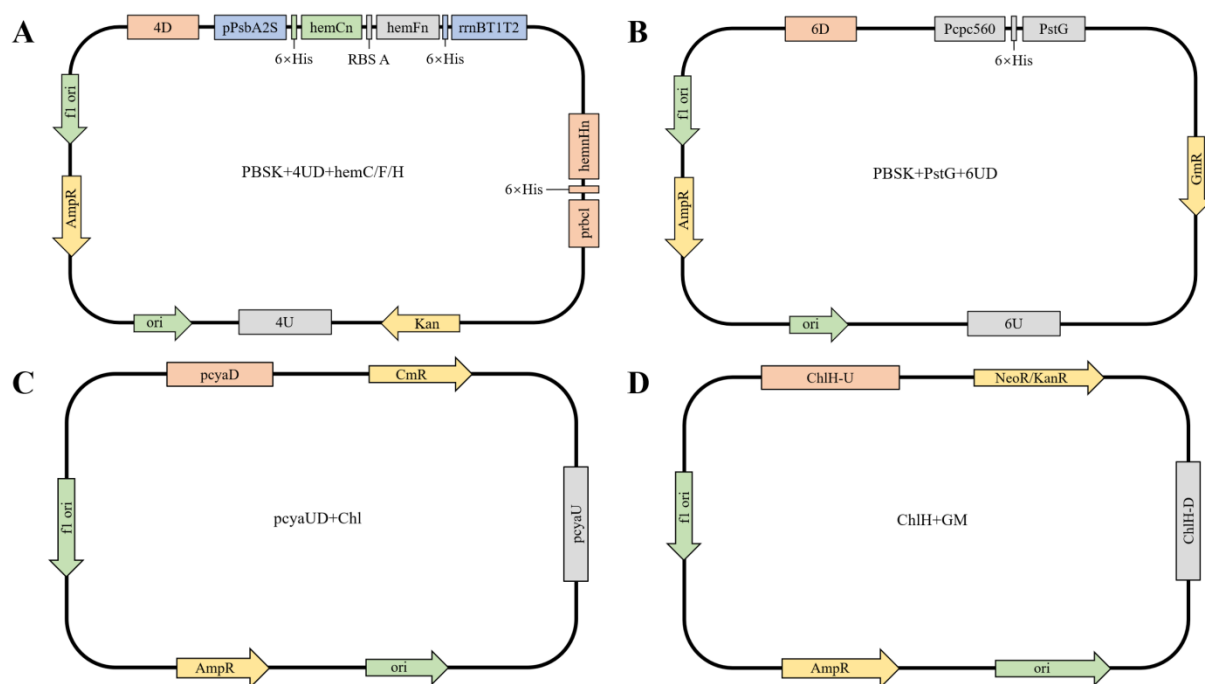
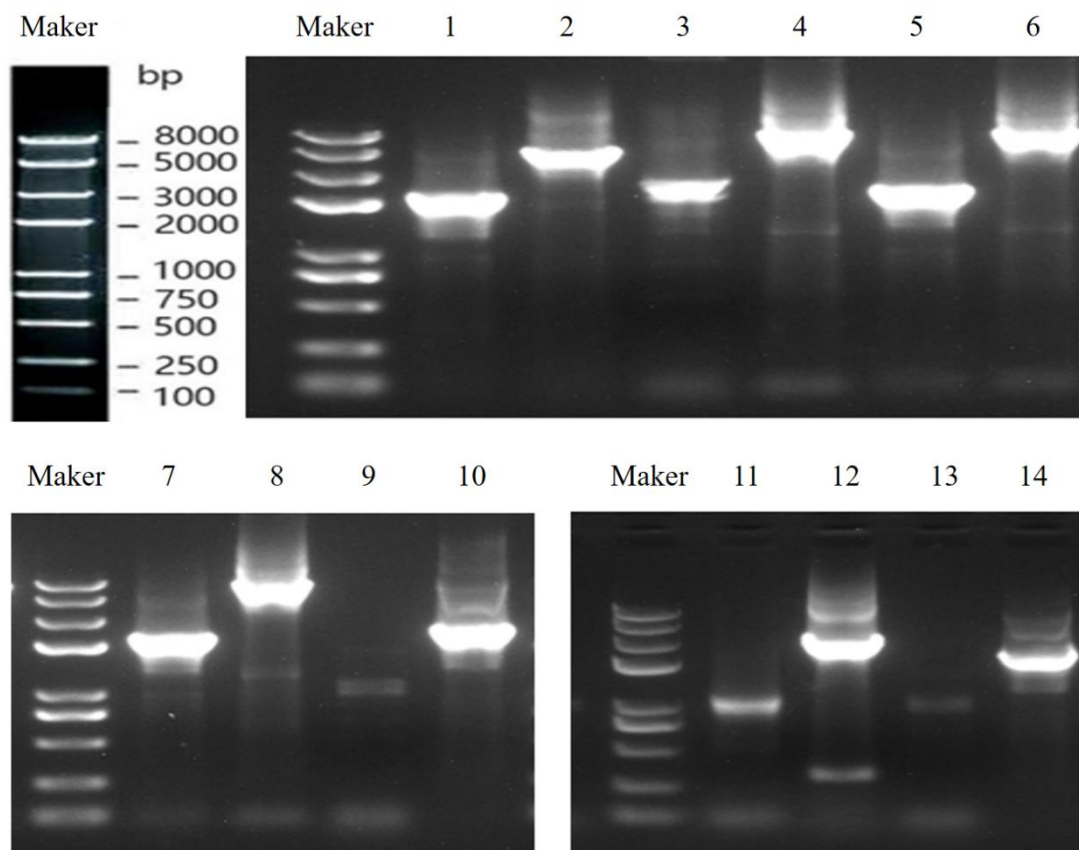


## Supplementary materials



**Figure S1. Plasmid mapping.** (A) *hemC*, *hemF* and *hemH* co-expression plasmid profile. (B) *ptsG* overexpression plasmid profile. (C) *pcyA* homologous recombination knockout plasmid profile. (D) *chlH* homologous recombination knockout plasmid profile



**Figure S2. PCR detection of *Synechocystis* sp. PCC 6803 wild-type and combined transformants.** Lanes 2, 4, and 6: mutant strain glbn-ptsG-CFH; bands contain homology arm + target gene expression cassette. Lanes 1, 3, and 5: controls of lanes 2, 4, and 6, respectively; bands include homology arm only. Lanes 8 and 10: mutant strain CFH-PcyA, with controls presented in lanes 7 and 9, respectively. Lanes 12 and 14: mutant strain ChlH-PcyA, with controls shown in lanes 11 and 13, respectively.

**Table S1. Strains used in this study.**

<b>Strain name</b>	<b>Relevant strain and genotype</b>	<b>Source</b>
<i>Synechocystis</i>		
<i>sp.</i> PCC 6803 (wild-type)	N/A	The Institute of Hydrobiology
PtsG	<i>ptsG</i>	This study
$\Delta$ PcyA	$\Delta$ <i>pcyA</i>	This study
$\Delta$ ChlH	$\Delta$ <i>chlH</i>	This study
Gln-ptsG	<i>Gln</i> and <i>ptsG</i>	This study
CFH	<i>hemC</i> , <i>hemF</i> , and <i>hemH</i>	This study
PcyA-ChlH	$\Delta$ <i>pcyA</i> and $\Delta$ <i>chlH</i>	This study
CFH-PcyA	<i>hemC</i> , <i>hemF</i> , <i>hemH</i> , and $\Delta$ <i>pcyA</i>	This study
gln-ptsG- CFH	<i>gln</i> , <i>ptsG</i> , <i>hemC</i> , <i>hemF</i> , and <i>hemH</i>	This study

**Table S2. BG-11 Media Formulatio.**

<b>Component</b>	<b>g/L</b>
NaNO <sub>3</sub>	1.5
K <sub>2</sub> HPO <sub>4</sub>	0.04
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.075
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.036
Citric Acid	0.006
Ammonium ferric citrate	0.006
EDTA-2Na	0.001
Na <sub>2</sub> CO <sub>3</sub>	0.02
1000 × trace elements	1.0 mL

Table S3. 1000 × trace elements.

<b>Component</b>	<b>g/L</b>
H <sub>3</sub> BO <sub>3</sub>	2.86
MnCl <sub>2</sub> ·4H <sub>2</sub> O	1.81
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.222
NaMoO <sub>4</sub> ·2H <sub>2</sub> O	0.39
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.079
Co (NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	0.0494

**Table S4. Primers used for construction of plasmids and cloning of target fragments.**

Carrier name	Primer	Primer sequence (5'→3')
4UD-HemC-HemF-HemH-KAN	4UD-HemC-HemF-HemH-KAN PCR primer	
	4UD-F	GGAATAATCCCGTTGCTGTT
	4UD-R	GGCCgcATCCGCAAAGGC
	Prbcl-F	GATGGTGATGATGCATAAACATTGAATAGCC TAGCTTTCTCCCAC
	Prbcl-R	caattccggCAGTCAATGGAGAGCATTGCC ATGCCTTTGCGGATgcGGCCCCGCCAGGTAA
	PpsbA2S-F	ACTCTTCTCAA
	PpsbA2S-R	GATGGTGATGGTGCATAACTGACTAAACTTA GTCTAAAGGATTAATGAGAGTTTTG
	HemC-F	CTAAGTTTAGTCAGTTATGCACCATCACCAT CACCAC
	HemC-R	GAGAGACGGTCATTAGTCCTGTGTGATTTAT CCCCGG
	HemF-F	TCACACAGGACTAATGACCGTCTCTCCCACA AC
	HemF-R	CTAATGATGATGATGATGGTGACTATTAACC CAGTCCTGGGGAACATAAG
	HemH-F	CCAAAACAGgAATTCTAAAGCAAGCCGACA AAATGCAAC
	HemH-R	ATGCATCATCACCATCACCATGGTCGTGTTG GGGTCTTACTGCTAAAT
	KAN-F	TCCATTGACTGCCGGAATTGCCAGCTGGG
	KAN-R	ACAGCAACGGGATTATTCCCGCGGATACATA TTTGAATGTATTTAGAA
	4T1T2-F	TCATCATTAGTGCAgAAGAGTTTGTAGAAAC GCA
	6UD-ptsG-GM PCR primer	
	PPSBA2L-F	ATAGCTCTAGGTGTTTCAGCACTTTAGCGTTC CAGTGGATATTTG
	PPSBA2L-R	TGATGCATATGTATTTGTTCGATGTTTCAGATT GG
	ptsG-F	CGACAAATACATATGCATCATCATCATC ATTTTAA

pcyaUD-Chl	ptsG-R	TCTGCAGTTAGTGGTTACGGATGTACTCATC CA
	6TT-F	CCGTAACCACTAACTGCAGAAGAGTTTGTAG AAACGC
	6TT-R	GTTCCGCGGAATTCCTGTTTTGGCGGATG
	GM-F	AAACAGGAATTCCGCGGAACCCCTATTTGTT
	GM-R	GCCCCAATTAACCTTGTCTGAAACGCAAAAG AAAATGCCG
	6UD-F	CAGACAAGGTTAATTGGGGCTAG
	6UD-R	TGCTGAACACCTAGAGCTATTAAATATTC
	pcyaUD-Chl PCR primer	
	PCYAD-F	ACTAGTGGATCCCCCggatcATTCCGGTTTTGA TGCTAACGG
	PCYAD-R	acgtgccgatcaACTCCACCGGGGCCGGCC
	PUC-F1	GGGCTGCAGGAATTCGATATC
	PUC-R1	GGGGGATCCACTAGTTCTAGAG
	Chl-F1	CCGGTGGAGTtgatcggcacgtaagaggttc
	Chl-R1	GCCGCACTTaataagacataageggctatttaacga
ChlHUD-Ch	pcyaD-F	tatgtctattAAGTGCGGCTATTGCGGAT
	pcyaD-U	ATATCGAATTCCTGCAGCCCGGGAATTAGTG ATGTGTGCATATTG
	ChlHUD-Chl PCR primer	
	GM-F1	AGACGTTACCGCGCGGAACCCCTATTTGTTT ATTTTCT
	GM-R1	TACGGTAAGGAaaaacgcaaaagaaaatgccgat
	ChlHD-F	GGGGTTCGCGCGGTAACGTCTTCATCGGTG T
	ChlHD-R	CTATAGGGCGAATTGGAGCTCAGGTATTCAA ACAGGGGTTTGA
	ChlHU-F	CTAAAGGGAACAAAAGCTGGATATGGGCAT TTGGCATCCC
	ChlHU-R	ttttgcgtttTCCTTACCGTAGATCAGTAAATTTTG C
	PUC-F2	CCAGCTTTTGTTCCCTTTAGTG
	PUC-R2	AGCTCCAATTCGCCCTATAG

**Table S5. qPCR primers and their sequences.**

<b>Primer</b>	<b>Primer sequence (5'→3')</b>
A-Q-F	AAGGAGCCACGGACATCACCAT
A-Q-R	ACAGCCAGTCAAATTCTCGCAGTT
C-Q-F	GCCAAGTGCCTATCGGTGTTAATAC
C-Q-R	CGCTAAGATTTCCGCTAGGATTTCC
F-Q-F	GGGCAAATCCCTACCTCCTTCAAT
F-Q-R	TCCACCTCCGAACCACCACA
glbn-Q-F	TGGAACCACCGCTGTCGATCTA
glbn-Q-R	TTTATCCGTACCGCCAAAGGCATAG
H-Q-F	GGGTGTTGGAGGAAATGTGGCATA
H-Q-R	AAATATGGGCTTGGTCTGGATTGGG
PcyA-Q-F	GTGGAGTAAGTGCGGCTATTGC
PcyA-Q-R	TGACATTGCTGGGACGGATGAA
rnpB-F	TTTAGAAAACAGCAACCAGT
rnpB-R	GGCAGGAAAAAGACCAACCT
chlM-Q-F	GGCATTGGTCTATGGCAGTGAT
chlM-Q-R	GCCTCCTCCGTTGGATAGTGAA
ChlH-Q-F	TCGGCACCCACGGTTCTTTG
ChlH-Q-R	GGATTGTTGGCGGCGTAGTAGT
ccmA-Q-F	CCTTGCATCAGGTACGCGACAG
ccmA-Q-F	GCCAGGGCTTCCAGACATTGTG
ptsG-Q-F	AATCGCTGATGCTGCCGGTATC
ptsG-Q-R	CCGCCTGCTTCTGCCATAACAT



**Table S6. PCR amplification procedure.**

<b>Step</b>	<b>Temperature</b>	<b>Time</b>
Initial denaturation	95 °C	30 s
Denaturation	95 °C	10 s
Annealing (metallurgy)	60 °C	30 s
Number of cycle	40	