Supplementary Information

Discovery of a super-strong promoter enables efficient production of heterologous proteins in cyanobacteria

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Table of Contents

I. **Table S1**. Transcription factor binding sites located in upstream of the initiation codon of *cpcB* gene.

II. **Table S2**. Strains and plasmids used in this study.

III. **Table S3.** Primers used in this study.

IV. **Fig. S1.** *Synechocystis* codon optimized version of *ter* gene from *T. denticola*.

V. **Fig. S2.** RT-PCR confirmation of transcription of *ter* gene from P_{cpc374} and P_{cpc560}.

VI. **Fig. S3.** Schematic diagram of construction of expression vectors.

Table S1 Transcription factor binding sites located in upstream of the initiation codon of *cpcB* gene.

TFBS (Species)	Start Position	End Position	Score	Sequence
igB (N14) | *Bacillus subtilis* (strain 168)	381	412	13.3	CGTGTTTCTCCCTGGATTTATTTAGGTAATAT
H-NS | *Escherichia coli* (strain K12)	393	402	5.56	TGGATTTATT
Crp | *Escherichia coli* (strain K12)	402	423	6.52	TTAGGTAATATCTCTCATAAAT
YhiX | *Escherichia coli* (strain K12)	402	419	6.29	TTAGGTAATATCTCTCAT
SigE (-35) | *Bacillus subtilis* (strain 168)	405	415	3.42	GGTAATATCTC
NarL | *Pseudomonas aeruginosa* (strain ATCC 15692 / PAO1)	410	416	5.28	TATCTCT
PvdS | *Pseudomonas aeruginosa* (strain ATCC 15692 / PAO1)	417	425	6.22	CATAAATCC
Fur | *Pseudomonas aeruginosa* (strain ATCC 15692 / PAO1)	443	461	8.73	GTTAATGGAGATCAGTAAC
IHF | *Escherichia coli* (strain K12)	473	488	5.95	GGTCATTACTTTGGAC
PucR | *Bacillus subtilis* (strain 168)	478	488	3.59	TTACTTTGGAC
SigB (-10) | *Bacillus subtilis* (strain 168)	503	514	5.78	CGGGGGAATTGT
SigH (-10) | *Bacillus subtilis* (strain 168)	505	514	7.15	GGGGAATTGT
OxyR | *Escherichia coli* (strain K12)	516	526	2.9	TTTAAGAAAAT
SigH (-35) | *Bacillus subtilis* (strain 168)	545	556	7.88	GTAGGAGATTAA

T**able S2** Strains and plasmids used in this study.

Strains and plasmids	**Relevant characteristics**	**Reference**
Strains		
E.coli DH5α	Commercial transformation host for cloning	Takara Co., Ltd.
Cyanobacteria		
S. 6803	*Synechocystis* sp. PCC 6803 wild-type	D.J. Shi
Δpta	pta::Km^r	This study
Δpta::P_{cpc560}ter	pta::$P_{cpc560}terT_{rbcL}$::Km^r	This study
Δpta::P_{cpc374}ter	pta::$P_{cpc374}terT_{rbcL}$::Km^r	This study
Δpta::DldhE	pta::Km^r::$P_{cpc560}DldhET_{rbcL}$	This study
Plasmids		
pSM2	pMD18-T derivate, Amp^r Km^r, containing *pta* knockout cassette	1
pSM2-P_{cpc560}ter	pMD18-T derivate, Amp^r Km^r, containing *pta* knockout cassette and $P_{cpc560}terT_{rbcL}$ expression cassette	This study
pSM2-P_{cpc374}ter	pMD18-T derivate, Amp^r Km^r, containing *pta* knockout cassette and $P_{cpc374}terT_{rbcL}$ expression cassette	This study
pSM2- P_{cpc560}DldhE	pMD18-T derivate, Amp^r Km^r, containing *pta* knockout cassette and $P_{cpc560}DldhET_{rbcL}$ expression cassette	This study

Abbreviations: Amp^r, ampicillin resistance; Km^r, kanamycin resistance.

Ref. 1. Zhou J., et. al. Designing and creating a modularized synthetic pathway in cyanobacterium *Synechocystis* enables production of acetone from carbon dioxide. *Metab Eng* **14**, 394-400 (2012).

Table S3 Primers used in this study.

Primers	Sequence (5'-3')	Used for plasmid
P_{cpc560}F(XhoI)	CTGACT**CTCGAG**ACCTGTAGAGAAGAGTCCCTGAA	pSM2-P_{cpc560}ter, pSM2-P_{cpc560}DldhE
PcpcR(ter)	GAACGCTTTG ATCGCATTTA A TGAATTAATCTCCTACTTGACTTTATG	pSM2-P_{cpc560}ter
TrbcF1	ACCGGTGTTTGGATTGTCGG	pSM2-P_{cpc560}ter, pSM2-P_{cpc560}DldhE
TrbcR1(XhoI)	CTGACT**CTCGAG**GCTGTCGAAGTTGAACATCAG	pSM2-P_{cpc560}ter, pSM2-P_{cpc560}DldhE
TerF	ATGATTGTGAAACCCATGGT	pSM2-P_{cpc374}ter
TerR(Trbc)	CCGACAATCCAAACACCGGTTTAAATGCGATCAAAGCGTTC	pSM2-P_{cpc560}ter
Pcpc374F(XhoI)	CTGACT**CTCGAG**TCTTCCCTTCCCAATCCAG	pSM2-P_{cpc560}ter
DldhEF	ATGAAACTCGCCGTTTATAGCA	pSM2-P_{cpc374}ter
DldhER(Trbc)	CCGACAATCCAAACACCGGTTTAAACCAGTTCGTTCGGGC	pSM2-P_{cpc560}DldhE
PcpcR(DldhE)	TGCTATAAACGGCGAGTTTCAT TGAATTAATCTCCTACTTGACTTTATG	pSM2-P_{cpc560}DldhE pSM2-P_{cpc560}DldhE pSM2-P_{cpc560}DldhE

Introduced restriction sites are in bold.

ATGATTGTGAAACCCATGGTGCGCAACAACATTTGTTTGAACGCCCACCCCCAAGGCTGTAAAAA
AGGCGTGGAAGATCAAATTGAATACACCAAAAAACGCATTACCGCCGAAGTGAAAGCCGGCGCCAAAGCCC
CCAAAAACGTGTTGGTGTTGGGCTGTTCCAACGGCTACGGCTTGGCCTCCCGCATTACCGCCGCCTTTGGC
TACGGCGCCGCCACCATTGGCGTGTCCTTTGAAAAAGCCGGCTCCGAAACCAAATACGGCACCCCCGGCTG
GTACAACAACTTGGCCTTTGATGAAGCCGCCAAACGCGAAGGCTTGTACTCCGTGACCATTGATGGCGATG
CCTTTTCCGATGAAATTAAAGCCCAAGTGATTGAAGAAGCCAAAAAAAAAAGGCATTAAATTTGATTTGATT
GTGTACTCCTTGGCCTCCCCCGTGCGCACCGATCCCGATACCGGCATTATGCACAAATCCGTGTTGAAACC
CTTTGGCAAAACCTTTACCGGCAAAACCGTGGACCCCTTTACCGGCGAATTGAAAGAAATTTCCGCCGAAC
CCGCCAACGATGAAGAAGCCGCCGCCACCGTGAAAGTGATGGGCGGCGAAGATTGGGAACGCTGGATTAAA
CAATTGTCCAAAGAAGGCTTGTTGGAAGAAGGCTGTATTACCTTGGCCTACTCCTACATTGGCCCCGAAGC
CACCCAAGCCTTGTACCGCAAAGGCACCATTGGCAAAGCCAAAGAACACTTGGAAGCCACCGCCCACCGCT
TGAACAAAGAAAACCCCTCCATTCGCGCCTTTGTGTCCGTGAACAAAGGCTTGGTGACCCGCGCCTCCGCC
GTGATTCCCGTGATTCCCTTGTACTTGGCCTCCTTGTTTAAAGTGATGAAAGAAAAAGGCAACCACGAAGG
CTGTATTGAACAAATTACCCGCTTGTACGCCGAACGCTTGTACCGCAAAGATGGCACCATTCCCGTGGATG
AAGAAAACCGCATTCGCATTGATGATTGGGAATTGGAAGAAGATGTGCAAAAAGCCGTGTCCGCCTTGATG
GAAAAAGTGACCGGCGAAAACGCCGAATCCTTGACCGATTTGGCCGGCTACCGCCACGATTTTTTGGCCTC
CAACGGCTTTGATGTGGAAGGCATTAACTACGAAGCCGAAGTGGAACGCTTTGATCGCATTTAA

Fig. S1. *Synechocystis* codon optimized version of *ter* gene from *T. denticola*. Start and stop codons are also indicated.

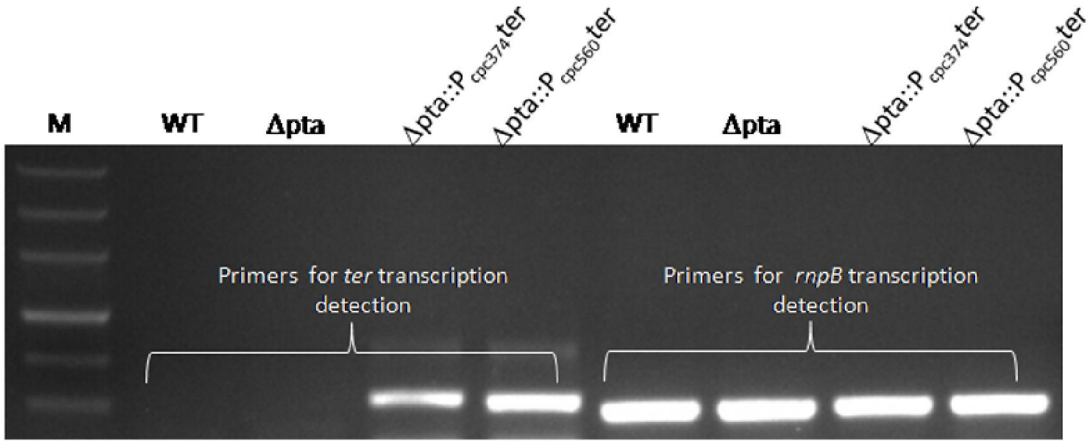

Fig. S2. RT-PCR confirmation of transcription of *ter* gene from P_{cpc374} and P_{cpc560}. Total RNA of *S.* 6803 wild-type (WT), Δpta, Δpta::P_{cpc374}ter and Δpta::P_{cpc560}ter cells was isolated using Redzol reagent (Qiagen, Beijing, China). Residual DNA in RNA preparations was eliminated by digestion with RNAse-free DNAse and reverse transcription reactions were performed using Reverse Transcription kit (Qiagen, Beijing, China). Reverse transcription products were amplified by PCR and analyzed by electrophoresis on 1.2% (w/v) agarose gels. M was DNA marker III. Transcription of *rnpB* was used as a positive control.

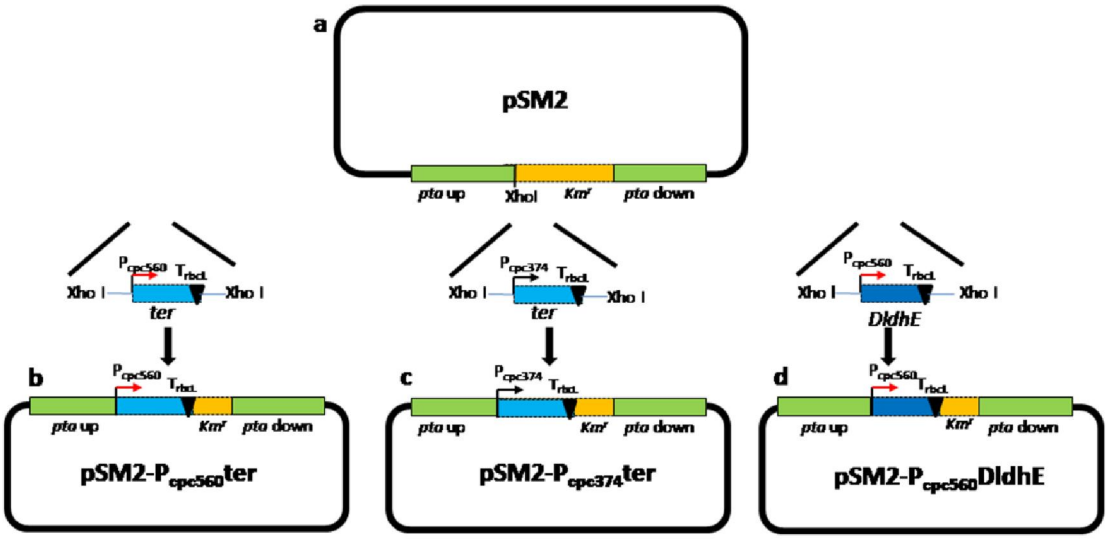

Fig. S3. Schematic diagram of construction of expression vectors.