Science Advances

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Supplementary Materials for

Complete enzyme set for chlorophyll biosynthesis in *Escherichia coli*

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Published 26 January 2018, *Sci. Adv.* **4**, eaaq1407 (2018)
DOI: 10.1126/sciadv.aaq1407

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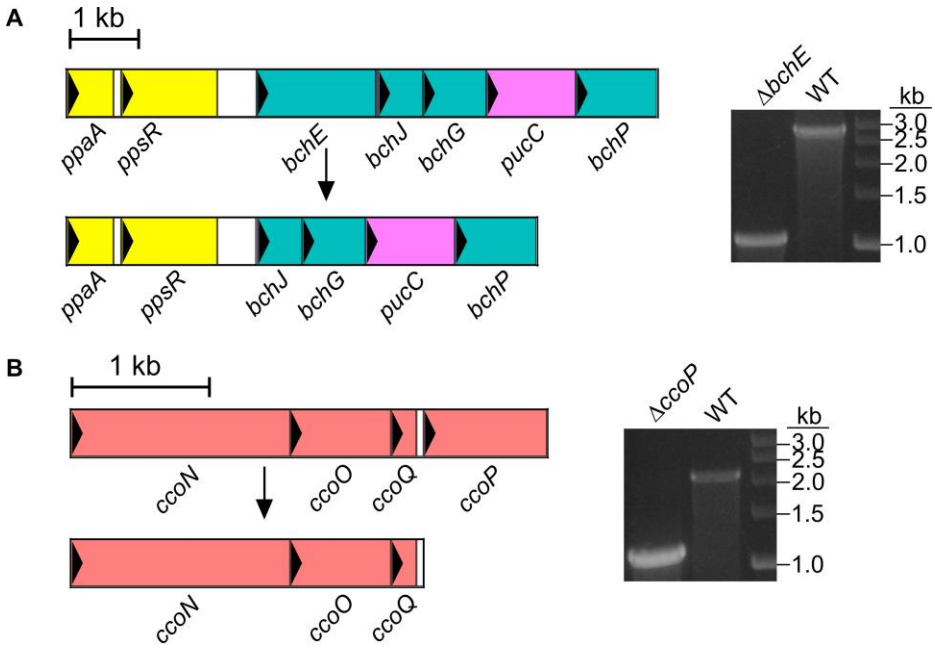

fig. S1. Deletions of the *bchE* and *ccoP* genes in *Rba. capsulatus*. (**A**) Deletion of the *bchE* gene. Lengths of PCR products: WT = 2762 bp; Δ*bchE* = 1046 bp. (**B**) Deletion of the *ccoP* gene. Lengths of PCR products: WT = 2046 bp; Δ*ccoP* = 1164 bp. Cyan: bacteriochlorophyll biosynthesis genes; magenta: assembly factors; yellow: regulatory genes; pink: cytochromes. Agarose gels of colony PCR products confirming the gene deletion are also shown.

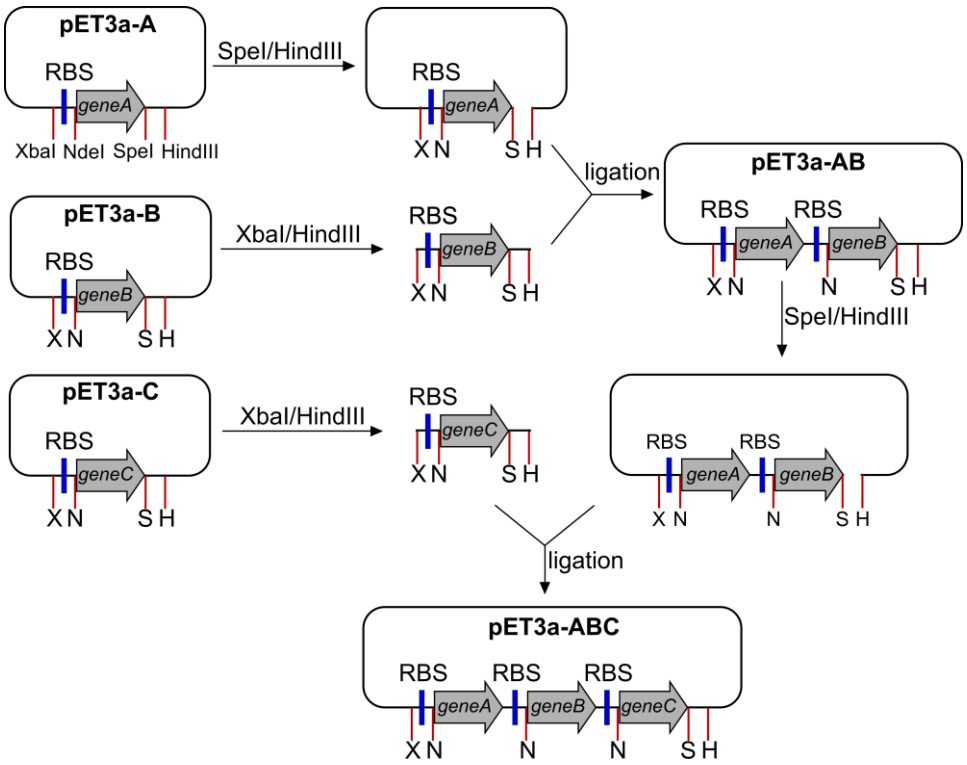

fig. S2. Diagram of the link-and-lock method for plasmid construction. An SpeI site was engineered to the pET3a vector to allow link and lock cloning. Here shows consecutive cloning of 3 genes as an example. Additional genes can be added using the same methodology. Genes to be cloned were first ligated into the NdeI/SpeI sites of the modified pET3a vector, resulting in the pET3a-A, pET3a-B, and pET3a-C plasmids. The pET3a-A plasmid serves as the master vector and is cut with SpeI/HindIII. The *geneB* fragment serves as the insert and is cut out from the pET3a-B plasmid with XbaI/HindIII. As the SpeI enzyme shares compatible cohesive ends with the XbaI enzyme, these two sites are eliminated upon ligation. The resulting pET3a-AB plasmid contains only one SpeI site. For the construction of the pET3a-ABC plasmid, the pET3a-AB plasmid serves as the master vector and the *geneC* fragment cut from the pET3a-C plasmid serves as the insert. RBS, ribosome binding site.

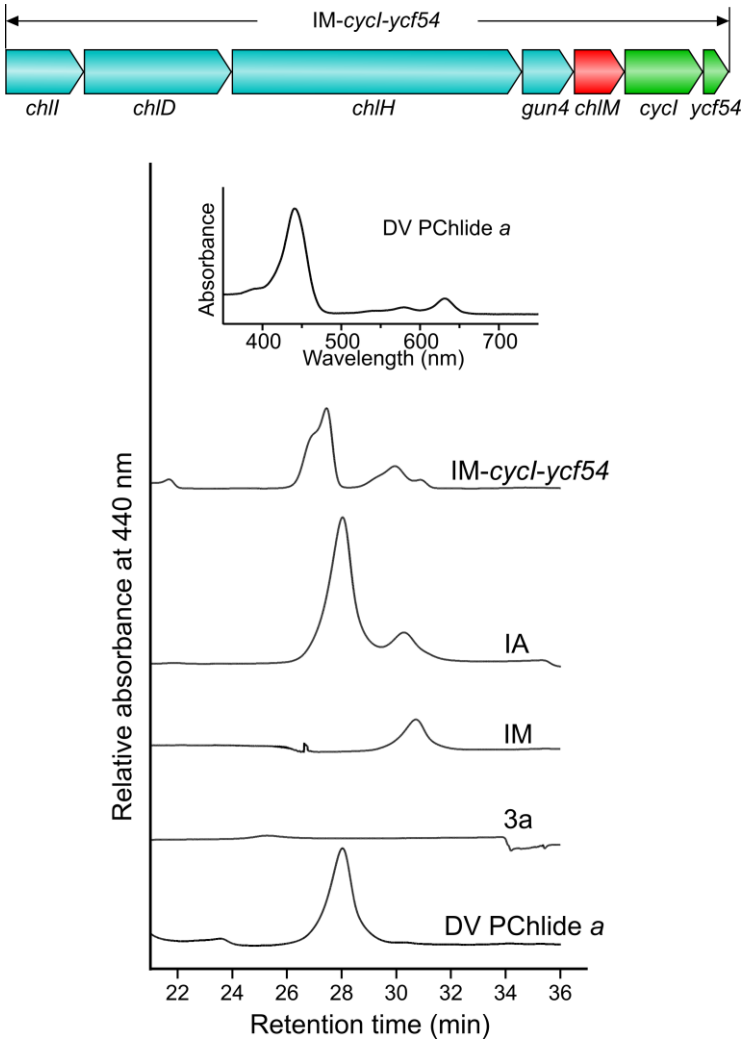

fig. S3. The production of DV PChlide a in the IA and IM-*cycI-ycf54* strains. A supplementary figure to Fig. 3C. Pigment accumulation in described *E. coli* strains was analyzed by HPLC with elution profiles monitored by absorbance at 440 nm. The in vivo activity of the *Synechocystis* cyclase is demonstrated by the accumulation of DV PChlide a in the IM-*cycI-ycf54* strain. The lack of alignment of the major elution peak of IM-*cycI-ycf54* with the other elution profiles arises from the use of a different HPLC column used to analyze the IM-*cycI-ycf54* sample. However, the diagnostic absorption of DV PChlide a shown in the inset, recorded for the major elution peak of the IM-*cycI-ycf54* sample, shows that the addition of *cycI-ycf54* to the IM construct confers cyclase activity on the *E. coli* strain.

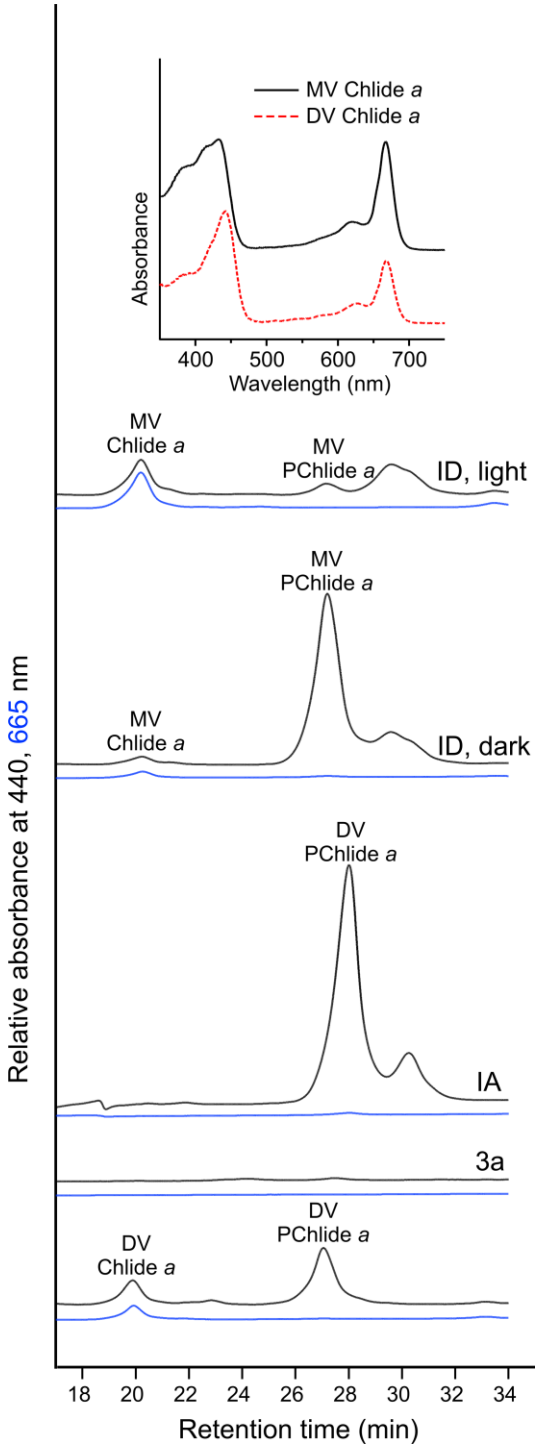

fig. S4. The light-dependent production of MV Chlide a in the ID strain. A supplementary figure to Fig. 3D. Pigment accumulation in described *E. coli* strains was analyzed by HPLC with elution profiles monitored by absorbance at 440 nm (shown in black) and 665 nm (shown in blue).

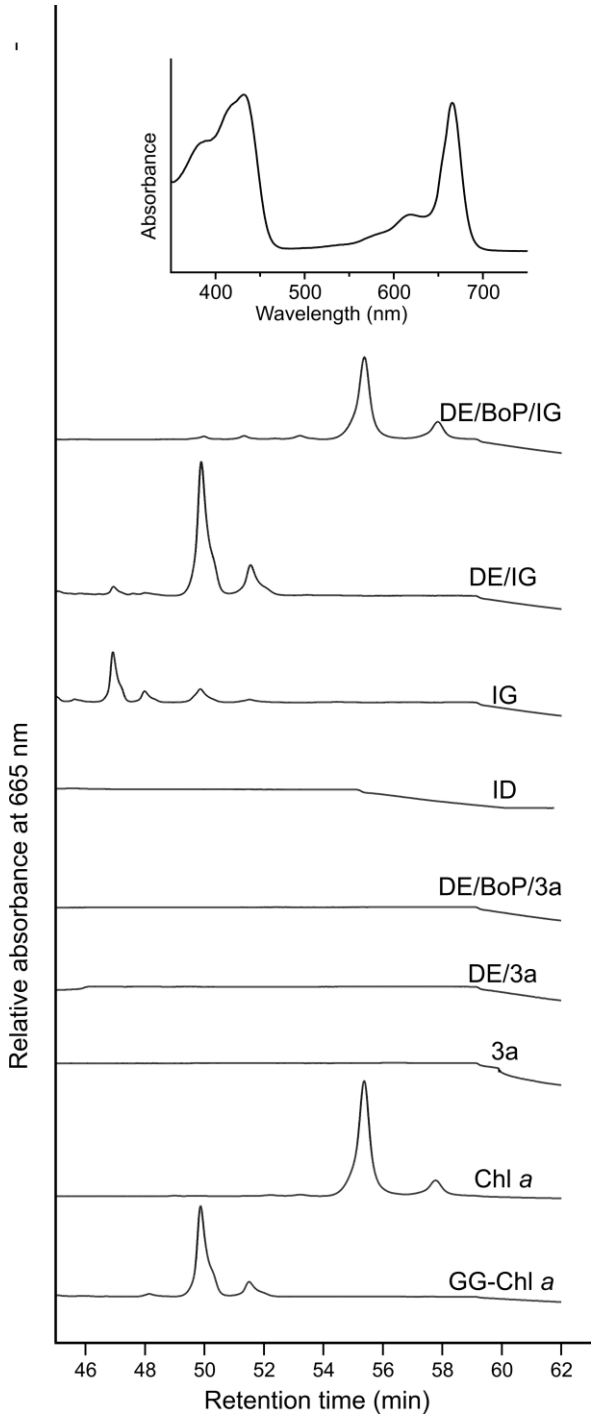

fig. S5. The production of GG–Chl a in the DE/IG strain and of Chl a in the DE/BoP/IG strain. A supplementary figure to Fig. 3E. Pigment accumulation in described *E. coli* strains was analyzed by HPLC with elution profiles monitored by absorbance at 665 nm.

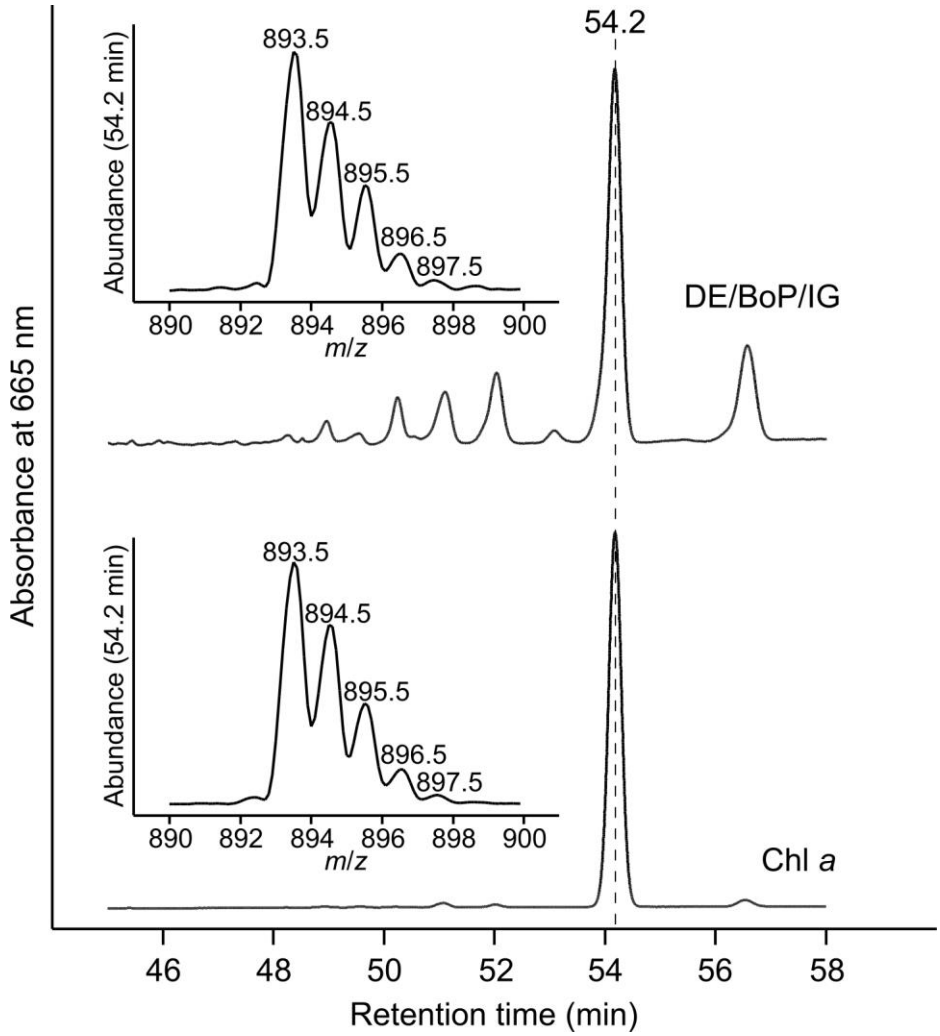

fig. S6. Verification of the production of Chl a in *E. coli* by LC-MS. The pigment extract from the DE/BoP/IG strain and the Chl a standard was analyzed. Mass spectra of the dominant peak present in the elution profiles are shown.

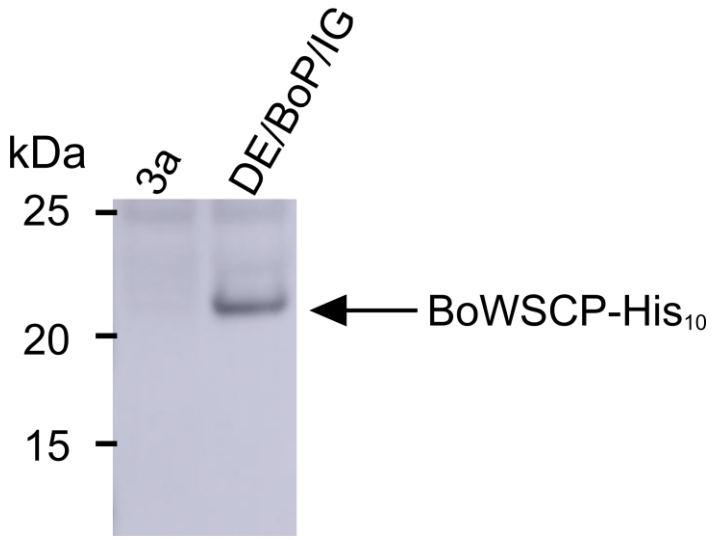

fig. S7. Western blot analysis of the BoWSCP-His_{10} expression in the DE/BoP/IG strain. Soluble fractions isolated from *E. coli* cell lysates by centrifugation, were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Resolved proteins were transferred onto a polyvinylidene fluoride membrane for immunodetection. The membrane was incubated with an anti-6-His primary antibody (Bethyl), and then with a secondary antibody conjugated with horseradish peroxidase (Sigma-Aldrich). The predicted molecular weight of the BoWSCP-His_{10} protein is 20.8 kDa.

table S1. List of genes used to assemble the Chl biosynthesis pathway in *E. coli*.

Gene	Locus	Organism	Annotation
chlI	slr1030	*Synechocystis* sp. PCC 6803	I subunit of magnesium chelatase
chlD	slr1777	*Synechocystis* sp. PCC 6803	D subunit of magnesium chelatase
chlH	slr1055	*Synechocystis* sp. PCC 6803	H subunit of magnesium chelatase
gun4	sll0558	*Synechocystis* sp. PCC 6803	porphyrin-binding protein that enhances magnesium chelatase
chlM	slr0525	*Synechocystis* sp. PCC 6803	magnesium-protoporphyrin IX methyltransferase
acsF	RGE_33550	*Rubrivivax gelatinosus* IL144	O_2-dependent magnesium-protoporphyrin IX monomethyl ester cyclase
por	slr0506	*Synechocystis* sp. PCC 6803	light-dependent protochlorophyllide oxidoreductase
bciB	slr1923	*Synechocystis* sp. PCC 6803	ferredoxin-dependent 8-vinyl reductase
chlP	sll1091	*Synechocystis* sp. PCC 6803	geranylgeranyl reductase
chlG	slr0056	*Synechocystis* sp. PCC 6803	chlorophyll *a* synthase
dxs	b0420	*Escherichia coli*	1-deoxy-D-xylulose-5-phosphate synthase
crtE	RGE_33730	*Rubrivivax gelatinosus* IL144	geranylgeranyl pyrophosphate synthase

table S2. Strains and plasmids described in this study. [*]Research Institute for Photosynthetic Hydrogen Production, Kanagawa University, Japan. [†]Institute of Microbiology, Department of Phototrophic Microorganisms, Třeboň, Czech Republic. [‡]Indiana University, USA. [§]Department of Biochemistry, University of Oxford, UK.

Strain/Plasmid	Characteristics	Source
E. coli		
JM109	Cloning strain for plasmid construction	Promega
S17-1	Conjugation strain for transfer of plasmid to *Rba. capsulatus*	(*37*)
C43(DE3)	Expression strain for *in vivo* assay and assembly of chlorophyll biosynthesis pathway	(*11*)
Rvi. gelatinosus		
WT	IL144	S. Nagashima[*]
Δ*bchE*Δ*acsF*	Unmarked deletion of the *bchE* and *acsF* genes in WT	(*5*)
Synechocystis		
WT	sp. PCC 6803, glucose tolerant	R. Sobotka[†]
Δ*chlP*	Em^R replacement of the *chlP* gene in WT	(*10*)
Rba. capsulatus		
WT	SB1003, Rif^R	C. Bauer[‡]
Δ*bchE*Δ*ccoP*	Unmarked deletion of the *bchE* and *ccoP* genes in WT	This study
Plasmid		
pK18mobsacB	Allelic exchange vector, Km^R	J. Armitage[§]
pK18Δ*bchE*	Upstream-NdeI-downstream of the *Rba. capsulatus bchE* gene cloned into XbaI/HindIII sites of pK18mobsacB, Km^R	This study
pK18Δ*ccoP*	Upstream-NdeI-downstream of the *Rba. capsulatus ccoP* gene cloned into XbaI/HindIII sites of pK18mobsacB, Km^R	This study
pBBRBB-$Ppuf_{843\text{-}1200}$	Expression vector carrying the 843-1200 region of the *puf* promoter of *Rba. sphaeroides*, Km^R	(*35*)
pBB[*acsF*]	The *Rvi. gelatinosus acsF* gene cloned into the BglII/NotI sites of pBBRBB-$Ppuf_{843–1200}$, Km^R	This study
pET3a-*acsF*	The *Rvi. gelatinosus acsF* gene with an added SpeI site cloned into the NdeI/BamHI sites of pET3a, Amp^R	This study
IM	Link and lock cloning, five genes *chlI-chlD-chlH-gun4-chlM* cloned into pET3a, Amp^R	This study
IA	Link and lock cloning, six genes *chlI-chlD-chlH-gun4-chlM-acsF* cloned into pET3a, Amp^R	This study
ID	Link and lock cloning, eight genes *chlI-chlD-chlH-gun4-chlM-acsF -por-dvr* cloned into pET3a, Amp^R	This study
IG	Link and lock cloning, nine genes *chlI-chlD-chlH-gun4-chlM-acsF -por-dvr-chlG* cloned into pET3a, Amp^R	This study
DE	The *E. coli dxs* and *Rvi. gelatinosus crtE* genes cloned into the NcoI/HindIII sites and NdeI/XhoI sites of the pCOLADuet1 vector, Km^R	This study
BoP	The BoWSCP-His_{10} coding sequence and *Synechocystis chlP* gene cloned into the NcoI/HindIII sites and NdeI/XhoI sites of the pACYCDuet1 vector, Cm^R	This study
IM-*cycI-ycf54*	Link and lock cloning, seven genes *chlI-chlD-chlH-gun4-chlM-cycI-ycf54* cloned into pET3a, Amp^R	This study

table S3. Oligonucleotide primers used in this study.

Primer	Sequence (5'-3')
bchEUpXbaIF	GCTCTAGAGGAGCTGATCCCGCCCTTCC
bchEUpR	GCCGTCACTCCTTCTTATTCGCGCATGGCTGACCCTCC
bchEDownF	GGAGGGTCAGCCATGCGCGAATAAGAAGGAGTGACGGC
bchEDownHindIIIR	GAGTCTAAGCTTTCGACCCGGAACCGC
bchEScreenF	GGAATAGCCTTTTTCCGGTGC
bchEScreenR	GGTTGTCATCGATGCGGAAG
ccoPUpXbaIF	GAGTCTTCTAGAGCTATCTGGCCAATGTGCCGC
ccoPUpR	GATCCGTTTGGCTGTTACTGGCTCATCTCCACGCCTCCT
ccoPDownF	AGGAGGCGTGGAGATGAGCCAGTAACAGCCAAACGGATC
ccoPDownHindIIIR	GAGTCTAAGCTTGCCAGATCTCGAGCCCGAAGA
ccoPScreenF	GCAATCGGTGGTGCCGGAATC
ccoPScreenR	CCAAGCCCGGCCATGATCAGA
acsFremoveBglIIF	GATCACCAACGAGATATCCAAGCAGGT
acsFremoveBglIIR	ACCTGCTTGGATATCTCGTTGGTGATC
acsFBglIIF	GAGTCTAGATCTATGCTCGCGACCCCGACGAT
acsFNotIR	GAGTCTGCGGCCGCTCACCATGCCGGGGCCATGC
acsFNdeIF	CGCCATATGCTCGCGACCCCGACGATCGAATC
acsFSpeIBamHIR	GCCGGATCCACTAGTTCACCATGCCGGGGCCATG
chlIremoveXbaIF	AAAGATCCTCTGGAGTCCATTGATTCC
chlIremoveXbaIR	AATCAATGGACTCCAGAGGATCTTTCC
chlIremoveHindIIIF	TTGTCGATGAGGCTTAACGTCG
chlIremoveHindIIIR	ACGTTAAGCCTCATCGACAACG
pETaddSpeIF	ATCCGGCTACTAGTAAAGCCCGAAAGGAAGC
pETaddSpeIR	TTCCTTTCGGGCTTTACTAGTAGCCGGATCC
gun4NdeIF	TCCATATGTCTGATAATTTGACC
gun4SpeIR	TCACTAGTTTACCAACCGTATTGGGACC
gun4removeXbaIF	AAACCCTCCGGAACCTAGAACAGG
gun4removeXbaIR	TTCCTGTTCTAGGTTCCGGAGGGTTTGG
gun4removeHindIIIF	AAGAATTTACCAAACTTTGGCCGAAAATTGG
gun4removeHindIIIR	AATTTTCGGCCAAAGTTTGGTAAATTCTTTTCC
chlMNdeIF	GCGCATATGACCAACGCCGCCCTAGACG
chlMSpeIBamHIR	GCCGGATCCACTAGTTAAGAGCGCACCGCCTCTAAAATACG
porNdeIF	GCCCATATGGAACAACCGATGAAACCCACGG
porSpeIBamHIR	GCCGGATCCACTAGTCTAAACCAGACCCACTAACTTTTC
porremoveHindIIIF	ATACGGAGCTAAGGCCTTAATTGAC
porremoveHindIIIR	GTCAATTAAAGCCTTAGCTCCGTAT
dvrNdeIF	GCGCATATGACCGTTCCTGCCCCCCACC
dvrSpeIBamHIR	GCGGGATCCACTAGTTATTGCTGGGGAAGTTTATACTGC
dvrremoveSpeIF	GGAAACTACTAGCAGATCGCCAGAAACG
dvrremoveSpeIR	CGTTTCTGGCGATCTGCTAGTAGTTTCC
chlGNdeIF	GCGCATATGTCTGACACACAAAATACC
chlGSpeIBamHIR	GCCGGATCCACTAGTCAAATCCCCGCATGGCCTAGG
chlPNdeIF	GCGCATATGGTATTACGGGTAGCAGTCG
chlPSpeIBamHIR	GCCGGATCCACTAGTTAAGGGGCTAAAGCGTTACC
chlPXhoIR	GGAACTCGAGTTAAGGGGCTAAAGCGTTACCC
dxsNcoIF	GGCCCATGGAGTTTTGATATTGCCAAAT
dxsHindIIIR	GGCAAGCTTTTATGCCAGCCAGGCCTTGATT
dxsremoveHindIII1R	GAAGAGTACAGCTTACCGGAAA
dxsremoveHindIII1F	TTTCCGGTAAGCTGTACTCTTC
dxsremoveHindIII2R	CAGGACCGGCAGCTTTTGAATCG
dxsremoveHindIII2F	CGATTCAAAAGCTGCCGGTCCTG
crtENdeIF	TCTCATATGAACACGATGACTCGCATCGA
crtEXhoIR	GGCCTCGAGTCAAGCGGTCTGGGTCGGAG
cycINdeIF	GCGCATATGGTTAATACCCTCGAAAAGCCCGGAT
cycISpeIBamHIR	GCGGGATCCACTAGTTAGCGCACAGCTCCAGCCAACTGA
ycf54NdeIF	GCGCATATGGCTACCTATTATTATGCTTTGGCAAG
ycf54SpeIBamHIR	GCGGGATCCACTAGTCTAATCCAGGGATGCAAGGGGGTC