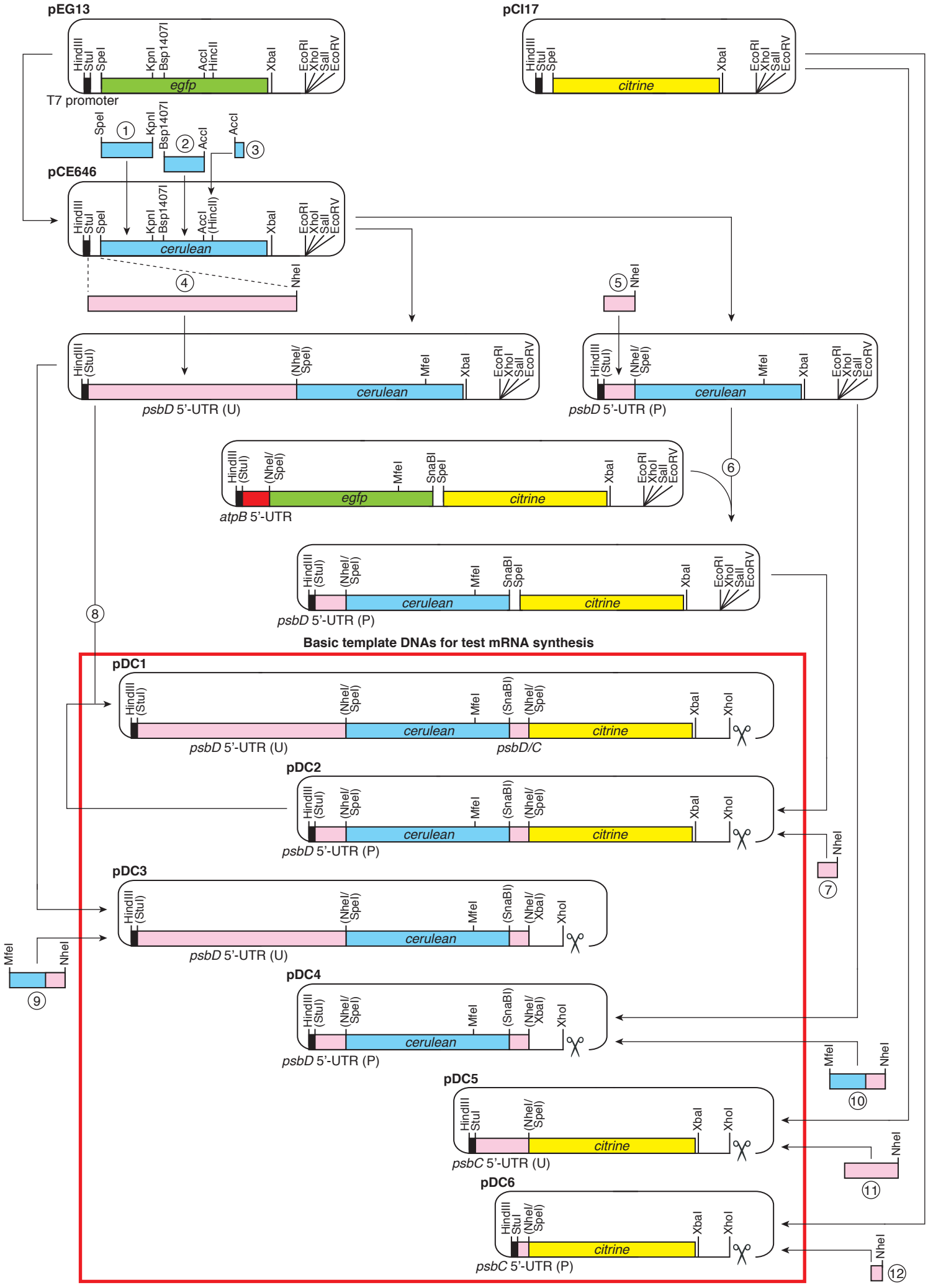


Supplementary Figure S1. Plasmid construction based on pUC18. Filled box, T7 promoter; cyan box, *citrine*; green box, *egfp*; yellow box, *citrine*; pink box, *psbD/C* portions; separated pink box, PCR products from the *psbD/C* in tobacco chloroplast DNA.

Supplementary Figure S2. Primers used in this study.

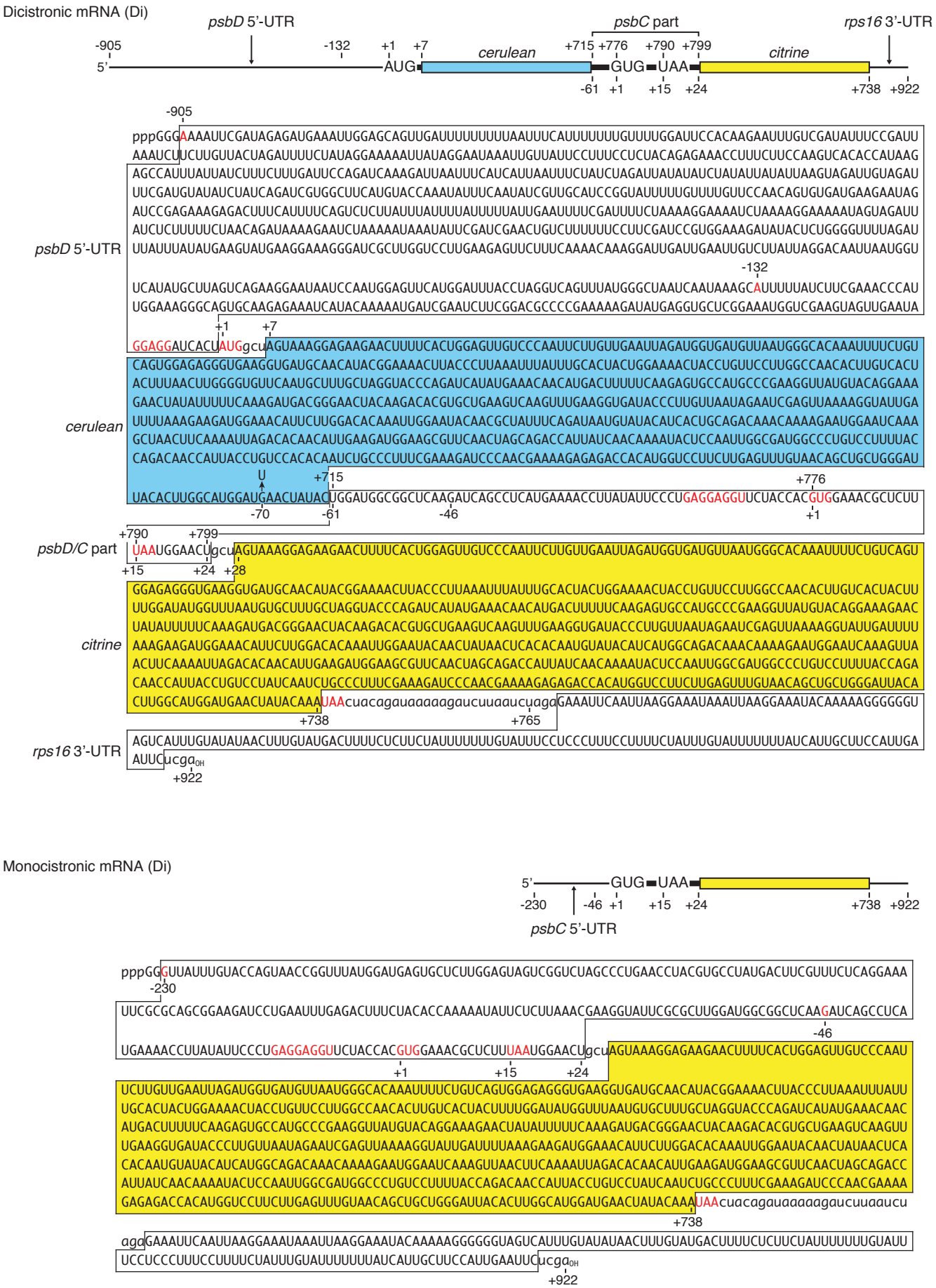
Supplementary Figure S1:

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)

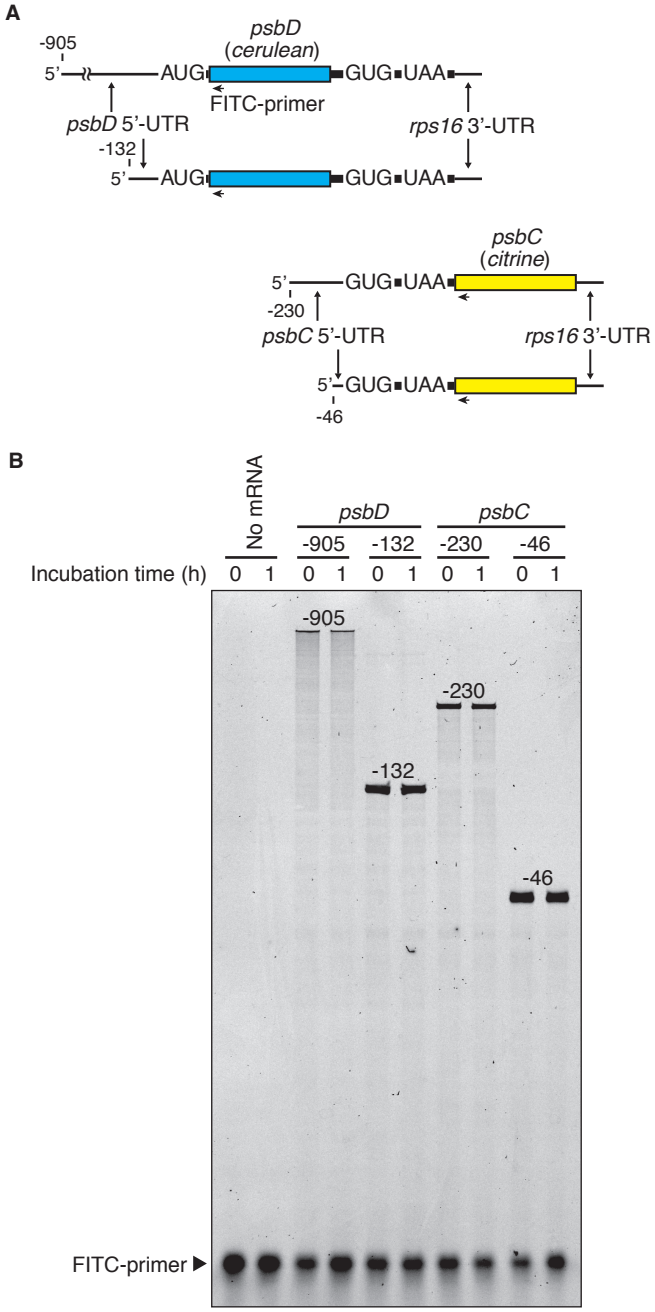
Figure 4:
No 5'-UTR/AUG: On-14 and On-15 (annealing)

Figure 5:
A point mutation: On-1 and On-16 (template: pEG13)

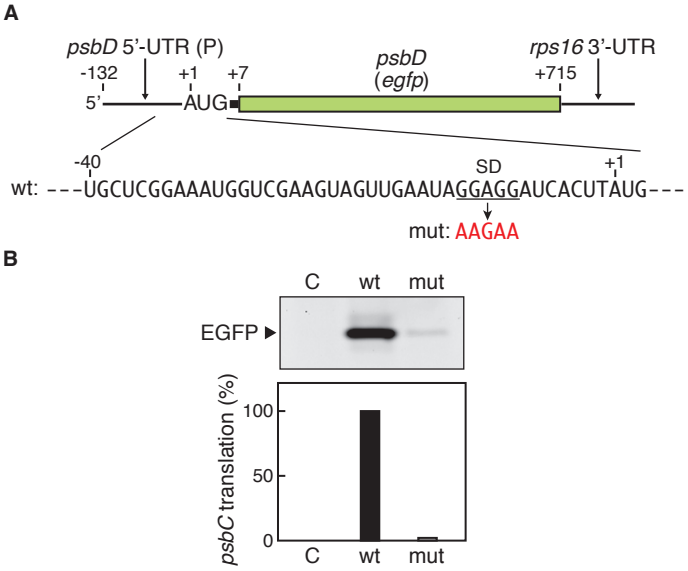
On-1: 5'-CAGATCATATGAAACAACATGACTTTTTCA
On-2: 5'-GTGATGTATACATTATCTGAAATAGCGTTGTATTCCAATTTGTGTC
On-3: 5'-CGCCAGGGTTTTCCCAGTCACGAC
On-4: 5'-CTGGGTACCTAGCAAAGCATTGAACACCCCAAGTTAAAGTAGTGAC
On-5: 5'-ATACATCACTGCAGACAAACAAAAGAATGGAATCAAAGCT
On-6: 5'-AGCTTTGATTCCATTCTTTTGTTTGTCTGCAGTGATGT
On-7: 5'-GAAAATTCGATAGAGATGAAATTGGAGC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



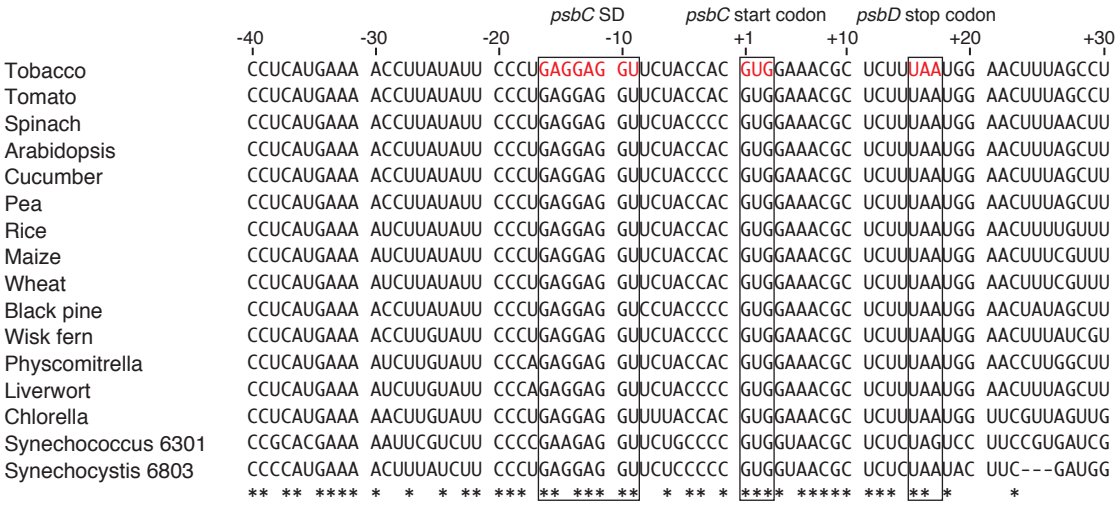
Supplementary Figure S3. The *psbD/C* test mRNA sequence. Deletion from +28 to +765 in the dicistronic mRNA provided the monocistronic *psbD* mRNA. T7 RNA polymerase adds two or three additional Gs at the 5'-end.



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.



Supplementary Figure S5. Role of an SD-like sequence on translation of the *psbD* cistron.



Supplementary Figure S6. Alignment of a sequence encompassing the *psbC* start codon.