

Supplementary material

Table S1. Plasmids used in this study.

Plasmids	Description	Source
pCL1920	rep _{pSC101} Spc ^R P _{lac}	[1]
cl- <i>rhtA</i>	rep _{pSC101} Spc ^R P _{lac-lacO-rhtA-terminator} BBa_B1006- P _{lac-lacO-rfp}	This study
P _{cysJ-rfp}	rep _{pSC101} Spc ^R P _{cysJ} B0034- <i>rfp</i>	This study
P _{cysD-rfp}	rep _{pSC101} Spc ^R P _{cysD} B0034- <i>rfp</i>	This study
P _{cysJH-rfp}	rep _{pSC101} Spc ^R P _{cysJH} B0034- <i>rfp</i>	This study
cl-JAR	rep _{pSC101} Spc ^R P _{cysJ} B0034- <i>rhtA-terminator</i> BBa_B1006- P _{cysJ} B0034- <i>rfp</i>	This study
cl-DAR	rep _{pSC101} Spc ^R P _{cysD} B0034- <i>rhtA-terminator</i> BBa_B1006- P _{cysD} B0034- <i>rfp</i>	This study
cl-JHAR	rep _{pSC101} Spc ^R P _{cysJH} B0034- <i>rhtA-terminator</i> BBa_B1006- P _{cysJH} B0034- <i>rfp</i>	This study
cl-100AR	rep _{pSC101} Spc ^R P _{J23100} B0034- <i>rhtA-terminator</i> BBa_B1006- P _{J23100} B0034- <i>rfp</i>	This study
cl-101AR	rep _{pSC101} Spc ^R P _{J23101} B0034- <i>rhtA-terminator</i> BBa_B1006- P _{J23101} B0034- <i>rfp</i>	This study
cl-109AR	rep _{pSC101} Spc ^R P _{J23109} B0034- <i>rhtA-terminator</i> BBa_B1006- P _{J23109} B0034- <i>rfp</i>	This study
cl-105AR	rep _{pSC101} Spc ^R P _{J23105} B0034- <i>rhtA-terminator</i> BBa_B1006- P _{J23105} B0034- <i>rfp</i>	This study
cl-110AR	rep _{pSC101} Spc ^R P _{J23110} B0034- <i>rhtA-terminator</i> BBa_B1006- P _{J23110} B0034- <i>rfp</i>	This study
cl-116AR	rep _{pSC101} Spc ^R P _{J23116} B0034- <i>rhtA-terminator</i> BBa_B1006- P _{J23116} B0034- <i>rfp</i>	This study
cl-JB	rep _{pSC101} Spc ^R P _{cysJ} B0034- <i>rhtB</i>	This study
cl-JC	rep _{pSC101} Spc ^R P _{cysJ} B0034- <i>rhtC</i>	This study

Table S2. Strains used in this study.

Strains	Description	Source
<i>E. coli</i> DH5 α	F- supE44 Δ lacU169 (ϕ 80 <i>lacZ</i> Δ M15) <i>hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i>	Invitrogen
<i>E. coli</i> K-12 MG1655	K12 F- <i>lambda- ilvG- rfb-50 rph-1</i>	Invitrogen
Tm	L-threonine production host cell	FuFeng Group
Tm-cl	Tm carrying pCL1920	This study
Tm-AR	Tm carrying <i>cl-rhtA</i>	This study
MG-cl	<i>E. coli</i> K-12 MG1655 carrying pCL1920	This study
MG-P _{cysJ}	<i>E. coli</i> K-12 MG1655 carrying <i>cl-J</i>	This study
MG-P _{cysD}	<i>E. coli</i> K-12 MG1655 carrying <i>cl-D</i>	This study
MG-P _{cysJH}	<i>E. coli</i> K-12 MG1655 carrying <i>cl-JH</i>	This study
Tm-P _{cysJ}	Tm carrying <i>cl-J</i>	This study
Tm-P _{cysD}	Tm carrying <i>cl-D</i>	This study
Tm-P _{cysJH}	Tm carrying <i>cl-JH</i>	This study
Tm-JAR	Tm carrying <i>cl-JAR</i>	This study
Tm-DAR	Tm carrying <i>cl-DAR</i>	This study
Tm-JHAR	Tm carrying <i>cl-JHAR</i>	This study
Tm-100AR	Tm carrying <i>cl-100AR</i>	This study
Tm-101AR	Tm carrying <i>cl-101AR</i>	This study
Tm-109AR	Tm carrying <i>cl-109AR</i>	This study
Tm-105AR	Tm carrying <i>cl-105AR</i>	This study
Tm-110AR	Tm carrying <i>cl-110AR</i>	This study
Tm-116AR	Tm carrying <i>cl-116AR</i>	This study
Tm-JB	Tm carrying <i>cl-JB</i>	This study
Tm-JC	Tm carrying <i>cl-JC</i>	This study

Table S3. Primers used in this study.

Primer name	Sequences (5'-3')
cl- <i>rhtA</i> reconstruction	
cl-F1	agcaaaataaaagaattagacattaattaaagcttgcatgcctgcaggt
cl-R1	gaccggcattttacgtaatgaaccaggcattggcgtaatcatggtcatact
cl- <i>rhtA</i> -F	atgcctgggttcattacgtaaaatgc
cl- <i>rhtA</i> -R	gcaagctttaattaatgtctaattc
P _{cysJ-rfp} reconstruction	
cl-F2	agcttgcatgcctgcaggt
cl-R2	gcctgggggtgcctaatgagtga
cysJ-F	gagttagctcactcattaggcaccagcggtgcgcaaaatcgctgatt
cysJ-R	ctagtatttctccttttcttagagtaagcaaagctgtttctgcg
RFP-F1	tctagagaaaggagagaaatactagatggcttctccgaagacgt
RFP-R1	ctctagagtcgacctgcaggcatgcaagctttaagcgtagtttctgcgtttgc
P _{cysD-rfp} reconstruction	
cl-F2	agcttgcatgcctgcaggt
cl-R2	gcctgggggtgcctaatgagtga
cysD-F	gagttagctcactcattaggcaccagcggtgccttaagcactttttg
cysD-R	ctagtatttctccttttcttagaatgtttcgactataggagcgt
RFP-F1	tctagagaaaggagagaaatactagatggcttctccgaagacgt
RFP-R1	ctctagagtcgacctgcaggcatgcaagctttaagcgtagtttctgcgtttgc
P _{cysJH-rfp} reconstruction	
cl-F2	agcttgcatgcctgcaggt
cl-R2	gcctgggggtgcctaatgagtga
cysJ-F	gagttagctcactcattaggcaccagcggtgcgcaaaatcgctgatt
cysJH-R	gtaagcaaagctgtttctgcg
cysJH-F	tgacagggcgcagaaacagctttgcttaccggaaatcctggcgtcgttgat
cysH-R	ctagtatttctccttttcttagatgccttgctgatgcgac
RFP-F1	tctagagaaaggagagaaatactagatggcttctccgaagacgt
RFP-R1	ctctagagtcgacctgcaggcatgcaagctttaagcgtagtttctgcgtttgc
cl-JAR reconstruction	
cl-JA-F	tctagagaaaggagagaaatactagatgcctgggttcattacgtaaaatgc
cysJ-R	ctagtatttctccttttcttagagtaagcaaagctgtttctgcg
cysJ-F2	ataaaagaattagacattaattaaacccccgccctgacagggcgggggttttttgggtgcgcaaaatcgctgattta

cl-JA-R	ttaattaatgtctaattctttattttgc
RFP-F2	tctagagaaaggagagaaatactagatggcttctccgaagcgt
RFP-R1	ctctagagtcgacctgcaggcatgcaagctttaagcgtagtttcgctgttgc
cl-F2	agcttgcctgcctgcaggt
cl-DAR reconstruction	
cysD-R	ctagtatttctcctcttctctagaatgttcgactataggagcgt
cl-JA-F	tctagagaaaggagagaaatactagatgcctggttcattacgtaaaatgc
cl-JA-R	ttaattaatgtctaattctttattttgc
cysD-F2	attagacattaataaaaaaaaaaacccgccctgacagggcggggttttttcgggtgccttaagcacttttg
RFP-F2	tctagagaaaggagagaaatactagatggcttctccgaagcgt
RFP-R1	ctctagagtcgacctgcaggcatgcaagctttaagcgtagtttcgctgttgc
cl-F2	agcttgcctgcctgcaggt
cl-JHAR reconstruction	
cysH-R	ctagtatttctcctcttctctagatgccttgctgatgcgac
cl-JA-F	tctagagaaaggagagaaatactagatgcctggttcattacgtaaaatgc
cl-JA-R	ttaattaatgtctaattctttattttgc
cysJ-F2	ataaaagaattagacattaataaaaaaaaaaacccgccctgacagggcggggttttttggcgcaaaatcgctgattta
cysH-R	ctagtatttctcctcttctctagatgccttgctgatgcgac
RFP-F2	tctagagaaaggagagaaatactagatggcttctccgaagcgt
RFP-R1	ctctagagtcgacctgcaggcatgcaagctttaagcgtagtttcgctgttgc
cl-F2	agcttgcctgcctgcaggt
cl-100AR reconstruction	
100AR-R	gagctagcactgtacctaggactgagctagccgtcaagcctggggtgcctaagagtga
100AR-F	ggctagctcagtcctaggtacagtgtagctctagagaaaggagagaaatactag
cl-rhtA-R4	ctaggactgagctagccgtcaaaaaaaaaaacccgccctgacagggcggggttttttttaattaatgtctaattc
100AR-F	ggctagctcagtcctaggtacagtgtagctctagagaaaggagagaaatactag
cl-101AR reconstruction	
101AR-R	gctagcataatacctaggactgagctagctgtaaagcctggggtgcctaagagtga
101AR-F	agctagctcagtcctaggtattatgctagctctagagaaaggagagaaatactag
cl-rhtA-R5	ctaggactgagctagctgtaaaaaaaaaaacccgccctgacagggcggggttttttttaattaatgtctaattc
101AR-F	agctagctcagtcctaggtattatgctagctctagagaaaggagagaaatactag
cl-109AR reconstruction	
109AR-R	gctagcacagtcctaggactgagctagctgtaaagcctggggtgcctaagagtga
109AR-F	agctagctcagtcctaggactgtgctagctctagagaaaggagagaaatactag

cl-rhtA-R6	ctaggactgagctagctgtaaaaaaaaaaaccgccctgtcagggcgggggttttttttaattaatgtctaattc
109AR-F	agctagctcagtcctaggactgtgctagctctagagaaaggagagaaatactag
cl-105AR reconstruction	
105AR-F	tcagtcctaggtactatgctagctctagagaaaggagagaaatactagatgcctgggttc
105AR-R	gagctagcatagtagctaggactgagctagccgtaaagcctggggtgcctaatagtga
cl-rhtA-F	atgcctgggttcattacgtaaaatgc
cl-rhtA-R2	ctaggactgagctagccgtaaaaaaaaaaaccgccctgtcagggcgggggttttttttaattaatgtctaattc
cl-rhtA-R3	ctagtatttctccttttcttagagctagcatagtagctaggactgagctagccgta
RFP-F2	tctagagaaaggagagaaatactagatggcttctccgaagcgt
cl-110AR reconstruction	
110AR-F	ggctagctcagtcctaggtacaatgctagctctagagaaaggagagaaatactag
110AR-F2	tcagtcctaggtagcaatgctagctctagagaaaggagagaaatactagatgcctgggttc
cl-rhtA-F	atgcctgggttcattacgtaaaatgc
110AR-R	gctagcattgtacctaggactgagctagccgtaaagcctggggtgcctaatagtga
cl-rhtA-R2	ctaggactgagctagccgtaaaaaaaaaaaccgccctgtcagggcgggggttttttttaattaatgtctaattc
cl-116AR reconstruction	
cl-rhtA-F	atgcctgggttcattacgtaaaatgc
116AR-F	tcagtcctaggactatgctagctctagagaaaggagagaaatactagatgcctgggttc
116AR-F2	tgacagctagctcagtcctaggactatgctagctctagagaaaggagagaaatactag
116AR-R	gagctagcatagtagctaggactgagctagctgtcaagcctggggtgcctaatagtga
cl-rhtA-R3	agtcctaggactgagctagctgtcaaaaaaaaaaaccgccctgtcagggcgggggttttttttaattaatgtctaattc
116AR-F2	tgacagctagctcagtcctaggactatgctagctctagagaaaggagagaaatactag
cl-JB reconstruction	
cl-F2	gcgctgtagcatcggcgaggcatgcgtgaagcttgcatgcctgcaggt
cl-rhtB-R	tcacgcatgcctcgccgatgct
cl-JC reconstruction	
cl-F3	tttggcattcatttgatttttcgcggtgaagcttgcatgcctgcaggtc
cl-rhtC-R	tcaccgcgaaataatcaaatgaatgcc

Table S4. Analysis of the initial sensing times and L-threonine concentrations of the three promoters in the L-threonine-producing strains.

	Tm-P_{cysJ}	Tm-P_{cysD}	Tm-P_{cysJH}
3 h	0.05 g/L	— —	0.05 g/L
5 h	— —	0.25 g/L	— —

1. Lerner, C.G. and Inouye, M. (1990) Low copy number plasmids for regulated low-level expression of cloned genes in *Escherichia coli* with blue/white insert screening capability. *Nucleic acids research* 18, 4631. 10.1093/nar/18.15.4631.