

Cis-Element Engineering Promotes the Expression of *Bacillus subtilis* Type I L-Asparaginase and Its Application in Food

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Supplementary Data

Supplementary Table S1: Description and sources of vectors with different promoters used

in this study.

Strains/Promoter	Sigma factor	Application	Source
P43	σ^A, σ^B	Recombinant vector	[12]
PaprE	σ^A	Recombinant vector	[52]
PyxiE	σ^A	Recombinant vector	[53]
PsecA	σ^A	Recombinant vector	[54]
Pylbp	σ^W	Recombinant vector	[55]
Phag	σ^D	Recombinant vector	[15]
PfusA	σ^A	Recombinant vector	[15]
PSacB	σ^B	Recombinant vector	[56]
PydzA	$\sigma^D, \sigma^E, \sigma^F$	Recombinant vector	[13]
PspoVG	σ^H	Recombinant vector	[57]
PsrfA	σ^H	Recombinant vector	[28]
PlytR	σ^A, σ^X	Recombinant vector	[57]
PftsH	σ^A	Recombinant vector	[57]
PyvyD	σ^B, σ^H	Recombinant vector	[57]
PsodA	σ^B	Recombinant vector	[13]
PamyE	σ^A	Recombinant vector	[52]
P43-P43	$\sigma^A, \sigma^B, \sigma^A, \sigma^B$	Recombinant vector	This work
Papre-P43	$\sigma^A, \sigma^A, \sigma^B$	Recombinant vector	This work

PyvyD-P43	$\sigma^B, \sigma^H\text{-}\sigma^A, \sigma^B$	Recombinant vector	This work
PspoVG-P43	$\sigma^H\text{-}\sigma^A, \sigma^B$	Recombinant vector	This work
PaprE-PaprE	$\sigma^A\text{-}\sigma^A$	Recombinant vector	This work
P43-PaprE	$\sigma^A, \sigma^B\text{-}\sigma^A$	Recombinant vector	This work
PyvyD-PaprE	$\sigma^B, \sigma^H\text{-}\sigma^A$	Recombinant vector	This work
PspoVG-PaprE	$\sigma^H\text{-}\sigma^A$	Recombinant vector	This work
PyvyD-PyvyD	$\sigma^B, \sigma^H\text{-}\sigma^B, \sigma^H$	Recombinant vector	This work
P43-PyvyD	$\sigma^A, \sigma^B\text{-}\sigma^B, \sigma^H$	Recombinant vector	This work
PaprE-PyvyD	$\sigma^A\text{-}\sigma^B, \sigma^H$	Recombinant vector	This work
PspoVG-PyvyD	$\sigma^H\text{-}\sigma^B, \sigma^H$	Recombinant vector	This work
PSpoVG-PspoVG	$\sigma^H\text{-}\sigma^H$	Recombinant vector	This work
P43-PspoVG	$\sigma^A, \sigma^B\text{-}\sigma^H$	Recombinant vector	This work
PaprE-PspoVG	$\sigma^A\text{-}\sigma^H$	Recombinant vector	This work
PyvyD-PspoVG	$\sigma^B, \sigma^H\text{-}\sigma^H$	Recombinant vector	This work

Supplementary Table S2: Description and sources of different RBS and spacer sequences used in this study.

Strains	Promoter	RBS and spacer sequences	Source	Translation rate (au)
RBS 0	Pap ^r E- PyvyD	GGGAGGCGTTCCTTG	PyvyD	4960.1
RBS 1	Pap ^r E- PyvyD	AGGGGGATTATT	PnprE	87846.79
RBS 2	Pap ^r E- PyvyD	GTAAGAGAGGAATGTACAC	P43	8881.85
RBS 3	Pap ^r E- PyvyD	AGGAGCGATTACAT	PhpaII	11469.9
RBS 4	Pap ^r E- PyvyD	AGGAGAGGGACGCGT	Pap ^r E	20612.74
RBS 5	Pap ^r E- PyvyD	AAAGGAGGAAGGATCA	pHT43(Pgrac)	85822.58
RBS 6	Pap ^r E- PyvyD	AGGAGGTGTGTGTA	pMA0911	55958.16
RBS 7	Pap ^r E- PyvyD	GGGAGGTATGACAAT	PsrfA	80498.66
RBS 8	Pap ^r E- PyvyD	AGGTGGTGAACACT	PspoVG	22194.66

RBS 9	PapE- PyvyD	GGAGTGTCAAG	PamyE	11168.34
RBS 10	PapE- PyvyD	AAGGAGATATACAT	pET30a	32138.63
RBS 11	PapE- PyvyD	GGAGGTTATTTCA	PgroES	118319.57
RBS 12	PapE- PyvyD	AGGAGATATACCATGGGCAGCAGC	pET28a	2985.39
RBS 13	PapE- PyvyD	GTGAGGTGGATGCA	Pveg	85648.93
RBS 14	PapE- PyvyD	GGAGAGAGGAA	PwapA	9090.42
RBS 15	PapE- PyvyD	GGAGGCGTAGGC	RBS Calculator	82241.74
RBS 16	PapE- PyvyD	GGAGGTAGGTAATAC	RBS Calculator	107421.95
RBS 17	PapE- PyvyD	AGGAGGCACAACAT	RBS Calculator	139574.81
RBS 18	PapE- PyvyD	TCAAGGAGGTTGGATT	RBS Calculator	187915.15
RBS 19	PapE- PyvyD	GGAGGTTATACG	RBS Calculator	266472.30

RBS 20	PapE-	GGAGGTCATATT	RBS Calculator	315092.76
	PyvyD			
RBS 21	PapE-	GGAGGTTTATTTT	RBS Calculator	360348.07
	PyvyD			
RBS 22	PapE-	GGAGGTTTTTTTA	RBS Calculator	409128.04
	PyvyD			

Supplementary Table S3: Oligonucleotides used in this study.

Primers	Sequence
PaprE-F	TTTTTTGAGCAACTGGATCCGTTCTTTTCTGTATGAAAATAGTTA TTTCG
PaprE-R	AGAGCTACTTTCTTTTTTCATTCTTTACCCTCTCCTTTTAAAAAAA TTCAG
PyxiE-F	TTTTTTGAGCAACTGGATCCAATTGAAGCGCGCGAAGC
PyxiE-R	AGAGCTACTTTCTTTTTTCATGCTCTTCCCGCCTTTCGG
PsecA-F	TTTTTTGAGCAACTGGATCCGGACATCGTCCGTCAGAAACG
PsecA-R	AGAGCTACTTTCTTTTTTCATTATAACGCTCCTCTATCATCACAC G
Pylbp-F	TTTTTTGAGCAACTGGATCCGTCACAACAGTCACGTCGTGATAA
Pylbp-R	AGAGCTACTTTCTTTTTTCATACAAATCTCCCCCTTTGTTGTTT
Phag-F	TTTTTTGAGCAACTGGATCCGGATTTTTTTTATTTTTGTATTAACA AAATCAGAGAC
Phag-R	AGAGCTACTTTCTTTTTTCATTGTTTTGTTCCCTCCCTGAATATGTT
PfusA-F	TTTTTTGAGCAACTGGATCCTTGATTTTGCCGCTTAAGTCAAG
PfusA-R	AGAGCTACTTTCTTTTTTCATTCTAAAATCCTCCTTAAGAGCTTTT AAT
PSacB-F	TTTTTTGAGCAACTGGATCCGATATTTTCTGAATTGTGATTAAA

	AAGGC
PSacB-R	AGAGCTACTTTCTTTTTCATCGTTCATGTCTCCTTTTTTATGTAC
	TG
PydzA-F	TTTTTTGAGCAACTGGATCCCCCGTTCTGTTACAGATGGAG
PydzA-R	AGAGCTACTTTCTTTTTCATAAATCGCCCTCCTTGTGGA
PspoVG-F	TTTTTTGAGCAACTGGATCCACGGACAATATTTTGACACTCAC
	AA
PspoVG-R	AGAGCTACTTTCTTTTTCATAGTAGTTCACCACCTTTTCCCTAT
	ATAA
PsrfA-F	AAGACAAATTCTGCTACTAATGATAGGTGGTATGTTTTCGCTTG
PsrfA-R	TCTTTGTCGCCGCTGCTGCACATGTGTACATTCCTCTCTTACCTA
	TAATG
PlytR-F	TTTTTTGAGCAACTGGATCCCTAACCCTACATAAGTACCTTCTT
	TTGTT
PlytR-R	AGAGCTACTTTCTTTTTCATCCTTGCACCTCGTCTGTAAAT
PftsH-F	TTTTTTGAGCAACTGGATCCAGATTAGGATTGCGAGCAGCA
PftsH-R	AGAGCTACTTTCTTTTTCATCATAACCCTCCAGCATGTTTAAAA
PyvyD-F	TTTTTTGAGCAACTGGATCCGATCAATTGGTCTCTTTCTCTTTT
	CC
PyvyD-R	AGAGCTACTTTCTTTTTCATCAAAGAACGCCTCCCTTTTATTA
PsodA-F	TTTTTTGAGCAACTGGATCCGAAATGCTGGCGGCAGGT
PsodA-R	AGAGCTACTTTCTTTTTCATGATAATTCCTCCTTAGTATATATGTA

	CTGAAATG
PamyE-F	TTTTTTGAGCAACTGGATCCTAGAGTGATTGTGATAATTTTAAAT GTAAGCG
PamyE-R	AGAGCTACTTTCTTTTTCATTCTTGACACTCCTTATTTGATTTTT G
V-F	ATGAAAAAGAAAGTAGCTCTTATTACAACA
V-R	GGATCCAGTTGCTCAAAAAAATC
P43-P43-F	TTTTTTGAGCAACTGGATCCTGATAGGTGGTATGTTTTCGCTTG
P43-P43-R	CGAAAACATACCACCTATCATTATATTTTACATAATCGCGCGC
PaprE-P43-F	TTTTTTGAGCAACTGGATCCGTTCTTTTCTGTATGAAAATAGTT ATTTCTG
PaprE-P43-R	CGAAAACATACCACCTATCACTTACTTAAAAGACTATTCTGTG AATTTATTGT
PyvyD-P43-F	TTTTTTGAGCAACTGGATCCGATCAATTGGTCTCTTTCTCTTTTC C
PyvyD-P43-R	CGAAAACATACCACCTATCAATTTCTCTTTTACCCTTTACAATTC CT
PspoVG-P43-F	TTTTTTGAGCAACTGGATCCTGCGGAAGTAAACGAAGTGACG
PspoVG-P43-R	CGAAAACATACCACCTATCGTGTATCAATTCCACGATTTTTTCTG
PaprE-PaprE-F	TTTTTTGAGCAACTGGATCCGTTCTTTTCTGTATGAAAATAGTTA TTTCG
PaprE-PaprE-R	ATTTTCATACAGAAAAGAACCTTACTTAAAAGACTATTCTGTGA

	ATTTATTGT
P43-PaprE-F	TTTTTTGAGCAACTGGATCCTGATAGGTGGTATGTTTTCGCTTG
P43-PaprE-R	ATTTTCATACAGAAAAGAAGTTATATTTTACATAATCGCGCGC
PyvyD-PaprE-F	TTTTTTGAGCAACTGGATCCGATCAATTGGTCTCTTTCTCTTTTC
	C
PyvyD-PaprE-R	ATTTTCATACAGAAAAGAAGTTCTCTTTTACCCTTTACAATTC
	CT
PspoVG-PaprE-F	TTTTTTGAGCAACTGGATCCTGCGGAAGTAAACGAAGTGACG
PspoVG-PaprE-R	ATTTTCATACAGAAAAGAAGTGTATCAATTCCACGATTTTTTCT
	G
PyvyD-PyvyD-F	TTTTTTGAGCAACTGGATCCGATCAATTGGTCTCTTTCTCTTTTC
	C
PyvyD-PyvyD-R	GAGAAAGAGACCAATTGATCATTCTCTTTTACCCTTTACAATTC
	CT
P43-PyvyD-F	TTTTTTGAGCAACTGGATCCTGATAGGTGGTATGTTTTCGCTTG
P43-PyvyD-R	GAGAAAGAGACCAATTGATCTTATATTTTACATAATCGCGCGC
PaprE-PyvyD-F	TTTTTTGAGCAACTGGATCCGTTCTTTTCTGTATGAAAATAGTTA
	TTTCG
PaprE-PyvyD-R	GAGAAAGAGACCAATTGATCCTTACTTAAAAGACTATTCTGTGA
	ATTTATTGT
PspoVG-PyvyD-F	TTTTTTGAGCAACTGGATCCTGCGGAAGTAAACGAAGTGACG
PspoVG-PyvyD-R	GAGAAAGAGACCAATTGATCGTGTATCAATTCCACGATTTTTTC

	TG
PSpoVG-PspoVG-F	TTTTTTGAGCAACTGGATCCTGCGGAAGTAAACGAAGTGACG
PSpoVG-PspoVG-R	AGTGTCAAAATATTGTCCGTGTGTATCAATTCCACGATTTTTTCT
	G
P43-PspoVG-F	TTTTTTGAGCAACTGGATCCTGATAGGTGGTATGTTTTCGCTTG
P43-PspoVG-R	AGTGTCAAAATATTGTCCGTTTATATTTTACATAATCGCGCGC
PaprE-PspoVG-F	TTTTTTGAGCAACTGGATCCGTTCTTTTCTGTATGAAAATAGTTA
	TTTCG
PaprE-PspoVG-R	AGTGTCAAAATATTGTCCGTCTTACTTAAAAGACTATTCTGTGA
	ATTATTGT
PyvyD-PspoVG-F	TTTTTTGAGCAACTGGATCCGATCAATTGGTCTCTTTCTCTTTTC
	C
PyvyD-PspoVG-R	AGTGTCAAAATATTGTCCGATTCTCTTTTACCCTTTACAATTCC
	T
Mutant 1-F	AGCAGGAATTGTAAAGGGTATAAGAG
Mutant 1-R	CCCTTTACAATTCCTGCTTAAACATA
Mutant 2-F	TGTAAAGGGTAAAAGAGAATTAGATACATATC
Mutant 2-R	CTCTTTTACCCTTTACAATACCTG
Mutant 3-F	GCAGGTATTGTAAAGGGTATAAGAGAATTAGATACATATC
Mutant 3-R	CCCTTTACAATACCTGCTTAAACATAAATCTTT
RT-BIA-F	TTCAGGACGGCAGCTATGAC
RT-BIA-R	TATATCTGGGCTGCACGCTG

16 S-F	GACGAAAGTCTGACGGAGCA
16 S-R	TGCCACCTACGTATTACCGC
RBS 1-F	AAGGGGGATTATTATGAAAAAGAAAGTAGCTCTTATTACAACA
RBS 1-R	CATAATAAATCCCCCTTTTATTAAGGATATGTATCTAATTCTCTT ATACC
RBS 2-F	GTAAGAGAGGAATGTACACATGAAAAAGAAAGTAGCTCTTATT ACAACA
RBS 2-R	GTACATTCCTCTCTTACTTTTATTAAGGATATGTATCTAATTCTCT TATACC
RBS 3-F	AAGGAGCGATTTACATATGAAAAAGAAAGTAGCTCTTATTACAA CA
RBS 3-R	TATGTAAATCGCTCCTTTTATTAAGGATATGTATCTAATTCTCTT ATACC
RBS 4-F	AAGGAGAGGGACGCGTATGAAAAAGAAAGTAGCTCTTATTACA ACA
RBS 4-R	TACGCGTCCCTCTCCTTTTATTAAGGATATGTATCTAATTCTCTT ATACC
RBS 5-F	AAAAGGAGGAAGGATCAATGAAAAAGAAAGTAGCTCTTATTAC AACA
RBS 5-R	TTGATCCTTCCTCCTTTTTTATTAAGGATATGTATCTAATTCTCT TATACC
RBS 6-F	AAGGAGGTGTGTGTAATGAAAAAGAAAGTAGCTCTTATTACAA

	CA
RBS 6-R	CATTACACACACCTCCTTTTTATTAAGGATATGTATCTAATTCTCT TATACC
RBS 7-F	AGGGAGGTATGACAATATGAAAAAGAAAGTAGCTCTTATTACA ACA
RBS 7-R	AGGGAGGTATGACAATATGAAAAAGAAAGTAGCTCTTATTACA ACA
RBS 8-F	AGGGAGGTATGACAATATGAAAAAGAAAGTAGCTCTTATTACA ACA
RBS 8-R	TAGTAGTTCACCACCTTTTTATTAAGGATATGTATCTAATTCTCTT ATACC
RBS 9-F	AAAGGAGTGTCAAGATGAAAAAGAAAGTAGCTCTTATTACAAC A
RBS 9-R	CATCTTGACACTCCTTTTTATTAAGGATATGTATCTAATTCTCTTAT ACC
RBS 10-F	AAAGGAGATATACATATGAAAAAGAAAGTAGCTCTTATTACAAC A
RBS 10-R	CATATGTATATCTCCTTTTTTATTAAGGATATGTATCTAATTCTCTT ATACC
RBS 11-F	AGGAGGTTATTTCAATGAAAAAGAAAGTAGCTCTTATTACAACA
RBS 11-R	CATTGAAATAACCTCCTTTTTATTAAGGATATGTATCTAATTCTCTT ATACC

RBS 12-F	AGATATACCATGGGCAGCAGCATGAAAAAGAAAGTAGCTCTTAT TACAACA
RBS 12-R	GCTGCCCATGGTATATCTCCTTTTTATTAAGGATATGTATCTAATT CTCTTATACC
RBS 13-F	AGTGAGGTGGATGCAATGAAAAAGAAAGTAGCTCTTATTACAA CA
RBS 13-R	CATTGCATCCACCTCACTTTTATTAAGGATATGTATCTAATTCTCT TATACC
RBS 14-F	AAAGGAGAGAGGAAATGAAAAAGAAAGTAGCTCTTATTACAA CA
RBS 14-R	CATTCCTCTCTCCTTTTTATTAAGGATATGTATCTAATTCTCTTATA CC
RBS 15-F	AAAAGGAGGCGTAGGCATGAAAAAGAAAGTAGCTCTTATTACA ACA
RBS 15-R	ATGCCTACGCCTCCTTTTTATTAAGGATATGTATCTAATTCTCTTAT ACC
RBS 16-F	AGGAGGTAGGTAATACATGAAAAAGAAAGTAGCTCTTATTACA ACA
RBS 16-R	TGTATTACCTACCTCCTTTTTATTAAGGATATGTATCTAATTCTCTT ATACC
RBS 17-F	AAGGAGGCACAACATATGAAAAAGAAAGTAGCTCTTATTACAA CA

RBS 17-R	CATATGTTGTGCCTCCTTTTATTAAGGATATGTATCTAATTCTCT TATACC
RBS 18-F	ATCAAGGAGGTTGGATTATGAAAAAGAAAGTAGCTCTTATTACA ACA
RBS 18-R	TAATCCAACCTCCTTGATTTTATTAAGGATATGTATCTAATTCTCT TATACC
RBS 19-F	AAAGGAGGTTATACGATGAAAAAGAAAGTAGCTCTTATTACAA CA
RBS 19-R	CATCGTATAACCTCCTTTTATTAAGGATATGTATCTAATTCTCTTAT ACC
RBS 20-F	AGGAGGTCATATTTATGAAAAAGAAAGTAGCTCTTATTACAACA
RBS 20-R	CATAAATATGACCTCCTTTTATTAAGGATATGTATCTAATTCTCTT ATACC
RBS 21-F	AGGAGGTTTATTTTATGAAAAAGAAAGTAGCTCTTATTACAACA
RBS 21-R	CATAAAATAAACCTCCTTTTATTAAGGATATGTATCTAATTCTCTT ATACC
RBS 22-F	AGGAGGTTTTTTTAAATGAAAAAGAAAGTAGCTCTTATTACAACA
RBS 22-R	CATTAAAAAACCTCCTTTTATTAAGGATATGTATCTAATTCTCTT ATACC

Supplementary Figure S1: Acrylamide levels in potato chips subjected to different treatments. (A) The control group; (B) The blanched group; (C) the BIAase treatment group; (D) the combined of blanching and BIAase treatment group.

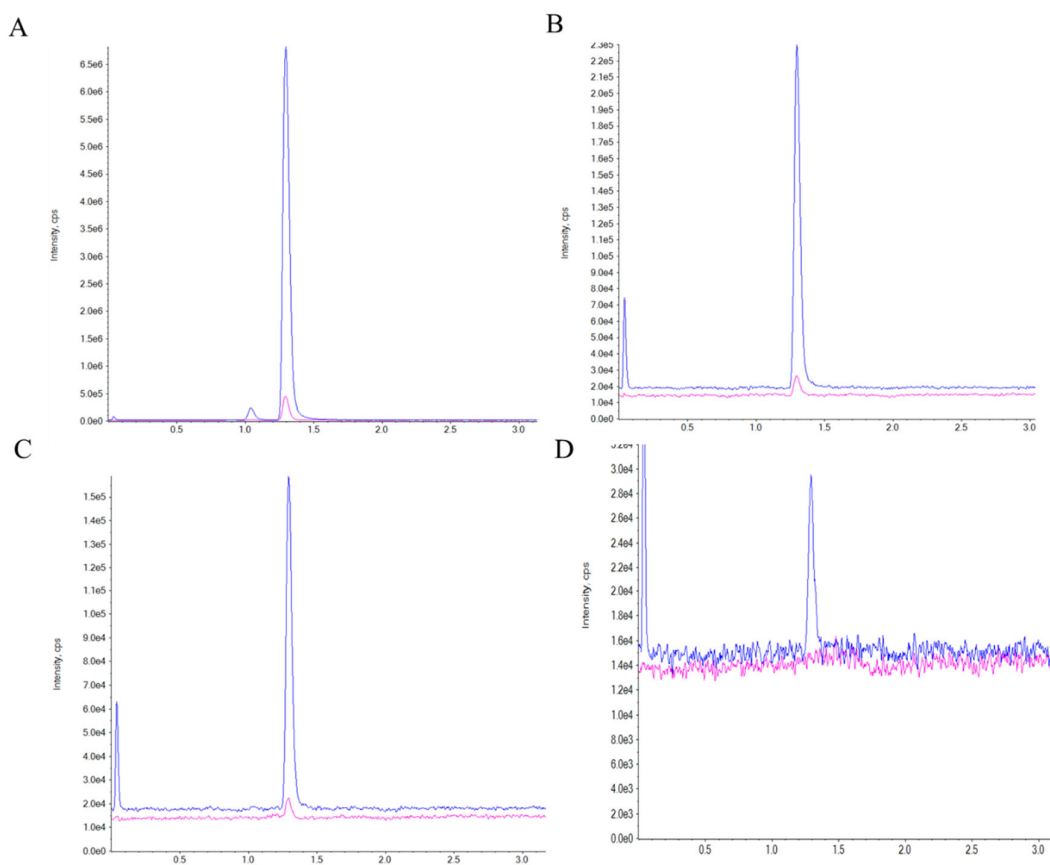


Figure S1