

Enhancing the Activity of a Self-Inducible Promoter in *Escherichia coli* through Saturation Mutation and High-Throughput Screening

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Supplementary Materials

Table S1. All the primers in this experiment.

Primers	Sequence
Verc-F	AGCGGGTTTACGGATTTCTGAATGGAAGCTTGGATTCTCAC
N20-R	CTAGCTCTAAAACCGCGGTAGAGAAGTTTGACCGCTAAGATCTGACTCCATAACAGAG
N20-F	TCTTAGCGGTCAAACCTTCTCTACCGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
Verc-R	GAAAGGCCAGTCTCAAGCGACAGGCCCATGGATTCTTCG
UP-F	AGAATCCATGGGCCTGTCGCTTGAGACTGGCCTTTCTG
UP-R	GATGATCGAGAGTAACTTGACGCGCATTACCTCGGTAGACAC
DOWN-F	TCTACCGAGGTAATGCGCTGCAAGTTACTCTCGATCATCCGG
DOWN-R	AGAATCCAAGCTTCCATTCAGAAATCCGTAAACCCGCTGC
YrpoS-F	CATCGGACCTTTTATTGTGCACAGA
YrpoS-R	AATCCGTAAACCCGCTGCGTTAT
P-L	CACGCCGAAACAAGCCCGCACTTCTGCTCTCC
Model I	AATTATTTCTAGAGGATTTGCCACAAGTATAAGCGGCAAATCCGGGTTNNNNNNGGAGAGCAGAAAGTGCGG
Model II	AATTATTTCTAGAGGATTTGCCACAAGTATANNNNNNNNNNNNNNNNNNNACGCCGGGAGAGCAGAAAGTGCGG
Model III	AATTATTTCTAGAGNNNNNNNNNNNAGTATAAGCGGCAAATCCGGGTTACGCCGGGAGAGCAGAAAGTGCGG
V-F	CCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA
V-R	GCTTGTTTCGGCGTGGGTAT
YpET28a-Pfic	CATTAGGAAGCAGCCCAGTAGTAGG
EcoRI-rfp-F	AAAGGATCCGAATTCCATATGGCGAGTAGCGAAGACG
SalI-rfp-R	AAAAAGCTTGTCGACTTAAGCACCGGTGGAGTGACG

Table S2. All the promoters screened in this experiment.

Num	Sequence	σ^S strength	σ^{70} strength	σ^S selectivity
fic	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTTGCCGCTTATACTTGTGGCAAAT	2981±314	7952±98	2.67
I-2	CCGCACTTCTGCTCTCCCGGGACAACCCGGATTTGCCGCTTATACTTGTGGCAAAT	3552±214	174±40	0.05

I-15	CCGCACTTCTGCTCTCC	TAAGTA	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	2497±466	2095±472	0.84
I-11	CCGCACTTCTGCTCTCC	GGGGGT	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	2669±92	2151±803	0.81
I-3	CCGCACTTCTGCTCTCC	ACGAGT	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	2476±275	2744±720	1.11
I-10	CCGCACTTCTGCTCTCC	ATAGCC	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	4655±553	2970±890	0.64
I-1	CCGCACTTCTGCTCTCC	AAACGT	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	1806±252	3072±282	1.70
I-5	CCGCACTTCTGCTCTCC	GGCCGG	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	2296±233	3208±243	1.40
I-14	CCGCACTTCTGCTCTCC	TGAGCT	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	2339±242	3577±341	1.53
I-12	CCGCACTTCTGCTCTCC	AAAGCA	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	2939±153	3681±570	1.25
I-13	CCGCACTTCTGCTCTCC	CAAACG	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	1719±193	4112±791	2.39
I-9	CCGCACTTCTGCTCTCC	CCCCAC	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	4299±196	9718±637	2.26
I-6	CCGCACTTCTGCTCTCC	ACACAT	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	3045±204	12953±403	4.25
I-4	CCGCACTTCTGCTCTCC	AAGCCA	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	4174±294	16691±534	4.00
I-8	CCGCACTTCTGCTCTCC	GGGATA	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	3675±170	19755±187	5.38
I-7	CCGCACTTCTGCTCTCC	AAGAAA	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	5227±373	21272±494	4.07
I-20	CCGCACTTCTGCTCTCC	CAGCCA	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	3501±89	36845±2442	10.52
I-19	CCGCACTTCTGCTCTCC	AGGCAG	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	3907±503	63212±1946	16.18
I-17	CCGCACTTCTGCTCTCC	CTGCAA	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	4829±77	69070±4025	14.30
I-16	CCGCACTTCTGCTCTCC	CTGGCA	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	9207±380	86809±1796	9.43
I-18	CCGCACTTCTGCTCTCC	GAACAA	AACCCGGATTG	CCGCTTATACTTGTGGCAAAT	9211±371	98286±2962	10.67
II-5	CCGCACTTCTGCTCTCCCGGCGT	TAAAAGTGTTAATAAG		TATACTTGTGGCAAAT	3467±290	247±150	0.07
II-16	CCGCACTTCTGCTCTCCCGGCGT	ACTCACTGTTTCGCGGG		TATACTTGTGGCAAAT	3613±477	545±348	0.15
II-1	CCGCACTTCTGCTCTCCCGGCGT	GACGCGAAGCCGCTGTG		TATACTTGTGGCAAAT	3576±353	824±171	0.23
II-9	CCGCACTTCTGCTCTCCCGGCGT	TGAGGAGATCGCAGAG		TATACTTGTGGCAAAT	3019±33	1499±147	0.50
II-15	CCGCACTTCTGCTCTCCCGGCGT	CGGAGAAACGGAGGATA		TATACTTGTGGCAAAT	2477±120	1570±531	0.63
II-8	CCGCACTTCTGCTCTCCCGGCGT	TTCCATACCGATAGCTA		TATACTTGTGGCAAAT	2601±99	2116±504	0.81
II-6	CCGCACTTCTGCTCTCCCGGCGT	CACTAATTCAAGCACGT		TATACTTGTGGCAAAT	3426±18	2544±615	0.74
II-7	CCGCACTTCTGCTCTCCCGGCGT	AACAGATTGAGAGTCGC		TATACTTGTGGCAAAT	3481±401	2835±56	0.81
II-2	CCGCACTTCTGCTCTCCCGGCGT	TTAAACTATCTGTGCTT		TATACTTGTGGCAAAT	3969±91	5901±623	1.49
II-3	CCGCACTTCTGCTCTCCCGGCGT	CGGATACAAGTAGGATG		TATACTTGTGGCAAAT	5700±263	7069±2913	1.24
II-12	CCGCACTTCTGCTCTCCCGGCGT	TAAAGAAGAAGCTAACA		TATACTTGTGGCAAAT	6589±105	7128±251	1.08
II-14	CCGCACTTCTGCTCTCCCGGCGT	CGTCAGAACCTTTAGTT		TATACTTGTGGCAAAT	6680±719	8181±136	1.22

II-22	CCGCACTTCTGCTCTCCCGGCGT	TCTCGACCAGTAAC	TGCTATACTTGTGGCAAAT	2209±234	10115±287	4.58
II-4	CCGCACTTCTGCTCTCCCGGCGT	AAAAGAAACGCGGT	TGCTATACTTGTGGCAAAT	2355±533	11641±2116	4.94
II-13	CCGCACTTCTGCTCTCCCGGCGT	ATAATACTATTGACAAC	TATACTTGTGGCAAAT	10575±661	12034±2187	1.14
II-11	CCGCACTTCTGCTCTCCCGGCGT	AAAAGACGCGATAAC	CGCTATACTTGTGGCAAAT	4278±229	22330±800	5.22
II-18	CCGCACTTCTGCTCTCCCGGCGT	GCTTCTTAGAATCGG	CTATACTTGTGGCAAAT	7047±926	24917±924	3.54
II-17	CCGCACTTCTGCTCTCCCGGCGT	ATTATATGTACGTGAT	CTATACTTGTGGCAAAT	7616±290	39738±2376	5.22
II-19	CCGCACTTCTGCTCTCCCGGCGT	TCATCGGAATATATAG	CTATACTTGTGGCAAAT	4830±642	42147±4469	8.73
II-24	CCGCACTTCTGCTCTCCCGGCGT	TAAACAAGAAGAGAGG	CTATACTTGTGGCAAAT	4440±318	45767±2939	10.31
II-10	CCGCACTTCTGCTCTCCCGGCGT	GTCGAGCTAAACATGG	CTATACTTGTGGCAAAT	3708±317	50073±2943	13.50
II-35	CCGCACTTCTGCTCTCCCGGCGT	GACTTGGGGGCTTAAG	CTATACTTGTGGCAAAT	3916±141	52489±3599	13.40
II-23	CCGCACTTCTGCTCTCCCGGCGT	CACACGAAGAAAGAGG	CTATACTTGTGGCAAAT	8591±754	56431±4027	6.57
II-25	CCGCACTTCTGCTCTCCCGGCGT	GTGGGGGAACCCGGT	GTCTATACTTGTGGCAAAT	6042±620	74336±5014	12.30
II-30	CCGCACTTCTGCTCTCCCGGCGT	CCTAAGGAATAACCAT	CTATACTTGTGGCAAAT	2930±247	75530±1397	25.77
II-26	CCGCACTTCTGCTCTCCCGGCGT	GATCAAGCTAAAGGAAC	CTATACTTGTGGCAAAT	2535±300	77585±3829	30.60
II-20	CCGCACTTCTGCTCTCCCGGCGT	CGCTCAACATTATCAT	CTATACTTGTGGCAAAT	6153±978	79257±5797	12.88
II-31	CCGCACTTCTGCTCTCCCGGCGT	GACATCACATAACGGAC	CTATACTTGTGGCAAAT	3393±586	93840±5936	27.66
II-21	CCGCACTTCTGCTCTCCCGGCGT	CTCCGCGATAAATCAAC	CTATACTTGTGGCAAAT	7889±863	90895±4223	11.52
II-29	CCGCACTTCTGCTCTCCCGGCGT	GGTGAGTGTGTGAATG	CTATACTTGTGGCAAAT	7777±803	95237±5665	12.25
II-27	CCGCACTTCTGCTCTCCCGGCGT	GCGGCTCCCGGCTAGAC	CTATACTTGTGGCAAAT	5139±544	96357±3205	18.75
II-36	CCGCACTTCTGCTCTCCCGGCGT	TTACGACGAGATGAGT	CTATACTTGTGGCAAAT	4342±160	103775±4274	23.90
II-32	CCGCACTTCTGCTCTCCCGGCGT	TTCGCGAGCCTATGGAC	CTATACTTGTGGCAAAT	4023±157	109645±2351	27.25
II-34	CCGCACTTCTGCTCTCCCGGCGT	GCTCACGCCAGTTCGT	CTATACTTGTGGCAAAT	5499±114	116282±1097	21.15
II-28	CCGCACTTCTGCTCTCCCGGCGT	ACGAACGAAATAGATAC	CTATACTTGTGGCAAAT	8171±250	137194±1322	16.79
II-33	CCGCACTTCTGCTCTCCCGGCGT	CCC CGGAACCGCGCGT	CTATACTTGTGGCAAAT	4327±627	160515±3171	37.10
III-2	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATT	TGCCGCTTATACT	CAGGCAGCAT	2202±227	8075±211	3.67
III-9	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATT	TGCCGCTTATACT	CACCGTACAG	2060±38	10878±306	5.28
III-1	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATT	TGCCGCTTATACT	GAAACGAAAA	2443±77	17199±864	7.04
III-10	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATT	TGCCGCTTATACT	TAACAAATTI	2950±155	21191±1386	7.18
III-4	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATT	TGCCGCTTATACT	AAGTAAACAG	15071±539	21968±4461	1.46
III-8	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATT	TGCCGCTTATACT	TGAGTAAGCG	1984±256	22609±3765	11.40
III-5	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATT	TGCCGCTTATACT	CAGATAGCAC	1719±35	24270±975	14.12
III-3	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATT	TGCCGCTTATACT	CTTTCCTGG	2299±27	27959±4051	12.16

III-6	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTGAAAGAACAG	1927±121	33543±2135	17.40
III-7	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTAAAAAAGGTA	2795±261	42266±4854	15.12
III-16	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTGTTAATTCCC	3207±172	44320±1977	13.82
III-17	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTTAATGGGAAC	3568±374	48473±3739	13.58
III-20	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTAGTATAGCTA	6145±507	51348±2314	8.36
III-13	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTATATAAGAAA	3865±240	57010±4153	14.75
III-15	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTATAAGAGATT	3799±153	71351±1735	18.78
III-18	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTATAGACCGTA	4093±345	78306±3422	19.13
III-12	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTTGAAATAGGA	3129±238	115957±4813	37.06
III-19	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTAAAAAGTTCC	4478±580	116684±3254	26.06
III-11	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTTGCAATGCCA	4606±270	133063±3430	28.89
III-14	CCGCACTTCTGCTCTCCCGGCGTAACCCGGATTGCGCGTTATACTATAATAGATC	3356±249	166863±2143	49.73

Note: yellow highlighted part is the mutation area.

Table S3. The promoter's improvement under stress conditions in other experiments.

Promoter	Stress conditions	Improvement (than original media)	Reference
PgorES	10g/L acetic acid	1-2×	[58]
pssA	high osmotic pressure (0.8 M NaCl)	3.5×	[59]
pspA	high osmotic pressure (0.8 M NaCl)	2.22×	[59]
rodA	high osmotic pressure (0.8 M NaCl)	1.73×	[59]
P21285	high osmotic pressure (0.5 M NaCl)	1.17×	[60]
P191	high osmotic pressure (0.5 M NaCl)	1.11×	[60]

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