

Supporting Information

**Refactoring the six-gene Photosystem II core in the chloroplast of the green algae
*Chlamydomonas reinhardtii***

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Supporting Table 1. DNA fragment lengths of the deleted PSII genes. Numbers represent base pairs with respect to the start codon (5') and stop codon (3') of the CDS. The *psbA* CDS length includes introns.

Gene	5'	CDS	3'	Comments
<i>psbA</i>	300	6639	1525	<i>psbA</i> has been previously deleted and complemented (1)
<i>psbD</i>	0	1059	0	<i>psaA</i> exon 3 is transcribed in the same operon downstream of <i>psbD</i> (2). 5' and 3' UTRs were not deleted in case they affected the trans-splicing of <i>psaA</i> .
<i>psbC</i>	546	1386	0	<i>psbC</i> 5' UTR is 546 bp long (3). The <i>psbC</i> promoter and 3' UTR were not deleted because they might drive the expression of the tRNA-His 273 bp downstream of <i>psbC</i> stop codon. ORF2971 is 943 bp upstream of <i>psbC</i> . The 5' deletion leaves a 393 bp 3'UTR for ORF2971.
<i>psbB</i>	0	1527	451	<i>psbB</i> is the first gene of an operon containing <i>psbT</i> and <i>psbH</i> (4). The deletion of the complete <i>psbB-psbT</i> intergenic region effectively exchanges the promoter/5'UTR of <i>psbT</i> with that of <i>psbB</i> .
<i>psbE</i>	0	248	0	<i>psbE</i> is not part of an operon (5, 6). There is a tRNA-Met 113 bp upstream of the <i>psbE</i> start codon. Not deleting the <i>psbE</i> 3' UTR was required for rescuing photosynthetic function of the knock-out strain.
<i>psbF</i>	0	135	0	<i>psbF</i> is transcribed at the beginning of an operon along with <i>psbL</i> (6). Not deleting the <i>psbF</i> 3' UTR was required for rescuing photosynthetic function of the knock-out strain.

Supporting Table 2. Amino acid sequence identities and codon adaption indexes (CAI) with respect to *C. reinhardtii* sequences. The numbers in parentheses correspond to the total of amino acids in the *C. reinhardtii* homologs. A CAI value of 1.00 would indicate that the codons are perfectly optimized for *C. reinhardtii* plastid codon preferences (7).

% Identity	PsbA (352)	PsbD (352)	PsbB (508)	PsbC (461)	PsbE (82)	PsbF (44)	Average
<i>C. reinhardtii</i>	100%	100%	100%	100%	100%	100%	100%
<i>V. carteri</i>	99%	99%	99%	98%	99%	95%	98%
<i>S. obliquus</i>	94%	99%	95%	95%	94%	82%	93%
CAI							
<i>C. reinhardtii</i>	0.67	0.77	0.81	0.80	0.87	0.83	0.79
<i>V. carteri</i>	0.65	0.73	0.76	0.78	0.71	0.87	0.75
<i>S. obliquus</i>	0.67	0.80	0.81	0.78	0.86	0.86	0.80

Supporting Table 3. DNA coordinates of the *C. reinhardtii* 5' and 3' regulatory regions cloned into our PSII expression vectors. Numbers represent base pairs with respect to the start codon (5') and stop codon (3') of the CDS. The CDS coordinates correspond to the ones from *C. reinhardtii*.

Gene	5'	CDS	3'	Comments
<i>psbA</i>	351	1059	346	<i>psbA</i> transcription starts 90 bp upstream of the start codon (8).
<i>psbD</i>	450	1059	211	<i>psbD</i> transcription starts 74 bp upstream of the start codon (9).
<i>psbC</i>	846	1386	237	<i>psbC</i> transcription starts 546 bp upstream of the start codon (3).
<i>psbB</i>	397	1527	324	<i>psbB</i> transcription starts 147 bp upstream of the start codon (4).
<i>psbE</i>	607	248	317	unknown transcription start
<i>psbF</i>	625	135	330	unknown transcription start

Supporting Table 4. USER cloning site (UC) sequences from vectors in Supporting Figure 1. Blue: Nt.BbvCI site; Red: PstI site; Underlined: unique sequences that allow for directional cloning.

Name	Sequence
UC1	GCTGAGG <u>GT</u> TTAATTAA <u>GA</u> CCTCAGC
UC2	GCTGAGG <u>GT</u> TTAATTAA <u>CT</u> CCTCAGC
UC3	GCTGAGG <u>CA</u> TTAATTAA <u>GA</u> CCTCAGC

Supporting Table 5. Primers used for the construction of the PSII knock-out vectors.

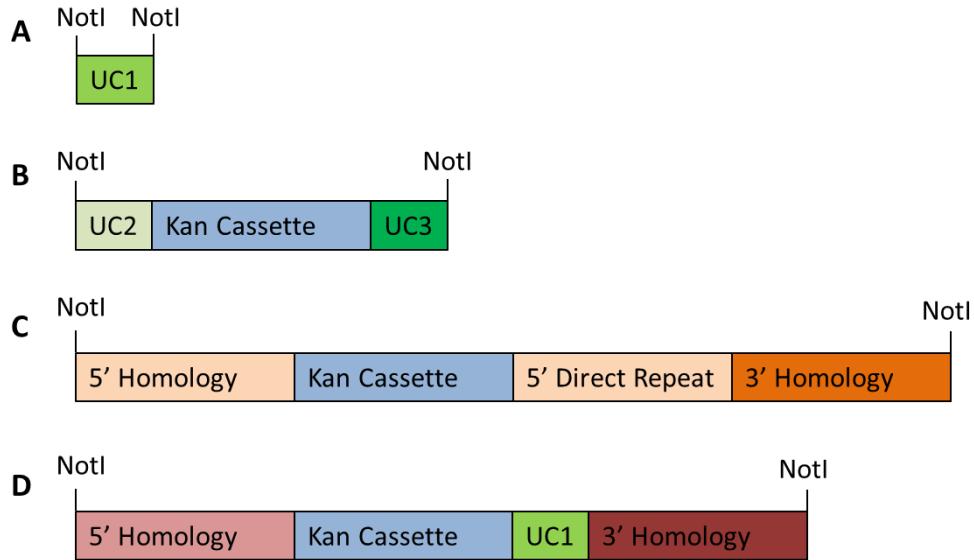
Knock-out constructs. HM: Homology region; DR: direct repeat; Numbers indicate whether it is the 5' or 3' regulatory region of the corresponding CDS	
Name	Sequence (5' -3'; USER cloning tails in bold)
psbA KO Construct	
psbA_HM5_fw	GGGTTAAUCAACACTTACTGT TTTATTTTGAGTTAC
psbA_HM5_rev	GGAGTTAAUTTAGTGCCCAGGGGTTAC
psbA_DR5_fw	GCGATTAUCAACACTTACTGT TTTATTTTGAGTTAC
psbA_DR5_rev	AGGATCCUTTAGTGCCCAGGGGTTAC
psbA_HM3_fw	AGGATCCUGGATCCGTATCCATGCTAGCAATATC
psbA_HM3_rev	GGTCTAAUATTCTAAGTTACTTGCCCAGG
psbD KO Construct	
psbD_HM5_fw	GGGTTAAUAAATATTAGTGGATATTATATACTCCG
psbD_HM5_rev	GGAGTTAAUTGCGTGTATCTCAAATAAAAAACAACTC
psbD_DR5_fw	GCGATTAUAAATATTAGTGGATATTATATACTCCG
psbD_DR5_rev	ATATATCGGTGUATCTCAAATAAAAAACAACTCATCG
psbD_HM3_fw	ACACGCATATAUTTTATATAAATTACCAACTAATTAGTATTGG
psbD_HM3_rev	GGTCTAAUTCGGATAAACTGCATAAAATTATAG
psbB KO construct	
psbB_HM5_fw	GGGTTAAUGTTAAGGTATAAAAAGCTTAACTTAG
psbB_HM5_rev	GGAGTTAAUAAAAAATTTTACTGATTTTTACTTAATTATTAC
psbB_DR5_fw	GCGATTAUCCGTCATCCGAAATTCAAATC
psbB_DR5_rev	ACTAAAGCTCCAUAAAAATTTTACTGATTTTTACTTAATTATTAC
psbB_HM3_fw	ATGGAAGCTTAGUATATACTTCTATTAGTTGGG
psbB_HM3_rev	GGTCTAAUATGGCTATCGTCTATCCATGAAG
psbC KO construct	
psbC_HM5_fw	GGGTTAAUAGCCAAAATTAGATACTTAATGTC
psbC_HM5_rev	ATAAAAAAATTTTAUTTATTATATGAAAAAAATTAAATG
psbC_DR3_fw	ATAAAAAATTTTAUTTATTAAAATTGGGTTTACCATG
psbC_DR3_rev	GGAGTTAAUCCGGCTTAATTATATAAGATAGCG
psbC_HM3_fw	GCGATTAUTTTTATTATTTAAAATTGGGTTTACCATG
psbC_HM3_rev	GGTCTAAUGCGTTGCTAAATATGTCATCTCC
psbE KO construct	
psbE_HM5_fw	GGGTTAAUATTAGTGGCAGTTGCCTCCTCCCT
psbE_HM5_rev	GGAGTTAAUAAAAAACCTCTAAGTGTGCTTACTTAGC
psbE_DR5_fw	GCGATTAUATTAGTGGCAGTTGCCTCCTCCCT
psbE_DR5_rev	AAAAAAAATACCTCUAAGTGTGCTTACTTAGC
psbE_HM3_fw	AGGAGGTATTTTTUAATCTAAAACTTTTACTTAGAAAATTTC
psbE_HM3_rev	GGTCTAAUTAATATCAGAACATTGGAATAGCTAACAGC
psbF KO construct	
psbF_HM5_fw_5u(1)	GGGTTAAUAGTAAACTTAAGAGGATGAAACTCTTGG
psbF_HM5_rev_3u(2)	GGAGTTAAUATTAATAAAAATAAGGGTAATTACCTTTG
psbF_DR5_fw_5u(2)	GCGATTAUAGTAAACTTAAGAGGATGAAACTCTTGG

psbF_DR5_revfus	AGGGACGAATACAATU AATAAAAATAAGGGTAATTTC
psbF_HM3_fwfus	AATTGTATTCGTCCUAAAAGAACGTTAG
psbF_HM3rev_3u(1)	GGTCTAAUGTCTTAGTGTAAACATAGAAAATAACCCC

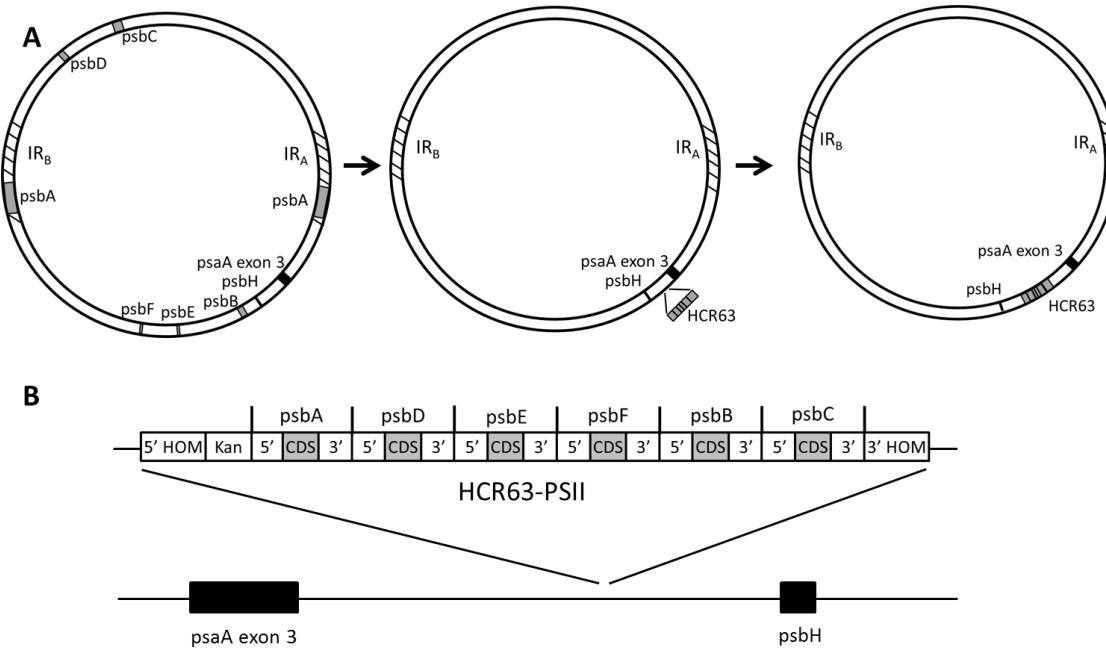
Supporting Table 6. Primers used for the construction of the PSII complementation vectors.

PSII complementation constructs	
Name	Sequence (5'-3'; USER cloning tails in bold)
HCR63-CrPSII (<i>C. reinhardtii</i>)	
psbA_prom_fw	GGGTTAAUCCACTGCCTGCTTCCTCC
psbA_3'UTR_rev	ATGTGTCGAGUGGTACGCCACTGC
psbD_prom_fw	ACTCGACACAU TTTTGAGTCATGGAGTCATATG
psbD_3'UTR_rev	AGAACGTCC TUCGGACGTGAAAATGCTGGTTAGTC
psbE_prom_fw	AAGGCACGTT CUTTACGGTCTATATATTC
psbE_3'UTR_rev	ATTAGTCATCUA GACTAGTGCCAGTTGCTC
psbF_prom_fw	AGATGACTAAU AGGGCTTACCCCTGGTTCAATTACC
psbF_3'UTR_rev	ATCCCCGAGAU ATTAATAGCCTGCCCTCGG
psbB_prom_fw	ATCTCGGGGAU GTCCTTCGCATAATG
psbB_3'UTR_rev	ACACAAGTTUAAA ACTTGAAGAATTCAAGTGTATGG
psbC_prom_fw	AAA ACTTGTGUAGCCCTGTAAGGAACGTCC
psbC_3'UTR_rev	GGTCTAAUGC GATCGCACCCCTCAGCTACAATCCACTGCCTTACCACTTGG
HCR63-VcPSII (<i>V. carteri</i>)	
psbA_prom_fw	GGGTTAAUCCACTGCCTGCTTCCTCC
Chlamy_psbA_prom_rev	ATATGUTAATT TTAAAGTTAATTCTCC
Volvox_psbA_CDS_fw	AAAATUAACATAU GACAGCACTTAGAACGTGCGT
Volvox_psbA_CDS_rev	AGTTUAAAAAAA ATUAGTCATAGAAGAATTGTTAGAAC
Chlamy_psbA_3'UTR_fw	AATTTUTTTAAACU AAAATAATCTGGTTAAC
Chlamy_psbD_prom_rev	ATGCGUGTATC UCCAAAATAAAAAAC
Volvox_psbD_CDS_fw	AGAUACACGCAU GACAATAGCGATTGGTACATATCAAGA
Volvox_psbD_CDS_rev	ATATAAAAUA TATUAAAGAGCGTACACGTGGTAAACTT
Chlamy_psbD_3'UTR_fw	AATATATUTT TATAUAAAATTACCAATAACTAATTAGTATTGG
Chlamy_psbE_prom_rev	ATAAAAAAUACCU CCTAAGTGTGCTACTTAGC
Volvox_psbE_CDS_fw	AGGTATUTTTAU UAGGCTGGTAAACCGGTAGAAC
Volvox_psbE_CDS_rev	AGTTTUAGATTA ATUACCAGCTAATTTTAACTTGTCTA
Chlamy_psbE_3'UTR_fw	AATAATCUAAAACU TTTACTTAGAAAATT
Chlamy_psbF_prom_rev	ATAATTAAUAAAUA ATAGGGTAATTACCTTTG
Volvox_psbF_CDS_fw	ATTTTAUTAATTAU GACAACAAAAATCAGCTGAAG
Volvox_psbF_CDS_rev	AGGGACGAAUACTU ACGTTGAATAAATTGCATTGCAG
Chlamy_psbF_3'UTR_fw	AAGTATTG UCCCCUAAAAGAACGTTAGTAC
Chlamy_psbB_prom_rev	ATAAAAAAU TTTUA CTGAT TTTACTTAATTATTAC
Volvox_psbB_CDS_fw	AAAAAAU TTTTAU <u>GGGTTACCTGGTACGTG</u> TAC
Volvox_psbB_CDS_rev	AAAAGAAA ACTUAGAATGCTCACGTAAAGAAGAAGTG
Chlamy_psbB_3'UTR_fw	AAGTTTCTT U <u>CTGGCAGTTGGC</u>
Chlamy_psbC_prom_rev	ACTTGCAU ACCUCCGTACAAATTATTTG
Volvox_psbC_CDS_fw	AGGTAAU GCAAAGUGGAAACACTTTAATGGAACACTT
Volvox_psbC_CDS_rev	ATAATAUAAA ATUAGTCAAAGGACGCATTGAAAGAAC
Chlamy_psbC_3'UTR_fw	AATTTUATT ATTAUAAAATTGGTTGTTACCA

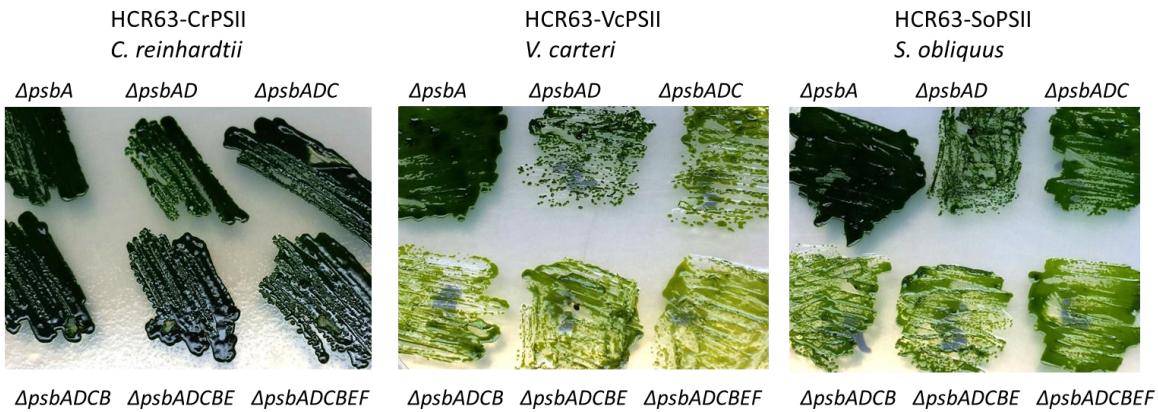
psbC_3'UTR_rev	GGTCTTAAUGCGATCGCACCCCTCAGCTACAATCCACTGCCTTACCACTTGG
HCR63-SoPSII (<i>S. obliquus</i>)	
psbA_prom_fw	GGGTTTAAUCCACTGCCTGCTTCCTCC
Chlamy_psbA_prom_rev	ATATGUTAATTUTTTAAAGTTTAATTCTCC
Scene_psbA_CDS_fw	AAAATUAACATAUGACAGCTTTAGCTAAAACGAAGCATCTAGCCTTGGG
Scene_psbA_CDS_rev	AGTTUAAAAAAAATUAAGCGTTACAGAAGGAGCTTC
Chlamy_psbA_3'UTR_fw	AATTTTUTTTAACUAAAATAACTGGTTAAC
Chlamy_psbD_prom_rev	ATGCGUGTATCUCCAAAATAAC
Scene_psbD_CDS_fw	AGAUACACGCAUGACTATTGCGATTGGTAGTACATAC
Scene_psbD_CDS_rev	ATATAAAAUAATATUATAGAGCGTACACGAGGTAATAC
Chlamy_psbD_3'UTR_fw	AATATATUTTTATAUAAAATTACCAATACTAATTAGTATTGG
Chlamy_psbE_prom_rev	ATAAAAAAUACCUCCTAAGTGTGCTTACTTAGC
Scene_psbE_CDS_fw	AGGTATUTTTAUGGCAGGAAAACCACTAGAACG
Scene_psbE_CDS_rev	AGTTTUAGATTAUATTGAGATAATTTTAACTTGTCTAATGC
Chlamy_psbE_3'UTR_fw	AATTAATCUAAAACUTTTTACTTAGAAAATT
Chlamy_psbF_prom_rev	ATAATTAUAAAUAATAGGGTAATTACCTTTG
Scene_psbF_CDS_fw	ATTTTAUTAATTAUAGACAACAAGAAAATCAGCTGAAGC
Scene_psbF_CDS_rev	AGGGACGAAUACTAACGTTGAATTGCATTGCTG
Chlamy_psbF_3'UTR_fw	AAGTATTGUCCCCUAAAAGAACGTTAGTAC
Chlamy_psbB_prom_rev	ATAAAAAAUUUUUUACTGATTTTTACTTAATTATTAC
Scene_psbB_CDS_fw	AAAAAAUUTTTTAUGGGCTTACCTGGTATCGT
Scene_psbB_CDS_rev	AAAAGAAAAACTUAGAAAAGCTCACGAACAGAACTTG
Chlamy_psbB_3'UTR_fw	AAGTTUTTCTTUCTGGCAGTTGGC
Chlamy_psbC_prom_rev	ACTTTGCAUTACCUCCGTACAAATTATTTG
Scene_psbC_CDS_fw	AGGTAAUGCAAAGUGGAAACGCTTTAACGGAACAC
Scene_psbC_CDS_rev	ATAATAUAAAATUAATCTAAAGGTCTCATAGATAATACTGG
Chlamy_psbC_3'UTR_fw	AATTTUATTATAUAAAATTGGGTGTTACCA
psbC_3'UTR_rev	GGTCTTAAUGCGATCGCACCCCTCAGCTACAATCCACTGCCTTACCACTTGG



Supporting Figure 1. USER cloning vectors from this study. **A.** HCR1. **B.** HCR2. **C.** Vector for gene deletions. **D.** HCR63. UC: USER Cloning site, the different colors refer to different overhangs, allowing for directional cloning (Supporting Table 4). NotI refers to the site in pBlueScript II (Agilent).



Supporting Figure 2. Integration of the HCR63-PSII vectors into the *C. reinhardtii* plastid genome. **A.** The original wild-type chloroplast genome contains all PSII genes. After the knock-out strains are generated, the HCR63 vectors are transformed and integrate by homologous recombination into a neutral site between *psaA* exon 3 and *psbH*. **B.** Close-up scheme of HCR63-PSII and its integration site. IR_A and IR_B refer to the inverted DNA repeats of the plastid genome.



Supporting Figure 3. Photosynthetic growth on plates of the knock-out strains complemented with synthetic PSII constructs. The complementation vector names are presented in the upper part of the figure. Each of the six patches per construct corresponds to a different knock-out strain recipient.

References

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