1 Supplement

TABLE S1 Plasmids used in this work.

Plasmid	Characteristics	Reference
pDrive	Cloning vector	Qiagen, Germany
13K19	pBeloBAC11 with DNA of chromosome	CUGI, Clemson
	17 including Cre17.g698750	University, USA (1)
31013	pBeloBAC11 with DNA of chromosome	CUGI, Clemson
	10 including <i>RAA8</i> (Cre10.g440000)	University, USA (1)
31013-1	Ligation of 36 kb derived from	This work
	31013: <i>Sgs</i> l	
31013-2	Ligation of 48 kb derived from	This work
	31013: <i>Sgs</i> l	
31013-3	Ligation of 34 kb derived from 31O13:Bln	This work
31013-4	Ligation of 36 kb derived from 31O13: Blnl	This work
31013-5	Ligation of 31O13:Sful, Clal	This work
31013-6	Subcloned DNA of <i>RAA8</i> ,	This work
	Cre10.g440050	
31013-7	Subcloned DNA of <i>RAA8</i>	This work
pDrive_PCR250B	0.70 kb DNA of <i>RAA8</i> in pDrive	This work
pDrive_PCR252A	1.45 kb DNA of <i>RAA8</i> in pDrive	This work
pDrive_PCR253	0.80 kb DNA of <i>RAA8</i> in pDrive	This work
pDrive_PCR256B	1.28 kb DNA of <i>RAA8</i> in pDrive	This work
plG637.1	0.9 kb <i>Bg</i> /II/ <i>Nsi</i> l fragment for <i>tscA</i> probes	(2, 3)

	in Northern analyses	
3		

TABLE S2 Oligonucleotides used in PCR, RT-PCR or hybridization experiments.

Oligonucleotide	Sequence (5' \rightarrow 3')	Application, reference			
Cloning and sequencing of RAA8					
for_IP2_Ex2-2	CAGTTGGTGCGGGTGTTGG	Specific for RAA8, this work			
rev_IP2_Ex3	CTCAATGGCAGCGAGACCT	Specific for <i>RAA8</i> , this work			
rev_raa8-F1-2	GCCCTTCCTGTACATAACC	Specific for <i>RAA8</i> , this work			
for-2-IP2_Ex3	GCTGGCGGCGCTGTCACAC	Specific for RAA8, this work			
rev_IP2_Ex4	TCGGGCTGGTAGCCCAGAC	Specific for <i>RAA8</i> , this work			
for_raa8_F1	CTGGCATGCTCAGGGTCGC	Specific for RAA8, this work			
rev_raa8_F1.1	AGCCGTCTGTGAAGGTTTG	Specific for RAA8, this work			
for_raa8-F2-7	GACACACCGCAGGAGTTG	Specific for RAA8, this work			
raa8_F3-for	TAGCATGGAGTGGCTACAG	Specific for <i>RAA8</i> , this work			
rev_raa8-F2-4	TGCGGACCTCGCACTGAAG	Specific for <i>RAA8</i> , this work			
for-raa8-F6-3	GGGCTACCAGCCCGAGGAC	Specific for <i>RAA8</i> , this work			
for-raa8-Ex2-3	CGGCTGCTTACAAACCTTC	Specific for <i>RAA8</i> , this work			
for_Raa8_Mutati	TTGGCGGCGGCAGGTGTAG	Specific for <i>RAA8</i> , this work			
on					
rev_Raa8_Mutati	TTTGCGCTTTCGCATGGG	Specific for RAA8, this work			
on					
rev-raa8-F6-4	ACTTCTTGCTTGCGCATCC	Specific for <i>RAA8</i> , this work			
raa8-F2-for	GCAGGCGGTTATGTACAGG	Specific for <i>RAA8</i> , this work			
rev-raa8-F2-4	TGCGGACCTCGCACTGAAG	Specific for <i>RAA8</i> , this work			
for-raa8-F2-5	GGCGCTGCTTCAGTGCGAG	Specific for <i>RAA8</i> , this work			

rev-raa8-F2-6	AACTCCTGCGGTGTGTCC	Specific for <i>RAA8</i> , this work
for-raa8-F6-4	GATGCGCAAGCAAGAAGTG	Specific for <i>RAA8</i> , this work
rev_raa8-F5-2	GTTGTGCATCGCTATCTC	Specific for <i>RAA8</i> , this work
for-raa8-F2-3	GAGATAGCGATGCACAAC	Specific for <i>RAA8</i> , this work
rev-raa8-F6-2	GTCCACACGCCGGGCCTTG	Specific for <i>RAA8</i> , this work
for-raa8-F6	TGCCGTGCAGGCATGCAG	Specific for <i>RAA8</i> , this work
rev-raa8-F6-3	TGACACGCCAACATGCTC	Specific for <i>RAA8</i> , this work
for-raa8-F6-2	CAAGGCCCGGCGTGTGGAC	Specific for <i>RAA8</i> , this work
rev_IP2_Ex7	GGTCTGCCTGCTGCTCCTC	Specific for <i>RAA8</i> , this work
for-raa8-F7	AGCATGTTGGCGTGTCAG	Specific for <i>RAA8</i> , this work
rev_IP2-Ex8	CTGCAGCGCCAGCACCAG	Specific for <i>RAA8</i> , this work
rev-raa8-F8	CGTGTGGTTGTGCATGTG	Specific for <i>RAA8</i> , this work
for-raa8-F9	ACGAACTCGCACACGTTG	Specific for <i>RAA8</i> , this work
rev_IP2_Ex9	ACGGAGGCACCAGCTTGTC	Specific for <i>RAA8</i> , this work
RT-PCR analyses	1	
for_psaAEx1	AGTACTCCAGAGCGCGAAG	RT-PCR of <i>psaA</i> (4)
	С	
for_psaAEx2	TTGGAACCTTCACGCAGAT	RT-PCR of <i>psaA</i> (4)
	G	
Oligo 19	TTGGTTAAGTGACCCTACTC	RT-PCR of <i>psaA</i> (4)
	A	
Oligo 50	GTATTCAGTGCACACTTTGG	RT-PCR of <i>psaA</i> (4)
	TCAATTAGG	

Oligo 53	AAAAGTAAAGATTGCGGTTG	RT-PCR	of	psaA,	Northern
	AT	analysis (4)		



FIG S1 Functional complementation of *trans*-splicing mutant F19. Test for
photoautotrophic growth of wild type 137C, *trans*-splicing mutant F19, and selected
transformants. Cells were spread onto acetate containing (TAP) or acetate lacking (HS)
agar plates at cell densities as indicated and incubated under low light (LL).



FIG S2 Molecular characterization of F19 and complemented strains. PCR analysis
using cDNA of 137C, F19, two transformants derived from transformation with 31O13-6
(T6-8/1, -8/2), and two transformants of 31O13-7 (T6-10/1, -10/2). Different primer
combinations were used to amplify *psaA* exon 1 - exon 2, exon 2 - exon 3, and exon 1 exon 3.

Sequence A GAGPADLAACVSGLGRLLVLPGGHWVAALCEATRPLLLSRGTSLAELAA Secondary structure SS confidence Disorder confidence	Confidence Key High(9) Low (0) ? Disordered (37%) A Alpha helix (58%) Beta strand (2%)
Sequence L P P P Q WQ R D L E A A T A Q R MA R A A A E A A Q L A A A G V A E A P G S G R G G S R S A G G G Secondary structure SS confidence Disorder 7333333333333333333333333333333333333	G G G G G G L R S A
Sequence A D L K A L D Q L L T A Q A L WWR L S S G S S T S S S S S S S S S S S S S S	RKDSGAEAAA ?????????
Sequence V T E L R P S P G WL D A L V E C A A A L MA A L G P A A S P Q S A L A G L P Y T L H V L G H D P G Secondary structure Disorder confidence	PDFISALLAA
Sequence C S R Y L P L L P L A R D P G L A H E H T A P G A P T P T L S P T P N A N P G G G G G G G G T Q Q Q Q Secondary structure SS confidence Disorder ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?	A Q E A A G P H V V
Sequence V Q V P A S A P A F T G P Q L S R L A L G L A G L G Y Q P E D A WV R C L Q ME S A L R L S G C D P Secondary structure SS confidence Disorder	MELADLLEGL
Sequence LLLAEGCEAGGGSSSSSASGSSSTSGSSGEAQWVDSRLVPAFLGCWWAAA Secondary structure Disorder	E I A MHNDTA V
Sequence A A A A L E R R A R R V D L R L A T A MR A E L R A R L S D R S G A G A A WG Y WS E Q L P G L D P Secondary structure SS confidence Disorder Disorder?? _????????	DRLFEMSDQD
Sequence I E A E L A A L Q F P V F D A G M A L A A A Q E E Q Q A D Q Q E Q E E Q E Q E Q G R T G R W R P Q Secondary structure SS confidence Disorder Disorder confidence	P D G G S G G G G G G G G G G G G G G G G
Sequence P I K D G N K V S F D V G MP V L QR Y T L WAIL R G G G S S S T A A G E A S E P A G R I P G P L Secondary structure SS confidence Disorder confidence	PEGAAAWVGS

21 Continued on next page

Sequence Secondary structure SS confidence Disorder Disorder confidence	LAVRSVALLSGPRAAPAADGRLTAAQLQQLAAAAAAPGSAVVVAPPAFWLESMLACQAAA	Confidence Key High(9) Low (0) ? Disordered (37%) A Alpha helix (58%) Beta strand (2%)
Sequence Secondary structure SS confidence Disorder Disorder confidence	MML GAYGGA GSL SLAEYESAVGHDADGA EAGEP WRRQLLQRRAAAAQRAALLQAAAAQRA 	
Sequence Secondary structure SS confidence Disorder Disorder confidence	ALLQAAAAAARAWALPPVPEWGEAFLRASRLAFLSPPPPPLPQPSRQSSPPAAGVGPGASA 	
Sequence Secondary structure SS confidence Disorder Disorder confidence	A D A A D T A A A V T G P G G G D G S G G D G G S G G S G S G S G	
Sequence Secondary structure SS confidence Disorder Disorder confidence	S VDLMR GGGGS GVGPEEAVALVLALQRLGHTPSRLWAEAVLERMAQGLP TPTQLSEPGAP	
Sequence Secondary structure SS confidence Disorder Disorder confidence	GAYATAAPSVTGNPAPMLGAAAAAAEAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
Sequence Secondary structure SS confidence Disorder Disorder confidence	ALAALHERHG DLPAPLRARLLAALPQLDVTPSAVWLGPAVEAFWEGVPLLLEPLRQRLRQ	
Sequence Secondary structure SS confidence Disorder Disorder confidence	L S R G G T E T G A A A G A A G A A G A S S D T Q P G L E A A G E G A G A P G D A A A A L V A P L V S G L T A V S E	
Sequence Secondary structure SS confidence Disorder Disorder confidence	L G V P V K Q Q L TALL G R L S D L MAP P P TAAAAAAAAAAAAAAA AAAA R S P GAATTS SAAATTT AAAAT ?	
Sequence Secondary structure SS confidence Disorder Disorder confidence	LAAAEVGRVLVCLAAMRRATPSRANQRVLDSLFAAAAERLPLSGPLTAELLDAWDKLVPP	

23 FIG S3 Secondary structure prediction of a partial Raa8 sequence (from A1,546 to 24 **P2,745).** Prediction of α -helices (green), β -strands (blue arrows), and coil regions (faint lines) by the program Phyre² (5). Scale of confidence key is given in highest (9, red) to 25 lowest (0, blue). Predicted OPR repeats are marked as black boxes. Residues were 26 coloured according to a property based scheme (A,S,T,G,P small/polar; M,I,L,V 27 hydrophobic; K,R,E,N,D,H,Q charged; W,Y,F,C aromatic/cysteine). Question marks 28 29 indicate the prediction of disordered regions.

- 30 Supplemental references
- Kim UJ, Birren BW, Slepak T, Mancino V, Boysen C, Kang HL, Simon MI,
 Shizuya H. 1996. Construction and characterization of a human bacterial artificial
 chromosome library. Genomics 34:213-218.
- Herdenberger F, Holländer V, Kück U. 1994. Correct *in vivo* RNA splicing of a
 mitochondrial intron in algal chloroplasts. Nucleic Acids Res 22:2869-2875.
- Holländer V, Kück U. 1998. Splicing of the mitochondrial group II intron *rl1*:
 conserved intron-exon interactions diminish splicing efficiency. Curr Genet 33:117 123.
- Glanz S, Jacobs J, Kock V, Mishra A, Kück U. 2012. Raa4 is a *trans*-splicing
 factor that specifically binds chloroplast *tscA* intron RNA. Plant J 69:421-431.
- 41 5. Kelley LA, Sternberg MJ. 2009. Protein structure prediction on the Web: a case
 42 study using the Phyre server. Nat Prot 4:363-371.