

## Construction of a novel anaerobic pathway in *Escherichia coli* for propionate production

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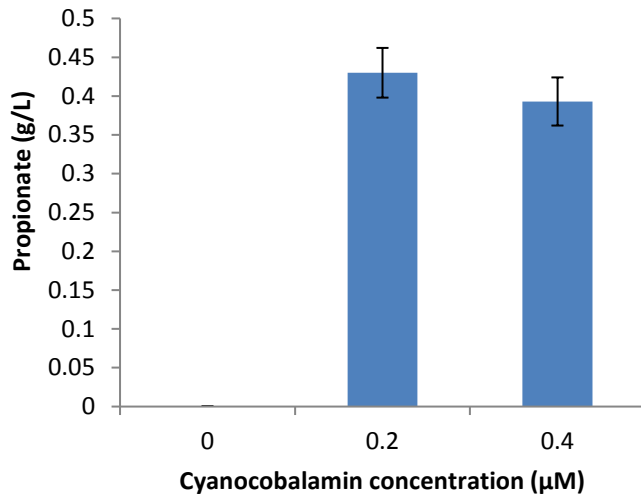


Figure S1. propionate production of strain T110 (plac-Sbm) with various concentration of cyanocobalamin. Propionate production was dependent on supplementation of cyanocobalamin, and titer of propionate reached peak value at concentrations of 0.2  $\mu$ M. Strains were cultivated micro-aerobically in the dark at 37°C, until the cultures reached at an OD600 of 0.5. Then culture was added IPTG (1 mM) with appropriate concentration and cultivated anaerobically in dark for 72 h. All experiments were performed in triplicate.

Table S1. propionate production of strain T110 (plac-Sbm) with various concentration of cyanocobalamin

g/L	propionate	propionate	propionate
cyanocobalamin ( $\mu$ M)	0	0.2	0.4
average value	0	0.430	0.393
standard deviation	0	0.032	0.031

Table S2. Propionate and fermentation products of engineered strains with sbm operon under different promoters. Strains in glass screw-cap tubes with NBS (20 g/L glucose) media were cultivated micro-aerobically in the dark at 37°C, until the cultures reached at an O.D600 of 0.5. Then added IPTG with appropriate concentration and cultivated anaerobically in the dark for 72 h. All experiments were performed in triplicate.

titer (g/L)	Succinate	Acetate	propionate
T110 (pbba-Sbm)	9.30±0.18	1.39±0.12	2.34±0.08
T110 (ptac-Sbm)	11.21±0.12	1.68±0.11	0.66±0.01
T110 (plac-Sbm)	9.99±0.12	1.56±0.09	0.43±0.07

Table S3. The effects of expression of *M. extorquens* AM1 Methylmalonyl CoA mutase on propionate production. Strains were cultivated micro-aerobically in glass screw-cap tubes in dark at 37°C, until the cultures reached at an OD600 of 0.5. Then IPTG with appropriate concentration was added and cultivated anaerobically in the dark for 72 h at 30°C. All experiments were

performed in triplicate.

titer (g/L)	succinate	acetate	propionate
T110 (pbba-Sbm)	9.30±0.18	1.39±0.12	2.34±0.08
T110(pbba-Sbm, plac-ABb)- 0.02mM IPTG	9.76±0.19	0.64±0.17	2.39±0.21
T110(pbba-Sbm, plac-ABb)- 0.08mM IPTG	9.26±0.13	0.47±0.07	2.51±0.18
T110(pbba-Sbm, plac-ABb)- 0.2mM IPTG	10.32±0.11	0.48±0.08	2.61±0.20
T110(pbba-Sbm, plac-ABb)- 1mM IPTG	9.98±0.13	0	2.26±0.19
T110(pbba-Sbm,pbba-ABb)	6.09±0.08	0.53±0.06	2.72±0.12

Table S4. PCR primers used in this work

Primers	Sequence (5' → 3')	Sources
c-lac-pet28a	ACCAGGTCTCACGGTCCGCTCATGAATTAATTCTTAGAA AAACTCATCGA; ACCAGGTCTCAGAGCACCGGATCTCGACCG	This study
c-lac-lac	ACCAGGTCTCAGCTCCTTGCGGCAAACCTTACATTAATT GC; ACCAGGTCTCATTAGGATGGCCTCCTTTGAATTCAATTG TTATCCGC	This study
c-lac-sbm	ACCAGGTCTCACTAATGTCTAACGTGCAGGAGTGGC; ACCAGGTCTCAATGACGAGTCATTAACCCAGCATCGAG C	This study
c-lac-dbl term	ACCAGGTCTCATCATCTCCAGGCATCAAATAAACGAA AGGC; ACCAGGTCTCAACCGCAGCCGAACGCCCTAG	This study
c-tac-sbm1	CACACCAGGTCTCAAATCCCTGCGAACGAAGGAGTAA AAATGG; CACACCAGGTCTCACGAGCCGATGATTAATTGTCAAAA CGCAATTAATGTAAGTTTGCCGCC	This study
c-tac-sbm2	CACACCAGGTCTCACTCGTATAATGTGTGGAATTGTGAT TGAATTCAAAGGAGGCCATCC; CACACCAGGTCTCAGATTAATGACCAACGAAATTAGGT TTACG	This study
c-bba-sbm1	CACACCAGGTCTCAAATCCCTGCGAACGAAGGAGTAA AAATGG; CACACCAGGTCTCATAGGACTGAGCTAGCTAACGCAAT TAATGTAAGTTTGCCGCC	This study
c-bba-sbm2	CACACCAGGTCTCACCTAGGTATTATGCTAGCATTGAAT TCAAAGGAGGCCATCC; CACACCAGGTCTCAGATTAATGACCAACGAAATTAGGT TTACG	This study
c-pACYC184	TGAGCCGTCTGCTCTCCAGGCATCAAATAAACGAAAG	This study

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	GCTCATCGTGCGTCTGACGAAATTCCTTTTTAACGTTCA	
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c-mcmA	CGTTAAAAAGGAATTCGTCAGACGCACGATGAGCTCC	This study
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	GCGACAACCGACTTTACTGGAAAAGCGGACTACTCGG	
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c-mcmB	GTCCGCTTTTCCAGTAAAGTCGGTTGTGCGCCTGCCC;	This study
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Plasmid and sequence data  
 plac-Sbm



plac-Sbm

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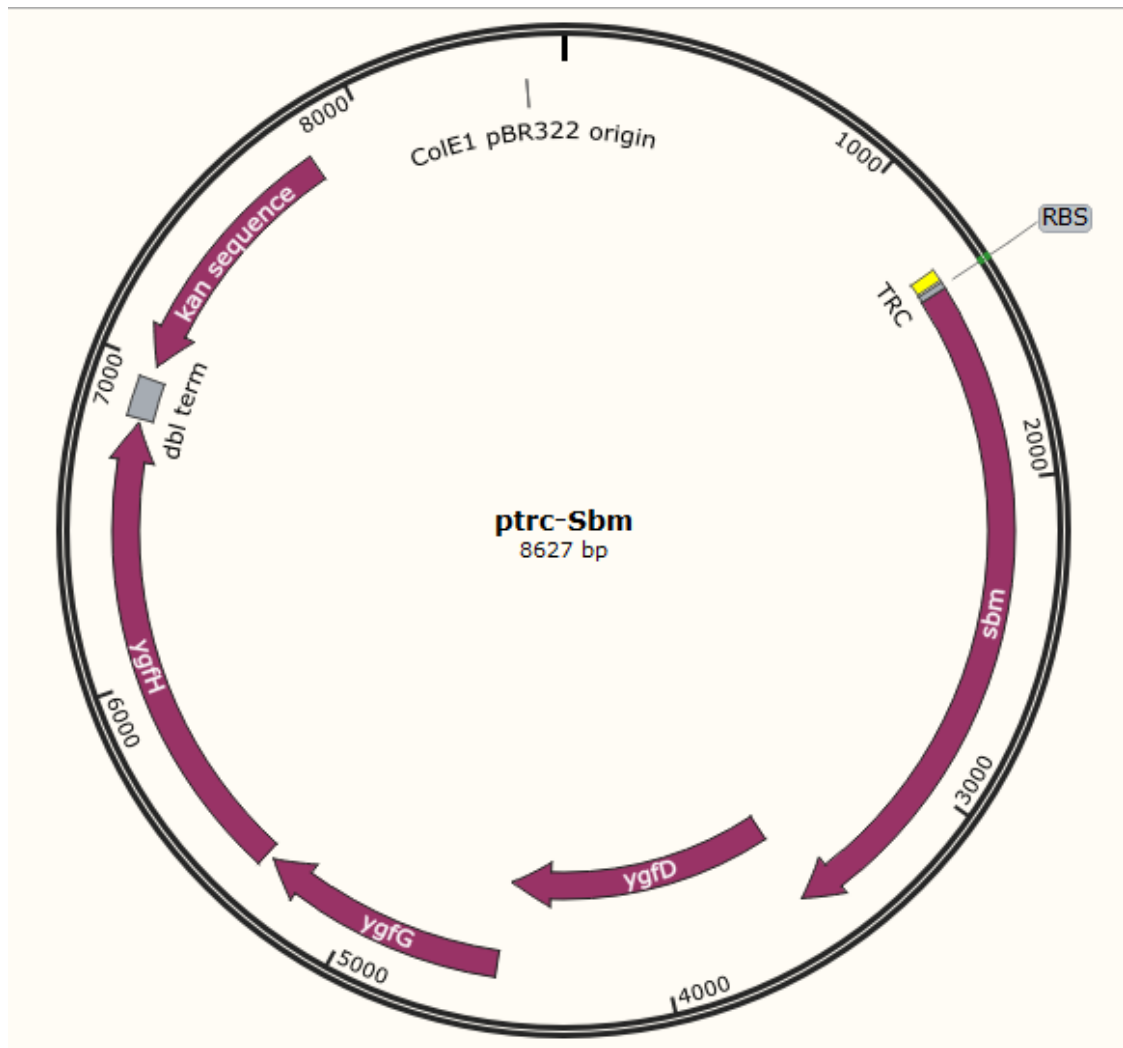
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Ptc-Sbm



Ptc-Sbm

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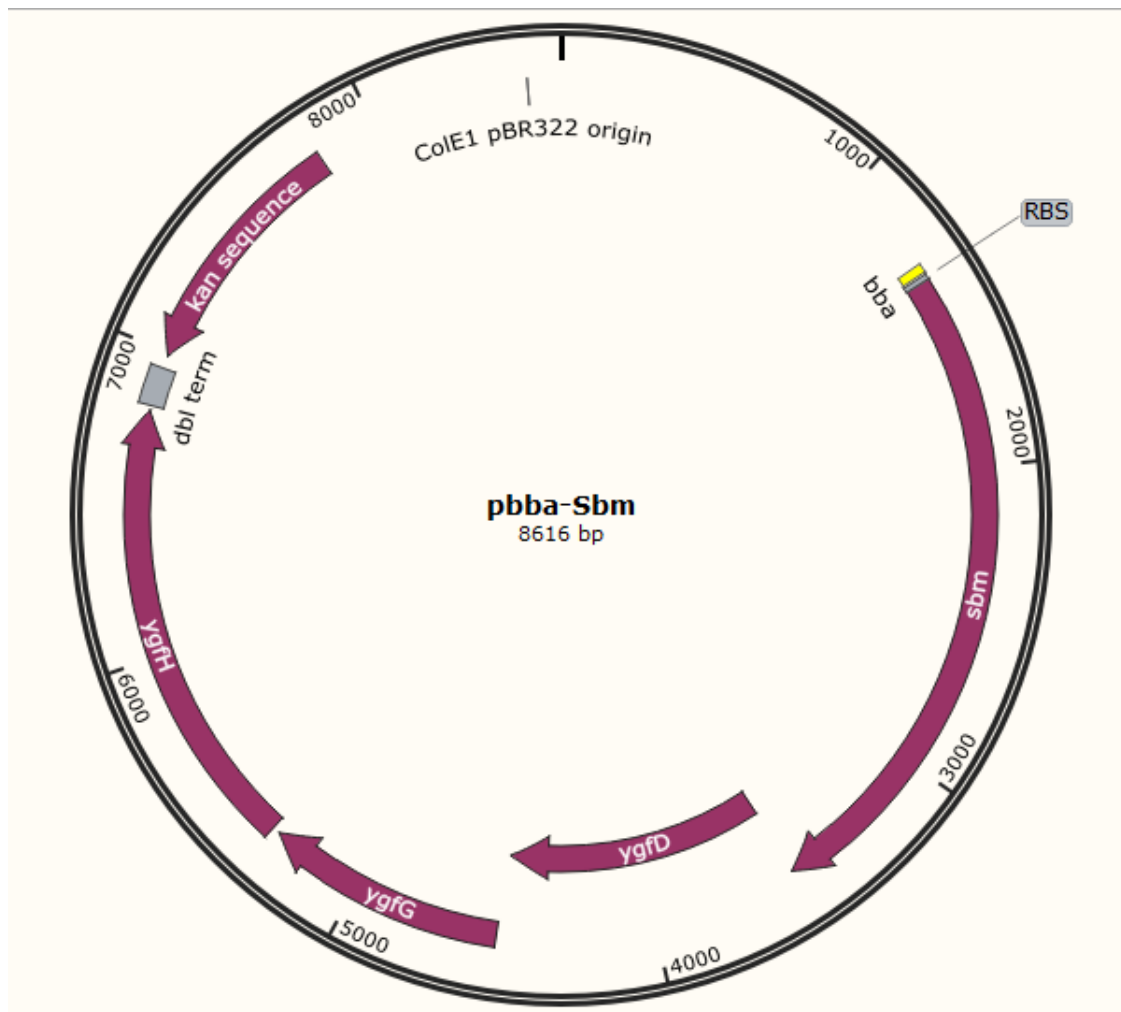
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