A Novel Method to Screen Strong Constitutive Promoters in *Escherichia coli* and *Serratia marcescens* for Industrial Applications

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Table S1. Strains and plasmids used in this study

Strains/Plasmids	Characteristics	Source
Strains		
Escherichia coli JM109	Host for cloning	Lab stock
E. coli MG1655	Wild-type strain	Lab stock
Corynebacterium glutamicum ATCC13032	Wild-type strain	Lab stock
Bacillus subtilis 168	Wild-type strain	Lab stock
Serratia marcescens JNB5-1	Wild-type strain	Lab stock
Val01	Strain obtained by ARTP mutagenesis derived from *E. coli* W3110	This study
Val02	Val01 derivative with expression of *ilvCDE*, controlled by P_{BS76}-50 promoter	This study
Val03	Val01 derivative with expression of *ilvCDE*, controlled by P_{BS76}-75 promoter	This study
Val04	Val01 derivative with expression of *ilvCDE*, controlled by P_{BS76}-85 promoter	This study
Val05	Val01 derivative with expression of *ilvCDE*, controlled by P_{BS76}-100 promoter	This study

SM01	JNB5-1 derivative with expression of *pigFN*, controlled by P_{pig} promoter	This study
SM02	JNB5-1 derivative with expression of *pigFN*, controlled by P_{RpIJ} promoter	This study
SM03	JNB5-1 derivative with expression of *pigFN*, controlled by P_{SM} promoter	This study
Plasmids		
pUC19	Lac promoter, pBR322 origin, Amp^R	Lab stock
pUC19-P_{BBa_23118}-*gfp*	pUC19 derivative with *gfp* driven by constitutive promoter P_{BBa_J23118}	This study
pUC19-P_{EC}-*gfp*	pUC19 derivative with *gfp* driven by promoter P_{EC} screened from *E. coli* MG1655	This study
pUC19-P_{BS}-*gfp*	pUC19 derivative with *gfp* driven by promoter P_{BS} screened from *B. subtilis* 168	This study
pUC19-P_{CG}-*gfp*	pUC19 derivative with *gfp* driven by promoter P_{CG} screened from *C. glutamicum* ATCC13032	This study
pUC19-P_{BS76}-*gfp*	pUC19 derivative with *gfp* driven by promoter P_{BS76} truncated from promoter P_{BS}	This study
pUC19-$P_{BS76\text{-}variant}$-*gfp*	pUC19 derivative with *gfp* driven by promoter $P_{BS76\text{-}variant}$ optimized based on promoter P_{BS76}	This study
pUC19-P1-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P1 derived from promoter P_{BS}	This study
pUC19-P2-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P2 derived from promoter P_{BS}	This study

pUC19-P3-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P3 derived from promoter P_{BS}	This study
pUC19-P4-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P4 derivedfrom promoter P_{BS}	This study
pUC19-P5-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P5 derived from promoter P_{BS}	This study
pUC19-P6-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P6 derived from promoter P_{BS}	This study
pUC19-P7-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P7 derived from promoter P_{BS}	This study
pUC19-P8-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P8 derived from promoter P_{BS}	This study
pUC19-P9-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P9 derived from promoter P_{BS}	This study
pUC19-P10-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P10 derived from promoter P_{BS}	This study
pUC19-P11-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P11 derived from promoter P_{BS}	This study
pUC19-P12-*gfp*	pUC19 derivative with *gfp* driven by truncated promoter P12 derived from promoter P_{BS}	This study
pTrc99a	Expression vector, trc promoter, Amp^R	Lab stock
pTrc99a-P_{BS}-50-*ilvCDE*	Expressing *ilvCDE* via pTrc99a under the control of promoter P_{BS}-50	This study
pTrc99a-P_{BS}-75-*ilvCDE*	Expressing *ilvCDE* via pTrc99a under the control of promoter P_{BS}-75	This study

pTrc99a-P_{BS}-85-*ilvCDE*	Expressing *ilvCDE* via pTrc99a under the control of promoter P_{BS}-85	This study
pTrc99a-P_{BS}-100-*ilvCDE*	Expressing *ilvCDE* via pTrc99a under the control of promoter P_{BS}-100	This study
pUCP18	Broad-host-range shuttle vector, Amp^R	Lab stock
pUCP18-P_{SM}-*gfp*	pUCP18 derivative with *gfp* driven by promoter P_{SM} screened from *S. marcescens* JNB5-1	This study
pUCP18-P_{pig}-*gfp*	pUCP18 derivative with *gfp* driven by the native promoter P_{pig}	This study
pUCP18-P_{pig}-*pigFN*	pUCP18 derivative with *pigFN* driven by native promoter P_{pig}	This study
pUCP18-P_{RpIJ}-*pigFN*	pUCP18 derivative with *pigFN* driven by constitutive promoter P_{rpIJ}	This study
pUCP18-P_{SM}-*pigFN*	pUCP18 derivative with *pigFN* driven by promoter P_{SM} screened from *S. marcescens* JNB5-1	This study

Table S2. Primers used in this study

Primers	Sequences (5'-3')	Function
p19-GFP-F1	AGTACCATGATTACGCCAAGCTTG	Primers used to construction of the plasmid pUC19-P_{BBa_23118}-*gfp*
p19-GFP-R1	TCACAATTCCACACATTATACGAGCCGGAT GATTAATTGTCAAGCCTGGGGTGCCTAATG AGT	
p19-GFP-F2	TAATGTGTGGAATTGTGAGCGGATAACAAT TTCACACAGGAAACAGCTATGAGTAAAGGA	
p19-GFP-R2	GAAGAACTTTTCACTGGAGT TGGCGTAATCATGGTCATCTATTTGTATAGT TCATCCATGCCATGTGTAATCC	
promoter-BS-F1(116)	TAAAGAGGAGAAAGGTACCCGCATAATAAA GGAAAAAGCAGGCGCATG	Primers used to identify the core region of the promoter P_{BS}

promoter-BS-F1(96)	TAAAGAGGAGAAAGGTACCCGGCGCATGGATATAAGGCGC
promoter-BS-F1(76)	TAAAGAGGAGAAAGGTACCCCTGCTTTTTTATTGTTGAAAGCGCTTTATTTTTCCC
promoter-BS-F1(56)	TAAAGAGGAGAAAGGTACCCGCGCTTTATTTTTCCCCTACAATAGATGAAAACG
promoter-BS-F1(36)	TAAAGAGGAGAAAGGTACCCAATAGATGAAAACGGCGTGTAAGGGAG
promoter-BS-F1(16)	TAAAGAGGAGAAAGGTACCCAAGGGAGGAGCGATCCATGAGTAAA
promoter-BSTY-R1	CTATTTGTATAGTTCATCCATGCCATGTGTAATCC

promoter-BSTY-F2	GGATGAACTATACAAATAGCCGGGTACCGAGCTCGAATTCA
promoter-BSTY-R2	GGGTACCTTTCTCCTCTTTAATGAATTCGC
promoter-BS76TY-F1	ATGAGTAAAGGAGAAGAACTTTTCACTGGAGT
promoter-BS76TY-R1	CTATTTGTATAGTTCATCCATGCCATGTGTAATCC
promoter-BS76TY-F2	GGATGAACTATACAAATAGCCGGGTACCGAGCTCGAATTCA
promoter-BS76-R2(56)	AGTTCTTCTCCTTTACTCATGCCGTTTTCATCTATTGTAGGGGAAAAATAAAG
promoter-BS76-R2(36)	AGTTCTTCTCCTTTACTCATGGGAAAAATAAAGCGCTTTCAACAATAAAAAAGC

promoter-BS76-R2(16)	AGTTCTTCTCCTTTACTCATAACAATAAAAA AGCAGGGGTACCTTTCTCC	
p19-BS(76)10and1NN-F1	CTTTATTTTTCCCCTACAATNNNNNNNNNACG GCGTGTAAGGGAGGAG	Primers used to construction of a gradient promoter library *via* modifying promoter P_{BS76}
p19-BS(76)10NN-F1	AAAGCGCTTTATTTTTCCCCNNNNNNAGAT GAAAACGGCGTGTAAGGGAG	
p19-BS(76)35and10NN-F1	CTGCTTTTTTATTGTTGAAANNNNNNNNNN NNNNNNNTACAATAGATGAAAACGGCGTGT AAGGGAG	
p19-BS(76)35NN-F1	CCAGGACTGCTTTTTTATTGNNNNNNGCGCT TTATTTTTCCCCTACAATAGATGAAAACG	
p19-BS(76)10and1NN-R2	ATTGTAGGGGAAAAATAAAGCGCTTTCAAC A	

p19-BS(76)10NN-R2	GGGGAAAAATAAAGCGCTTTCAACAATAAAAAAGC	
p19-BS(76)35and10NN-R2	TTTCAACAATAAAAAAGCAGGCCTGGGG	
p19-BS(76)35NN-R2	CAATAAAAAAGCAGGCCTGGGGTG	
p19-BS(76)TY-R1	CTATTTGTATAGTTCATCCATGCCATGTGTAATCCC	
p19-BS(76)TY-F2	TGGATGAACTATACAAATAGCCGGGTACC	
promoter-BS-F1(76)	CTGCTTTTTTATTGTTGAAAGCGCTTTATTTTTCCC	Primers used to construction of the plasmid pUC19-P$_{BS76}$-*gfp*
promoter-BS-R1(76)	CTATTTGTATAGTTCATCCATGCCATGTGTAATCCC	
promoter-BS-F2(76）	TGGATGAACTATACAAATAGCCGGGTACC	

promoter-BS-R2(76)	TTTCAACAATAAAAAAGCAGGCCTGGGGTG CCTAATGAGT	
18-Ppig-pigFN-F1	AAAACGACGGCCAGTGCCAAGCTTTTTTTCC TCCGGAATGCTCCTGC	Primers used to construction of the plasmid pUCP18-P_{pig}-*pigFN*
18-Ppig-pigFN-R1	TCTTGCTTGGTTAAAGGCATTGGGTTGAGAG ATTAAATTAGCTAATATTTCTAGTTTGGAGG	
18-Ppig-pigFN-F2	TAATTTAATCTCTCAACCCAATGCCTTTAAC CAAGCAAGATGCC	
18-Ppig-pigFN-R2	AAAGCAATCCATACATTCATTTATTTTTCGC CGACGATCAGGGT	
18-Ppig-pigFN-F3	TGATCGTCGGCGAAAAATAAATGAATGTAT GGATTGCTTTGGCCGT	

18-Ppig-pigFN-R3	TTCGAGCTCGGTACCCGGGGATCCTTACAG CACGAAAGGAATGAAACACTTAACCT	
18-PrplJ-pigFN-F1	AAAACGACGGCCAGTGCCAAGCTTTCGCAC TTGCGATTATCGCTTTG	Primers used to construction of the plasmid pUCP18-P_{rplJ}-*pigFN*
18-PrplJ-pigFN-R1	TCTTGCTTGGTTAAAGGCATTAGCTTTTTGC TCCTGGATTAGCCG	
18-PrplJ-pigFN-F2	AATCCAGGAGCAAAAAGCTAATGCCTTTAA CCAAGCAAGATGCC	
18-PrplJ-pigFN-R2	AAAGCAATCCATACATTCATTTATTTTTCGC CGACGATCAGGG	
18-PrplJ-pigFN-F3	TGATCGTCGGCGAAAAATAAATGAATGTAT GGATTGCTTTGGCCG	

18-PrplJ-pigFN-R3	TTCGAGCTCGGTACCCGGGGATCCTTACAG CACGAAAGGAATGAAACACTTAACC	
18-PSM-pigFN-F1	AAAACGACGGCCAGTGCCAAGCTTGCCTGC CTTCCGTTCGTC	Primers used to construction of the plasmid pUCP18-P$_{SM}$-*pigFN*
18-PSM-pigFN-R1	TCTTGCTTGGTTAAAGGCATGAGACCAGAG CTCCAATTATTTATAAACGTAAATAATTACT C	
18-PSM-pigFN-F2	ATAATTGGAGCTCTGGTCTCATGCCTTTAAC CAAGCAAGATGCC	
18-PSM-pigFN-R2	AAAGCAATCCATACATTCATTTATTTTTCGC CGACGATCAGGG	
18-PSM-pigFN-F3	TGATCGTCGGCGAAAAATAAATGAATGTAT GGATTGCTTTGGCCG	

18-PSM-pigFN-R3	TTCGAGCTCGGTACCCGGGGATCCTTACAG CACGAAAGGAATGAAACACTTAACC	
18-Ppig-gfp-F1	AAAACGACGGCCAGTGCCAAGCTTTTTTTCC TCCGGAATGCTCCTGC	Primers used to construction of the plasmid pUCP18-P_{pig}-*gfp*
18-Ppig-gfp-R1	AGTTCTTCTCCTTTACTCATTGGGTTGAGAG ATTAAATTAGCTAATATTTCTAGTTTGGAGG	
18-Ppig-gfp-F2	TAATTTAATCTCTCAACCCAATGAGTAAAG GAGAAGAACTTTTCACTGGAGT	
18-Ppig-gfp-R2	TTCGAGCTCGGTACCCGGGGATCCCTATTTG TATAGTTCATCCATGCCATGTGTAATCCC	
18-PrplJ-gfp-F1	AAAACGACGGCCAGTGCCAAGCTTTCGCAC TTGCGATTATCGCTTTG	Primers used to construction of the plasmid pUCP18-P_{rplJ}-*gfp*

18-PrplJ-gfp-R1	AGTTCTTCTCCTTTACTCATTAGCTTTTTGCT CCTGGATTAGCCG	
18-PrplJ-gfp-F2	AATCCAGGAGCAAAAAGCTAATGAGTAAAG GAGAAGAACTTTTCACTGGAGT	
18-PrplJ-gfp-R2	TTCGAGCTCGGTACCCGGGGATCCCTATTTG TATAGTTCATCCATGCCATGTGTAATCCC	
18-PSM-gfp-F1	AAAACGACGGCCAGTGCCAAGCTTGCCTGC CTTCCGTTCGTC	Primers used to construction of the plasmid pUCP18-P$_{SM}$-*gfp*
18-PSM-gfp-R1	AGTTCTTCTCCTTTACTCATGAGACCAGAGC TCCAATTATTTATAAACGTAAATAATTACTC	
18-PSM-gfp-F2	ATAATTGGAGCTCTGGTCTCATGAGTAAAG GAGAAGAACTTTTCACTGGAGT	

18-PSM-gfp-R2	TTCGAGCTCGGTACCCGGGGATCCCTATTTG TATAGTTCATCCATGCCATGTGTAATCCC	
99a-BS50-ilvCDE-F1	AATCGAAACTGGGGGGTTAAGCTGTTTTGG CGGATGAGAGAAG	Primers used to construction of the plasmid pTrc99a-P$_{BS50}$-*ilvCDE*
99a-BS50-ilvCDE-R1	AGGGGAAAAATAAAGCGCCTTGATCAATAA AAAAGCAGGCTCATTTCAGAATATTTGCCA GAACCG	
99a-BS50-ilvCDE-F2	AGGCGCTTTATTTTTCCCCTACAATAGATGA AAACGGCGTGTAAGGGAGGAGCGATCCATG GCTAACTACTTCAATACACTGAATCTGC	
99a-BS50-ilvCDE-R2	TCAGCTTTCTTCGTGGTCATTTAACCCGCAA CAGCAATACGTT	

99a-BS50-ilvCDE-F3	GTATTGCTGTTGCGGGTTAAATGACCACGAAGAAAGCTGATTACATTT	
99a-BS50-ilvCDE-R3	TTAACCCCCCAGTTTCGATTTATCGC	
99a-BS75-ilvCDE-F1	GCTGTTTTGGCGGATGAGAGAAGTAGGGGAAAAATAAAGCGCTTTCAACAATA	Primers used to construction of the plasmid pTrc99a-P_{BS75}-*ilvCDE*
99a-BS75-ilvCDE-R1	AAAAAGCAGGCTCATTTCAGAATATTTGCCAGAACCG	
99a-BS75-ilvCDE-F2	AGCGCTTTATTTTTCCCCTACAATAACGTGCCACGGCGTGTAAGGGAGGAGCGATCCATGGCTAACTACTTCAATACACTGAATCTGC	
99a-BS75-ilvCDE-R2	CAGCTTTCTTCGTGGTCATTTAACCCGCAACAGCAATACGTT	

99a-BS75-ilvCDE-F3	CGTATTGCTGTTGCGGGTTAAATGACCACG AAGAAAGCTGATTACATTT	
99a-BS75-ilvCDE-R3	CTCTCATCCGCCAAAACAGCTTAACCCCCC AGTTTCGATTTATCGC	
99a-BS85-ilvCDE-F1	AGCTGTTTTGGCGGATGAGAGAAG	Primers used to construction of the plasmid pTrc99a-P_{BS85}-*ilvCDE*
	TGTAGGGGAAAAATAAAGCGCCTTGATCAA	
99a-BS85-ilvCDE-R1	TAAAAAAGCAGGCTCATTTCAGAATATTTG CCAGAACCG	
	CGCTTTATTTTTCCCCTACAATAGATGAAAA	
99a-BS85-ilvCDE-F2	CGGCGTGTAAGGGAGGAGCGATCCATGGCT AACTACTTCAATACACTGAATCTGC	
99a-BS85-ilvCDE-R2	AATCAGCTTTCTTCGTGGTCATTTAACCCGC AACAGCAATACGTT	

99a-BS85-ilvCDE-F3	ATTGCTGTTGCGGGTTAAATGACCACGAAG AAAGCTGATTACATTT	
99a-BS85-ilvCDE-R3	TCTCATCCGCCAAAACAGCTTAACCCCCCA GTTTCGATTTATCGC	
99a-BS100-ilvCDE-F1	TAAGCTGTTTTGGCGGATGAGAGAAG	Primers used to construction of the plasmid pTrc99a-P$_{BS100}$-*ilvCDE*
99a-BS100-ilvCDE-R1	TTGTAGGGGAAAAATAAAGCGCTTTCAACA ATAAAAAAGCAGGCTCATTTCAGAATATTT GCCAGAACCG	
99a-BS100-ilvCDE-F2	GCTTTATTTTTCCCCTACAATAGATGAAAAC GGCGTGTAAGGGAGGAGCGATCCATGGCTA ACTACTTCAATACACTGAATCTGC	
99a-BS100-ilvCDE-R2	TAATCAGCTTTCTTCGTGGTCATTTAACCCG CAACAGCAATACGTT	

99a-BS100-ilvCDE-F3	TTGCTGTTGCGGGTTAAATGACCACGAAGAAAGCTGATTACATTT
99a-BS100-ilvCDE-R3	TCATCCGCCAAAACAGCTTAACCCCCCAGTTTCGATTTATCGC