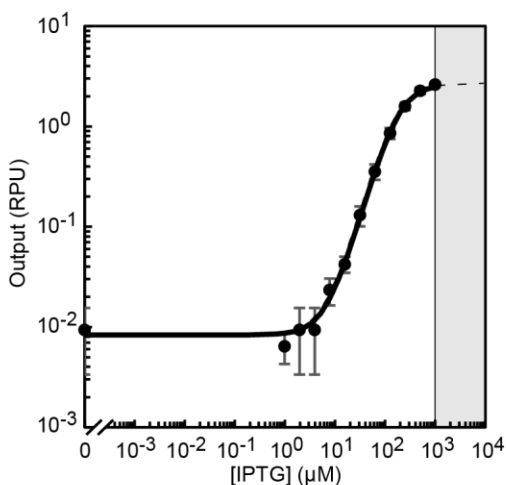
LacI^{AM}:P_{Tac}:IPTG	Inducer Isopropyl-β-D-thiogalactoside Source Gold - I2481C Stock 1 M Solvent Water Max induction 1 mM Storage -20 °C	Notes - LacI is a repressor from <i>Escherichia coli</i> ; native gene is present in <i>E. coli</i> MG1655, DH10B, and BL21, including Marionette strains - AraC ^{AM} :P _{BAD} :Ara induction is 1.5-fold inhibited by 1 mM IPTG
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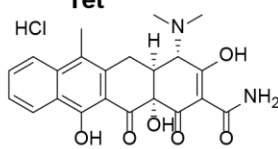


Plasmid-encoded regulator

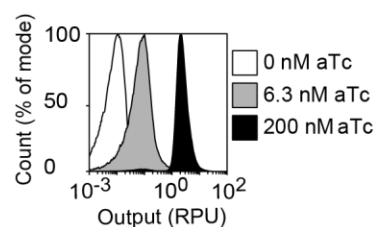
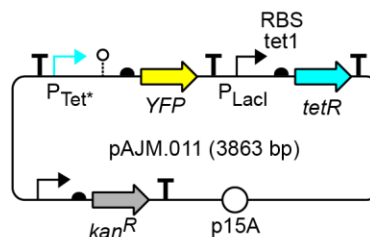
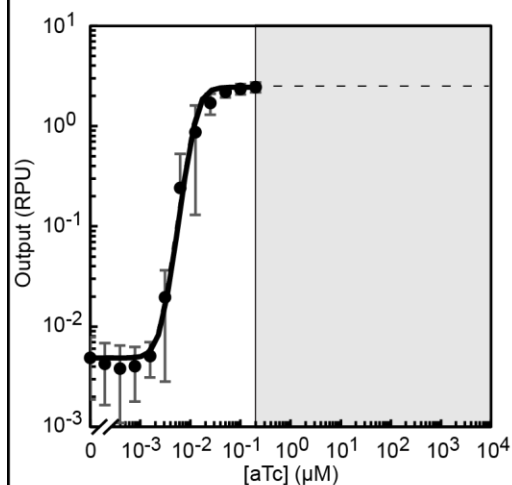


Genome-encoded regulator

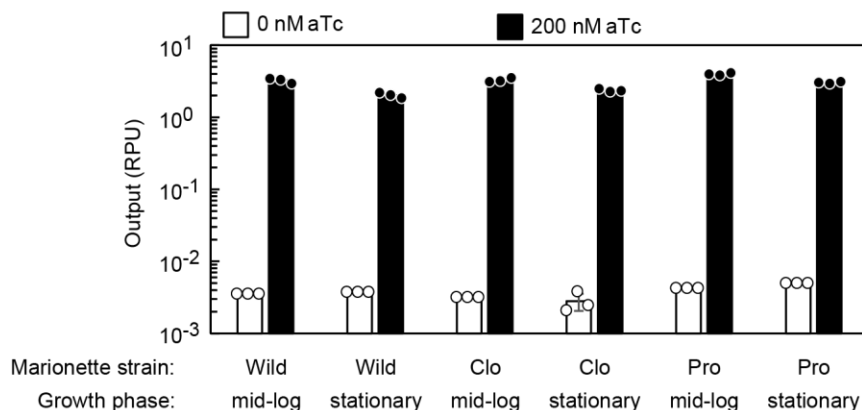
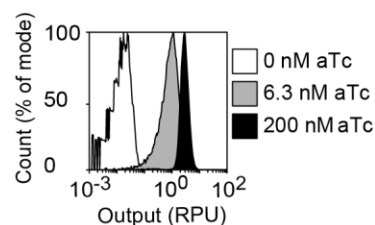
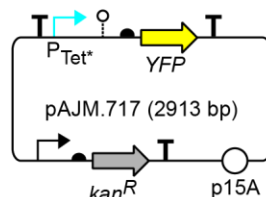
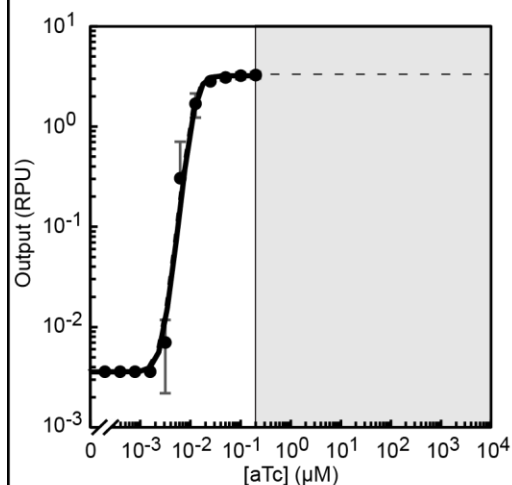


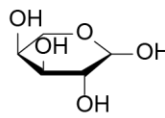
TetR:P_{Tet}[*]:aTc 	Inducer Source Stock Solvent Max induction Storage	Anhydrotetracycline HCl Sigma 37919 200 μ M 1:1 Water:EtOH 200 nM -20 $^{\circ}$ C	Notes -TetR is a repressor from an <i>Escherichia coli</i> mobile element; not present in <i>E. coli</i> MG1655, DH10B, or BL21
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Plasmid-encoded regulator

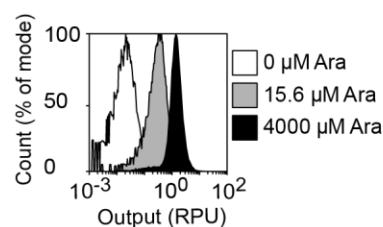
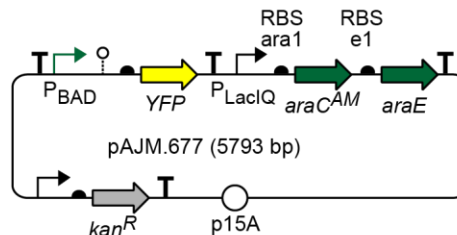
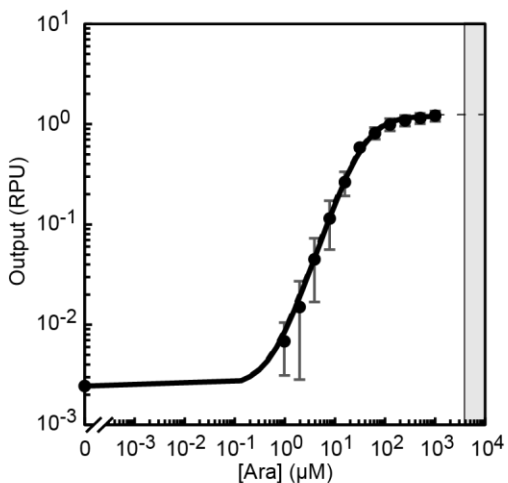


Genome-encoded regulator

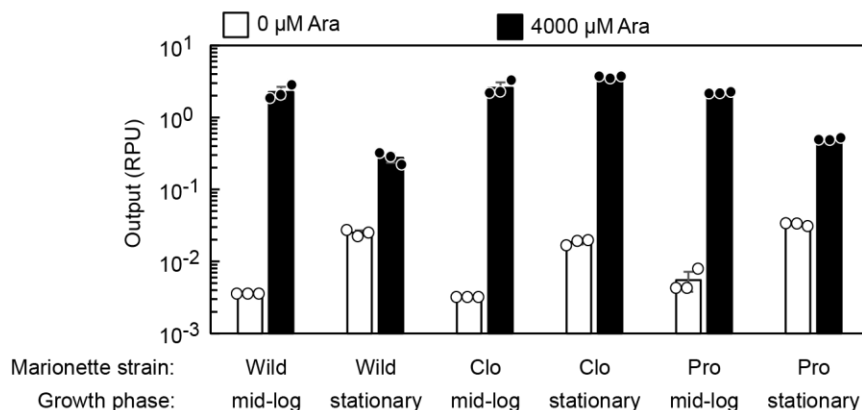
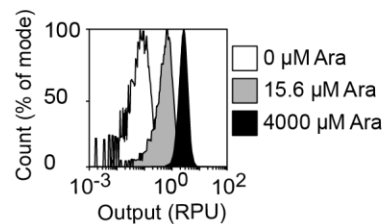
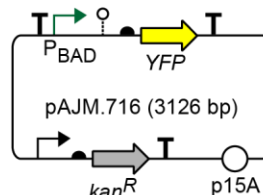
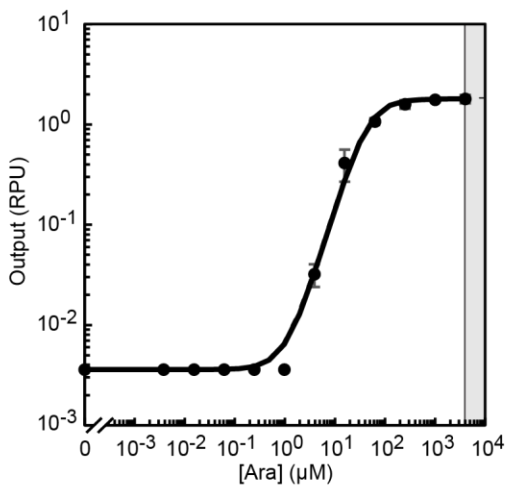


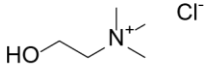
AraC^{AM}:P_{BAD}:Ara 	Inducer	L-Arabinose	Notes -AraC is an activator/repressor from <i>Escherichia coli</i> ; native gene is present in <i>E. coli</i> MG1655 and BL21 and Marionette-Wild and -Pro; these strains can also catabolize Ara -AraC ^{AM} :P _{BAD} :Ara induction is 1.5-fold inhibited by 1 mM IPTG
	Source	Sigma A3256	
	Stock	1 M	
	Solvent	Water	
	Max induction	4 mM	
	Storage	-20 °C	

Plasmid-encoded regulator

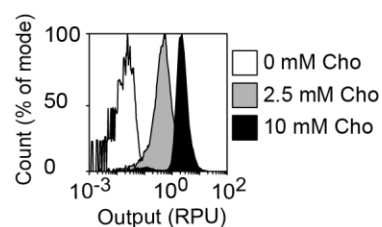
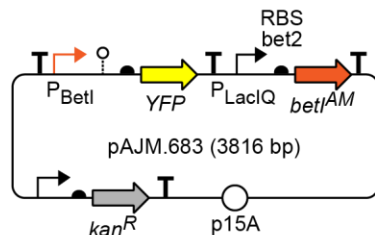
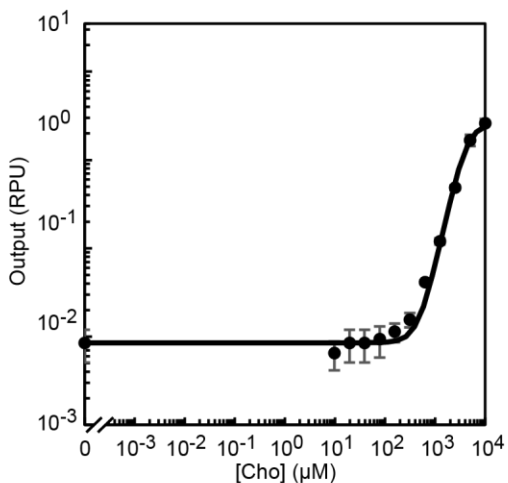


Genome-encoded regulator

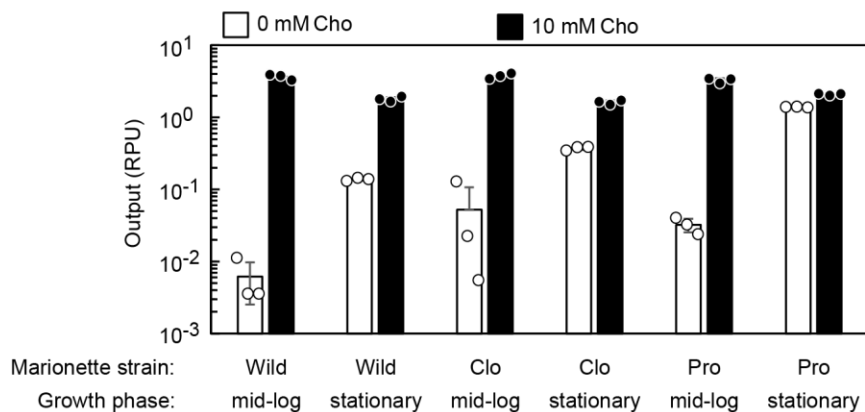
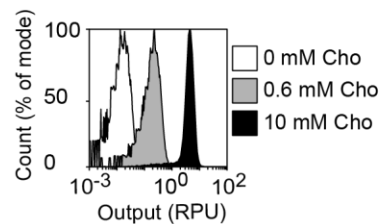
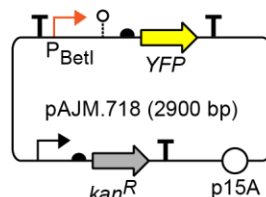
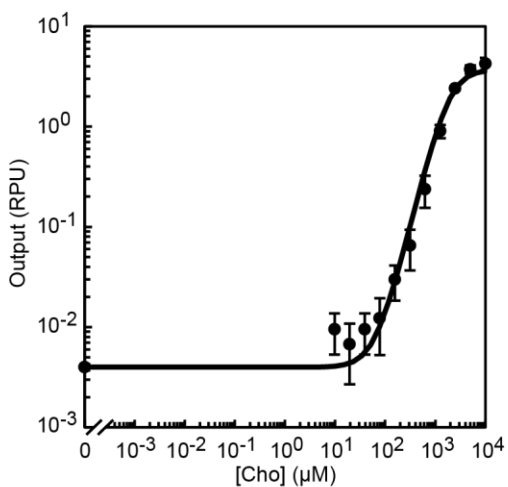


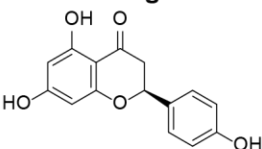
BetI^{AM}:P_{BetI}:Cho 	Inducer	Choline chloride	Notes -BetI is a TetR family repressor from <i>Escherichia coli</i> ; native gene is present in <i>E. coli</i> MG1655, DH10B, and BL21, including Marionette strains - <i>Escherichia coli</i> produces Cho, resulting in induction of P _{BetI} in stationary phase
	Source	Sigma C7017	
	Stock	1 M	
	Solvent	Water	
	Max induction	10 mM	
	Storage	-20 °C	

Plasmid-encoded regulator

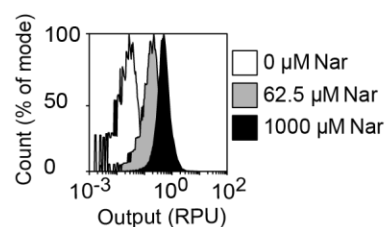
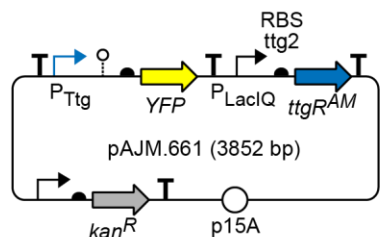
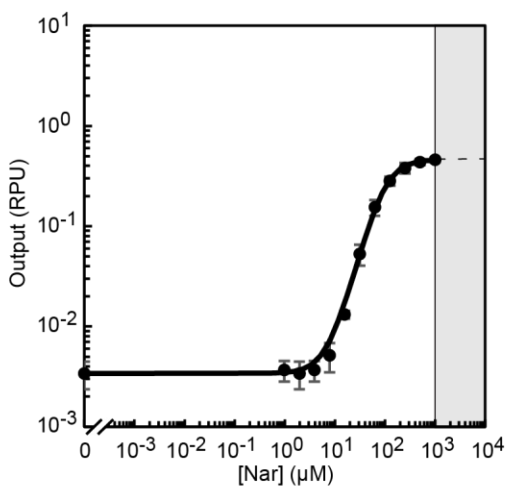


Genome-encoded regulator

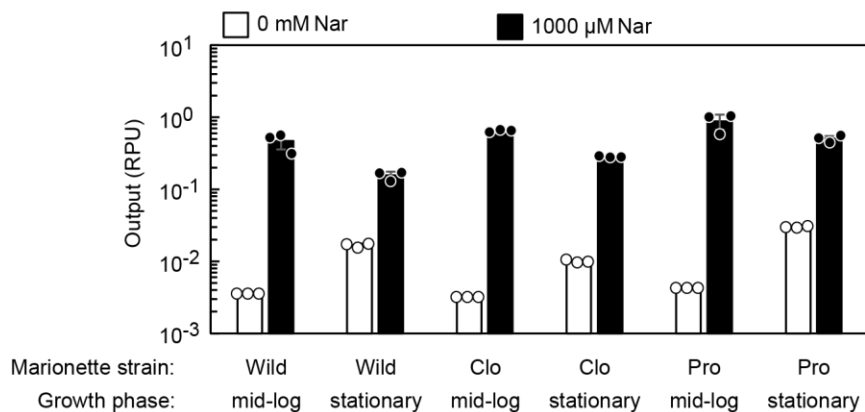
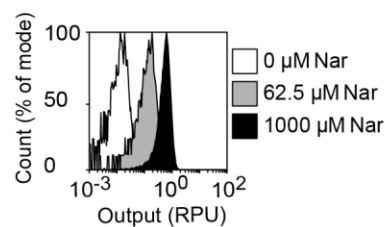
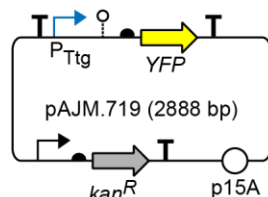
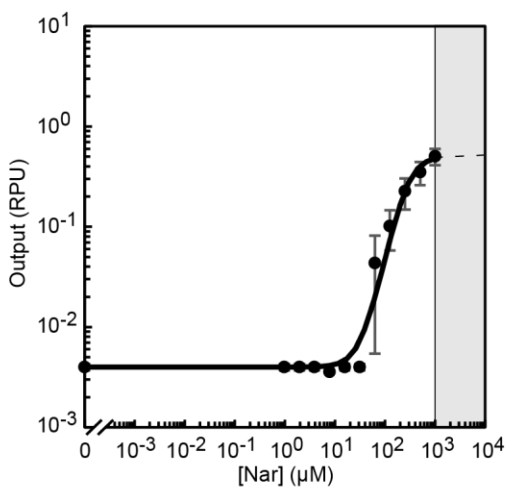


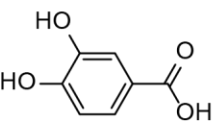
TtgRAM::P_{Ttg}::Nar 	Inducer	Naringenin	Notes -TtgR is a TetR family repressor from <i>Pseudomonas putida</i> -Nar forms a precipitate in media; this does not affect induction
	Source	Sigma N5893	
	Stock	1 M	
	Solvent	DMSO	
	Max induction	1 mM	
	Storage	-20 °C	

Plasmid-encoded regulator

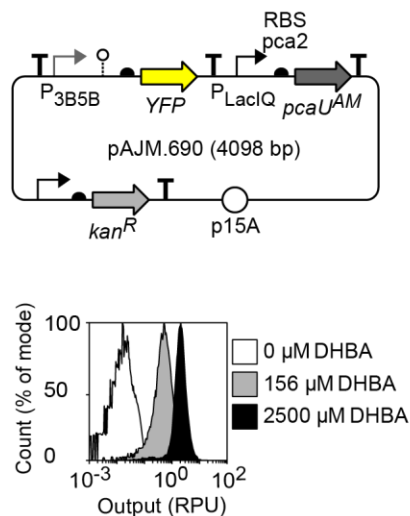
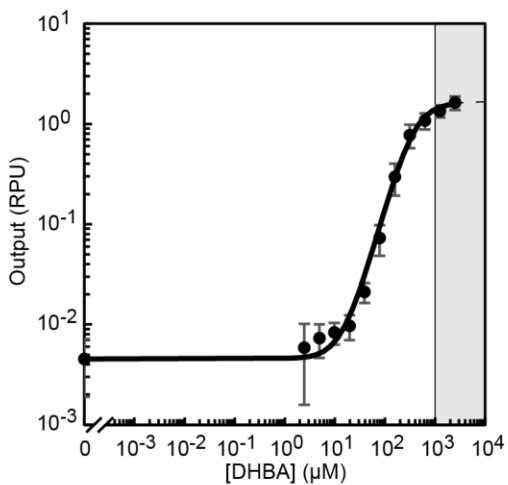


Genome-encoded regulator

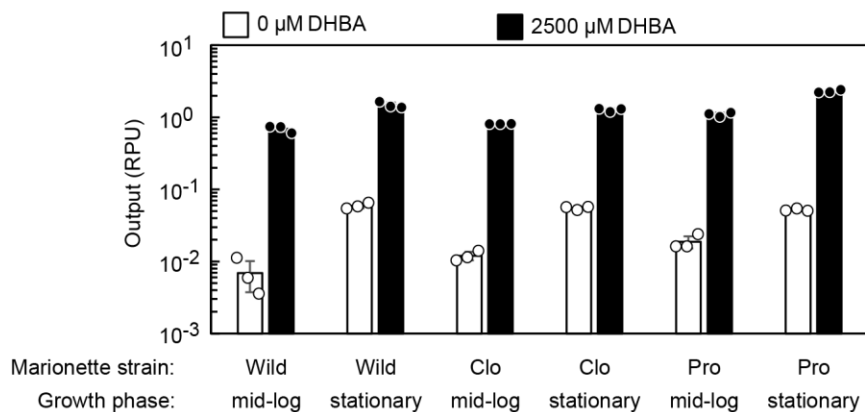
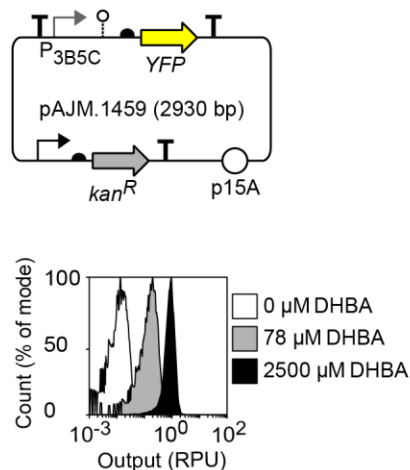
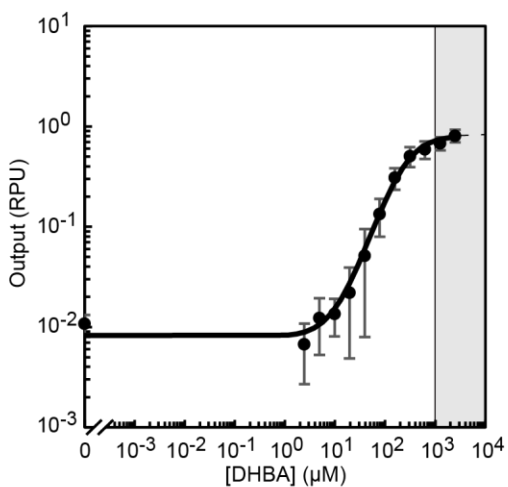


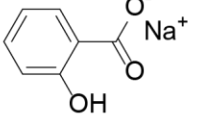
PcaU^{AM}:P_{3B5}:DHBA 	Inducer	3,4-Dihydroxybenzoic acid	Notes -PcaU is a IclR family repressor from <i>Acinetobacter</i> sp. <i>ADP1</i> -PcaU ^{AM} :P _{3B5} is 2.2-fold induced by 100 μ M Van -VanR ^{AM} :P _{VanCC} is 1.3-fold induced by 1 mM DHBA
	Source	Sigma 37580	
	Stock	1 M	
	Solvent	EtOH	
	Max induction	1 mM	
	Storage	-20 $^{\circ}$ C	

Plasmid-encoded regulator

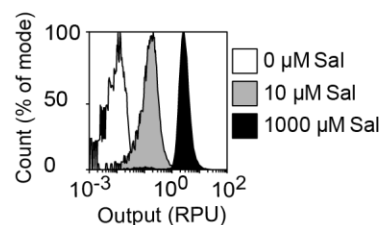
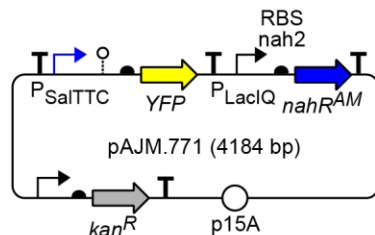
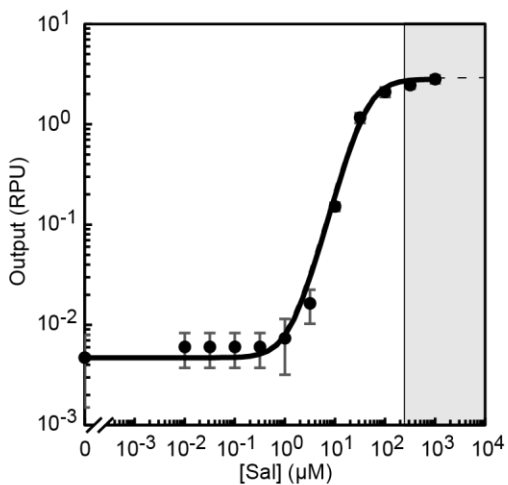


Genome-encoded regulator

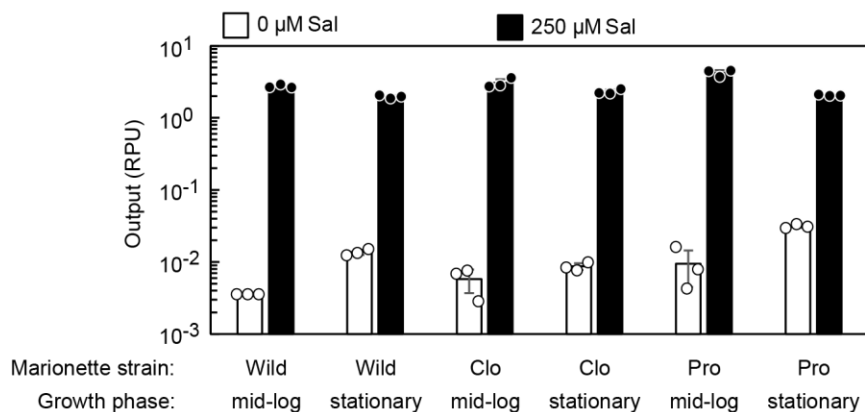
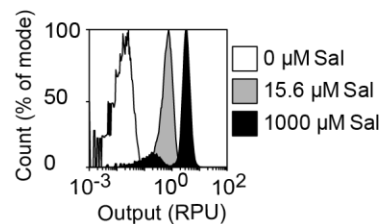
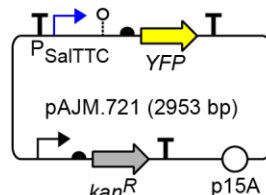
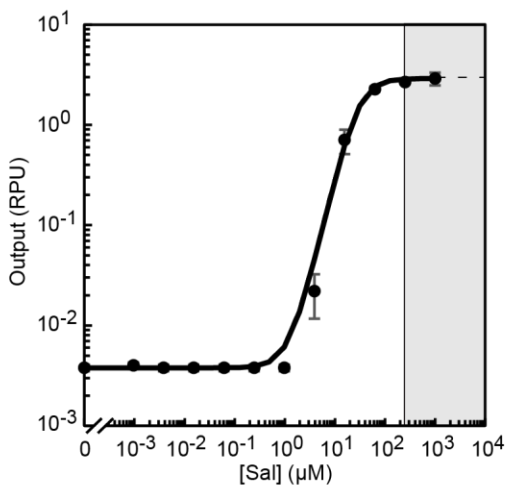


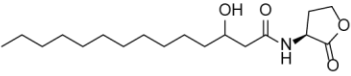
NahR^{AM}:P_{Sal}TTC:Sal 	Inducer	Sodium salicylate	Notes -NahR is a LysR family activator from <i>Pseudomonas putida</i> -PhlF ^{AM} :P _{PhlF} is 2.6-fold induced by 100 μM Sal -CymR ^{AM} :P _{CymRC} :Cuma induction is 13-fold antagonized by 100 μM Sal
	Source	Sigma S3007	
	Stock	1 M	
	Solvent	Water	
	Max induction	100 μM	
	Storage	-20 °C	

Plasmid-encoded regulator

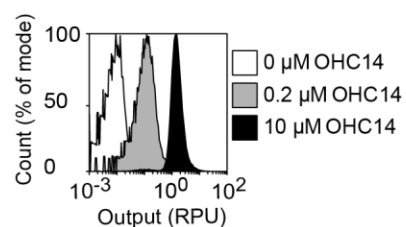
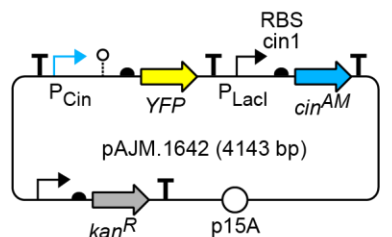
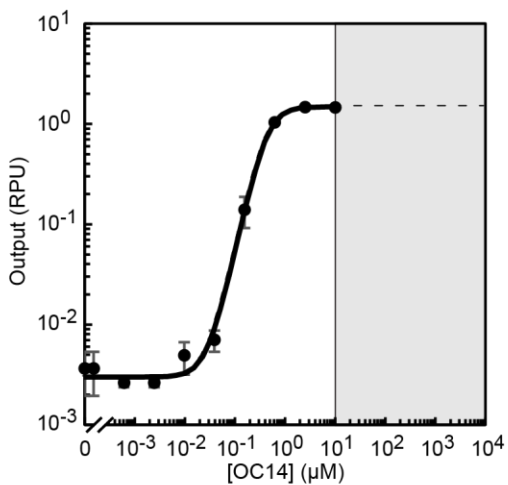


Genome-encoded regulator

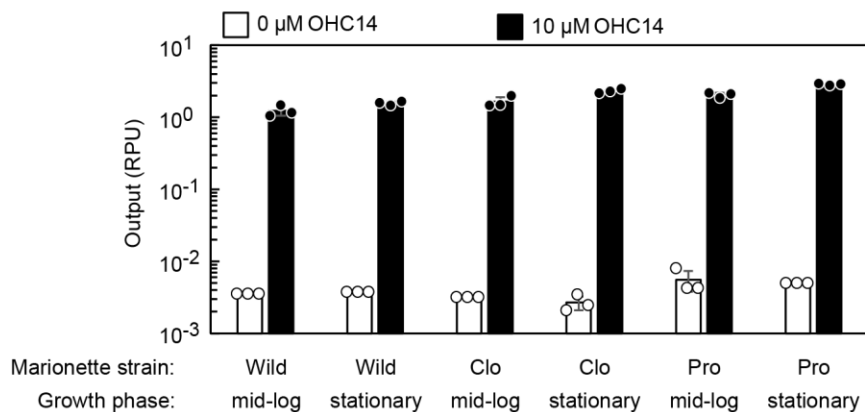
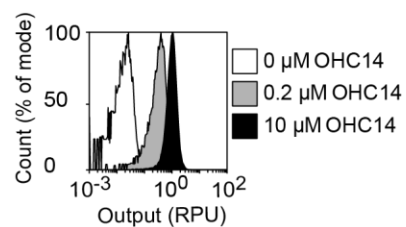
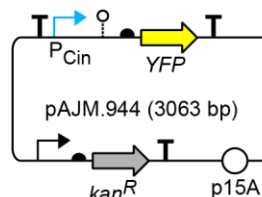
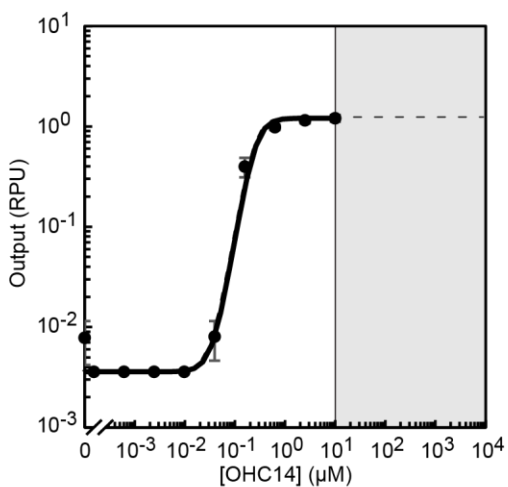


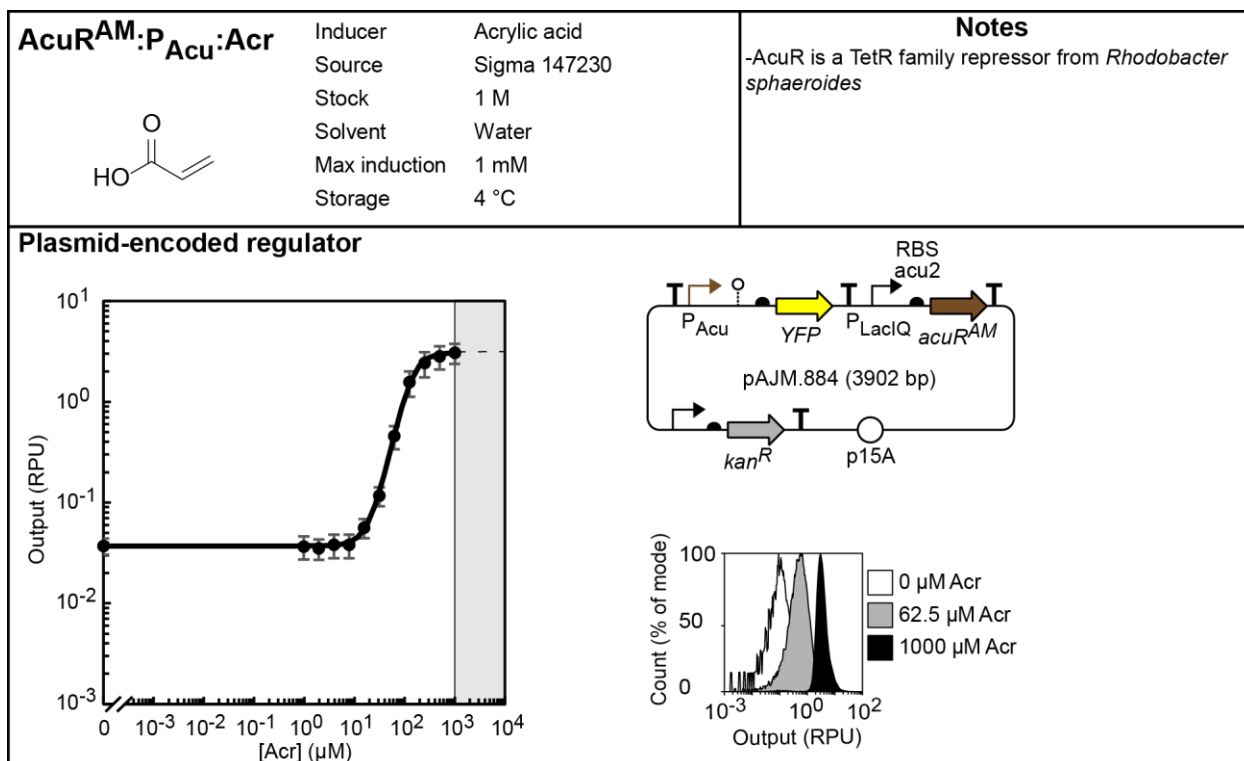
CinRAM:P_{Cin}:OHC14 	Inducer	3OHC14-HSL	Notes -CinR is a LuxR family activator from <i>Rhizobium leguminosarum</i>
	Source	Sigma 51481	
	Stock	10 mM	
	Solvent	DMF	
	Max induction	10 μM	
	Storage	-20 °C	

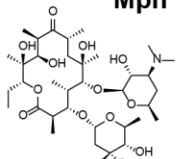
Plasmid-encoded regulator



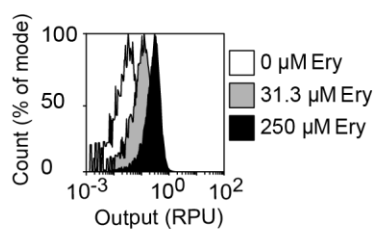
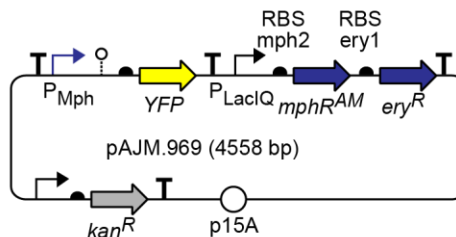
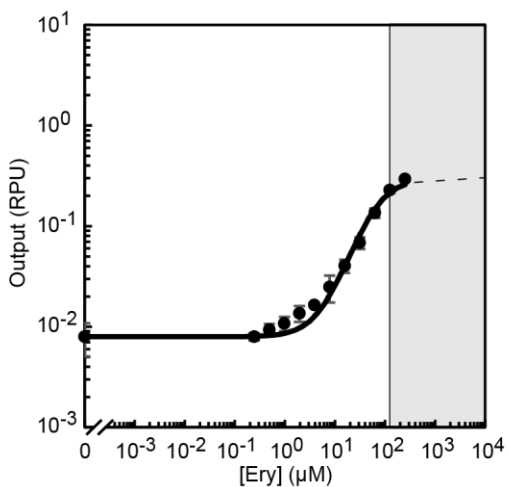
Genome-encoded regulator





MphRAM:P_{Mph}:Ery 	Inducer	Erythromycin	Notes -MphR is a TetR family repressor from <i>Escherichia coli</i> clinical isolates; not present in <i>E. coli</i> MG1655, DH10B, or BL21 -Ery inhibits translation in <i>Escherichia coli</i> -Ery ^R methylates the ribosome, conferring Ery resistance up to 125 µM
	Source	Sigma E5389	
	Stock	100 mM	
	Solvent	EtOH	
	Max induction	125 µM	
	Storage	4 °C	

Plasmid-encoded regulator

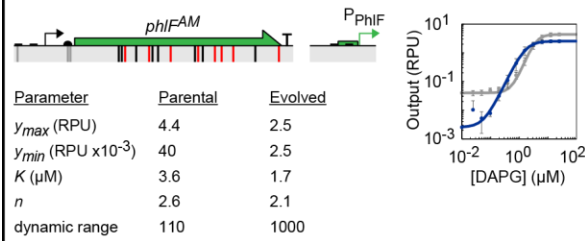


Supplementary Note 2: Sensor evolution

Each page details the process of directed evolution to optimize 12 sensors.

Regulator:P_{Out}:Inducer The genetic design of each sensor is shown, with non-synonymous (red) and non-coding or synonymous (grey/black) mutations noted. Mutations in grey were also applied to the parental sensor. Response functions of the evolved (blue) and parental (grey) sensors are shown. All data are from experiments using <i>E. coli</i> DH10B cells in mid-log growth in LB media. The mean of three replicates from different days is shown and error bars represent the standard deviation. Response function parameters are provided for parental and evolved sensors, definition are provided in the main text.	Directed evolution interventions The selection parameters for each round of selection are noted. R1 refers to Round 1 of selection. DNAP refers to DNA polymerase (error-prone PK6 vs high-fidelity KOD). “Shuffle” indicates that libraries were subject to gene shuffling prior to the next round of selection. The first two rounds of the LacI selection utilized the positive selection plasmid and the negative selection plasmid, respectively. All other rounds utilize dual selection plasmids (Supplementary Figure 6).
Initial library Degeneracy (orange) was added to the regulator CDS, regulator RBS, and the output promoter. In the case of LacI, positions were partially randomized using multiple degenerate oligonucleotides mixed at the percentage shown. Black diamonds denote BbsI restriction sites. N=A25:C25:T25:G25; W=A50:T50; S=C50:G50; M=A50:C50; K=G50:T50; D=33A:33G:33T; V=33A:33C:33G.	
On/off screen RPU values for each clone with inducer(s) added as noted (Methods). All data are from experiments using <i>E. coli</i> DH10B cells in mid-log growth in LB media. The experiment was performed as a single replicate.	
Sequencing Non-synonymous mutations are listed as amino acid changes while synonymous mutations are not listed (<i>i.e.</i> , Q18M denotes that the 18 th amino acid mutated from glutamine to methionine). Blue denotes that the exact mutation appears in the final mutant (Regulator ^{AM}). * denotes a stop codon.	
Consensus mutant testing Response functions for parental (WT) sensors, evolved sensors, and notable sensors from the literature (LacI Q18M ²⁹ , AraC C280* ³⁰ , and BetI I22T ³¹) are shown. Where relevant, response functions with cross-reactive inducers or antagonistic inducers are shown. Nonsynonymous mutations are shown. Full sequences of promoters and proteins (parental and evolved) are provided in Supplementary Tables 1 and 4. All data are from experiments using <i>E. coli</i> DH10B cells in mid-log growth in LB media. The mean of three replicates from different days is shown and error bars represent the standard deviation.	

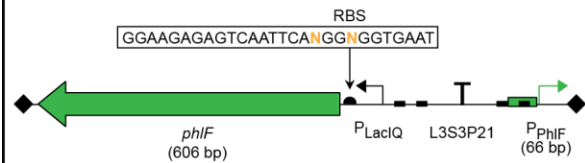
PhIF:P_{PhIF}:DAPG



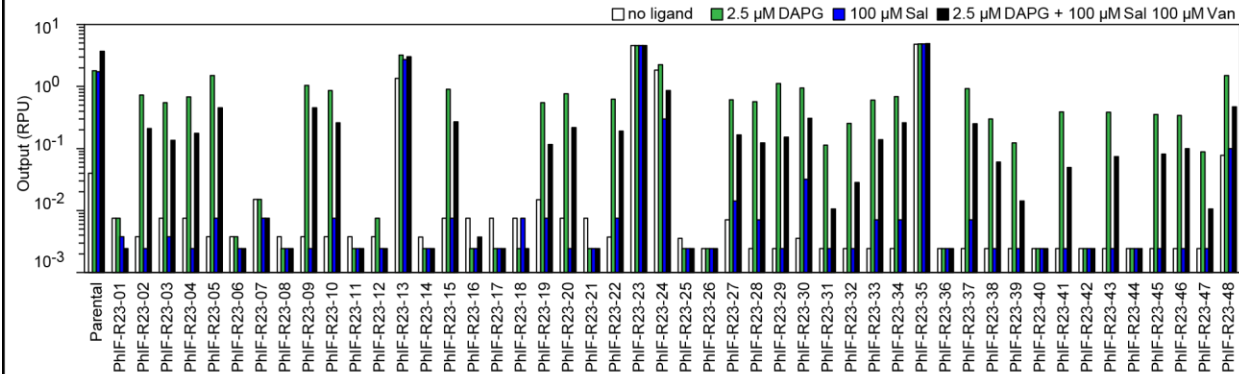
Directed evolution interventions

PhIF	[Cl-Phe]	[Negative ligand]	DNAP	[Positive ligand]	Notes
R1	2 mM		PK6	2.5 μM DAPG	
R2	2 mM		PK6	2.5 μM DAPG	
R3	2 mM		PK6	2.5 μM DAPG	
R4	2 mM		PK6	2.5 μM DAPG	
R5	4 mM		PK6	2.5 μM DAPG	
R6	4 mM		PK6	2.5 μM DAPG	
R7	4 mM		KOD	2.5 μM DAPG	
R8	4 mM		KOD	2.5 μM DAPG	
R9	4 mM	10 μM Sal	PK6	10 μM Sal + 2.5 μM DAPG	
R10	4 mM	10 μM Sal	PK6	10 μM Sal + 2.5 μM DAPG	
R11	4 mM	10 μM Sal	PK6	10 μM Sal + 2.5 μM DAPG	
R12	4 mM	20 μM Sal	PK6	20 μM Sal + 2.5 μM DAPG	
R13	4 mM	30 μM Sal	PK6	30 μM Sal + 2.5 μM DAPG	Shuffle
R14	4 mM	40 μM Sal	PK6	40 μM Sal + 2.5 μM DAPG	
R15	4 mM	40 μM Sal	PK6	40 μM Sal + 2.5 μM DAPG	
R16	4 mM	50 μM Sal	PK6	50 μM Sal + 2.5 μM DAPG	
R17	4 mM	60 μM Sal	PK6	60 μM Sal + 2.5 μM DAPG	
R18	4 mM	75 μM Sal	PK6	2.5 μM DAPG	
R19	4 mM	100 μM Sal	KOD	2.5 μM DAPG	
R20	4 mM	100 μM Sal	KOD	100 μM Sal + 2.5 μM DAPG	
R21	4 mM	100 μM Sal	KOD	100 μM Sal + 2.5 μM DAPG	Shuffle
R22	4 mM	100 μM Sal	KOD	100 μM Sal + 100 μM Van + 2.5 μM DAPG	
R23	4 mM	100 μM Sal	KOD	100 μM Sal + 100 μM Van + 2.5 μM DAPG	

Initial library (889 bp)



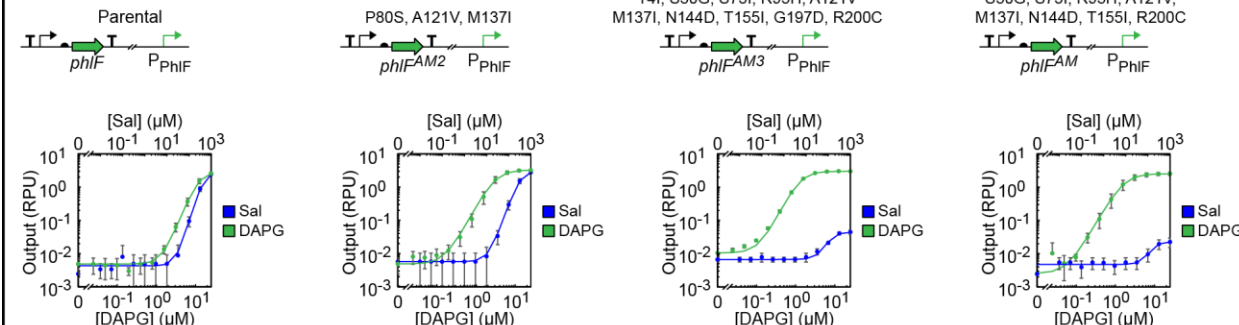
On/off screen

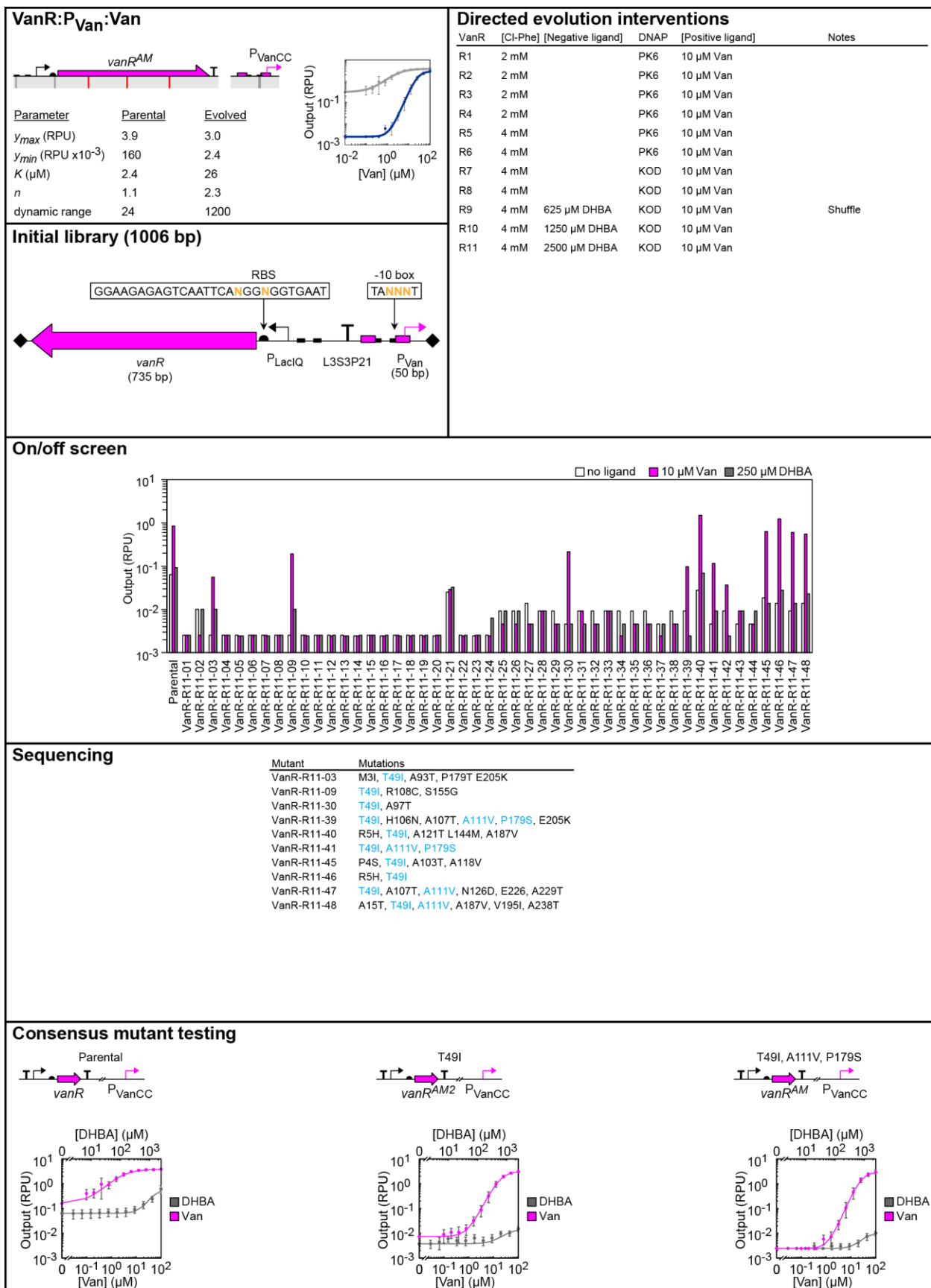


Sequencing

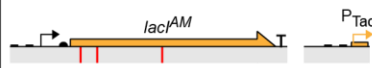
Mutant	Mutations
PhIF-R23-02	S50G, S73I, R94H, A121V, M137I, G197D
PhIF-R23-05	S50G, S73I, C111S, A121V, Q125L, M137I, G197C
PhIF-R23-09	T4I, S50G, S73I, R94H, A121V, M137I, N144D, R200C
PhIF-R23-10	S50G, S73I, A121V, M137I
PhIF-R23-15	S50G, S73I, A121V, M137I
PhIF-R23-20	S50G, S73I, R94C, C111S, A121V, Q125L, M137I, V179I, G197R
PhIF-R23-22	S50G, S73I, M137I, K153E, T155I
PhIF-R23-28	L38M, S50G, S73I, A121V, M137I, G197D, R200C
PhIF-R23-33	L38M, S50G, S73I, M137I, T155I
PhIF-R23-34	T4I, S50G, S73I, A121V, T124A, M137I, N144I, S148N
PhIF-R23-37	S50G, S73I, R94H, L123M, M137I, T155I, P196Q
PhIF-R23-46	S50G, S73I, M137I, V179A

Consensus mutant testing

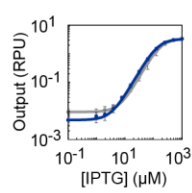




LacI:P_{Tac}:IPTG



Parameter	Parental	Evolved
y_{max} (RPU)	3.4	3.3
y_{min} (RPU $\times 10^{-3}$)	9.2	4.8
K (μ M)	170	140
n	1.8	1.8
dynamic range	370	700



Directed evolution interventions

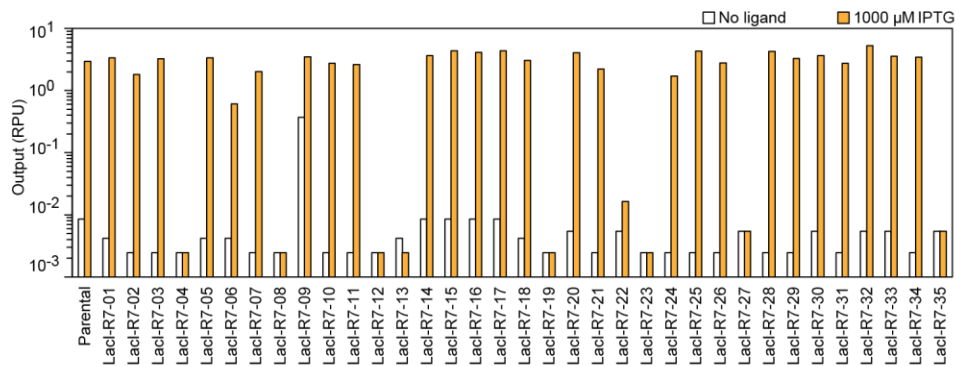
LacI	[Cl-Phe]	[Negative ligand]	DNAP	[Positive ligand]	Notes
R1	-	-	PK6	50 μ M IPTG	Positive selection
R2	-	0 μ M IPTG	PK6	-	Negative selection
R3	4 mM	-	PK6	50 μ M IPTG	Dual selection
R4	4 mM	-	PK6	50 μ M IPTG	Dual selection
R5	4 mM	-	PK6	50 μ M IPTG	Dual selection
R6	4 mM	-	KOD	50 μ M IPTG	Dual selection
R7	4 mM	-	KOD	50 μ M IPTG	Dual selection

Initial library (1359 bp)

L296	Q291	W220	F161	Q18
WKS	43%:VMG 57%:WKS	43%:VMG 57%:WKS	27%:VMG 55%:NDC 18%:WKG	27%:VMG 55%:NDC 18%:WKG



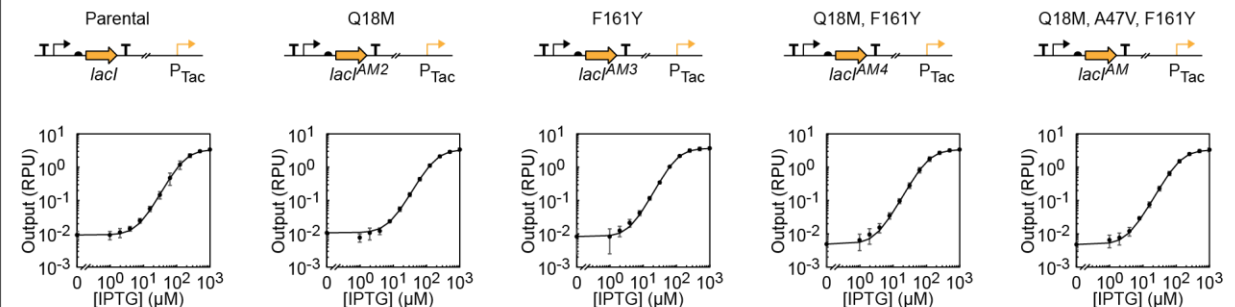
On/off screen



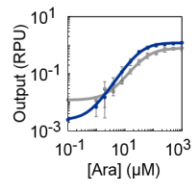
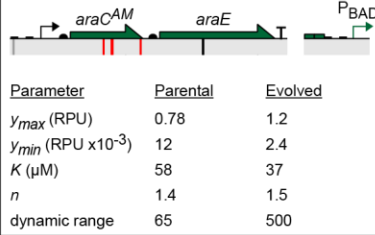
Sequencing

Mutant	Q18	A43	F161	W220	Q291	L296	Mutant	Q18	A43	F161	W220	Q291	L296
LacI-R7-01	Q	A	W	W	Q	M	LacI-R7-20	Q	A	Y	W	Q	L
LacI-R7-03	M	V	Y	W	Q	L	LacI-R7-21	Q	A	Y	W	Q	L
LacI-R7-05	M	A	Y	W	Q	M	LacI-R7-25	M	V	Y	W	Q	L
LacI-R7-07	Q	A	Y	W	Q	L	LacI-R7-28	M	V	Y	W	Q	L
LacI-R7-10	M	V	Y	W	Q	L	LacI-R7-29	Q	A	Y	W	Q	L
LacI-R7-11	Q	A	Y	W	Q	L	LacI-R7-30	Q	A	Y	W	Q	L
LacI-R7-14	Q	A	Y	W	Q	L	LacI-R7-31	H	A	Y	W	Q	I
LacI-R7-16	Q	A	Y	W	Q	L	LacI-R7-32	Q	V	Y	W	Q	L
LacI-R7-17	Q	A	Y	W	Q	L	LacI-R7-33	Q	V	W	W	Q	I
LacI-R7-18	Q	A	Y	W	Q	L							

Consensus mutant testing



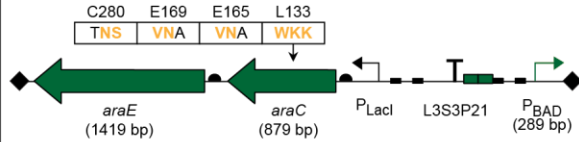
AraC:P_{BAD}:Ara



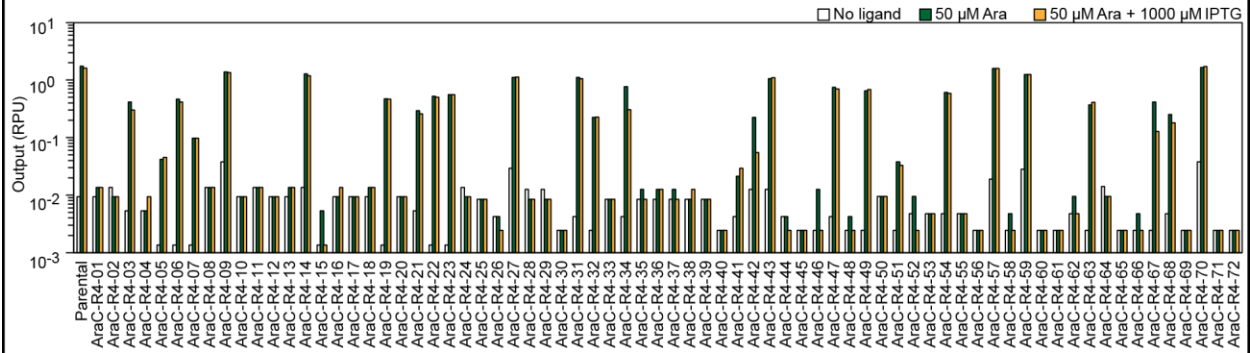
Directed evolution interventions

AraC	[Cl-Phe]	[Negative ligand]	DNAP	[Positive ligand]	Notes
R1	2 mM	1000 μ M IPTG	PK6	1000 μ M IPTG + 50 μ M Ara	
R2	2 mM	1000 μ M IPTG	PK6	1000 μ M IPTG + 50 μ M Ara	
R3	4 mM	1000 μ M IPTG	PK6	1000 μ M IPTG + 50 μ M Ara	
R4	4 mM	1000 μ M IPTG	PK6	1000 μ M IPTG + 50 μ M Ara	

Initial library (2848 bp)



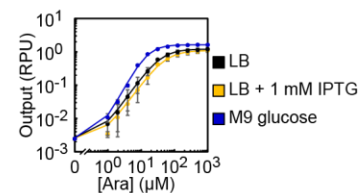
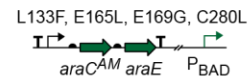
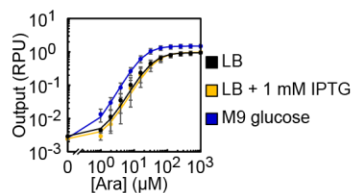
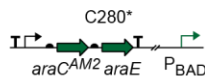
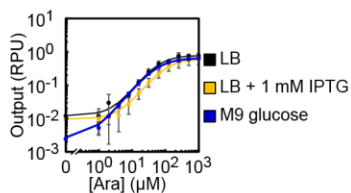
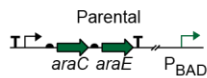
On/off screen



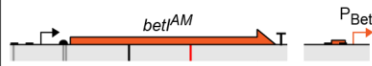
Sequencing

Mutant	L133	E165	E169	C280
AraC-R4-06	R	L	K	W
AraC-R4-14	L	R	G	C
AraC-R4-19	I	P	Q	C
AraC-R4-21	F	A	A	S
AraC-R4-22	S	R	G	L
AraC-R4-23	W	G	I	L
AraC-R4-31	F	L	G	L
AraC-R4-32	R	I	L	W
AraC-R4-43	M	E	G	W
AraC-R4-47	M	R	A	F
AraC-R4-49	F	L	L	Y
AraC-R4-54	S	V	V	L
AraC-R4-57	I	V	E	F
AraC-R4-63	W	G	E	L

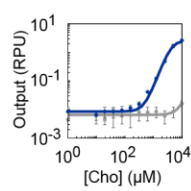
Consensus mutant testing



Betl:P_{Betl}:Cho



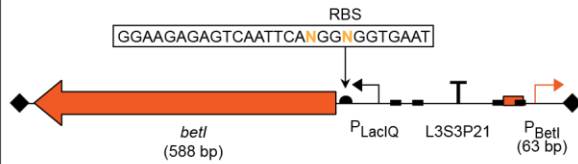
Parameter	Parental	Evolved
y_{max} (RPU)	0.03	2.6
y_{min} (RPU $\times 10^{-3}$)	6.5	8.5
K (μ M)	9000	4100
n	2.5	2.7
dynamic range	5	310



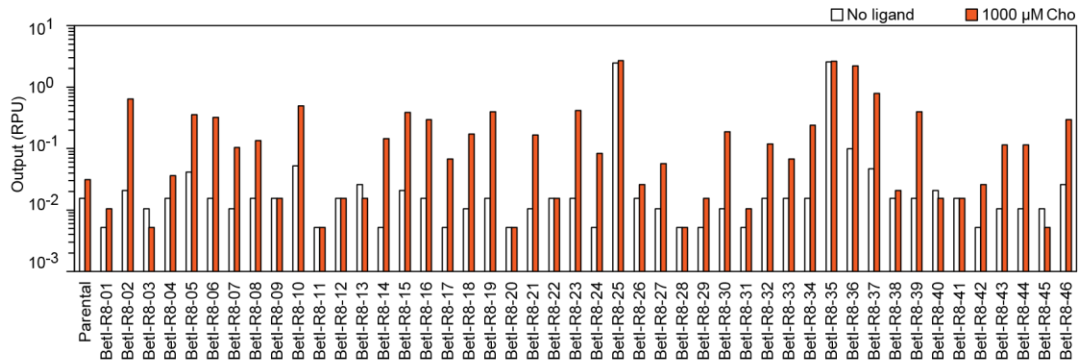
Directed evolution interventions

Betl	[Cl-Phe] [Negative ligand]	DNAP	[Positive ligand]	Notes
R1	2 mM	PK6	5 mM Cho	
R2	2 mM	PK6	5 mM Cho	
R3	4 mM	PK6	5 mM Cho	
R4	4 mM	PK6	4 mM Cho	
R5	4 mM	PK6	3 mM Cho	
R6	4 mM	PK6	2 mM Cho	
R7	4 mM	KOD	1 mM Cho	
R8	4 mM	KOD	1 mM Cho	

Initial library (871 bp)



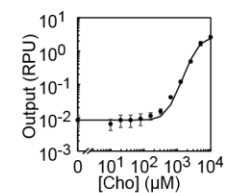
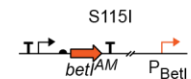
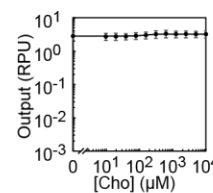
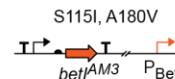
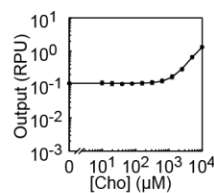
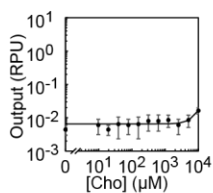
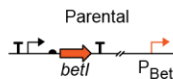
On/off screen



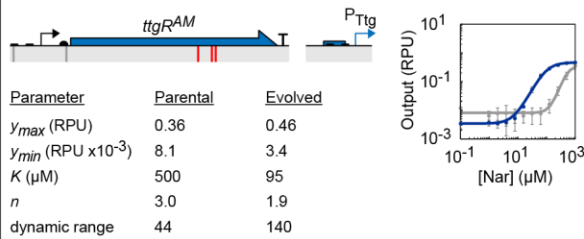
Sequencing

Mutant	Mutations
Betl-R8-02	M121I, Q127H, A180V
Betl-R8-06	R37C, S115I
Betl-R8-14	S115I, D195N, *196RYGSA*
Betl-R8-15	S115I, K177E, H186Q
Betl-R8-16	S115I, K173E
Betl-R8-19	D70N, S115N, A180V
Betl-R8-23	H77Y, S115I
Betl-R8-30	G93D, Q149H, A180V, R185H
Betl-R8-36	I32V, S115I, S182N, T189I
Betl-R8-39	R51H, H77L, S102N, A180V, D195N, *196G*

Consensus mutant testing



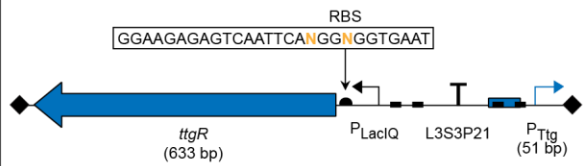
TtgR:P_{Ttg}:Nar



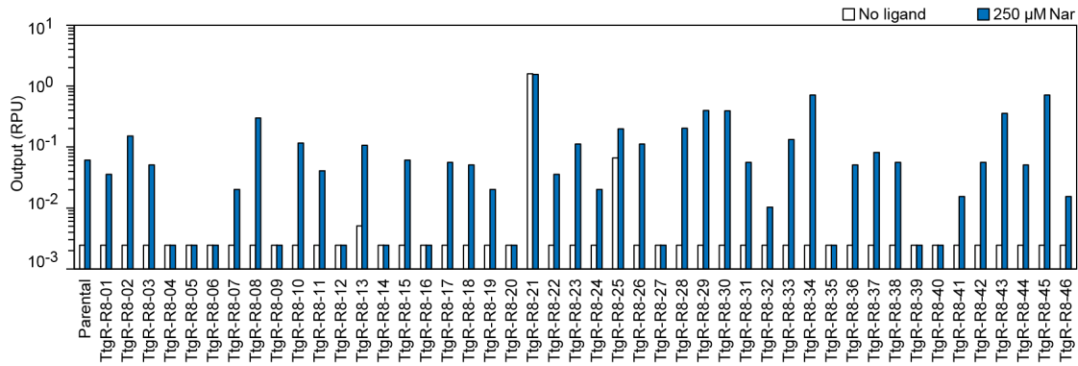
Directed evolution interventions

TtgR	[Cl-Phe]	[Negative ligand]	DNAP	[Positive ligand]	Notes
R1	2 mM		PK6	250 μM Nar	
R2	2 mM		PK6	250 μM Nar	
R3	4 mM		PK6	250 μM Nar	
R4	4 mM		PK6	250 μM Nar	
R5	4 mM		PK6	250 μM Nar	
R6	4 mM		PK6	250 μM Nar	
R7	4 mM		KOD	250 μM Nar	
R8	4 mM		KOD	250 μM Nar	

Initial library (904 bp)



On/off screen



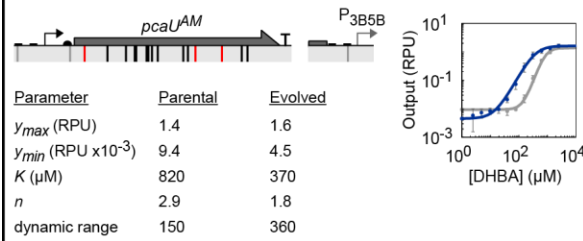
Sequencing

Mutant	Mutations
TtgR-R8-08	R90H, R130H, A144T
TtgR-R8-29	A61T, H67L, G140A, E161G
TtgR-R8-30	A61T, R130C, A144T, R151H, K193N, A207T
TtgR-R8-34	I17V, H67R, D71N, A144T, A146V, A148V
TtgR-R8-43	R130H, A148V
TtgR-R8-45	S79N, R130H, A144T, A148V, *211Q*

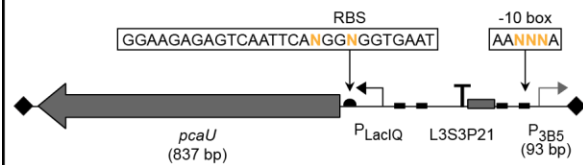
Consensus mutant testing



PcaU:P_{3B5}:DHBA



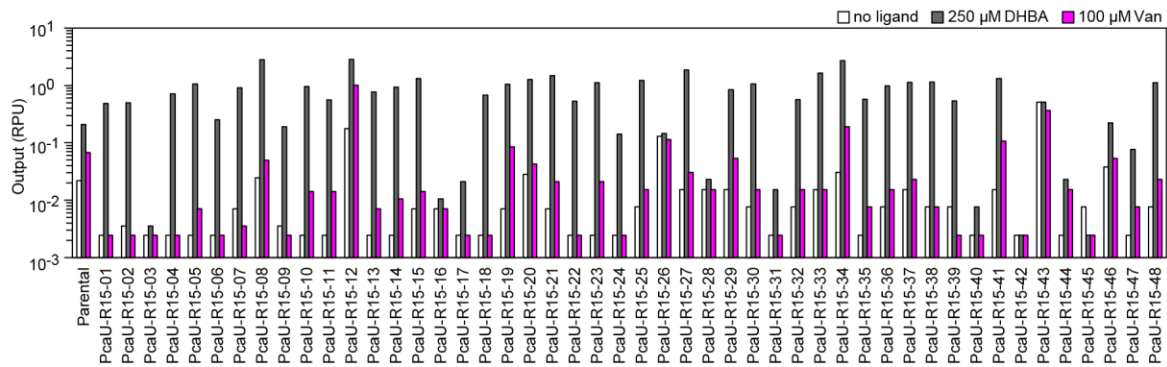
Initial library (1153 bp)



Directed evolution interventions

PcaU	[Cl-Phe]	[Negative ligand]	DNAP	[Positive ligand]	Notes
R1	2 mM		PK6	500 μ M DHBA	
R2	2 mM		PK6	500 μ M DHBA	
R3	4 mM		PK6	500 μ M DHBA	
R4	4 mM	10 μ M Van	PK6	10 μ M Van + 250 μ M DHBA	
R5	4 mM	20 μ M Van	PK6	20 μ M Van + 250 μ M DHBA	
R6	4 mM	20 μ M Van	PK6	20 μ M Van + 250 μ M DHBA	
R7	4 mM	30 μ M Van	PK6	30 μ M Van + 250 μ M DHBA	
R8	4 mM	40 μ M Van	PK6	40 μ M Van + 250 μ M DHBA	
R9	4 mM	50 μ M Van	PK6	50 μ M Van + 250 μ M DHBA	
R10	4 mM	75 μ M Van	PK6	250 μ M DHBA	
R11	4 mM	100 μ M Van	KOD	250 μ M DHBA	
R12	4 mM	100 μ M Van	KOD	100 μ M Van + 250 μ M DHBA	
R13	4 mM	100 μ M Van	KOD	250 μ M DHBA	Shuffle
R14	4 mM	100 μ M Van	KOD	200 μ M DHBA	
R15	4 mM	100 μ M Van	KOD	100 μ M DHBA	

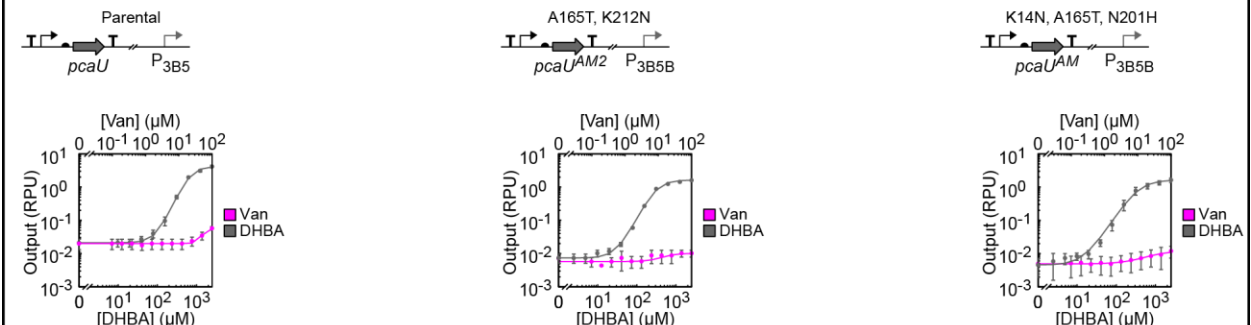
On/off screen



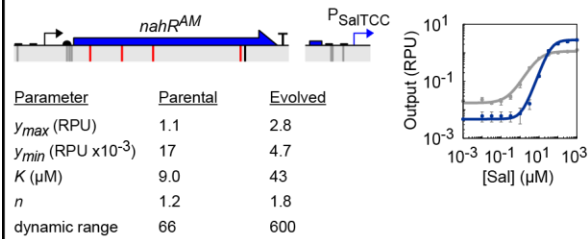
Sequencing

Mutant	Mutations
PcaU-R11-04	K14N, A165T, N201H
PcaU-R15-01	G102S, A165T, K212N, K256E
PcaU-R15-02	G98A, G102S, P107S, P162S, V241I
PcaU-R15-04	A165T, K212N
PcaU-R15-05	G102S, A165T, S209N
PcaU-R15-07	A165T, K212N
PcaU-R15-13	A165T, Q181K, K212N, Q237R
PcaU-R15-18	A165T, K212N
PcaU-R15-22	A165T, N201H, R206C, Q261H
PcaU-R15-25	A165T, Q192R, I260V
PcaU-R15-27	A165T, K212M
PcaU-R15-30	A165T, L193V, K212E
PcaU-R15-33	A165T, V241I, N276I
PcaU-R15-36	A165T
PcaU-R15-37	T20N, A165T, S122R, R206C, Q237R, V241I
PcaU-R15-38	K9R, A165T, A174T, Q261R, H262Y
PcaU-R15-48	M1I, A165T, Q192R

Consensus mutant testing



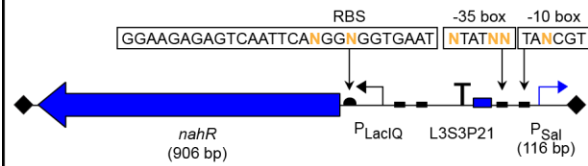
NahR:P_{Sal}:Sal



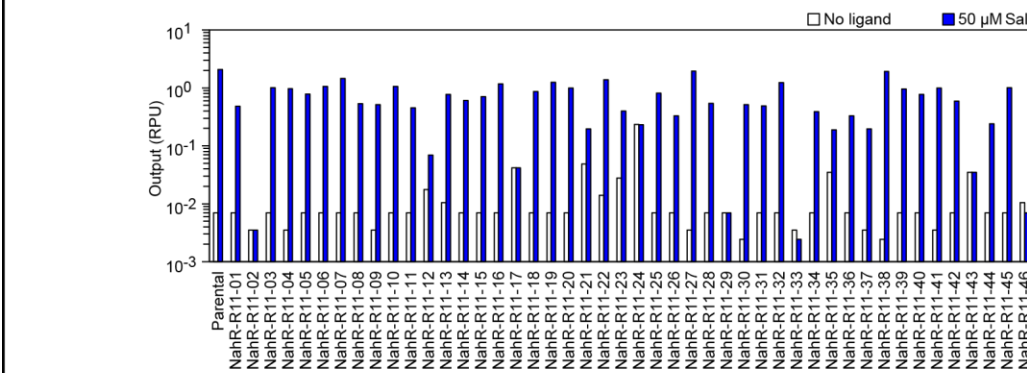
Directed evolution interventions

NahR	[Cl-Phe]	[Negative ligand]	DNAP	[Positive ligand]	Notes
R1	2 mM		PK6	50 μ M Sal	
R2	2 mM		PK6	50 μ M Sal	
R3	2 mM		PK6	50 μ M Sal	
R4	2 mM		PK6	50 μ M Sal	
R5	4 mM		PK6	50 μ M Sal	
R6	4 mM		PK6	50 μ M Sal	
R7	4 mM		KOD	50 μ M Sal	
R8	4 mM		KOD	50 μ M Sal	
R9	4 mM		KOD	30 μ M Sal	Shuffle
R10	4 mM		KOD	20 μ M Sal	
R11	4 mM		KOD	10 μ M Sal	

Initial library (1240 bp)



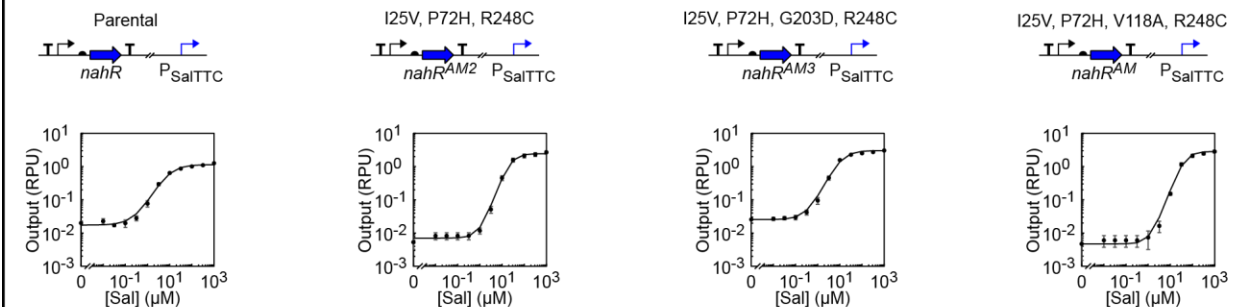
On/off screen



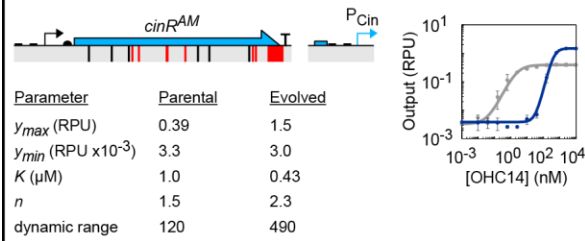
Sequencing

Mutant	Mutations
NahR-R11-04	P72H, N144D, A230T, R248C, S260C
NahR-R11-07	P72H, R248C
NahR-R11-16	V118A, Q168K, R248L
NahR-R11-19	P72H, R248C, D251N
NahR-R11-27	L6M, I25V, P72H, V118A, L154H, R183C, R248C
NahR-R11-38	L6M, I25V, P72H, V118A, L154H, I200V, R248C
NahR-R11-41	I25V, Q49R, V118A, V197A, R248C

Consensus mutant testing



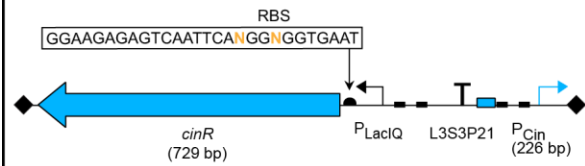
CinR:P_{Cin}:OHC14



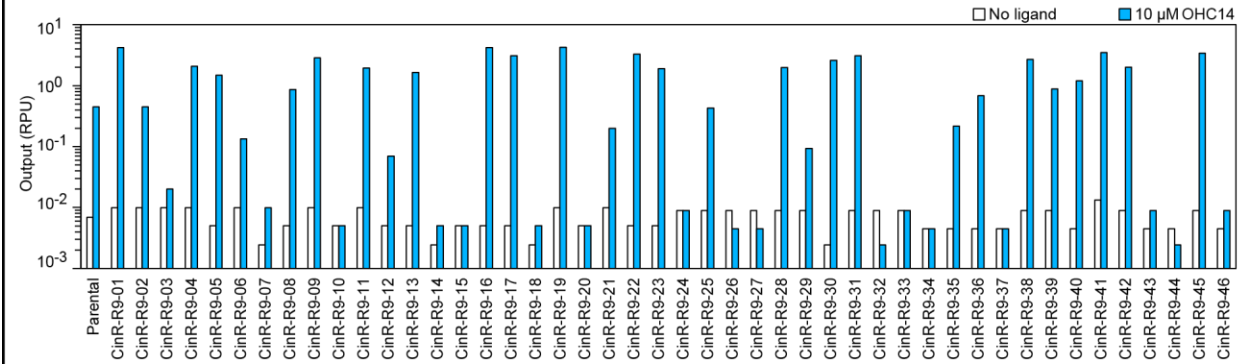
Directed evolution interventions

CinR	[Cl-Phe] [Negative ligand]	DNAP	[Positive ligand]	Notes
R1	2 mM	PK6	5 μ M OHC14	
R2	2 mM	PK6	5 μ M OHC14	
R3	2 mM	PK6	5 μ M OHC14	
R4	2 mM	PK6	1.25 μ M OHC14	
R5	2 mM	PK6	1.25 μ M OHC14	
R6	4 mM	PK6	1.25 μ M OHC14	
R7	4 mM	10 μ M OC12	PK6	1.25 μ M OHC14
R8	4 mM	10 μ M OC12	PK6	1.25 μ M OHC14
R9	4 mM	10 μ M OC12	KOD	1.25 μ M OHC14

Initial library (1172 bp)



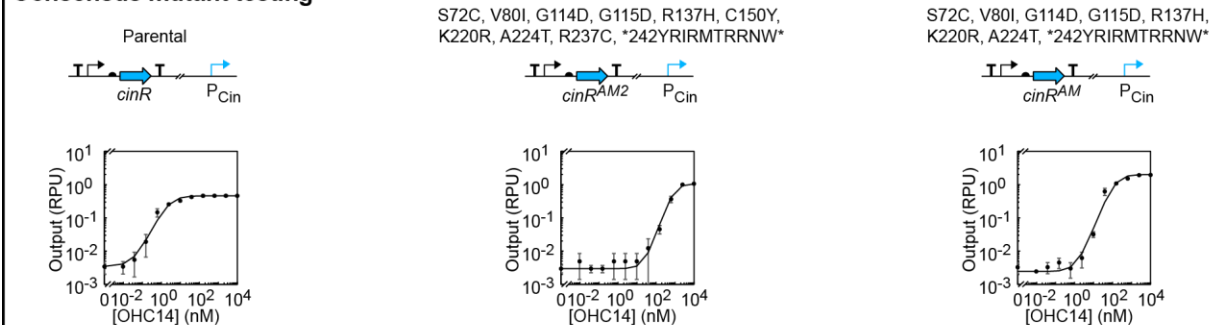
On/off screen



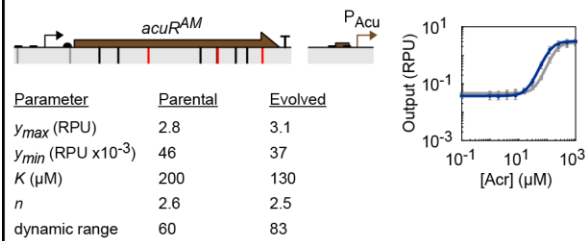
Sequencing

Mutant	Mutations
CinR-R9-01	R86H, R151H, P177S, K220R
CinR-R9-05	A103V, A136V, R149C, H170Y, L187M, K220R, *242YRIRMTRRNW*
CinR-R9-08	A19V, R137P, P139S, E142K, A178V, G204D, K220R, *242YRIRMTRRNW*
CinR-R9-11	L70I, Q87L, G115D, T144I, L146F, R149C, Y196N, K220R, *242YRIRMTRRNW*
CinR-R9-13	R28H, K50M, Q87P, V106L, H111Y, P121T, Q127R, D141N, K220R, *242YRIRMTRRNW*
CinR-R9-16	N71S, T98A, A136V, K197N, A230V, *242YRIRMTRRNW*
CinR-R9-17	S72C, V80I, G114D, G115D, R137H, K220R, A224T, *242YRIRMTRRNW*
CinR-R9-28	M1I, A63T, R67C, P97L, I138M, E142K, R149H, G204D, N240D, *242YKDPHDAT*
CinR-R9-30	S72C, V80I, G114D, G115D, R137H, K220R, A224T, *242YRIRMTRRNW*
CinR-R9-31	Y73H, I138M, V166I, *242YRIRMTRRNW*
CinR-R9-36	A32T, Y73H, A103T, G115S, L146F, K220R, *242YRIRMTRRNW*
CinR-R9-39	T98M, E100G, P175L, K220R, *242YRIRMTRRNW*
CinR-R9-40	T5I, G204D, K220R, *242YRIRMTRRNW*
CinR-R9-42	Q16R, A25V, A48T, A63D, K220R, Q235R, R237H
CinR-R9-45	K18M, A19T, K81R, D141E, N152D, K220R, A230T, *242YRIRMTRRNW*

Consensus mutant testing



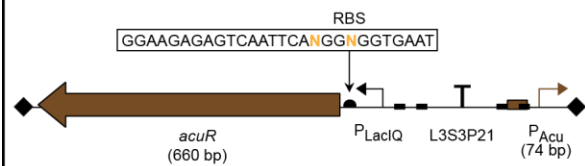
AcuR:P_{Acu}:Acr



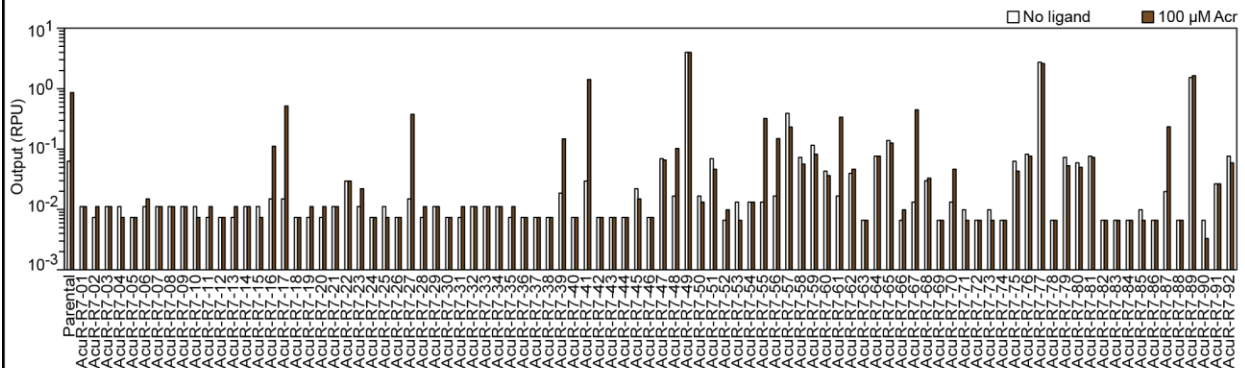
Directed evolution interventions

AcuR	[Cl-Phe]	[Negative ligand]	DNAP	[Positive ligand]	Notes
R1	2 mM		PK6	100 μ M Acr	
R2	2 mM		PK6	100 μ M Acr	
R3	4 mM		PK6	100 μ M Acr	
R4	4 mM		PK6	100 μ M Acr	
R5	4 mM		PK6	100 μ M Acr	
R6	4 mM		PK6	100 μ M Acr	
R7	4 mM		KOD	100 μ M Acr	

Initial library (957 bp)



On/off screen



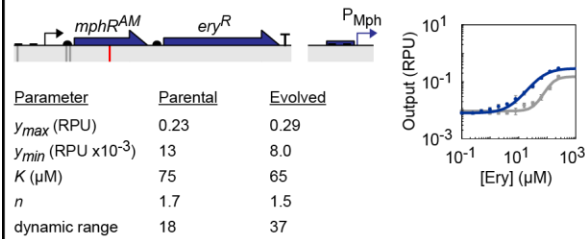
Sequencing

Mutant	Mutations
AcuR-R7-16	G147C, R161H, Q218L
AcuR-R7-17	V10D, P138Q, E150K, A171V
AcuR-R7-21	T6I, A117V
AcuR-R7-39	A28V, D73N, R211H
AcuR-R7-41	P138S
AcuR-R7-48	Q30R, A87V
AcuR-R7-55	P138Q, Q218R, E219N, *220NDKDPHDAT*
AcuR-R7-56	R34H, G49S, G167S
AcuR-R7-61	T4I, D24E, A117V
AcuR-R7-67	E80K, R154H, L202M
AcuR-R7-87	D24N, R122S

Consensus mutant testing



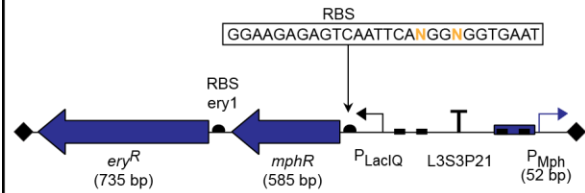
MphR:P_{Mph}:Ery



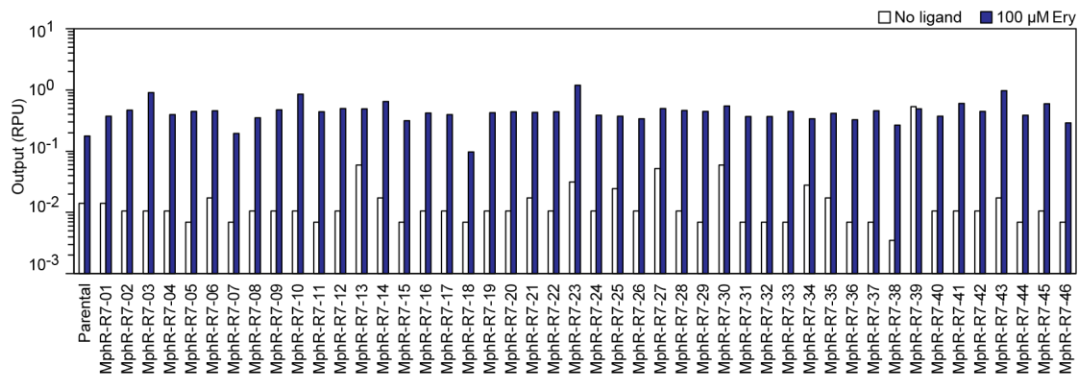
Directed evolution interventions

MphR	[Cl-Phe]	[Negative ligand]	DNAP	[Positive ligand]	Notes
R1	2 mM		PK6	100 μ M Ery	
R2	2 mM		PK6	100 μ M Ery	
R3	4 mM		PK6	100 μ M Ery	
R4	4 mM		PK6	100 μ M Ery	
R5	4 mM		PK6	100 μ M Ery	
R6	4 mM		PK6	100 μ M Ery	
R7	4 mM		KOD	100 μ M Ery	

Initial library (1613 bp)



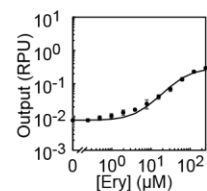
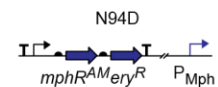
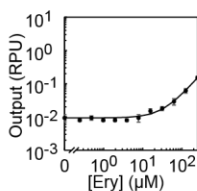
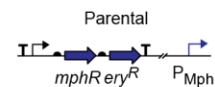
On/off screen



Sequencing

Mutant	Mutations
MphR-R7-03	N94D
MphR-R7-05	E11K, N94D, N97D
MphR-R7-10	L30M, N94D, N97D, P182Q, D186N
MphR-R7-11	N94D, R96S, Q188R
MphR-R7-29	P4L, G76S, N94D
MphR-R7-31	N94D, A174V
MphR-R7-32	T53I, N94D, H193R
MphR-R7-33	N94D, A142V
MphR-R7-37	K7I, N94D, D160N
MphR-R7-38	E11D, N94D, Q188R
MphR-R7-41	N94D, A192V
MphR-R7-43	N50D, V88I, A171T, E183G, Q191R, H193N
MphR-R7-44	N50D, P136L
MphR-R7-45	N94D

Consensus mutant testing



Supplementary Note 3: Plasmids parts list

Reporter only plasmids ^a									
Plasmid	Addgene accession	Genbank accession	Output promoter	Reporter				Origin	Resistance
pAJM.711	108512	MH101715	P _{PhIF}	<i>YFP</i>				p15A	Kan
pAJM.712	108513	MH101716	P _{CymRC}	<i>YFP</i>				p15A	Kan
pAJM.713	108514	MH101717	P _{LuxB}	<i>YFP</i>				p15A	Kan
pAJM.714	108515	MH101718	P _{VanCC}	<i>YFP</i>				p15A	Kan
pAJM.715	108516	MH101719	P _{Tac}	<i>YFP</i>				p15A	Kan
pAJM.717	108517	MH101720	P _{Tet*}	<i>YFP</i>				p15A	Kan
pAJM.716	108518	MH101721	P _{BAD}	<i>YFP</i>				p15A	Kan
pAJM.718	108519	MH101722	P _{BetI}	<i>YFP</i>				p15A	Kan
pAJM.719	108520	MH101723	P _{Ttg}	<i>YFP</i>				p15A	Kan
pAJM.1459	108521	MH101724	P _{3B5C}	<i>YFP</i>				p15A	Kan
pAJM.721	108522	MH101725	P _{SalTTC}	<i>YFP</i>				p15A	Kan
pAJM.944	108523	MH101726	P _{Cin}	<i>YFP</i>				p15A	Kan
Reporter and regulator plasmids ^a									
Plasmid	Addgene accession	Genbank accession	Output promoter	Reporter	Promoter	RBS	Regulator	Origin	Resistance
pAJM.847	108524	MH101727	P _{PhIF}	<i>YFP</i>	P _{LacIQ}	phl2	<i>phlF^{AM}</i>	p15A	Kan
pAJM.657	108525	MH101728	P _{CymRC}	<i>YFP</i>	P _{LacIQ}	cym1	<i>cymR^{AM}</i>	p15A	Kan
pAJM.474	108526	MH101729	P _{LuxB}	<i>YFP</i>	P _{Lac}	lux1	<i>luxR</i>	p15A	Kan
pAJM.773	108527	MH101730	P _{VanCC}	<i>YFP</i>	P _{LacIQ}	van2	<i>vanR^{AM}</i>	p15A	Kan
pAJM.336	108528	MH101731	P _{Tac}	<i>YFP</i>	P _{LacI}	lac1	<i>lacI^{AM}</i>	p15A	Kan
pAJM.011	108529	MH101732	P _{Tet*}	<i>YFP</i>	P _{LacI}	tet1	<i>tetR</i>	p15A	Kan
pAJM.677	108530	MH101733	P _{BAD}	<i>YFP</i>	P _{LacIQ}	ara1/e1	<i>araC^{AM} / araE</i>	p15A	Kan
pAJM.683	108531	MH101734	P _{BetI}	<i>YFP</i>	P _{LacIQ}	bet2	<i>betI^{AM}</i>	p15A	Kan
pAJM.661	108532	MH101735	P _{Ttg}	<i>YFP</i>	P _{LacIQ}	ttg2	<i>ttgR^{AM}</i>	p15A	Kan
pAJM.690	108533	MH101736	P _{3B5B}	<i>YFP</i>	P _{LacIQ}	pca2	<i>pcaU^{AM}</i>	p15A	Kan
pAJM.771	108534	MH101737	P _{SalTTC}	<i>YFP</i>	P _{LacIQ}	nah2	<i>nahR^{AM}</i>	p15A	Kan
pAJM.1642	108535	MH101738	P _{Cin}	<i>YFP</i>	P _{LacI}	cin1	<i>cinR^{AM}</i>	p15A	Kan
pAJM.884	108536	MH101739	P _{Acu}	<i>YFP</i>	P _{LacIQ}	acu2	<i>rhIR^{AM}</i>	p15A	Kan
pAJM.969	108537	MH101740	P _{Mph}	<i>YFP</i>	P _{LacIQ}	mph2	<i>mphR^{AM}</i>	p15A	Kan/Ery

a) The DNA sequence of all plasmids are provided in Supplementary Note 4.

[illegible]

CAAAATTATTGAAGGCTCCCTAACGGGGGGCCTTTTTTGTGTTCTGGTCTCCGCTTAACAGTACGTTGGCTGAGAAACCAATTGTCATATTGTCATCAGACATTGCCGTCAC

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