

**Additional file 1**

**Green light-induced production of isobutanol and 3-methyl-1-butanol by metabolically engineered cyanobacteria**

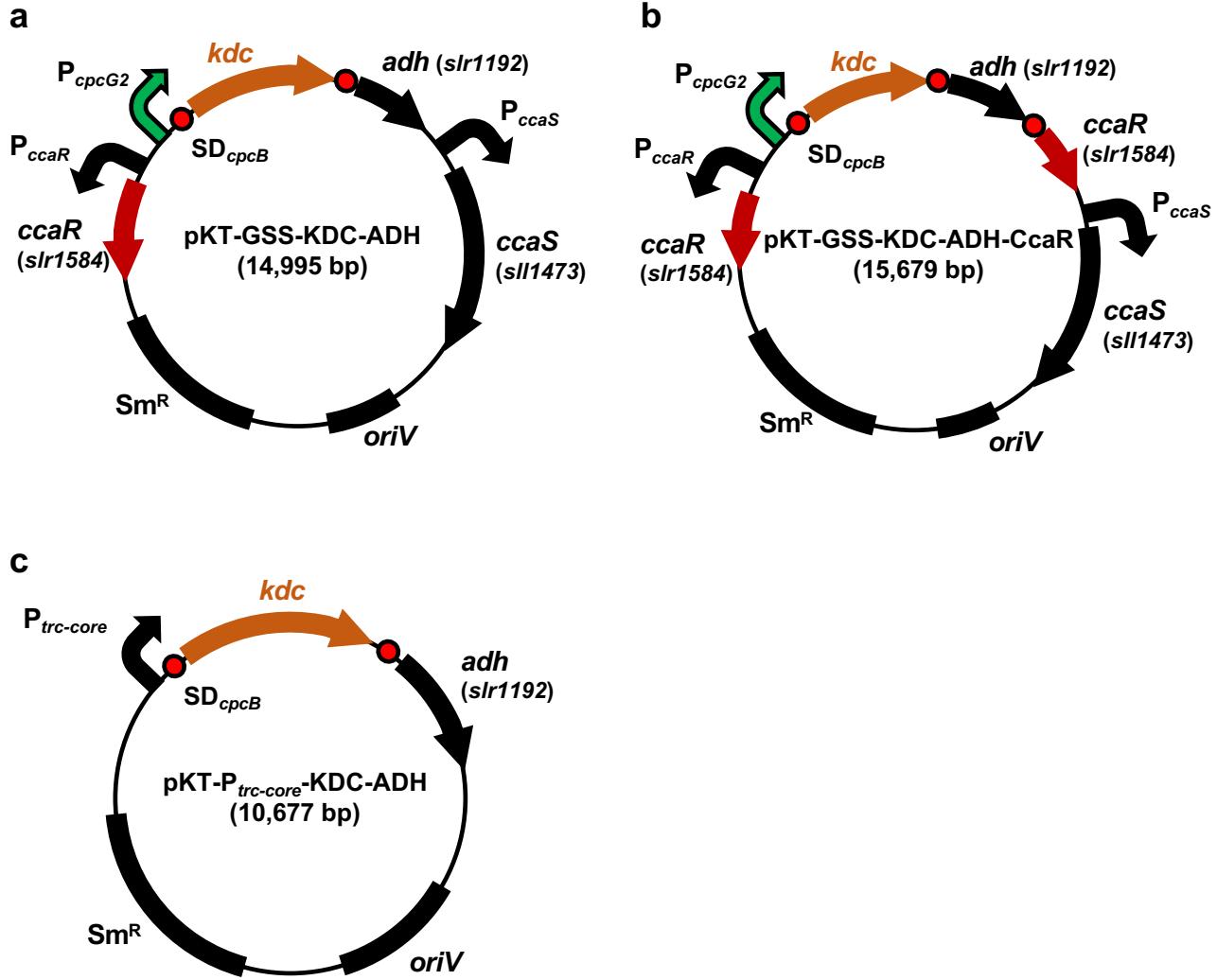
**Shunichi Kobayashi<sup>1</sup>, Shota Atsumi<sup>2</sup>, Kazunori Ikebukuro<sup>1</sup>, Koji Sode<sup>3</sup>, and Ryutaro Asano<sup>1</sup><sup>§</sup>**

<sup>1</sup>Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 2-24-16, Naka-cho, Koganei, Tokyo 184-8588, Japan

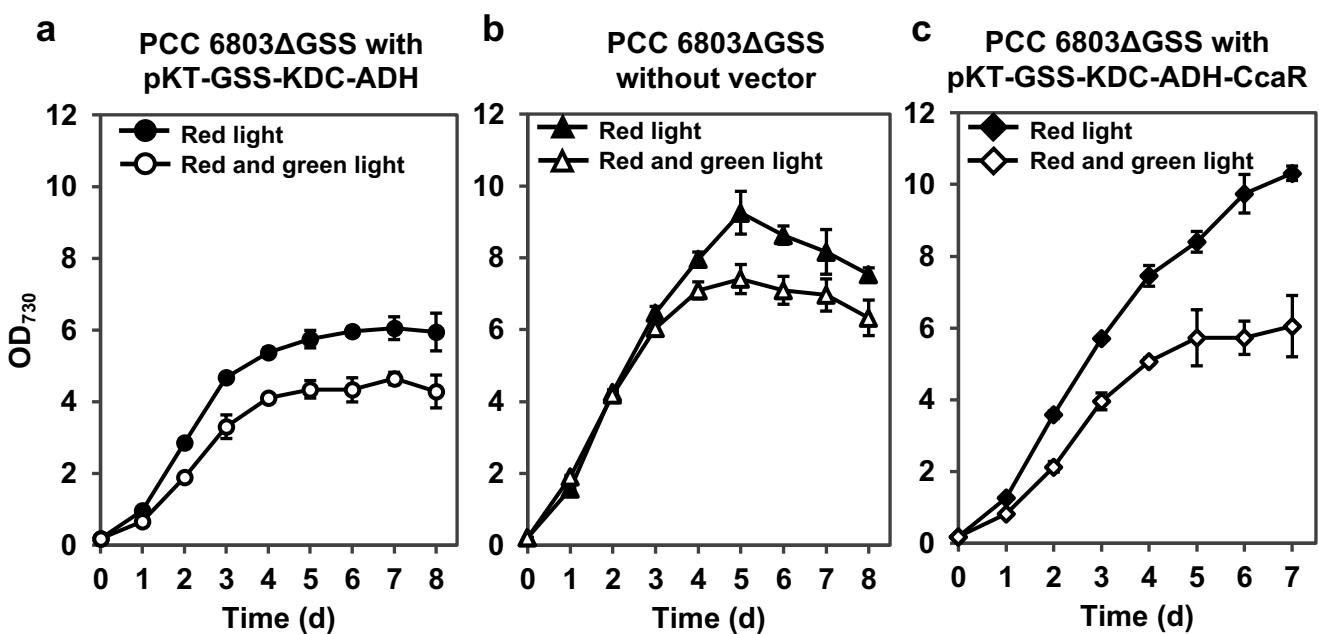
<sup>2</sup>Department of Chemistry, University of California Davis, One Shields Avenue, Davis, CA 95616, United States

<sup>3</sup>Joint Department of Biomedical Engineering, The University of North Carolina at Chapel Hill and North Carolina State University, Chapel Hill, NC 27599, United States

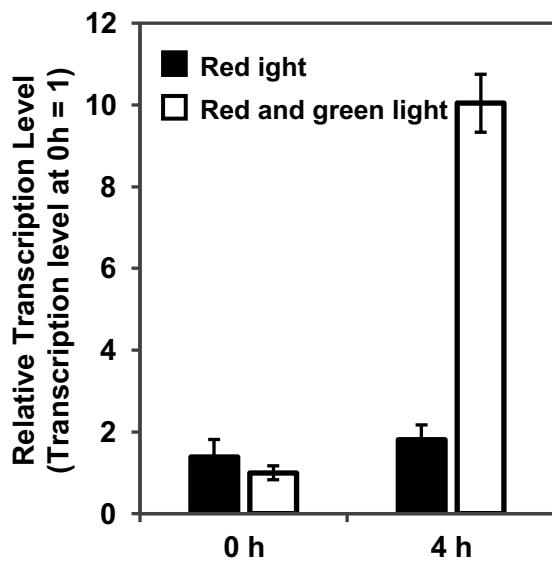
<sup>§</sup> Corresponding author; Ryutaro Asano (ryutaroa@cc.tuat.ac.jp)



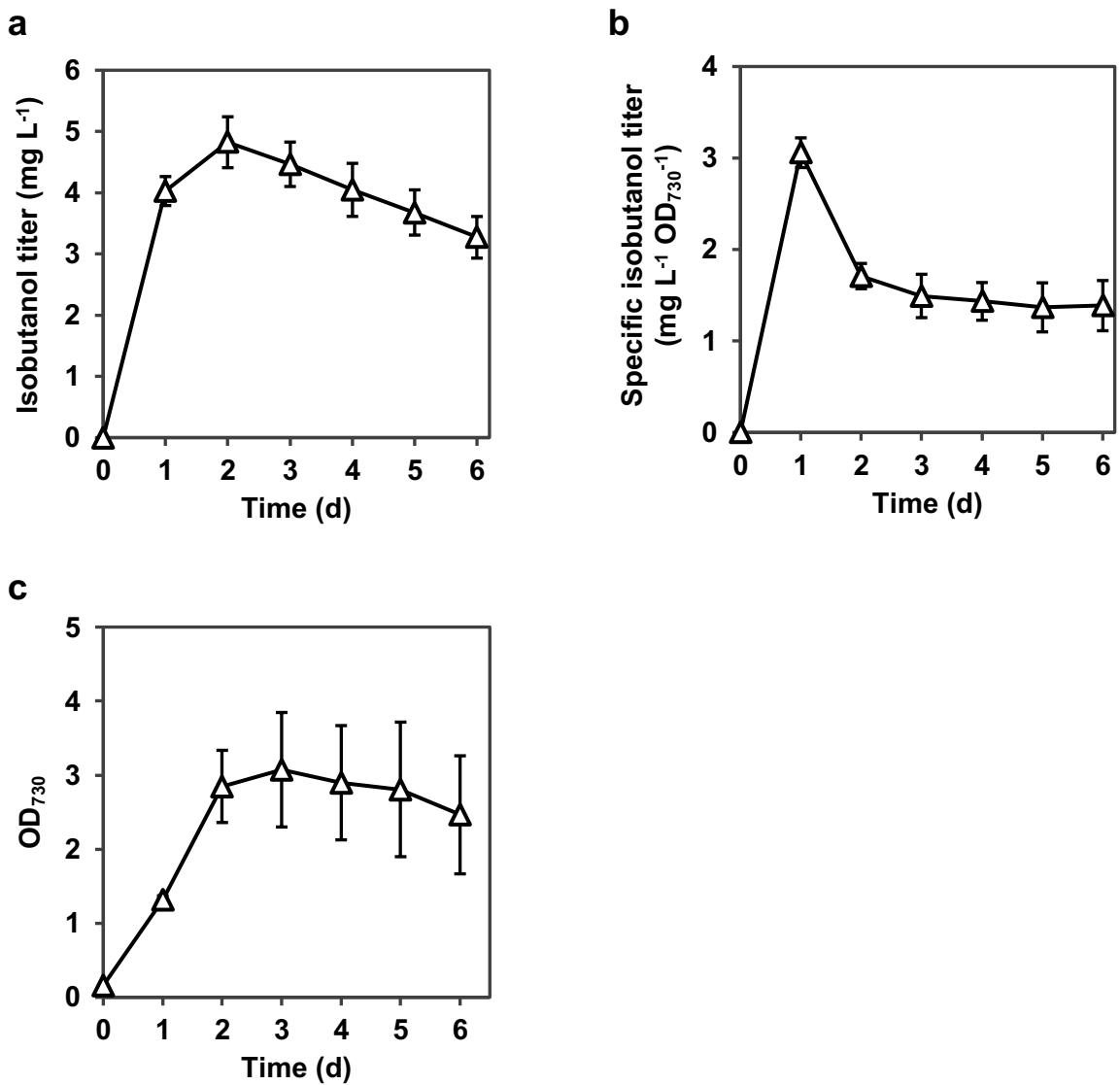
**Fig. S1** Design of vectors used in this study. **(a)** pKT-GSS-KDC-ADH for the application of CcaS/CcaR system to produce alcohol in cyanobacteria. pKT-GSS-KDC-ADH was constructed by inserting *kdc* and *adh* into the downstream of *P<sub>cpcG2</sub>* on pKT-GSS. **(b)** pKT-GSS-KDC-ADH-CcaR to tune the CcaS/CcaR system up for the enhancement of alcohol production. pKT-GSS-KDC-ADH-CcaR was constructed by inserting the structural genes of *kdc*, *adh*, and *ccaR* into the downstream of *P<sub>cpcG2</sub>* on pKT-GSS. **(c)** pKT-P<sub>trc-core</sub>-KDC-ADH for the constitutive activation of alcohol production in cyanobacteria. The *oriV* origin is replicated in *Synechocystis* sp. PCC 6803. Gene abbreviations are as follows: *P<sub>ccaS</sub>*, *ccaS* promoter; *P<sub>ccaR</sub>*, *ccaR* promoter; *P<sub>cpcG2</sub>*, *cpcG2* promoter; *kdc*, keto-acid decarboxylase; *adh*, alcohol dehydrogenase; *Sm<sup>R</sup>*, streptomycin-resistance gene; *SD<sub>cpcB</sub>*, Shine Dalgarno-like sequence of *cpcB* derived from *Synechococcus* sp. PCC 7002.



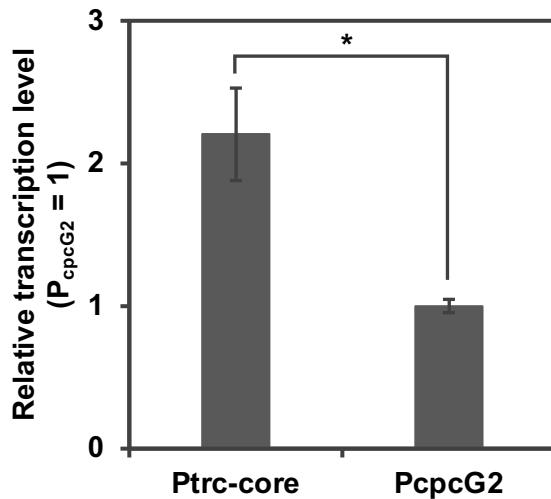
**Fig. S2** Growth curves of each strain used in this study. Growth curves of (a) the PCC 6803 $\Delta$ GSS strain with pKT-GSS-KDC-ADH, (b) the PCC 6803 $\Delta$ GSS strain, and (c) the PCC 6803 $\Delta$ GSS strain with pKT-GSS-KDC-ADH-CcaR. All strains were cultivated under the red light illumination alone (black markers) and under the red and green light illumination (white markers). All analyses were performed in triplicate. Error bars represent the standard deviation (SD) calculated from triplicate experiments using one clone.



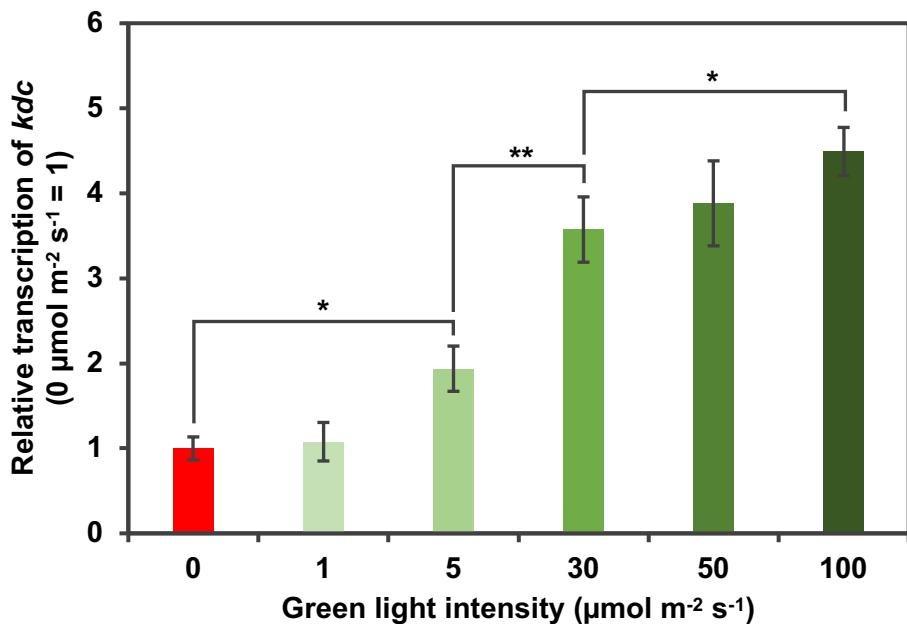
**Fig. S3** Transcriptional analysis of *kdc* gene in the *Synechocystis* sp. PCC 6803 $\Delta$ GSS strain. The relative gene transcription levels of *kdc* in the transformants of *Synechocystis* sp. PCC 6803 $\Delta$ GSS integrated with pKT-GSS-KDC-ADH cultivated under the red light illumination alone (black bar) and under the red and green light illumination (white bar) are shown. All analyses were performed in triplicate. Error bars represent the SD calculated from triplicate experiments using one clone.



**Fig. S4** Evaluation of alcohol production utilizing the  $P_{trc\text{-core}}$  system in *Synechocystis* sp. PCC 6803 $\Delta$ GSS. **(a)** Production titer of isobutanol in the culture supernatant of *Synechocystis* sp. PCC 6803 $\Delta$ GSS transformed with pKT- $P_{trc\text{-core}}$ -KDC-ADH cultivated under the red and green light illumination. **(b)** Specific titer of isobutanol under the red and green light illumination. **(c)** Growth curve of the transformant cultivated under the red and green light illumination. All analyses were performed in triplicate. Error bars represent the SD calculated from triplicate experiments using one clone.



**Fig. S5** Comparison of transcription level of *kdc* gene between the  $P_{trc\text{-core}}$  and the  $P_{cpcG2}$ . Transcription analysis was conducted after 24 h of cultivation of the PCC 6803ΔGSS strain transformed with pKT- $P_{trc\text{-core}}$ -KDC-ADH or pKT-GSS-KDC-ADH under red and green light illumination. All analyses were performed in triplicate. Error bars represent the SD calculated from triplicate experiments using one clone. Statistical analysis was performed using Student's *t*-test: \*  $p < 0.05$ .



**Fig. S6** Comparison of transcription level of *kdc* gene from the  $P_{cpcG2}$  under the various intensities of green light. Transcription level of *kdc* in the PCC 6803ΔGSS strain transformed with pKT-GSS-KDC-ADH under green light illumination ( $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ , or  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with red light illumination ( $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was shown. All analyses were performed in triplicate. Error bars represent the SD calculated from triplicate experiments using one clone. Statistical analysis was performed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test: \*  $p < 0.05$ , \*\*  $p < 0.005$ .

**Table S1** Comparison of the isobutanol production titer in cyanobacteria expressing keto-acid decarboxylase (*kdc*) and alcohol dehydrogenase (*adh*) in this study with that in previous studies.

Strain	Promoter (Inducer)	Genes	Titer	Productivity*	Production time	Ref.
PCC 7942	P <sub>trc</sub> (IPTG)	<i>kdc, yqhD</i>	50 mg L <sup>-1</sup>	17 mg L <sup>-1</sup> d <sup>-1</sup>	3 d	[3]
PCC 6803	P <sub>tac</sub> (No inducer)	<i>kdc, adhA</i>	90 mg L <sup>-1</sup>	15 mg L <sup>-1</sup> d <sup>-1</sup>	6 d	[26]
PCC 6803	P <sub>trc-core</sub> (No inducer)	<i>kdc</i> (S286T)	911 mg L <sup>-1</sup>	20 mg L <sup>-1</sup> d <sup>-1</sup>	46 d	[28]
PCC 6803ΔGSS	P <sub>cpcG2</sub> (Green light)	<i>kdc, slr1192</i>	238 mg L <sup>-1</sup>	48 mg L <sup>-1</sup> d <sup>-1</sup>	5 d	This study

\* The productivity was calculated as the average production level per day by dividing the titer by the number of days required for production.

**Table S2** Comparison of the isobutanol production in this study with the highest titer and productivity achieved in previous studies.

Strain	Promoter (Inducer)	Genes	Titer	Productivity*	Production time	Ref.
PCC 7942	P <sub>trc</sub> , P <sub>LlacO1</sub> (IPTG)	<i>kdc, yqhD, alsS, ilvC, ilvD</i>	450 mg L <sup>-1</sup>	75 mg L <sup>-1</sup> day <sup>-1</sup>	6 d	[3]
PCC 7942ΔglgC	P <sub>trc</sub> , P <sub>LlacO1</sub> (IPTG)	<i>kdc, yqhD, alsS, ilvC, ilvD</i>	550 mg L <sup>-1</sup>	69 mg L <sup>-1</sup> day <sup>-1</sup>	8 d	[24]
PCC 7942	P <sub>trc</sub> , P <sub>LlacO1</sub> (IPTG)	<i>kdc, adhA, alsS, ilvC, ilvD</i>	637 mg L <sup>-1</sup>	32 mg L <sup>-1</sup> day <sup>-1</sup>	20 d	[25]
PCC 6803	P <sub>trc-core</sub> (No inducer)	<i>kdc</i> (S286T)	911 mg L <sup>-1</sup>	20 mg L <sup>-1</sup> day <sup>-1</sup>	46 d	[28]
PCC 6803ΔGSS	P <sub>cpcG2</sub> (Green light)	<i>kdc, slr1192</i>	238 mg L <sup>-1</sup>	48 mg L <sup>-1</sup> day <sup>-1</sup>	5 d	This study

\* The productivity was calculated as the average production level per day by dividing the titer by the number of days required for production.

**Table S3** Comparison of the 3-methyl-1-butanol titer in cyanobacteria obtained in this study with that in previous studies.

Strain	Promoter (Inducer)	Genes	Titer	Productivity*	Production time	Ref.
PCC 7942Δ <i>glgC</i>	P <sub>trc</sub> , P <sub>LlacO1</sub> (IPTG)	<i>kdc</i> , <i>yqhD</i> , <i>alsS</i> , <i>ilvC</i> , <i>ilvD</i>	70 mg L <sup>-1</sup>	10 mg L <sup>-1</sup> d <sup>-1</sup>	7 d	[20]
PCC 6803	P <sub>trc-core</sub> (No inducer)	<i>kdc</i> (S286T)	225 mg L <sup>-1</sup>	5 mg L <sup>-1</sup> d <sup>-1</sup>	46 d	[23]
PCC 6803ΔGSS	P <sub>cpcG2</sub> (Green light)	<i>kdc</i> , <i>slr1192</i>	75 mg L <sup>-1</sup>	15 mg L <sup>-1</sup> d <sup>-1</sup>	5 d	This study

\* The productivity was calculated as the average production level per day by dividing the titer by the number of days required for production.

**Table S4** Sequences of the vector inserts used in this study.

pKT-GSS-KDC-ADH
CTGCAGTGCCACTGACGTAAAGAAAAGGAATTTCAGCAATTGCCCGTGCCAAGAAAGGCCAACCGT GAAGGTGAGCCAGTGAGTTGATTGCTACGTAAATTAGTTAGCTAGCCCTAGTGACTCGAATTCGCGGCCGCT TCTAGGCGAACGTTCAAATCCGCTCCGGCGATTGCTACTCAGGAGAGCGTTCACCGACAAACAACA GATAAAACGAAAGGCCAGTCAGTCTCGACTGAGCCTTCGTTTATTGATGCCTGGCAGTCCCTACTCTCG CATGGGGAGACCCCACACTACCATCGCGCTACGGCGTTCACTCTGAGTCGGCATGGGTCAGGTGGGA CCACCGCGCTACTGCCGCCAGGCAAATTCTGTTTATCAGACCGCTCTGCGTTCTGATTTAATCTGTATCA GGCTGAAAATCTCTCATCCGCCAAAACAGCCAAGCTTAGTCTTCCCTGGCACAAGATTTTCCG TTAAATTGGCCAGACGATAACCAATGCCATGGACCGTTCAATGGCATCTGCTGATAAACCGGCACTTTTA ATTTTGTCGAAACTCCGCACATGCACCTAACCGTATCTTCCCTCTGGGGACTCTCAAACCTCCAGATAAC TATCGATAATCATGCTCCGACTTAGCACCCGACGCCATTGCGGAGTAGTAATTCCAGAATGCTGATTCC TGCGGGTCAAAGACAAAACCTCATGTCATAACTAACCTCATAGGTGCTTGGATCCAACCTGATTGGCCCC ACTCTAAAACGGTTGGCACGTTGCACAACCCGACGCAACAAAGCTCGCACCCGGAAATACTCCCCA AATCCACTGGCTTGACCACATAATCATCCGCCCCGATCCAAGGCCGTGATTATCATGGATCGTATCCC TGGCTGTCATCATCAAAATTGGCATTAAATAACTGTGCGATGCCATTGGACAGAGGGTAATCCGTC ACTCCGGCAGCATCACATCCAAAATAACGAGGTATATTCCAGTCGGAGGCAAGTCCCAGGCAGGGAAAG CGTCGGTGGCAATATCAACGGTAAAGCTGGTACTCAATGCTTCAAGCAAGGGTTCCGCCAGGGCAAAT CATCCTCCACTAAAAGAATTCTCATAGCCATTGTCATTTCTATCAACCTCAGCTTACCTGAAGGGTG AACAGGTCTGGTTAATTATGTCGAAATGTAACAGTTAGTCGATCAGCTAACTTCCGATTCTTT ACGATTTCCTCCCCCTTTCTCAATTACTTGTAGGATCGATTAAATGCCAACACATACCAGTTA TTGGCTGGACATTAAACAACTTTAAGTTAATTACTAAGTATCTTAAAGTAGGAGATAAAAACATGTA TACGGTTGGAGATTATCTGCTTGATGCCGCATGAACTAGGCATTGAGGAATCTTGGTGTGTTCCGGGTGA TTACAATCTGCAATTCTGGATCAGATCATTAGTCGAAAGACATGAAATGGTTGGTAACGCCAATGAACT GAATGCCGTATATGGCTGACGGTTATGCCGTACCAAAAAAGCGGCTGCCCTTGTGACGACCTTGGTGT GGGAGAACTAGGCCGTAAATGGCTAGCCGGCAGCTATGCCAAAACCTGCCGTAGTGGAAATCGTTGG CTCTCCCACGTCAAAAGTGCAGAATGAAGGCAATTGTCATCATACCCGGCAGATGGCATTAAAGCA CTTATGAAAATGCATGAACCTGTGACAGCGGCCAGAACTCTGTTAACCGGGAAACGCCACCGTGTGAGAT CGACCGCGTACTAACGGCTTGCTAAAGAACGTAACCGGTGTATATCAATCTGCCGTAGATGTCGGC TGCAGAAGCCAAAAACCAAGTTACCGCTGAAGAAAGAGAAATAGCACATCTAACACCAGTGTAGGAAAT CCTCAACAAGATCCAGGAATCCCTGAAAATGCGAAAAGCCATCGTGTGATTACAGGACACGAAATTATT ATTGGGTGAGAAAACCGTGTCTCAGTTCAAAGCAAGACGAAACTGCCTATTACCCCTGAACTTTGG TAAATCGTCCGTGGATGAAGCGTTACCGTCTTTGGGATTAACTGCAAACGTCAGAGCCGAAATT GAAGGAATTCTGTGGAAAGCGCCATTTCATTCTGATGCTTGGCTGAAACTGACTGATAGTTGACGG CTTACTCATCACTAAACGAGAACAAAATGATCTCTCAACATTGACGAGGGCAAGATTTCACAGAGTC CATTAGAACCTCGACTCGAGTCTTGATTAGCAGTCTGCTGGATCTGCGGAAATGAAACAAAAGGGAA ATACATTGACAAGAACAGGAAGACTTGTACCGTCCAATGCAATTACTGAGTCAGATGCCGTG GGTTGAGAACCTACGAAAGCAACGAAACCATAGCGCGAACAGGTACGTGTTTTCGGAGCAAGCAG CATCTCCTGAAACCCAAATCACACTTTACGGTCAACCCTCTGGGTCTATTGGTACACCTTCCAGC AGCTCTCGGCTCACAGATAGCGATAAAGAGAGTCGACACCTGCTGTTATTGGCGATGGTTCTTGCAGT GACTGTTAGGAACTCGGCTAGCAATTGGGAGAAGATTAACCCATTGCTTCACTCATCAATAATGACGG GTATACCGTCAACGTGAAATCCATGGGCAAATCAAAGCTATAACGACATTCCGATGTTGAACTATTGAA ACTTCGGAACTGTTGGCGCAACTGAAGAACGTCGTGTCAAAATCGTGTGACAGAAACGAGTTGT CAGCGTTATGAAAGAAGCGCAGGCAGATCGCATGTACTGGATCGAGCTGATTCTGCCAAAGAAGA CGCTCCGAAAGTCCTGAAGAAAATGGGCAAACCTCTCGCAGAACAGAACAAATCTAAATATAAGTAGG AAAAACATGATTAAGCCTACGCTGCCCTGGAAGCCAACGGAAAACCTCAACCCCTTGAAATACGAC GCCCTGGGTGCTAATGAGGTGGAGATTGAGGTGCACTATTGTGGGGTGCCACAGTGATTGTC AATAACGAATGGGGCAATTCCAAATTACCCCTAGTGCCTGGTCACTGAGGTGGTGGTACTGT GGCGAAGGGGTGAACCATGTTGAGGTGGGGATTAGTGGGCTGGGTTGGCATCGGGCTACTGC TGCCATAGTTGTTATCTGGCTACCAACCTTGTGCCACGGCGAACGACATTGTGGCCACTAC GGCTTGGCGATGGGTGGGCCAGGGAGTCAGCGTGGTAAATTACCTAAAGGCATTGACCTAGCAGT GCCGGGCCCTTCTGTGGAGGAATTACCGTTTGCTAGTCCATGGTGGAACTGAGTTAAAGCCCAC AAAGTGGCAGTGATGCCATTGGGGCTTGGGCCATTAGCGGTGCAATTCTCCGGGCTGGGCTGT GTGACTGCCTTACCTCAGTGCAGGAAGCAACGGAAGTGTTGGAATTGGCGCTACCACAC TCCACCAATCCAGAGGGCAGTGCAGTGCGGAAGGAAATTGACTATATTATCTCCACTGT GAACCTGAAGCTTGACTGGAACATTACATCAGCACCGCTGGGCCAGGGACATT TTCACTTGTGGGTTGGAG

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#### pKT-GSS-KDC-ADH-CcaR

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CAGAGGACAAAAGCAAATTGGACCCCTTCATGGGGCAAAATGTCAGAAATTACCGGAACAGGAC  
TCGGTTAATGGTGCAGAACATGTGTTGACTTACACAGTGGCAGTATCTGCTAAAAGTGCAGTGAC  
**AGGGAAACAAACAGTTACTATCTGTTAAAAGCTATAACCATTGCTCGAGCTAGCGAAAAACCCGCT**  
TCGGCGGGTTTTCCGCTGCAG

**pKT-P<sub>trc-core</sub>-KDC-ADH**

CTGCAGGAGCTGTTGACAATTATCATCCGGCTCGTATAATGTTGGAGATCTTATAAGTAGGAGATAAAA  
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 CTACGGTGGCTTGGCGATGGGTTGGCCAAGGGAGTCAGCGTGGTGAATTACCTAAAGGCATTGACCT  
 AGCCAGTGCCGGGCCCTTTCTGTGGAGGAATTACCGTTTCAGTCCTATGGTGAACGTAGTTAAAGCC  
 CACTGCAAAAGTGGCAGTGATGGCATTGGGGCTGGCCATTAGCGGTGCAATTCTCCGGGCTGGGG  
 CTGTGAAGTGAUTGCCTTACCTCCAGTGCCAGGAAGCAAACGGAAGTGTGGAATTGGCGCTACCACAT  
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 TAGTCCCAGGCCACCATGCCACCATGTTGACTTGTGCTGCCCCATGACATTAAACCGTGGGAACAATT  
 TAGCTTGATCAGATCAACGAGGGCATGCCCATCTAGAAAGCGCAAAGCCCATTATCGGGTAGTGCCTCAG  
 CCATAGAAAAATTAGTCTAGACGCAAAAACCCGTTGGGGTTTTGCCCTGCAG

\*The regions encoding *ccaR* (Turquoise), P<sub>cpcG2</sub> (Green), Shine-Dalgarno-like sequence derived from *cpcB* from *Synechococcus* sp. PCC 7002 (Gray), *kdc* (Yellow), *adh* (Pink) and *ccaS* (Red), P<sub>trc-core</sub> (Dark red) were shown.

**Table S5** Primer sequences used in this study.

Primer No.	Sequence (5' to 3')
P01	GTTTAATTACTAACTAGATCTTATAAGTAGGAGATAAAAACATGTATACGGTTGGA GATTATCTGC
P02	TATCTCCTACTTATATTAGGATTGTTCTGTTCTGCGAAGAG
P03	CCTAATATAAGTAGGAGATAAAAACATGATTAAAGCCTACGCTGCC
P04	TTACCGTCAAAAAATCTAGACTAATTCTACTATGGCTGAGCACTACCC
P05	GTTTAATTACTAACTAGATCTTATAAGTAGGAGATAAAAACATG
P06	CTACTTATACTAATTCTACTATGGCTGAGCACTACCC
P07	GTAAAAATTAGTATAAGTAGGAGATAAAAACATGAGAATTCTTGTG
P08	TTACCGTCAAAAAATCTAGACTAGTTCCCTGGCACAAAG
P09	AATTCTGCAGGAGCTGTTGACAATTATCATCCGGCTCGTATAATGTGTGGAAGAT CTTATAAGTAGGAGATAAAAACATGTATACGG
P10	AATTCTGCAGGCGAAAAACCCGCCGAAGCGGGTTTTGCGTAGACTAATT TTTACTATGGCTGAGCAC
P11	GGTTGGTAACGCCAATGAAC
P12	ACCAAAGGTCGTCAAGAACG
P13	GCTAATACCCAATGTGCCGA
P14	CACTGCTGCCCTCCGTAG