

Supporting Information for

High-resolution mapping of Sigma Factor DNA Binding Sequences using Artificial Promoters, RNA aptamers and Deep Sequencing.

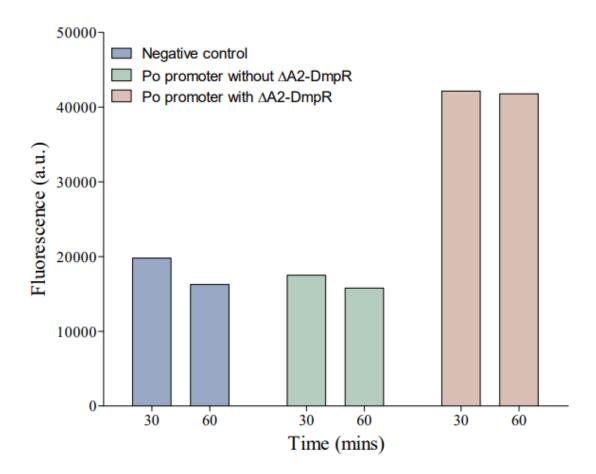
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This PDF file includes:

Figures S1 to S3 Tables S1 to S3 Benchling links of plasmid maps

Fig. S1. Importance of Δ A2-DmpR for *P. putida* σ ⁵⁴-Dependent IVT Reaction

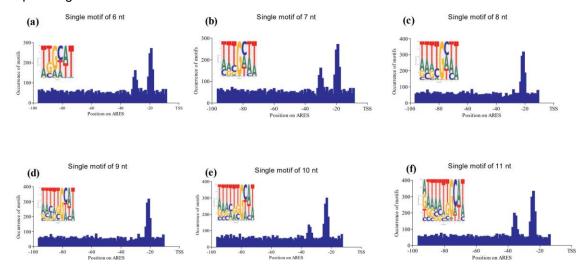


Fluorescence of the Mango aptamer was used to measure RNA synthesis. The results are presented for three experimental groups:

- 1. **Negative Control:** No template added, showing baseline fluorescence.
- 2. **Po Promoter without \triangle A2-DmpR:** Template of the Po promoter without $\triangle A2$ -DmpR, showing fluorescence levels similar to the negative control.
- 3. **Po Promoter with \triangle A2-DmpR:** Template of the Po promoter with $\triangle A2$ -DmpR, showing significantly higher fluorescence levels compared to the negative control.

The data demonstrate that RNA synthesis does not occur in the absence of Δ A2-DmpR, as indicated by the similar fluorescence levels of the negative control and the Po promoter without Δ A2-DmpR. In contrast, significant RNA synthesis, indicated by increased fluorescence, is observed in the presence of both the Po promoter template and Δ A2-DmpR. This highlights the essential role of Δ A2-DmpR in initiating transcription from the σ 54 dependent Po promoter.

Fig. S2. Distribution of single motif within a 97 bp Region Upstream of the TSS in the Highest Expressing ARES Subset

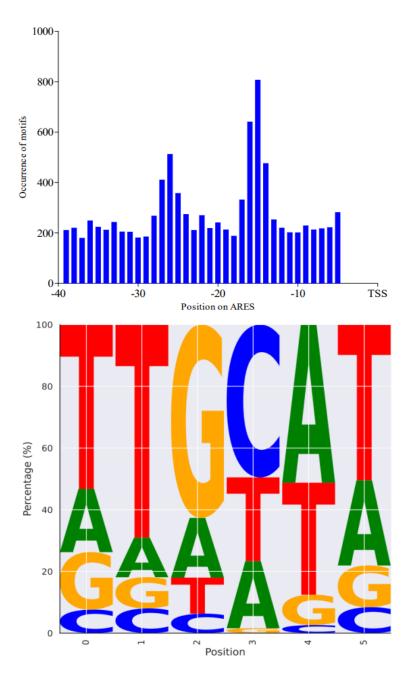


This figure illustrates the distribution of a single conserved motif within a 97 bp region upstream of the Transcription Start Site (TSS) across the highest expressing ARES library for motifs ranging from 6 to 11 nucleotides in length. The x-axis represents the position relative to the TSS, and the y-axis indicates the occurrence frequency of each motif.

- (a) The 6 bp motif (TTGCAT) is identified at the -12 position, consistent with previous core promoter analyses.
- (b) The 7 bp motif shows a sequence extension, adding a T at the beginning (TTTGCTT).
- (c) The 8 bp motif extends further with an additional T (TTTTGCTT).
- (d) The 9 bp motif continues this pattern (TTTTTGCAT).
- (e) The 10 bp motif follows the same trend (TTTTTTGCAT).
- (f) The 11 bp motif (ATTTTTTGCAT) displays the final extension in this analysis.

Each motif is consistently located at the same position upstream of the TSS, with the sequence extension demonstrating high conservation across the lengths studied.

Fig. S3. Distribution of single motif within the 40 bp Region Upstream of the TSS in the Highest Expressing ARES Subset



This figure illustrates the distribution of a single conserved motif within a 40 bp region upstream of the TSS among the highest expressing ARES subset. The x-axis represents the position relative to the TSS, and the y-axis indicates the occurrence frequency of the motif. Analysis reaffirmed the presence of the conserved 6 bp motif (TTGCAT) at the -12 position.

Table S1. Primers used in this study

Primer	Sequence			
name				
	Cloning of double-stranded 200 nt long ARES to pHH100-dBroccoli backbone			
BBa-Prefix- F	GAATTCGCGGCCGCTTCTAGAG			
BBa-Suffix- R	CTGCAGCGGCCGCTACTAGTA			
BB1-F	TCGCACGATATACAGGATTTTGCGGATTTGTCCTACTCAGGAG			
BB1-R	GTCAGCTTTATGCTTGTAAACCGACCGAACAGGCTTATGTC			
BB2-F	CGGTTTACAAGCATAAAGCTG			
BB2-R	GCAAAATCCTGTATATCGTGC			
BB3-F	CGTAATCAAGCCACTTCCTTTT			
BB3-R	GTACGCGTACGGTCTCTGGCATCCGACACCCTGCGTCAATG			
GG-Col-F	GCGGATTTGTCCTACTCA			
GG-Col-R	CTGGATCATCAGAGTATGTG			
For validation	on, 18 ARES, each 200 nt long cloned into the Mango-pSEVA2311 plasmid			
p1-Frag-F	GGCTACGGTCTCCAAGATATCCCGTATAACATTTGTACACTCCGTTATGG			
p1-Frag-R	GGCTACGGTCTCTTGGCAACATTTACATTCAGCAGGCACCAGCGCA			
p1-BB-F	GGCTACGGTCTCTGCCATGTGTATGTGGGCG			
p1-BB-R	GGCTACGGTCTCCTCTTAATTAAAGGCATCAAATAAAACGAAAGG			
p2-Frag-F	GGCTACGGTCTCGACGACTGTCTTAAGGTTAAG			
p2-Frag-R	GGCTACGGTCTCACCACGAGACATATACAATTAC			
p2-BB-F	GGCTACGGTCTCAGTGGATGTAAATGTTGCCATGTG			
p2-BB-R	GGCTACGGTCTCGTCGTTAATTAAAGGCATCAAATAAAACG			
p3-Frag-F	GGCTACGGTCTCCTTCGTGATAATGAGCTGAG			
p3-Frag-R	GGCTACGGTCTCGGGGTAAAATTATATTTAAAGCCG			
p3-BB-F	GGCTACGGTCTCGACCCCTGATGTAAATGTTGCCATGTG			
p3-BB-R	GGCTACGGTCTCCCGAAATTTAATTAAAGGCATCAAATAAAACG			
p4-Frag-F	GGCTACGGTCTCGTAATTGGATTTATTCTACTCTTCC			
p4-Frag-R	GGCTACGGTCTCTCGAAAGAATATCACAATGTAG			
p4-BB-F	GGCTACGGTCTCTTTCGGGGGGTATGTAAATGTTGCCATGTG			
p4-BB-R	GGCTACGGTCTCGATTATAACATTAATTAAAGGCATCAAATAAAACG			
p5-Frag-F	GGCTACGGTCTCGCCTTTAATTAAGTTGTCCTGATTATGGTAGTACTAGTAG			
p5-Frag-R	GGCTACGGTCTCTTGGCAACATTTACATATGCCCCGCCTCATCATATC			
p5-BB-F	GGCTACGGTCTCTGCCATGTGTATGTGGGCG			
p5-BB-R	GGCTACGGTCTCGAAGGCATCAAATAAAACGAAAGGC			
p6-Frag-F	GGCTACGGTCTCTGAGATTCATTAAATATTATTGGGGAAGAATTGG			
p6-Frag-R	GGCTACGGTCTCATGGACACCACCGTATCCGATG			
p6-BB-F	GGCTACGGTCTCATCCATGTAAATGTTGCCATGTG			
p6-BB-R	GGCTACGGTCTCTCCACTTTTAATTAAAGGCATCAAATAAAACG			
p7-Frag-F	GGCTACGGTCTCACCTTTAATTAATAAGCGAGATTCAAGAATTTTTAG			
p7-Frag-R	GGCTACGGTCTCCTAGTATAATTCCATTCCACAG			
p7-BB-F	GGCTACGGTCTCCACTACACGCTGATGTAAATGTTGCCATGTG			
p7-BB-R	GGCTACGGTCTCAAAGGCATCAAATAAAACGAAAG			
p8-Frag-F	GGCTACGGTCTCCAAGGATTTTGAATAAAGTGGTAAAG			
p8-Frag-R	GGCTACGGTCTCACAACATTTACATCCGCTATATGGCTAAATTAG			
p8-BB-F	GGCTACGGTCTCAGTTGCCATGTGTATGTGG			
p8-BB-R	GGCTACGGTCTCCCCTTTAATTAAAGGCATCAAATAAAACG			

p9-Frag-F	GGCTACGGTCTCTGAGTTTACTTCGGGTAAC
p9-Frag-R	GGCTACGGTCTCCCACATGGCAACATTTACATCTACCTATACCACCTAAGAG
p9-BB-F	GGCTACGGTCTCCTGTGTATGTGGGCGTACG
p9-BB-R	GGCTACGGTCTCTACTCCATTTAATTAAAGGCATCAAATAAAACGAAAG
p10-Frag-F	GGCTACGGTCTCAAAAGAGTATGATCCCATTGG
p10-Frag-R	GGCTACGGTCTCGACCATTGATAGGATAGAAAATAAC
p10-BB-F	GGCTACGGTCTCGTGGTTTGAATGTAAATGTTGCCATGTG
p10-BB-R	GGCTACGGTCTCACTTTAATTAAAGGCATCAAATAAAACG
p11-Frag-F	GGCTACGGTCTCCCTTTAATTAAAATTGTTTTTTTGAATGATATGGTTG
p11-Frag-R	GGCTACGGTCTCTTGATCGATTGCCTATTAAC
p11-BB-F	GGCTACGGTCTCTATCAGATCTTGCGATGTAAATGTTGCCATGTG
p11-BB-R	GGCTACGGTCTCTAAGGCATCAAATAAAACGAAAG
p12-Frag-F	GGCTACGGTCTCAACTAGTATACTATTTCAAAACATATTTG
p12-Frag-R	GGCTACGGTCTCGCAACATTTACATTCCTCATTTTCCATCATC
p12-BB-F	GGCTACGGTCTCGGTTGCCATGTGTATGTGG
p12-BB-R	GGCTACGGTCTCATAGTGTCTTTCTTAATTAAAGGCATCAAATAAAACG
p13-Frag-F	GGCTACGGTCTCACTTAAATAATGTAATACGAGCTCG
p13-Frag-R	GGCTACGGTCTCCATACCTCAGGTTTGC
p13-BB-F	GGCTACGGTCTCGGGAATGTAAATGTTGCCATGTG
p13-BB-R	GGCTACGGTCTCATAAGTTACAACTTAATTAAAGGCATCAAATAAAACG
p14-Frag-F	GGCTACGGTCTCTTACGTAGTTTGTGTCCAGTATCC
p14-Frag-R	GGCTACGGTCTCCCAACATTTACATCAAAACAGGTGCAGCGGC
p14-BB-F	GGCTACGGTCTCCGTTGCCATGTGTATGTGG
p14-BB-R	GGCTACGGTCTCCGTAAAATATGAACAATTTTAATTAAAGGCATCAAATAAAACG
p15-Frag-F	GGCTACGGTCTCGCGTAAATATGAACAATTTTAATTAAAGGCATCAAATAAACG
p15-Frag-R	GGCTACGGTCTCGCCTTTAATTAATGAACTTATATCTTCTGAACTTTTTTAACGTCTTG
p15-Flag-R	GGCTACGGTCTCGTGGCAACATTTACATGTCGGATACCACCGAGCC
p15-BB-R	GGCTACGGTCTCGGCCATGTGTGTGGGCG
•	
p16-Frag-F	GGCTACGGTCTCGCAACACGCTCTTCCAATC
p16-Frag-R	GGCTACGGTCTCGACACCCGTCTTGGAATG
p16-BB-F	GGCTACGGTCTCGTGGATGTAAATGTTGCCATGTG
p16-BB-R	GGCTACGGTCTCGTTTGCCCCCGCATCTTAATTAAAGGCATCAAATAAAACG
p17-Frag-F	GGCTACGGTCTCAACCCTCTTTTTGGAATCCTG
p17-Frag-R	GGCTACGGTCTCAATCGGAAGCGAGAAAATCGC
p17-BB-F	GGCTACGGTCTCACGATGTAAATGTTGCCATGTG
p17-BB-R	GGCTACGGTCTCAGGGTATTTTAATTAAAGGCATCAAATAAAACG
p18-Frag-F	GGCTACGGTCTCTTTTCGGCGGATTGATAG
p18-Frag-R	GGCTACGGTCTCTCCCTTGGCTAATGCGAATATAAAAC
p18-BB-F	GGCTACGGTCTCTGGAGATGTAAATGTTGCCATGTG
p18-BB-R	GGCTACGGTCTCTAAGAAATAGTTAAACCTTAATTAAAGGCATCAAATAAAACG
pO54-Frag- F	GGCTACGGTCTCAGCGGCTTAATTTCGCTCG
pO54-Frag- R	GGCTACGGTCTCTCACATGGCAACATTTACATATGTTCATGACTCCATTATTATTGTAC
pO54-BB-F	GGCTACGGTCTCTTGTGTATGTGGGCGTACG
pO54-BB-R	GGCTACGGTCTCACCGCGCTTGCGCAGATTAATTAAAGGCATCAAATAAAACGAAAG
p-Col-F	GTCTTTCGACTGAGCCTTTCG
p-Col-R	CCACATACCAAACCTTCCTTCG
Construction	n of Mango-pSEVA2311 plasmid
Lib1-F	CCTTTAATTAAATGTAAATGTTGCCATGTGTATGTG
Lib2-R	TCCAAGACTAGTACGCGCTACATCCGCTTTAG
Lib3-F	GGATGTAGCGCGTACTAGTCTTGGACTCCTGTTG
Lib4-R	GCAACATTTACATTAAAGGCATCAAATAAAACGAAAG
LIDT IX	

Lib7-F	GTCCTACTCAGGAGAGCGTTC
Lib8-R	GCCATGAATGATCCCGAAGG
Sequencing	of ARES plasmid library
SeqDNA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTN 5-7-GACGCAGGGTGTCGGATG
forward	
SeqDNA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTN 5-7-TGGTGCTCGAGCTTGTACAG
reverse	
Sequencing	of in vitro transcribed RNA
SeqRNA	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCGAG
forward	
SeqRNA1	AGACGTGTGCTCTTCCGATCTCCGACCGTCTCAGATGGACC
reverse	
SeqRNA2	CAAGCAGAAGACGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
reverse	

Table S2. The sequences and original IDs of the 18 randomly selected ARES, each 200 nt in length, are listed below for the validation experiment.

ID	Original IDs	Sequence
p1	ARES00610543	gatatcccgtataacatttgtacactccgttatggtctgtaataaaatttgggatcgttttttgcgttctaatttacA
		gatataggctagagtaactactcgcaaacagtgaagatgtgcagtaagggtcaaccggcatagaattgg
		ctcgtgagtacgtcgatctaaagagactggtgtgcgctggtgcctgctga
P2	ARES00171465	cgactgtcttaaggttaagacaatcttattgtgaggtactggggtattggatgcagcatgggtacatttggact
		acattatggatgggggttctgctacagtgtctacttgggacaatatttgcatcactataatgtGcaaaaaga
		aaagtcgggcccttgtttagaaggtatggtcatgtaattgtatatgtctcgtgg
P3	ARES00389971	atttcgtgataatgagctgagttccttgataaagcagtttgtgtaatgaatg
		attgacggctccttataacactccatatgattttgggaacggtgtatgcacaacgctgaggttGgaagtaat
		ggtgattatgatagttaggtggaataaacggctttaaatataattttacccctg
P4	ARES00141878	tgttataattggatttattctactcttcctcataaccgggcttgggaacgaatctagcatcaaacttggcGgag
		agatgtcgtttaggagtatgcatatatttctggaaatttcgggatttttgttaaggaatttccagtaggtgttgtttc
		gtcaggaatgaaactatagcagctacattgtgatattctttcgggggt
P5	ARES00013532	gttgtcctgattatggtagtactagtagagacaacgtcatcctataaatcgtccaatatataggtgttttggga
1.0	71112000010002	actgtcgaattgtgcgtacctatGatcgtcttttccgtaactaagacaagtcagcatattgggggtcgcatgt
		aacgattatcgcacagaagtgagtagtctaggtgatatgatgaggcggggcat
P6	ARES00256315	aagtgagattcattaaatattattggggaagaattggcttgcgagtggaaaaaatgactgatattaggataa
1 0	AIRE000230313	tcttttttgggatcaggtttagcatcagagttagatGagggcaagcaggtgttgattggaggtgcggaacag
		tactataactaccatgcgctgcttgtgggttttagacatcggatacggtggtgtcc
P7	ARES00413161	taagcgagattcaagaatttttagctttatcgtcgtggtgctgtcatgtagtaagta
Γ/	AINE300413101	ctgttgggagtcggggtagttaaagagttgtGaatgatcaaaggtatgtctatgactcagtccatagatcgg
		gaatagtgtggtcttccacctgtcactgtggaatggaat
P8	ARES00523631	aggattttgaataaagtggtaaagttgttgttgctagtgtctgaagatggtatgtttcgtaacgtctgcattgcca
Γ0	ANE300323031	aaataagtttgagcttatttactttcgggtgaatttgggacaataatttactggttcataaattGggggtaagta
P9	ARES00074902	gttacaatatcagaagtacgttggatgtactaatttagccatatagcgg
ГЭ	ANESUUU14902	aattgttcatattttacgtagtttgtgtccagtatcccagatacagaccgattagtttgcacgatctgtaaggag
		ggatgggattttgcatgccggtacgcttttgatttaaatGggacgaagctggtgcagcgattcttgatcaagt
P1	ARES00116280	atagggagagttagggggtatgtatgtgtactagccgctgcacctgttttg gatgcgggggcaaactttaaatgtgcctcgtgaacacgcggttgaacaattgaaatgtgagaggtgaggt
0	ARE300110200	tgtttagtgtcattggaaatgattttcgattaaactgggcgttagtggatGcgtcaaatttgtaaactaaggtg
U		ttgtcgacaatcgtgtcaggaaagctgccttgtaggcattccaagacggtgttgg
P11	ARES00237232	gaaagacactagtatactatttcaaaacatatttgtggaatgaaattgcttatgccgtgatctacagtgaagg
FII	ANE300231232	gcggcgttctttccggaaagctgttggggacaccagattgatt
		ggtactaaattataacacttaccttgctagctagcggatgatggaaaatgagga
P1	ARES00287266	agagtatgatcccattggttctatagggaatgaaacatttgttaacatacagtggtatgctgatttttgcacggt
2	ARE300201200	
2		ctgtgcatgtaggccgtctGtctgtaatgctgtgtgaaacaagaatcgtcgagtgcggttaatagggggctt
D1	ADEC00006654	gttttcgctttccgatcgcgagaatgttattttctatcctatcaatggtttga
P1 3	ARES00086654	tgaacttatatcttctgaactttttaacgtcttgttggttaccattagattaaaatagctatagaagcggtagatc
3		gtacgttcctcctaagagatgtcaaatatgtattcattacagtggattttgatactttgttgaaagttcgaaaaG
D4	ADEC00400700	gtattaagatggttggtttagggttgttaaggctcggtggtatccgac
P1	ARES00422720	gttgtaacttaaataatgtaatacgagctcggtagtatctttagcagtatttatcacttggttgg
4		aatgcgaaagaacaggggggaacatgatttttttccaggaggtcacgccaagattgtaaGagggctga
D4	ADEC00050070	gagagtcggaaagtgtatacatttaaaggatgtccccgcaaacctgaggtatgga
P1	ARES00952673	ggtttaactatttcttttcggcggattgatagtttaacatctcgtgtgttgatggccaaggtgttagaaggatcgt
5		gtcttgagggccgtggattgttctgatgatttttggcaggaaagttaacaaaaagcaaGgatcttatagtatg
D4	ADEC00470407	cattatgcttatggttttatattcgcattagccaagggag
P1	ARES00173427	aataccctctttttggaatcctgccatgatcggactttgtctaggtgggagcttttttcaaacgattttagagtaat
6		gatgacgtactttttgtatGggctcaattgtgtgtttaggacctttcagccatagtattctcaggctattacagac
D4	ADE000400540	gagtacacttcattgcataactgtaaagggcgattttctcgcttccg
P1	ARES00432513	aattgttttttgaatgatatggttgcattcatatttacatttacatccatatttgtgtatagcgattttgtaatatggta
7		gtctatgatataaatactGattgatattgccgaaacgaggtaaaaaacatttggtgagccattacatcttttg
		acagatttatcgaagatggttaataggcaatcgatcagatcttgcg

P1	ARES00438390	atggagtttacttcgggtaacattggttatatttgagatttgacacatgaccgcactagacttcactacgtgggt
8		ttaaacgtttactggttattttcagattttgtatgaaagttgaatatagggataggGacgctagggctggagtg
		ccgcagcctgataaagccgatacggggatatctcttaggtggtataggtag
pO ⁵	Pos-Control	atgagctctctgcgcaagcgcggcttaatttcgctcgctc
4		aacaataccttgaagtctgttttcagaccttggcacagccgttgcttgatgtcctgcgcaacatgtacaataat
		aatggagtcatgaacatatgg

Table S3: Annotation of IVT-generated σ^{54} -RNA polymerase binding motifs in the upstream regions of genes in the *P. putida* genome.

Gene	Product	-24 motifs	-12 motifs	p-value
PP_2783	3-oxoacyl-ACP reductase family protein	TGGGCT	TGGCAT	0,00405
PP_4970	cytochrome c	GTGGCT	GTGTTT	0,00353
PP_1666	DUF2066 domain-containing protein	GTGGTT	GTGTAT	0,003
eutC	ethanolamine ammonia-lyase subunit EutC	TTGGCA	TTGCAT	0,000286
PP_1786	glycosyltransferase	ATGGCA	TTGCAT	0,000286
PP_1030	hypothetical protein	TGGGCT	TTGATT	0,000918
PP_1810	hypothetical protein	TTGGCT	TTGTAT	0,00126
PP_1923	hypothetical protein	ATGGCT	TGGCAT	0,00405
PP_0091	lipoprotein	CTGGCA	TGGCTT	0,00462
PP_1622	peptidoglycan DD-metalloendopeptidase family protein	TTGGTT	TTGCAA	0,00329
PP_4204	type II toxin-antitoxin system MqsA family antitoxin	TTGGCA	GTGTAT	0,003
PP_1394	5-guanidino-2-oxopentanoate decarboxylase	ATGGCT	GTGTTT	0,00353
PP_4577	5-oxoprolinase subunit PxpA	TGGGTA	GTGTTT	0,00353
PP_0859	amidohydrolase	CTGGTA	GTGCTT	0,0015
PP_3521	DMT family transporter	GTGGTT	TTGTAT	0,00126
PP_1210	Dps family protein	TCGGCA	TTGAGT	0,00381
PP_1584	DUF2514 domain-containing protein	TGGGCA	GTGCAT	0,00174
PP_3248	Dyp-type peroxidase	TTGATA	TTGCAA	0,00329
motA	flagellar motor stator protein MotA	TTGGTT	TGGCAT	0,00405
purT	formate-dependent phosphoribosylglycinamide formyltransferase	GTGGCT	TGGCAT	0,00405
pgi	glucose-6-phosphate isomerase	TTGATA	TGGCTT	0,00462
PP_4543	GNAT family N-acetyltransferase	TTGTTA	TTGAGT	0,00381
PP_1159	hypothetical protein	TTGGCT	TTGCAT	0,000286
PP_5488	hypothetical protein	ATGGCT	GTGTAT	0,003
PP_0182	hypothetical protein	ATGGCA	TTGCAT	0,000286
PP_2952	LysR family transcriptional regulator	GTGGTT	TGGCAT	0,00405
PP_3132	oligosaccharide flippase family protein	TGGGCA	TAGCAT	0,00381
PP_5043	PhoPQ-activated pathogenicity-related family protein	TTGGCT	TTGCTT	0,000286
edd	phosphogluconate dehydratase	GTGGCA	TTGTTT	0,00208
PP_4924	S8 family serine peptidase	GTGGCA	TTGTTT	0,00208
PP_0146	TerC family protein	CTGGTA	GTGCAT	0,00174
PP_2420	TonB-dependent receptor	ATGGTT	TTGCGT	0,00267
yegQ	tRNA 5-hydroxyuridine modification protein YegQ	ATGGCT	GTGATT	0,00271
dusA	tRNA dihydrouridine(20/20a) synthase DusA	GTGGTA	GTGAAT	0,00248
tssG	type VI secretion system baseplate subunit TssG	TCGGCA	GTGTTT	0,00353

containing protein cls cardiolipin synthase TCGGCA ATGCAT 0,00491 PP_1188 dicarboxylate/amino acid:cation symporter TTGGCA TTGCTA 0,0041 PP_3989 DNA cytosine methyltransferase ATGGTA TGGCAT 0,00405 PP_2810 DUF1329 domain-containing protein CTGGCA TTGCGT 0,00267 PP_5363 DUF3617 domain-containing protein GTGGTA ATGCAT 0,00491 flgA flagellar basal body P-ring formation chaperone TCGGCA TTGCTT 0,00286 FlgA flagellar basal body rod protein FlgB TTGGCA TTGCTT 0,000286 flgG flagellar basal-body rod protein FlgF TTGGTT TTGCTA 0,0041 flgF flagellar basal-body rod protein FlgG ATGGCT TTGCAA 0,00329 fliE flagellar basal-body complex protein FliE CTGGCA TTGCTT 0,000286 FP_2836 fumarylacetoacetate hydrolase family protein GTGGCT TTGCAA 0,00329 PP_1946 glucose 1-dehydrogenase TTGGCA TTGCCA 0,00329 PP_1946 glucose 1-dehydrogenase TTGGCA TTGCAA 0,00329 PP_2089 GlxA family transcriptional regulator TAGGCA TTGCGT 0,0027 PP_2889 MurR/RpiR family transcriptional regulator ATGGCT TTGCAA 0,00329 rtrB nitrogen regulation protein NR(II) GTGGTT TTGCAA 0,00329 rP_3952 RNA polymerase factor sigma-54 AAGGCA TTGCTT 0,000286 PP_3288 universal stress protein TCGGTA TTGCAA 0,00329 PP_2209 2-aminoethylphosphonatepyruvate transaminase TGGCA TTGCAA 0,00329 PP_2140 Urrea ABC transporter substrate-binding protein TAGGCA TTGCAA 0,00329 PP_21950 ABC transporter substrate-binding protein TGGGCT TGGCAT 0,000266 PP_31141 branched-chain amino acid ABC transporter TGGCA TTGCAA 0,00329 PP_3146 ABC transporter substrate-binding protein TGGGCA TTGCAT 0,000266 PP_3170 hypothetical protein GTGGCA TTGGCAT 0,000267 regulatory protein BcsB	PP_2433	antitoxin Xre/MbcA/ParS toxin-binding domain-	CTGGCA	TGGCTT	0,00462
PP_1188 dicarboxylate/amino acid:cation symporter TTGGCA TTGCTA 0,0041 PP_3989 DNA cytosine methyltransferase ATGGTA TGGCAT 0,00405 PP_2810 DUF1329 domain-containing protein CTGGCA TTGCGT 0,00267 PP_36363 DUF3617 domain-containing protein GTGGTA ATGCAT 0,00491 fligA flagellar basal body P-ring formation chaperone TCGCA TTGCTT 0,00286 fligA flagellar basal body rod protein FlgB TTGGCA TTGCTA 0,0041 fligF flagellar basal-body rod protein FlgG ATGGCT TTGCAA 0,00329 fligE flagellar basal-body rod protein FlgG ATGGCT TTGCAA 0,00329 fligE flagellar bosk-basal body complex protein Flig CTGGCA TTGCAA 0,00329 fligE flagellar basal-body rod protein FlgG ATGGCT TTGCAA 0,00329 fligE flagellar basal-body rod protein FlgG ATGGCA TTGCAT 0,00286 PP_2836 flumarylacetoacetate hydrolase family protein GTGGCT TTGCAA 0,0	cls		TCGGCA	ATGCAT	0.00491
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PP_1141branched-chain amino acid ABC transporter substrate-binding proteinTGGGCTTGGCAT0,00405bcsBcellulose biosynthesis cyclic di-GMP-binding regulatory protein BcsBGTGGTTTTGCGT0,00267ppxexopolyphosphataseATGGCATTGCAT0,000286PP_3703hypothetical proteinTGGGCAGTGTAT0,003PP_0052MBL fold metallo-hydrolaseGTGGCATGGCAT0,00405PP_3539MerR family DNA-binding transcriptional regulatorGTGGTTTTGAGT0,00381PP_3060phage major tail tube proteinGTGGTTGTGCTT0,0015PP_3778pyrroline-5-carboxylate reductaseTTGACATTGCTA0,0041PP_1450HlpA activation/secretion protein HlpBGTGGCTTAGCAT0,00381PP_5722type II toxin-antitoxin system PemK/MazF family toxinCTGGTATTGAAT0,000631	PP_2209		ATGGCA	TTGCAA	0,00329
substrate-binding protein bcsB cellulose biosynthesis cyclic di-GMP-binding regulatory protein BcsB ppx exopolyphosphatase ATGGCA TTGCAT 0,000286 PP_3703 hypothetical protein TGGGCA GTGTAT 0,003 PP_0052 MBL fold metallo-hydrolase GTGGCA TGGCAT 0,00405 PP_3539 MerR family DNA-binding transcriptional regulator PP_3060 phage major tail tube protein GTGGTT GTGCTT 0,0015 PP_3778 pyrroline-5-carboxylate reductase TTGACA TTGCTA 0,0041 PP_1450 HlpA activation/secretion protein HlpB GTGGCT TAGCAT 0,000631 PP_5722 type II toxin-antitoxin system PemK/MazF amily toxin TTGAAT 0,000631	PP_3486		ATGGCT	GTGCTT	0,0015
regulatory protein BcsB ppx exopolyphosphatase ATGGCA TTGCAT 0,000286 PP_3703 hypothetical protein TGGGCA GTGTAT 0,003 PP_0052 MBL fold metallo-hydrolase GTGGCA TGGCAT 0,00405 PP_3539 MerR family DNA-binding transcriptional regulator PP_3060 phage major tail tube protein GTGGTT GTGCTT 0,0015 PP_3778 pyrroline-5-carboxylate reductase TTGACA TTGCTA 0,0041 PP_1450 HlpA activation/secretion protein HlpB GTGGCT TAGCAT 0,00381 PP_5722 type II toxin-antitoxin system PemK/MazF CTGGTA TTGAAT 0,000631	PP_1141	substrate-binding protein		TGGCAT	0,00405
PP_3703 hypothetical protein TGGGCA GTGTAT 0,003 PP_0052 MBL fold metallo-hydrolase GTGGCA TGGCAT 0,00405 PP_3539 MerR family DNA-binding transcriptional regulator PP_3060 phage major tail tube protein GTGGTT GTGCTT 0,0015 PP_3778 pyrroline-5-carboxylate reductase TTGACA TTGCTA 0,0041 PP_1450 HlpA activation/secretion protein HlpB GTGGCT TAGCAT 0,00381 PP_5722 type II toxin-antitoxin system PemK/MazF CTGGTA TTGAAT 0,000631	bcsB	regulatory protein BcsB			
PP_0052 MBL fold metallo-hydrolase GTGGCA TGGCAT 0,00405 PP_3539 MerR family DNA-binding transcriptional regulator PP_3060 phage major tail tube protein GTGGTT GTGCTT 0,0015 PP_3778 pyrroline-5-carboxylate reductase TTGACA TTGCTA 0,0041 PP_1450 HlpA activation/secretion protein HlpB GTGGCT TAGCAT 0,00381 PP_5722 type II toxin-antitoxin system PemK/MazF amily toxin CTGGTA TTGAAT 0,000631	ррх		ATGGCA	TTGCAT	0,000286
PP_3539 MerR family DNA-binding transcriptional regulator PP_3060 phage major tail tube protein PP_3778 pyrroline-5-carboxylate reductase PP_1450 HlpA activation/secretion protein HlpB PP_5722 type II toxin-antitoxin system PemK/MazF family toxin GTGGTT TTGAGT 0,00381 GTGGTT TTGAGT 0,00381 TTGAGT 0,00381	PP_3703	hypothetical protein	TGGGCA	GTGTAT	0,003
regulator PP_3060 phage major tail tube protein PP_3778 pyrroline-5-carboxylate reductase PP_1450 HlpA activation/secretion protein HlpB PP_5722 type II toxin-antitoxin system PemK/MazF family toxin FROM STREET STR	PP_0052		GTGGCA	TGGCAT	0,00405
PP_3778 pyrroline-5-carboxylate reductase TTGACA TTGCTA 0,0041 PP_1450 HlpA activation/secretion protein HlpB GTGGCT TAGCAT 0,00381 PP_5722 type II toxin-antitoxin system PemK/MazF CTGGTA TTGAAT 0,000631 family toxin	PP_3539	regulator	GTGGTT	TTGAGT	0,00381
PP_1450 HlpA activation/secretion protein HlpB GTGGCT TAGCAT 0,00381 PP_5722 type II toxin-antitoxin system PemK/MazF CTGGTA TTGAAT 0,000631 family toxin	PP_3060	phage major tail tube protein	GTGGTT	GTGCTT	0,0015
PP_5722 type II toxin-antitoxin system PemK/MazF CTGGTA TTGAAT 0,000631 family toxin	PP_3778	1,,	TTGACA	TTGCTA	0,0041
family toxin	PP_1450	HlpA activation/secretion protein HlpB	GTGGCT	TAGCAT	0,00381
xdhA xanthine dehydrogenase small subunit TGGGCA GTGCAT 0,00174	PP_5722	family toxin	CTGGTA	TTGAAT	0,000631
<u> </u>	xdhA	xanthine dehydrogenase small subunit	TGGGCA	GTGCAT	0,00174

Plasmid maps

1) N200 ARES

 $\underline{https://benchling.com/s/seq-wG6faFrPGndrWYAhEMqq?m=slm-wg6jqKmiUQcNzv7zXjAx}$

2) pHH100-dBroccoli construct

https://benchling.com/s/seq-bD8GNHJIVsiaienqUajK?m=slm-NNHWA6VcrijY2MuG6UKe

3) N200-pHH100-dBroccoli construct

 $\underline{https://benchling.com/s/seq-VxVk2xeLeUp684jSTSbb?m=slm-LhFMu9GY5rYQlaGJzR3s}$

4) Mango-pSEVA2311 construct

 $\underline{https://benchling.com/s/seq-CfaFXRxx76D39VVFn3LS?m=slm-Q88cA84fxmZQlkUefZ72}$