

**Supplementary Figure S1.** Plasmid construction based on pUC18. Filled box, T7 promoter; cyan box, *citrine*; green box, *egfp*; yellow box, *citrine*; pink box, *psbD/C* portions; separated pink box, PCR products from the *psbD/C* in tobacco chloroplast DNA.

**Supplementary Figure S2.** Primers used in this study.

**Supplementary Figure S1:**

- ① On-1 and On-2 (template: plasmid pEG13)
- ② On-3 and On-4 (template: plasmid pEG13)
- ③ On-5 and On-6 (annealing)
- ④ On-7 and On-8 (template: chloroplast DNA)
- ⑤ On-9 and On-8 (template: chloroplast DNA)
- ⑦ On-10 and On-11 (template: chloroplast DNA)
- ⑨ and ⑩: On-1 and On-11 (template: plasmid pDC2)
- ⑪ On-12 and On-11 (template: chloroplast DNA)
- ⑫ On-13 and On-11 (template: chloroplast DNA)

**Figure 4:**

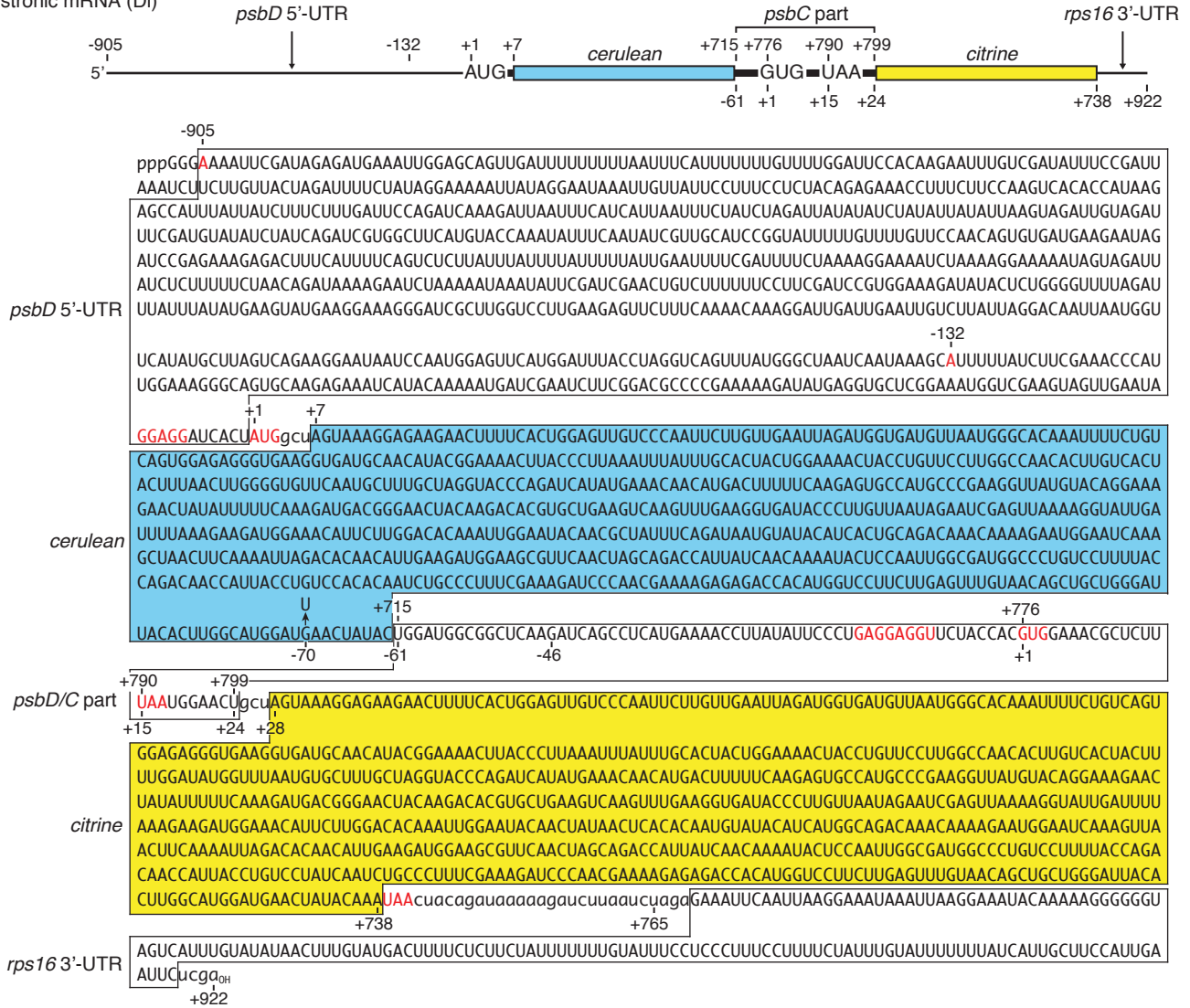
No 5'-UTR/AUG: On-14 and On-15 (annealing)

**Figure 5:**

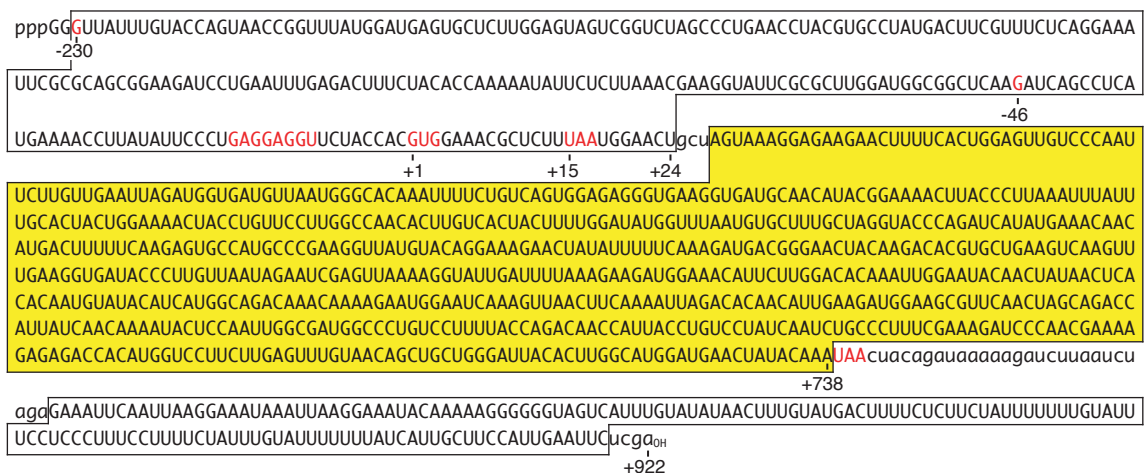
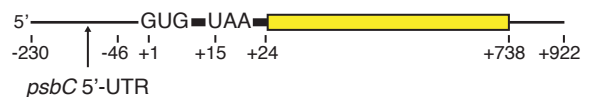
A point mutation: On-1 and On-16 (template: pEG13)

On-1: 5'-CAGATCATATGAAACAACATGACTTTTTTCA  
On-2: 5'-GTGATGTATACATTATCTGAAATAGCGTTGTATTCCAATTTGTGTC  
On-3: 5'-CGCCAGGGTTTTCCCAGTCACGAC  
On-4: 5'-CTGGGTACCTAGCAAAGCATTGAACACCCCAAGTTAAAGTAGTGAC  
On-5: 5'-ATACATCACTGCAGACAAACAAAAGAATGGAATCAAAGCT  
On-6: 5'-AGCTTTGATTCCATTCTTTTGTCTGCTGCAGTGATGT  
On-7: 5'-GAAAATTCGATAGAGATGAAATTGGAGC  
On-8: 5'-CCGCTAGCCATAGTGATCCTCCTATTCAACTA  
On-9: 5'-GATTTTTATCTTCGAAACCCATTGGAAAG  
On-10: 5'-TGGATGGCGGCTCAAGATCAGC  
On-11: 5'-CCGCTAGCAGTTCCATTAAGAGCGTTTCCAC  
On-12: 5'-GTTATTTGTACCAGTAACCGGTTTATGG  
On-13: 5'-GATCAGCCTCATGAAAACCTTATATTC  
On-14: 5'-AGCTTGCTGTAATACGACTCACTATAGGG  
On-15: 5'-CTAGCCCTATAGTGAGTCGTATTACAGCA  
On-16: 5'-CCTCTAGATTATACGTATAGTTAATCCATGCCAAGTG

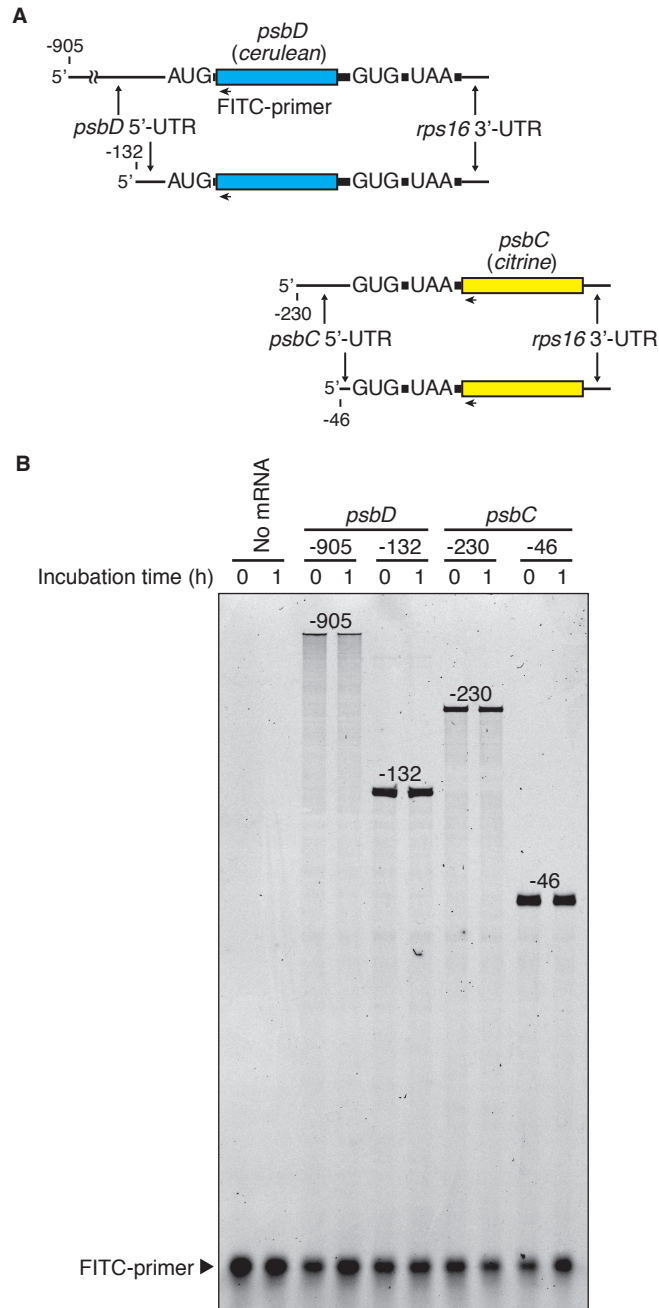
Dicistronic mRNA (Di)



Monocistronic mRNA (Di)



**Supplementary Figure S3.** The *psbD/C* test mRNA sequence. Deletion from +28 to +765 in the dicistronic mRNA provided the monocistronic *psbD* mRNA. T7 RNA polymerase adds two or three additional Gs at the 5'-end.



**Supplementary Figure S4.** Primer extension analysis of 5'-UTRs of the test mRNAs during 1-h *in vitro* translation reactions. **(A)** Monocistronic *psbD* and *psbC* test mRNAs and positions of FITC-labelled primers (left arrows)(see Figures 2A and 3A, respectively). **(B)** Gel patterns of the extended products. After incubation for 0 or 1 h, total RNA was isolated and subjected to primer extension.



	-40	-30	-20	<i>psbC</i> SD		<i>psbC</i> start codon		<i>psbD</i> stop codon		+30
				-10		+1	+10	+20		
Tobacco	CCUCAUGAAA	ACCUUUAUAAU	CCCU	GAGGAG	GU	UCUACCAC	GUGGAAACGC	UCUUJAAJGG	AACUUUAGCCU	
Tomato	CCUCAUGAAA	ACCUUUAUAAU	CCCUGAGGAG	GUUCUACCAC		GUGGAAACGC	UCUUJAAJGG	AACUUUAGCCU		
Spinach	CCUCAUGAAA	ACCUUUAUAAU	CCCUGAGGAG	GUUCUACCCC		GUGGAAACGC	UCUUJAAJGG	AACUUUAACUU		
Arabidopsis	CCUCAUGAAA	ACCUUUAUAAU	CCCUGAGGAG	GUUCUACCAC		GUGGAAACGC	UCUUJAAJGG	AACUUUAGCUU		
Cucumber	CCUCAUGAAA	ACCUUUAUAAU	CCCUGAGGAG	GUUCUACCCC		GUGGAAACGC	UCUUJAAJGG	AACUUUAGCUU		
Pea	CCUCAUGAAA	ACCUUUAUAAU	CCCUGAGGAG	GUUCUACCAC		GUGGAAACGC	UCUUJAAJGG	AACUUUAGCUU		
Rice	CCUCAUGAAA	AUCUUAUAAU	CCCUGAGGAG	GUUCUACCAC		GUGGAAACGC	UCUUJAAJGG	AACUUUUGUUU		
Maize	CCUCAUGAAA	AUCUUAUAAU	CCCUGAGGAG	GUUCUACCAC		GUGGAAACGC	UCUUJAAJGG	AACUUUCGUUU		
Wheat	CCUCAUGAAA	AUCUUAUAAU	CCCUGAGGAG	GUUCUACCAC		GUGGAAACGC	UCUUJAAJGG	AACUUUCGUUU		
Black pine	CCUCAUGAAA	ACCUUUAUAAU	CCCUGAGGAG	GUCCUACCCC		GUGGAAACGC	UCUUJAAJGG	AACUUAJAGCUU		
Wisk fern	CCUCAUGAAA	ACCUUGUAUU	CCCUGAGGAG	GUUCUACCCC		GUGGAAACGC	UCUUJAAJGG	AACUUUAUCGU		
Physcomitrella	CCUCAUGAAA	AUCUUGUAUU	CCCAGAGGAG	GUUCUACCAC		GUGGAAACGC	UCUUJAAJGG	AACCUUGGCUU		
Liverwort	CCUCAUGAAA	AUCUUGUAUU	CCCAGAGGAG	GUUCUACCCC		GUGGAAACGC	UCUUJAAJGG	AACUUUAGCUU		
Chlorella	CCUCAUGAAA	AACUUGUAUU	CCCUGAGGAG	GUUUUACCAC		GUGGAAACGC	UCUUJAAJGG	UUCGUUAGUUG		
Synechococcus 6301	CCGCACGAAA	AAUUCGUCUU	CCCCGAAGAG	GUUCUGCCCC		GUGGUAACGC	UCUCUAGJCC	UUCCGUGAUCG		
Synechocystis 6803	CCCCAUGAAA	ACUUUAUCUU	CCCUGAGGAG	GUUCUCCCC		GUGGUAACGC	UCUCUAAJAC	UUC--GAUGG		
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**Supplementary Figure S6.** Alignment of a sequence encompassing the *psbC* start codon.