

Supplementary information to

Developing a riboswitch-mediated regulatory system for metabolic flux control in thermophilic *Bacillus methanolicus*

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Supplementary Table S1. List of primers used in this study.

Primer name	Sequence	Function
VJFW	TCTAATCCTTCTAAAAAATATAATTTAGAA AACTAAG	sequencing primer for pTH1mp: FW
VJRW	GGTGCGGGCCTCTTCGCTATTACG	sequencing primer for pTH1mp; RV
sfGFP- pTH1mp_FW	TAAATAGGAGGTAGTACATATGCGTAAAG GCGAAGAGC	pTH1mp- <i>sfGFP</i> was constructed by amplifying <i>sfGFP</i> from sfGFP-pBAD with primers sfGFP-pTH1mp_FW and sfGFP-pTH1mp_RW, and joining the resulting PCR product with XbaI and AflIII digested pTHmp by means of the isothermal DNA assembly method
sfGFP- pTH1mp_RW	GACCTATGGCGGGTACCATATCATTTGTAC AGTTCATCCATACC	pTH1mp- <i>sfGFP</i> was constructed by amplifying <i>sfGFP</i> from sfGFP-pBAD with primers sfGFP-pTH1mp_FW and sfGFP-pTH1mp_RW, and joining the resulting PCR product with XbaI and AflIII digested pTHmp by means of the isothermal DNA assembly method
PSGF	ATGCGTAAAGGCGAAGAGCTGTT	Forward primer for amplification of vector backbone pTHmp- <i>sfGFP</i>
PSGR	AAAAGTAGTTTAAATGCTAATCTGGATGTT TGTC	Reverse primer for amplification of vector backbone pTHmp- <i>sfGFP</i>
LRIF	GATTAGCATTTAACTAGTTTTGTAGTTGA ATAATGATTCTCATCAG	Forward primer for amplification of lysine riboswitches derived from <i>B. methanolicus</i> to create plasmid pTH1mplrBM- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
LRIR	AGCTCTTCGCCTTTACGCATATTTCTTCTCC TCTCATGTT	Reverse primer for amplification of lysine riboswitches derived from <i>B. methanolicus</i> to create plasmid pTH1mplrBM- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM01	GTAAACAATTACATAAATAGGAGGTAGT	Forward primer for amplification of vector backbone pTHmp- <i>sfGFP</i> for constructs pTH1p05- <i>sfGFP</i> , pTH1p12- <i>sfGFP</i> and pTH1p18- <i>sfGFP</i>
PROM02	AATCTTTTGAAAATAAGCGAGAGAA	Reverse primer for amplification of vector backbone pTHmp- <i>sfGFP</i> for constructs pTH1p05- <i>sfGFP</i> , pTH1p12- <i>sfGFP</i> and pTH1p18- <i>sfGFP</i>
PROM09	TCGCTTATTTTCAAAAGATTTTAAAGCTATA AGCTCTTT	Forward primer for amplification of promoter (p04) derived from <i>B. methanolicus</i> to create plasmid pTH1p04- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM10	CTATTTATGTAATTGTTTACATATTATTGCA TAAAATG	Reverse primer for amplification of promoter (p04) derived from <i>B. methanolicus</i> to create plasmid pTH1p04- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM11	TCGCTTATTTTCAAAAGATTTAAAAAATAT TGAAGTTGTCTTA	Forward primer for amplification of promoter (p05) derived from <i>B. methanolicus</i> to create plasmid pTH1p05- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM12	CTATTTATGTAATTGTTTACAACTATTATTT TAGTTTTCAAAAAT	Reverse primer for amplification of promoter (p05) derived from <i>B. methanolicus</i> to create plasmid pTH1p05- <i>sfGFP</i> through assembly with PCR-amplified vector backbone

PROM23	TCGCTTATTTTCAAAAGATTAAAATTTGAA ATGGCAGCCACATTTT	Forward primer for amplification of promoter (p11) derived from <i>B. methanolicus</i> to create plasmid pTH1p11- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM24	CTATTTATGTAATTGTTTACATTTATCATTA TATCCTTTCTTAACTTTTCT	Reverse primer for amplification of promoter (p11) derived from <i>B. methanolicus</i> to create plasmid pTH1p11- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM25	TCGCTTATTTTCAAAAGATTAAATCGCATTTT CTTCCATTA	Forward primer for amplification of promoter (p12) derived from <i>B. methanolicus</i> to create plasmid pTH1p12- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM26	CTATTTATGTAATTGTTTACCTTTCAAATAT AGACCTTTTCAC	Reverse primer for amplification of promoter (p12) derived from <i>B. methanolicus</i> to create plasmid pTH1p12- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM31	TCGCTTATTTTCAAAAGATTATACTATCATA AAATTTTCGGTTTTTTCAT	Forward primer for amplification of promoter (p15) derived from <i>B. methanolicus</i> to create plasmid pTH1p15- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM32	CTATTTATGTAATTGTTTACTTTTAAATTAT ATCACTACAAAAAGAGAGTACAA	Reverse primer for amplification of promoter (p15) derived from <i>B. methanolicus</i> to create plasmid pTH1p15- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM35	TCGCTTATTTTCAAAAGATTGGAAGTTTTTA TCTATCTACAACCTGAA	Forward primer for amplification of promoter (p17) derived from <i>B. methanolicus</i> to create plasmid pTH1p17- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM36	CTATTTATGTAATTGTTTACAATCATTATTG TATATAAATTAAGTATTTTTCTATTAC	Reverse primer for amplification of promoter (p17) derived from <i>B. methanolicus</i> to create plasmid pTH1p17- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM37	TCGCTTATTTTCAAAAGATTGTAAACCTTC AACTGAATAAGTTCGGTTATATCC	Forward primer for amplification of promoter (p18) derived from <i>B. methanolicus</i> to create plasmid pTH1p18- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM38	CTATTTATGTAATTGTTTACTGTATTATTTTA ACATATTGATGGGAAGCTGGCCTGA	Reverse primer for amplification of promoter (p18) derived from <i>B. methanolicus</i> to create plasmid pTH1p18- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM39	TCGCTTATTTTCAAAAGATTAAAGTTCTAG TTAATAAATGAGTTTAAAT	Forward primer for amplification of promoter (p19) derived from <i>B. methanolicus</i> to create plasmid pTH1p19- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM40	CTATTTATGTAATTGTTTACACTATAAAATA TGTTTCTAAAAAGAAATC	Reverse primer for amplification of promoter (p19) derived from <i>B. methanolicus</i> to create plasmid pTH1p19- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM41	TCGCTTATTTTCAAAAGATTAAATAGATTAC GGGAACCGTCAT	Forward primer for amplification of promoter (p20) derived from <i>B. methanolicus</i> to create plasmid pTH1p20- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
PROM42	CTATTTATGTAATTGTTTACCGTCCTAATAT TTCGACAAAAGGAG	Reverse primer for amplification of promoter (p20) derived from <i>B. methanolicus</i> to create plasmid pTH1p20- <i>sfGFP</i> through assembly with PCR-amplified vector backbone
LR11	GTAGTTGAATAATGATTCCTCATCAG	Forward primer for amplification of vector backbone pTHmplrBM- <i>sfGFP</i> for creation of constructs pTH1p01lrBM- <i>sfGFP</i> , pTH1p05lrBM- <i>sfGFP</i> , pTH1p12lrBM- <i>sfGFP</i> and pTH1p18lrBM- <i>sfGFP</i> . PROM02 used as reverse primer
PROM03	TCGCTTATTTTCAAAAGATTATTCTTTCC CTTTAAACTT	Forward primer for amplification of promoter p01 derived from <i>B. methanolicus</i> to create plasmid pTH1p01lrBM- <i>sfGFP</i> through assembly with PCR-amplified vector backbone. LR12 used as reverse primer

LR12	AGGAATCATTATTCAACTACACTGTTAATA TACTATCTATCA	Reverse primer for amplification of promoter p01 derived from <i>B. methanolicus</i> to create plasmid pTH1p01lrBM- <i>sfGFP</i> through assembly with PCR-amplified vector backbone. PROM03 used as forward primer
LR20	AGGAATCATTATTCAACTACAACTATTATT TTAGTTTTCAAAAAT	Reverse primer for amplification of promoter p01 derived from <i>B. methanolicus</i> to create plasmid pTH1p05lrBM- <i>sfGFP</i> through assembly with PCR-amplified vector backbone. PROM11 used as forward primer
LR14	AGGAATCATTATTCAACTACCTTTCAAATA TAGACCTTTTCAC	Reverse primer for amplification of promoter p01 derived from <i>B. methanolicus</i> to create plasmid pTH1p12lrBM- <i>sfGFP</i> through assembly with PCR-amplified vector backbone. PROM25 used as forward primer
LR15	AGGAATCATTATTCAACTACTGTATTATTTT AACATATTGATGGGAAGCTGGCCTGA	Reverse primer for amplification of promoter p01 derived from <i>B. methanolicus</i> to create plasmid pTH1p18lrBM- <i>sfGFP</i> through assembly with PCR-amplified vector backbone. PROM37 used as forward primer
PGF2	GTAAACAATTACATAAATAGGAGGTAGT	Forward primer for amplification of vector backbone pTHmp- <i>sfGFP</i> for creation of construct pTH1mpApr- <i>sfGFP</i> . PSGR used as reverse primer
APR01	GATTAGCATTTAACTAGTTTTGGAAACGA ATCAATTAAATA	Forward primer for amplification of <i>B. subtilis</i> -derived <i>pbuE</i> riboswitch to create plasmid pTH1mpApr- <i>sfGFP</i> through assembly with PCR-amplified vector backbone.
APR02	CTATTTATGTAATTGTTTACTTTACTTAAAT GTTTTGATAAAT	Reverse primer for amplification of <i>B. subtilis</i> -derived <i>pbuE</i> riboswitch to create plasmid pTH1mpApr- <i>sfGFP</i> through assembly with PCR-amplified vector backbone.
APR03	ACTGTTAATATACTATCTATCA	Reverse primer for amplification of promoter p01 derived from <i>B. methanolicus</i> to create plasmid pTH1p01Apr- <i>sfGFP</i> through assembly with PCR-amplified <i>B. subtilis</i> -derived <i>pbuE</i> riboswitch and vector backbone. PROM03 used as forward primer.
APR04	ATAGATAGTATATTAACAGTGGAAACGAA TCAATTAAATA	Forward primer for amplification of <i>B. subtilis</i> -derived <i>pbuE</i> riboswitch to create plasmid pTH1p01Apr- <i>sfGFP</i> through assembly with PCR-amplified promoter p01 derived from <i>B. methanolicus</i> and vector backbone. APR02 used as reverse primer
APR05	AAATAGCTATTAAATTTTGTATAACCTC	Forward primer for site directed mutagenesis to introduce mutation in P1 region of <i>B. subtilis</i> -derived <i>pbuE</i> riboswitch. pTH1mpApr- <i>sfGFP</i> used as template.
APR06	GAGGTTATACAAAATTTAATAGCTATTT	Reverse primer for site directed mutagenesis to introduce mutation in P1 region of <i>B. subtilis</i> -derived <i>pbuE</i> riboswitch. pTH1mpApr- <i>sfGFP</i> used as template

Supplementary Table S2. Relative mean sfGFP fluorescence in pellets of *B. methanolicus*, *B. subtilis*, or *E. coli* recombinant strains carrying empty vector (pTH1mp), vector with sfGFP gene under the control of the P_{mdh} (pTH1mp-sfGFP) and vector with sfGFP gene under the control of the P_{mdh} and lysine riboswitch (pTH1mplrBM-sfGFP). OD₆₀₀ normalised fluorescence from strains carrying the empty vector and vector pTH1mp-sfGFP are used as controls. OD₆₀₀ normalised sfGFP fluorescence from cultures without or with 20 mM lysine added is shown (raw data for Figure 4).

Strain	Plasmid	sfGFP fluorescence [FU/OD ₆₀₀]	
		0 mM L-lysine	20 mM L-lysine
<i>Bacillus methanolicus</i>	pTH1mp	509±40	459±36
	pTH1mp-sfGFP	25387±338	19109±541
	pTH1mplrBM-sfGFP	7157±342	1054±79
<i>Bacillus subtilis</i>	pTH1mp	87±11	78±11
	pTH1mp-sfGFP	51698±1169	65639±2988
	pTH1mplrBM-sfGFP	3606±45	1442±33
<i>Escherichia coli</i>	pTH1mp	250±19	248±25
	pTH1mp-sfGFP	2294092±44389	2375738±85985
	pTH1mplrBM-sfGFP	124424±3531	82622±1396

Supplementary Table S3. Effect of different amino acids on the activity of the lysine riboswitch. OD₆₀₀ normalised sfGFP fluorescence from cultures without addition of amino acid or with 10 mM L-lysine, cadaverine, L-methionine, L-ornithine or L-proline is shown. The standard deviation of technical triplicates is shown.

Condition	MGA3(pTH1mp-sfGFP)		MGA3(pTH1mplrBM-sfGFP)	
	sfGFP fluorescence [FU/OD ₆₀₀]	Ratio to control condition	sfGFP fluorescence [FU/OD ₆₀₀]	Ratio to control condition
No supplementation (control)	32009±675	1.00	6492±258	1.00
10 mM L-lysine	27697±652	0.87	876±38	0.13
10 mM cadaverine	38275±879	1.20	6922±566	1.07
10 mM L-methionine	26893±559	0.84	4521±129	0.70
10 mM L-ornithine	31612±823	0.99	8321±173	1.28
10 mM L-proline	35608±486	1.11	7209±265	1.11

Supplementary Table S4. L-lysine final titers for MGA3 and M168-20 strains carrying different plasmids for controlled sfGFP production. The means of triplicates with standard deviations are shown.

Strain	Plasmid	L-lysine titer [mg L ⁻¹]
<i>Bacillus methanolicus</i> MGA3	pTH1mp	0.2±0.0
	pTH1mp-sfGFP	0.3±0.0
	pTH1mplrBM-sfGFP	0.3±0.0
<i>Bacillus methanolicus</i> M160-20	pTH1mp	20.1±2.6
	pTH1mp-sfGFP	33.2±1.3
	pTH1mplrBM-sfGFP	37.2±3.0