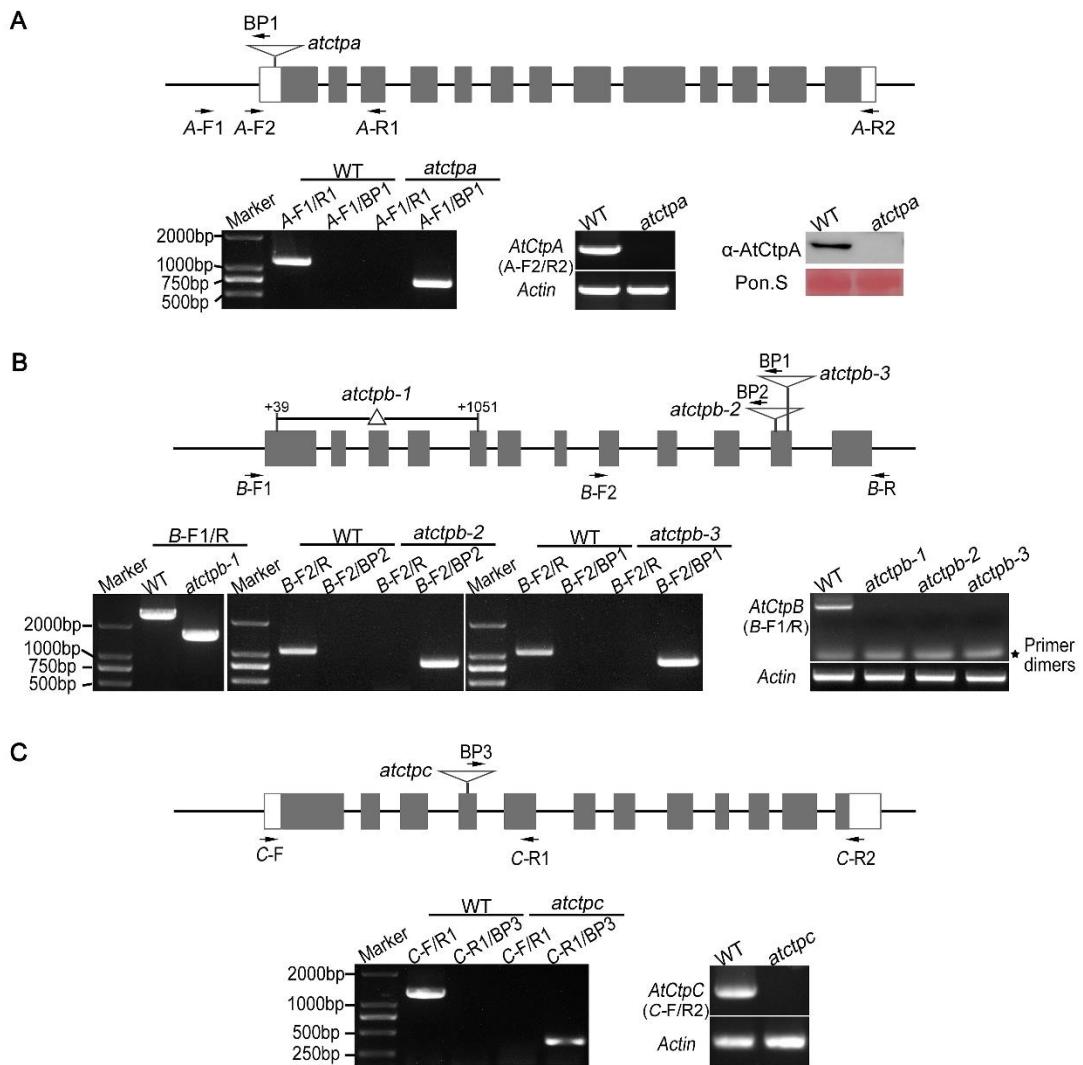


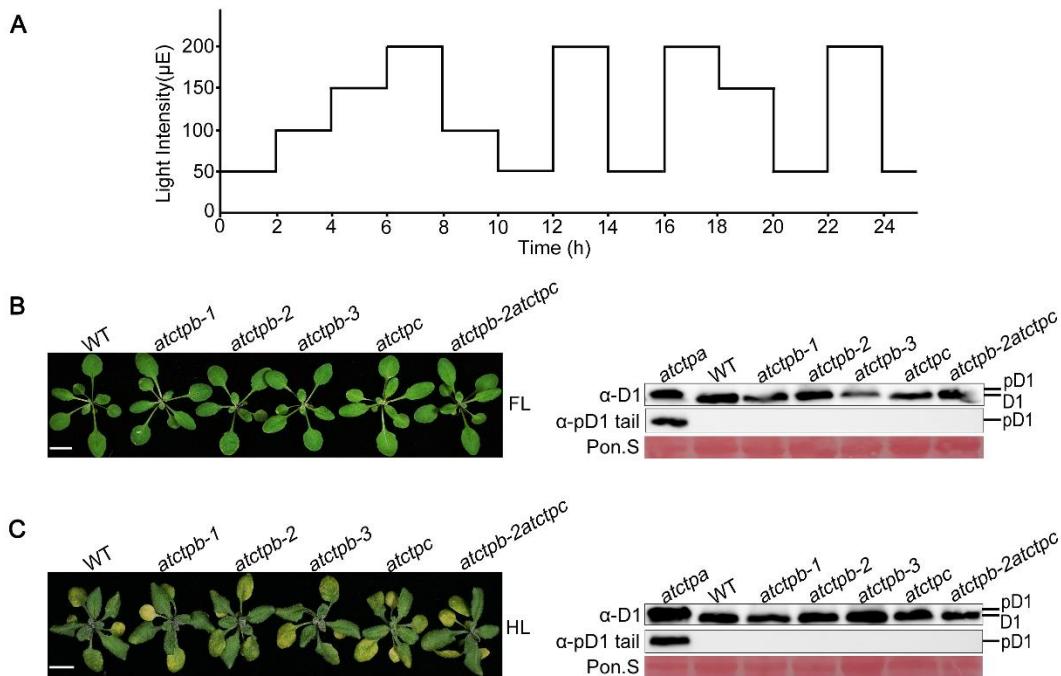
**Supplementary Figure S1. Phylogenetic tree constructed by Maximum-Likelihood method.**

To make the relationships of Ctp clades in Figure 1 more trustworthy, 286 Ctp proteins analyzed in figure 1 were also used to construct phylogenetic tree by MAGA6 using the method of Maximum-Likelihood, with the bootstrapping value set at 500 replications. The new tree displays the same topology as in figure 1.



**Supplementary Figure S2. Molecular characterizations of *atctpa* (A), *atctpb* (B), and *atctpc* (C) mutants.**

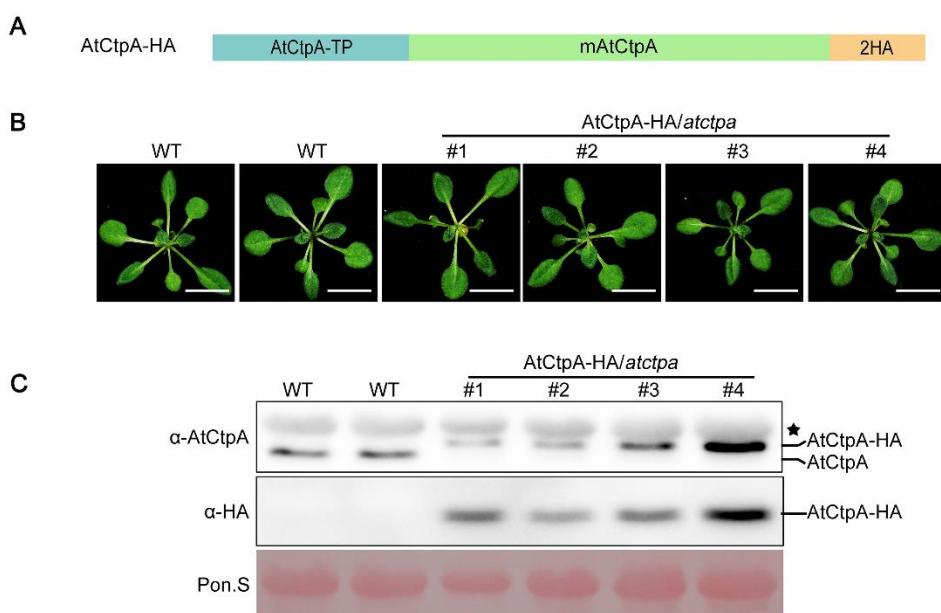
Upper panels in (A), (B), and (C) are schematic representations of different alleles of *atctpa*, *atctpb*, and *atctpc*. White boxes, the 5'- and 3'-UTR of genes; grey boxes, exons; black lines through boxes, the flanking regions and introns of genes; inverted triangles, T-DNA insertions; Δ, DNA deletions; horizontal arrows, PCR primers used for characterizing mutants. Molecular characterizations of mutants were placed below the schematics, including genotyping by PCR (left) and transcription level analyses by RT-PCR (right in (B) and (C) or middle in (A)), of which the forward primer (A-F2) for analysis of *AtCtpA* transcription level is located in the 5'-UTR region before the T-DNA insertion site. For *atctpa* mutant, AtCtpA protein level was further analyzed by immunoblot against α-AtCtpA antibody (right). The filters stained with Ponceau S (Pon. S) were used as loading controls.



**Supplementary Figure S3. Analyses of *atctpb* and *atctpc* mutants under FL and HL light conditions.**

(A) Schematic diagram of light intensity changes during a 24-hour period (Fluctuating light, FL).

(B) and (C) Characterizations of the phenotypes (left) and pD1 processing statuses (right) of *atctpb* and *atctpc* mutants under FL and HL conditions. The plants were grown in soil for three weeks as the following continuous light conditions: FL, under normal light (NL,  $80 \mu\text{mol/s/m}^2$ ) for one week and then under FL for two weeks; high light (HL,  $350 \mu\text{mol/s/m}^2$ ), under NL for two weeks and then under HL for one week. Scale bars denote 1 cm. After photographed, the pD1 processing statuses in plants were analyzed by immunoblot against  $\alpha$ -D1 (for D1 and pD1) and  $\alpha$ -pD1 tail (for pD1). The filters stained with Ponceau S (Pon. S) were used as loading controls.

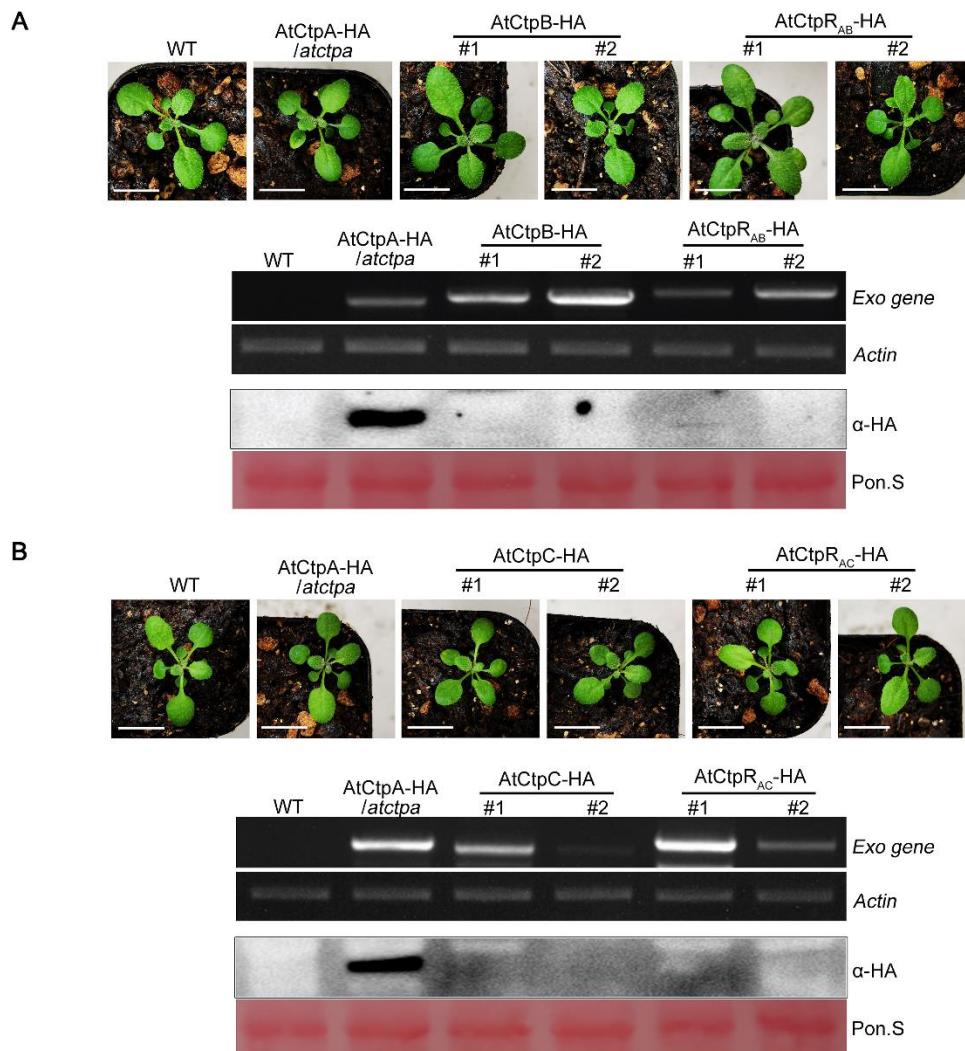


**Supplementary Figure S4. Complementation of *atctpa* mutant phenotypes with *AtCtpA*.**

**(A)** Schematic diagram of AtCtpA-HA for the complementation analysis. AtCtpA-TP is the chloroplastic transit peptide, mAtCtpA is the mature peptide; the 2HA indicates a double hemagglutinin tag.

**(B)** The phenotypes of transgenic *atctpa* plants carrying CaMV 35S promoter-driven *AtCtpA-HA*. The plants were grown on 1/2 MS medium with 1% sucrose under continuous low light (LL, 25  $\mu\text{mol}/\text{s}/\text{m}^2$ ) for three weeks. AtCtpA-HA #1-4 indicate different independent transgenic lines. Scale bars represent 1cm.

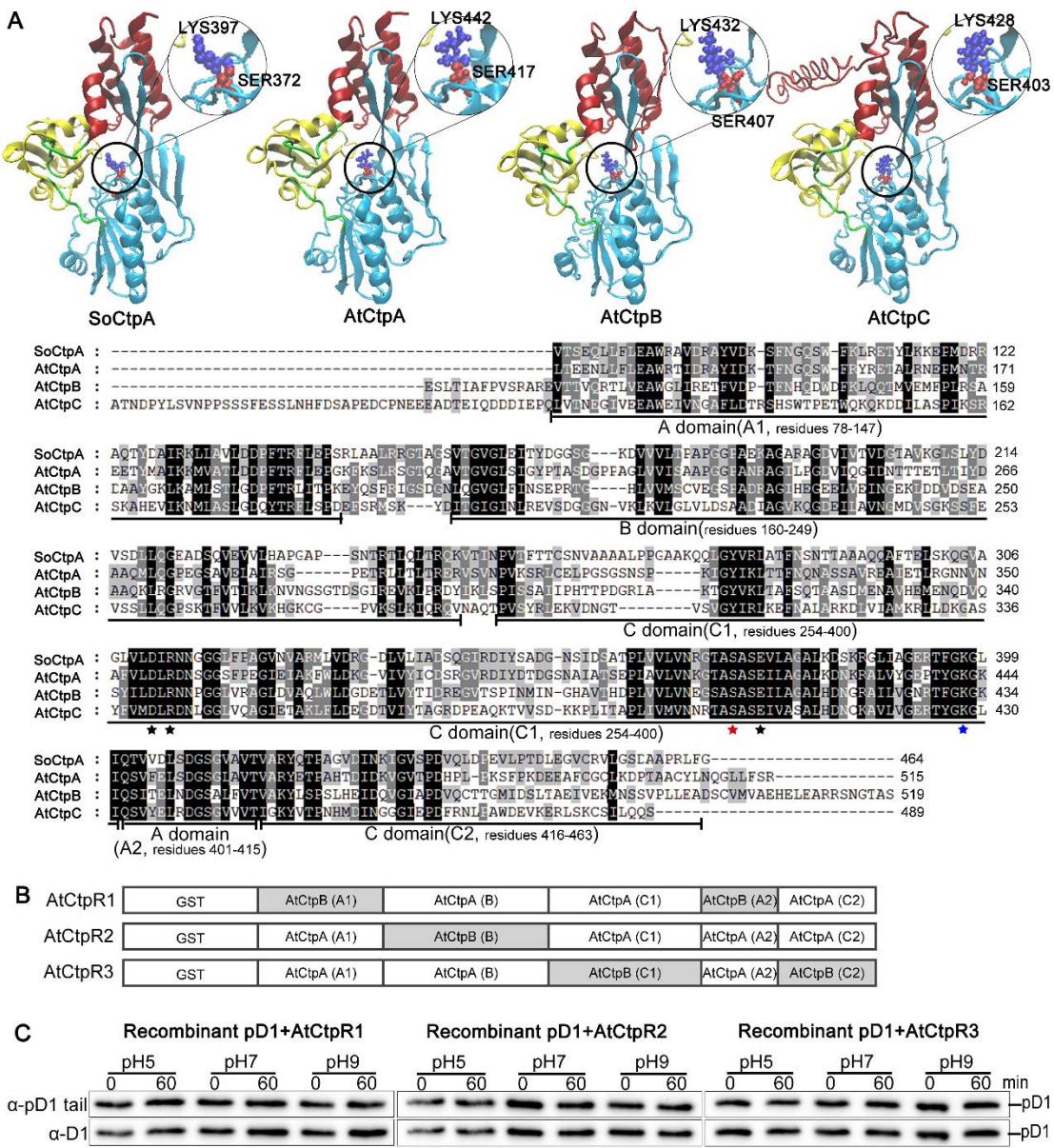
**(C)** The AtCtpA proteins levels of the plants in (B) were analyzed by immunoblot with  $\alpha$ -AtCtpA and  $\alpha$ -HA antibodies. The filter stained with Ponceau S (Pon. S) was used as loading control. Asterisk indicates unspecific bands.



**Supplementary Figure S5. Characterizations of transgenic plants carrying *AtCtpB* or *R<sub>AB</sub>* and *AtCtpC* or *R<sub>AC</sub>* driven by 35S promoter in wild type background.**

*AtCtpB-HA* or *AtCtpR<sub>AB</sub>-HA* and *AtCtpC-HA* or *AtCtpR<sub>AC</sub>-HA*, driven by CAMV 35S, were transformed into wild type (WT) Columbia through agrobacteria mediated method, and the diagrams for them were the same as Figure 6A. The upper panels in (A) and (B): T2 transgenic plants grown in soil under NL light (80  $\mu\text{mol}/\text{s}/\text{m}^2$ ) for two weeks. #1 and #2 represent two independent lines. Scale bars indicate 1 cm. The lower panels in (A) and (B): the expressions of exogenous transgenes (*Exo*) in transgenic

plants were analyzed at RNA (top two images) and protein (bottom two images) levels by semi-qRT-PCR and immunoblot. Native *Actin 2* (*AT3G18780*) and the filters stained with Ponceau S (Pon. S) were used as quantity controls, respectively.



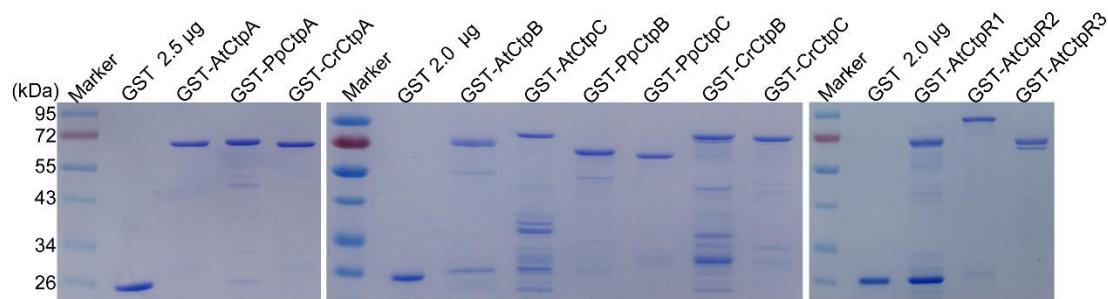
### Supplementary Figure S6. Domain swapping Analysis of AttcpA and ActpB.

**(A)** The structure (upper) and sequence (lower) comparisons of SoCtpA, AtCtpA, AtCtpB, and AtCtpC. The tertiary structure of *Scenedesmus obliquus* CtpA (SoCtpA) was obtained from PDB database (<https://www.rcsb.org/structure/1fcf>). The 3D structures of AtCtpA, AtCtpB, and AtCtpC were predicted by I-TASSER (Roy et al., 2012; Yang and Zhang, 2015; Zhang et al., 2017; Zhang, 2009). These four pdb files were uploaded in the Visual Molecular Dynamics program (Humphrey et al., 1996) to generate the presented structures. A domain is colored in red, B domain in yellow, C domain in blue, and loops between domains in green. The amino acids of catalytic dyad in structures were colored in blue (Lys) and red (Ser) as showed by magnification. Sequence comparisons were performed with ClustalW program in MEGA6. The structural domains are marked with black straight lines below the

corresponding sequences. The conserved amino acids essential for catalytic activity of CtpA (Inagaki et al., 2001; Che et al. 2013) are indicated by asterisks, in which Lys and Ser composing the catalytic dyad are marked with blue and red asterisks, respectively.

**(B)** Schematic diagrams for domain swapping experiment between AtCtpA and AtCtpB. The A, B, and C domains in GST fused AtCtpA were respectively replaced by their counterparts in AtCtpB (grey boxes) to form recombinant proteins AtCtpR1, AtCtpR2, and AtCtpR3. Un-swapped domains in each protein were drawn in white boxes.

**(C)** *In vitro* protease activity tests of AtCtpR1, AtCtpR2, and AtCtpR3 were carried out by using synthesized pD1 substrate as that in figure 4.



**Supplementary Figure S7. SDS-PAGE analyses of Ctp proteins used in the protease activity assays.**

After purification, the amounts of GST-Ctps in elutes were evaluated by SDS-PAGE analyses relative to standard GST protein. Ten µg of Ctp proteins were used in subsequent protease activity assays.

**Supplementary Table 1** Protein accession numbers in phylogenetic tree analyses.

| pD1<br>Type | Organism  | CtpA                |                 |       | CtpB    |                     |                 | CtpC  |         |                     | Database        |     |
|-------------|---|---------------------|-----------------|-------|---------|---------------------|-----------------|-------|---------|---------------------|-----------------|-----|
|             |   | Accession<br>number | coverage<br>(%) | Score | E-value | Accession<br>number | coverage<br>(%) | Score | E-value | Accession<br>number | coverage<br>(%) |     |
| Group I     | Cyanophyta  |                     |                 |       |         |                     |                 |       |         |                     |                 |     |
| (16)        | (Prokaryotic)   |                     |                 |       |         |                     |                 |       |         |                     |                 |     |
|             | Synechocystis sp.<br><i>PCC 6803</i>                      | WP_010873417        | —               | —     | —       | WP_010871361        | —               | —     | —       | WP_010872128        | —               | —   |
|             | Synechococcus<br><i>elongatus PCC 7942</i>                | ABB58360.1          | 88              | 480   | 3E-170  | ABB56548.1          | 82              | 365   | 4E-124  | ABB56744.1          | 100             | 543 |
|             | Leptolyngbya<br><i>boryana</i>                            | WP_017287738.1      | 86              | 458   | 3E-161  | WP_017289088.1      | 84              | 417   | 5E-144  | WP_026148389.1      | 98              | 567 |
|             | Geitlerinema sp. PCC<br>7407                              | WP_015173572.1      | 97              | 494   | 2E-176  | WP_015172436.1      | 93              | 431   | 1E-150  | WP_015170194.1      | 98              | 536 |
|             | Leptolyngbya <i>ohadii</i>                                | WP_088893494.1      | 96              | 474   | 2E-167  | WP_088894311.1      | 82              | 390   | 2E-133  | WP_088892848.1      | 97              | 531 |
|             | Pleurocapsa minor   | WP_015144827.1      | 95              | 565   | 0       | WP_015143772.1      | 84              | 532   | 0       | WP_015142506.1      | 99              | 639 |
|             | Limnoraphis robusta<br><i>CS-951</i>                      | KKD37180.1          | 95              | 499   | 1E-177  | KKD35691.1          | 85              | 412   | 5E-142  | KKD37108.1          | 98              | 545 |
|             | Rippkaea orientalis                                       | WP_012593970.1      | 96              | 564   | 0       | WP_012594914.1      | 88              | 563   | 0       | WP_15957337.1       | 98              | 634 |
|             | Gloeocapsa sp. PCC<br>7428                                | WP_015188917.1      | 90              | 493   | 7E-175  | WP_015186501.1      | 82              | 419   | 5E-145  | WP_015190966.1      | 98              | 557 |
|             | Oscillatoriales<br><i>cyanobacterium</i><br><i>JSC-12</i> | WP_009555551.1      | 95              | 471   | 2E-166  | WP_009555425.1      | 82              | 425   | 2E-147  | WP_009557210.1      | 98              | 559 |
|             | Leptolyngbya sp.<br><i>PCC 6406</i>                       | WP_027268870.1      | 96              | 471   | 1E-166  | WP_071992215.1      | 82              | 367   | 9E-125  | WP_008316952.1      | 99              | 530 |

|  |                |    |     |        |                |    |     |        |                |    |     |   |      |
|--|----------------|----|-----|--------|----------------|----|-----|--------|----------------|----|-----|---|------|
| <i>Chroogloeocystis siderophila</i>                | WP_073548808.1 | 90 | 489 | 8E-174 | WP_073549695.1 | 82 | 419 | 4E-145 | WP_073548177.1 | 98 | 559 | 0 | NCBI |
| <i>Planktothrix tepida</i>                         | WP_072717855.1 | 95 | 503 | 5E-179 | WP_072717382.1 | 82 | 420 | 2E-145 | WP_083580024.1 | 95 | 551 | 0 | NCBI |
| <i>Oscillatoria sp. PCC 10802</i>                  | WP_017716499.1 | 95 | 495 | 8E-176 | WP_017717799.1 | 82 | 430 | 2E-149 | WP_026098110.1 | 97 | 555 | 0 | NCBI |
| <i>Oscillatoriales cyanobacterium CG2_30_40_61</i> | OIP73180.1     | 95 | 500 | 4E-178 | OIP67295.1     | 82 | 419 | 2E-145 | OIP68024.1     | 99 | 561 | 0 | NCBI |
| <i>Planktothrix sp. PCC 11201</i>                  | WP_079679774.1 | 95 | 507 | 1E-180 | WP_079678415.1 | 82 | 416 | 9E-144 | WP_079680804.1 | 95 | 547 | 0 | NCBI |
| <i>Planktothrix agardhii</i>                       | WP_042153271.1 | 95 | 490 | 4E-173 | WP_042155587.1 | 82 | 417 | 7E-144 | WP_042154560.1 | 99 | 558 | 0 | NCBI |
| <i>Planktothrix paucivesiculata PCC 9631</i>       | VXD20545.1     | 95 | 502 | 1E-178 | VXD25008.1     | 82 | 425 | 2E-147 | VXD16497.1     | 99 | 560 | 0 | NCBI |
| <i>Roseofilum reptotaenium AO1-A</i>               | OJJ24040.1     | 93 | 472 | 6E-167 | OJJ25102.1     | 94 | 406 | 3E-140 | OJJ25011.1     | 95 | 524 | 0 | NCBI |
| <i>Phormidium ambiguum</i>                         | WP_073593457.1 | 90 | 490 | 5E-174 | WP_073591607.1 | 82 | 364 | 2E-123 | —              | —  | —   | — | NCBI |

#### Group II Chlorophyta

|                                  |                     |    |     |          |                    |    |     |         |                    |    |     |         |     |
|----------------------------------|---------------------|----|-----|----------|--------------------|----|-----|---------|--------------------|----|-----|---------|-----|
| (9)                              | <b>(Eukaryotic)</b> |    |     |          |                    |    |     |         |                    |    |     |         |     |
| <i>Chlamydomonas reinhardtii</i> | Cre10.g420550.t1.1  | 96 | 357 | 1.4E-118 | Cre10.g453807.t2.1 | 96 | 355 | 4E-116  | Cre06.g265850.t1.1 | 93 | 267 | 1.2E-80 | JGI |
|                                  |                     |    |     |          | Cre10.g426050.t1.2 | 79 | 229 | 3E-40   | Cre17.g739150.t1.2 | 83 | 261 | 5E-30   | JGI |
| <i>Dunaliella salina</i>         | Dusal.0111s00010.   | 96 | 359 | 1.1E-119 | Dusal.0015s00042.  | 83 | 241 | 1.4E-74 | Dusal.1339s00002.  | 88 | 289 | 4E-91   | JGI |
|                                  | 1                   |    |     |          | 1                  |    |     |         | 1                  |    |     |         |     |
| <i>Volvox carteri</i>            |                     | 96 | 372 | 5.3E-124 | Vocar.0030s0105.1  | 96 | 355 | 3E-116  | Vocar.0002s0139.1  | 93 | 241 | 9E-71   | JGI |

|                              |                   |    |     |          |                   |    |       |         |                   |    |     |          |      |  |  |
|------------------------------|-------------------|----|-----|----------|-------------------|----|-------|---------|-------------------|----|-----|----------|------|--|--|
|                              | Vocar.0016s0059.2 |    |     |          |                   |    |       |         |                   |    |     |          |      |  |  |
|                              |                   |    |     |          | Vocar.0005s0358.1 | 76 | 278   | 4E-41   | Vocar.0003s0289.1 | 74 | 183 | 3E-32    | JGI  |  |  |
| <i>Coccomyxa</i>             | XP_005648207.1    | 94 | 413 | 1.3E-141 | XP_005645927.1    | 98 | 372.9 | 2E-125  | XP_005652247.1    | 98 | 292 | 3.6E-94  | NCBI |  |  |
| <i>subellipsoidea C-169</i>  |                   |    |     |          | XP_005648181.1    | 94 | 197   | 4.9E-58 | XP_005647465.1    | 85 | 125 | 1.3E-32  | NCBI |  |  |
| <i>Micromonas pusilla</i>    | XP_003056496.1    | 94 | 364 | 1.1E-121 | —                 | —  | —     | —       | XP_003063156.1    | 87 | 271 | 4.5E-87  | NCBI |  |  |
| <i>Micromonas sp.</i>        | XP_002502055.1    | 95 | 364 | 1.2E-121 | —                 | —  | —     | —       | XP_002506449.1    | 94 | 310 | 9.1E-101 | NCBI |  |  |
| <i>RCC299</i>                |                   |    |     |          |                   |    |       |         |                   |    |     |          |      |  |  |
| <i>Ostreococcus</i>          | XP_001415400.1    | 96 | 352 | 1.5E-118 | —                 | —  | —     | —       | XP_001417628.1    | 89 | 305 | 5.1E-99  | NCBI |  |  |
| <i>lucimarinus</i>           |                   |    |     |          |                   |    |       |         |                   |    |     |          |      |  |  |
| <i>Scenedesmus</i>           | O04073.1          | 95 | 392 | 1E-136   | —                 | —  | —     | —       | —                 | —  | —   | —        | NCBI |  |  |
| <i>obliquus</i>              |                   |    |     |          |                   |    |       |         |                   |    |     |          |      |  |  |
| <i>Monoraphidium</i>         | XP_013900651.1    | 97 | 388 | 1E-133   | XP_013901188.1    | 85 | 298   | 1E-97   | —                 | —  | —   | —        | NCBI |  |  |
| <i>neglectum</i>             |                   |    |     |          |                   |    |       |         |                   |    |     |          |      |  |  |
| <i>Micractinium</i>          | PSC76991.1        | 94 | 386 | 6E-132   | PSC70752.1        | 93 | 315   | 2E-100  | PSC70735.1        | 88 | 328 | 1E-108   | NCBI |  |  |
| <i>conductrix</i>            |                   |    |     |          |                   |    |       |         |                   |    |     |          |      |  |  |
| <i>Chlorella sorokiniana</i> | PRW34015.1        | 91 | 382 | 1E-126   | PRW57074.1        | 99 | 323   | 3E-106  | PRW57137.1        | 86 | 320 | 3E-98    | NCBI |  |  |
| <i>Chlorella variabilis</i>  | XP_005849285.1    | 94 | 363 | 1E-124   | XP_005847884.1    | 97 | 341   | 2E-113  | XP_005847877.1    | 83 | 282 | 5E-91    | NCBI |  |  |
| <i>Chlamydomonas</i>         | GAX73199.1        | 96 | 365 | 9E-124   | GAX76907.1        | 88 | 332   | 3E-109  | GAX80856.1        | 90 | 290 | 1E-92    | NCBI |  |  |
| <i>eustigma</i>              |                   |    |     |          |                   |    |       |         |                   |    |     |          |      |  |  |
| <i>Raphidocelis</i>          | GBF95448.1        | 85 | 356 | 3E-120   | GBF96323.1        | 88 | 306   | 1E-99   | GBF93599.1        | 86 | 267 | 9E-83    | NCBI |  |  |
| <i>subcapitata</i>           |                   |    |     |          |                   |    |       |         | GBF93173.1        | 83 | 184 | 5E-51    | NCBI |  |  |
| <i>Ostreococcus tauri</i>    | XP_003074135.1    | 95 | 347 | 5E-117   | —                 | —  | —     | —       | XP_022838876.1    | 93 | 314 | 5E-104   | NCBI |  |  |
| <i>Bathycoccus prasinos</i>  | XP_007515489.1    | 92 | 326 | 1E-109   | —                 | —  | —     | —       | XP_007511360.1    | 90 | 321 | 1E-104   | NCBI |  |  |
| <i>Auxenochlorella</i>       | XP_011396732.1    | 94 | 308 | 1E-102   | XP_011397739.1    | 96 | 286   | 2E-86   | XP_011399412.1    | 86 | 237 | 2E-70    | NCBI |  |  |

|                              |                   |     |     |          |                   |     |     |        |                   |    |     |          |      |  |
|------------------------------|-------------------|-----|-----|----------|-------------------|-----|-----|--------|-------------------|----|-----|----------|------|--|
| <i>protothecoides</i>        |                   |     |     |          |                   |     |     |        |                   |    |     |          |      |  |
| <i>Gonium pectorale</i>      | —                 | —   | —   | —        | KXZ54634.1        | 88  | 337 | 1E-111 | KXZ42218.1        | 86 | 273 | 5E-86    | NCBI |  |
| <b>Embryophyte</b>           |                   |     |     |          |                   |     |     |        |                   |    |     |          |      |  |
| <b>(Eukaryotic)</b>          |                   |     |     |          |                   |     |     |        |                   |    |     |          |      |  |
| <b>Bryophyta</b>             |                   |     |     |          |                   |     |     |        |                   |    |     |          |      |  |
| <i>Physcomitrella patens</i> | XP_024385318.1    | 100 | 548 | 0        | XP_024391049.1    | 97  | 520 | 0      | XP_024398460.1    | 97 | 437 | 1.2E-148 | NCBI |  |
|                              |                   |     |     |          |                   |     |     |        | XP_024376785.1    | 93 | 440 | 2.5E-149 | NCBI |  |
|                              |                   |     |     |          |                   |     |     |        | XP_024384517.1    | 82 | 212 | 1.7E-54  | NCBI |  |
| <i>Marchantia</i>            | PTQ45047.1        | 94  | 542 | 0        | OAE24478.1        | 97  | 510 | 9E-177 | PTQ38413.1        | 95 | 453 | 7E-154   | NCBI |  |
| <i>polymorpha</i>            |                   |     |     |          |                   |     |     |        | PTQ29718.1        | 84 | 211 | 2.1E-47  | NCBI |  |
| <i>Sphagnum fallax</i>       | Sphfalx0044s0010. | 100 | 535 | 0        | Sphfalx0008s0198. | 97  | 539 | 0      | Sphfalx0014s0014. | 94 | 457 | 4.4E-158 | JGI  |  |
|                              | 1                 |     |     |          | 1                 |     |     |        | 2                 |    |     |          |      |  |
|                              |                   |     |     |          | Sphfalx0162s0014. | 97  | 513 | 2E-178 | Sphfalx0020s0021. | 83 | 166 | 3.3E-45  | JGI  |  |
|                              |                   |     |     |          | 1                 |     |     |        | 1                 |    |     |          |      |  |
| <b>Tracheophyte</b>          |                   |     |     |          |                   |     |     |        |                   |    |     |          |      |  |
| <i>Selaginella</i>           | XP_002979781.1    | 100 | 509 | 6.6E-180 | XP_024540786.1    | 96  | 485 | 7E-170 | XP_002965587.1    | 86 | 448 | 4E-160   | NCBI |  |
| <i>moellendorffii</i>        |                   |     |     |          |                   |     |     |        |                   |    |     |          |      |  |
| <b>Angiosperm</b>            |                   |     |     |          |                   |     |     |        |                   |    |     |          |      |  |
| <i>Amborella trichopoda</i>  | XP_011625922.1    | 100 | 619 | 0        | XP_006850171.1    | 100 | 659 | 0      | XP_011621275.1    | 83 | 429 | 2.2E-147 | NCBI |  |
| <b>Monocots</b>              |                   |     |     |          |                   |     |     |        |                   |    |     |          |      |  |
| <i>Ananas comosus</i>        | XP_020112923.1    | 100 | 608 | 0        | XP_020090596.1    | 100 | 498 | 1E-171 | XP_020098589.1    | 86 | 393 | 4.5E-133 | NCBI |  |
| <i>Musa acuminata</i>        | XP_009407843.1    | 100 | 630 | 0        | XP_009383212.1    | 100 | 630 | 0      | XP_009406283.1    | 97 | 542 | 0        | NCBI |  |
| <i>Spirodela polyrhiza</i>   | Spipo1G0011200    | 100 | 635 | 0        | Spipo12G0033700   | 100 | 637 | 0      | Spipo14G0041900   | 94 | 529 | 0        | JGI  |  |
| <i>Zostera marina</i>        | KMZ68881.1        | 100 | 602 | 0        | KMZ60132.1        | 98  | 577 | 0      | KMZ70100.1        | 92 | 516 | 0        | NCBI |  |
| <i>Brachypodium</i>          | XP_003578617.1    | 100 | 632 | 0        | XP_003563904.1    | 100 | 618 | 0      | XP_003569507.1    | 90 | 513 | 0        | NCBI |  |

|                           |                   |     |     |          |                   |     |     |   |                   |    |     |          |      |  |
|---------------------------|-------------------|-----|-----|----------|-------------------|-----|-----|---|-------------------|----|-----|----------|------|--|
| <i>distachyon3</i>        |                   |     |     |          |                   |     |     |   |                   |    |     |          |      |  |
| <i>Brachypodium</i>       | Brast05G210100.1  | 100 | 632 | 0        | Brast07G109600.1  | 100 | 617 | 0 | Brast01G192500.1  | 90 | 515 | 1.4E-180 | JGI  |  |
| <i>stacei3</i>            |                   |     |     |          |                   |     |     |   |                   |    |     |          |      |  |
| <i>Oryza sativa</i>       | XP_015626598.1    | 100 | 622 | 0        | XP_015643584.1    | 100 | 620 | 0 | XP_015622457.1    | 92 | 552 | 0        | NCBI |  |
| <i>Japonica Group</i>     |                   |     |     |          |                   |     |     |   |                   |    |     |          |      |  |
| <i>Oropetium thomaeum</i> | Oropetium_201501  | 75  | 446 | 2.7E-156 | Oropetium_201501  | 100 | 618 | 0 | Oropetium_201501  | 82 | 369 | 1.6E-124 | JGI  |  |
|                           | 05_04627A         |     |     |          | 05_23514A         |     |     |   | 05_11691A         |    |     |          |      |  |
| <i>Panum hallii</i>       | Pahal.A03864.2    | 100 | 634 | 0        | Pahal.D02099.1    | 100 | 625 | 0 | Pahal.E02231.1    | 92 | 529 | 0        | JGI  |  |
| <i>Panicum virgatum</i>   | Pavir.Ab03278.1   | 100 | 637 | 0        | Pavir.Da01617.1   | 100 | 623 | 0 | Pavir.Eb02683.1   | 90 | 521 | 0        | JGI  |  |
|                           | Pavir.Aa00118.1   | 91  | 637 | 0        |                   |     |     |   | Pavir.Ea02341.1   | 71 | 353 | 7.9E-120 | JGI  |  |
| <i>Setaria italica</i>    | Seita.1G364200.1  | 100 | 630 | 0        | Seita.4G138700.1  | 100 | 621 | 0 | —                 | —  | —   | —        | JGI  |  |
| <i>Sorghum bicolor</i>    | Sobic.004G343500. | 100 | 626 | 0        | Sobic.010G131700. | 100 | 618 | 0 | Sobic.003G247400. | 92 | 515 | 0        | JGI  |  |
|                           | 1                 |     |     |          | 1                 |     |     |   | 2                 |    |     |          |      |  |
| <i>Zea mays PH207</i>     | Zm00008a023329_   | 100 | 630 | 0        | Zm00008a034149_   | 100 | 591 | 0 | Zm00008a032213_   | 90 | 519 | 0        | JGI  |  |
|                           | T01               |     |     |          | T01               |     |     |   | T01               |    |     |          |      |  |
| <b>Eudicot</b>            |                   |     |     |          |                   |     |     |   |                   |    |     |          |      |  |
| <i>Aquilegia coerulea</i> | Aqcoe5G010500.1   | 100 | 662 | 0        | Aqcoe1G023500.1   | 100 | 671 | 0 | Aqcoe1G203500.1   | 94 | 538 | 0        | JGI  |  |
| <i>Amaranthus</i>         | AHYPO_003918-R    | 100 | 582 | 0        | AHYPO_000094-R    | 100 | 589 | 0 | AHYPO_004375-R    | 94 | 519 | 0        | JGI  |  |
| <i>hypochondriacus</i>    | A                 |     |     |          | A                 |     |     |   | A                 |    |     |          |      |  |
| <i>Daucus carota</i>      | DCAR_002290       | 100 | 642 | 0        | DCAR_013578       | 100 | 673 | 0 | DCAR_031456       | 95 | 480 | 5E-163   | JGI  |  |
| <i>Mimulus guttatus</i>   | —                 | —   | —   | —        | Migut.J01789.1    | 100 | 659 | 0 | Migut.M00953.1    | 70 | 351 | 2.4E-117 | JGI  |  |
| <i>Solanum</i>            | Solyc12g097030.1. | 100 | 651 | 0        | Solyc03g059260.2. | 100 | 663 | 0 | Solyc02g071190.2. | 87 | 512 | 2.8E-179 | JGI  |  |
| <i>lycopersicum</i>       | 1                 |     |     |          | 1                 |     |     |   | 1                 |    |     |          |      |  |
| <i>Solanumerosum</i>      | PGSC0003DMT40     | 100 | 651 | 0        | PGSC0003DMT40     | 100 | 670 | 0 | PGSC0003DMT40     | 91 | 534 | 0        | JGI  |  |
|                           | 0022218           |     |     |          | 0084308           |     |     |   | 0007927           |    |     |          |      |  |
| <i>Kalanchoe</i>          | Kaladp0024s0051.1 | 100 | 675 | 0        | Kaladp0087s0188.1 | 100 | 672 | 0 | —                 | —  | —   | —        | JGI  |  |

*fedtschenkoi*

|                             |                   |     |     |         |                   |     |     |        |                   |    |     |          |     |
|-----------------------------|-------------------|-----|-----|---------|-------------------|-----|-----|--------|-------------------|----|-----|----------|-----|
| <i>Kalanchoe laxiflora</i>  | Kalax.0130s0052.1 | 100 | 673 | 0       | Kalax.0322s0004.1 | 100 | 665 | 0      | Kalax.0835s0023.1 | 93 | 536 | 0        | JGI |
|                             | Kalax.1447s0004.1 | 84  | 554 | 0       | Kalax.0637s0013.1 | 99  | 660 | 0      | Kalax.0164s0012.3 | 93 | 535 | 0        | JGI |
| <i>Eucalyptus grandis</i>   | Eucgr.H02885.1    | 89  | 604 | 0       | Eucgr.A02402.1    | 96  | 651 | 0      | Eucgr.E02360.3    | 98 | 471 | 1.2E-163 | JGI |
| <i>Vitis vinifera</i>       | GSVIVT01001855    | 100 | 677 | 0       | GSVIVT01033332    | 100 | 691 | 0      | GSVIVT01021388    | 90 | 563 | 0        | JGI |
| <i>Genoscope</i>            | 001               |     |     |         | 001               |     |     |        | 001               |    |     |          |     |
| <i>Linum usitatissimum</i>  | Lus10004402       | 99  | 600 | 0       | Lus10040494       | 100 | 676 | 0      | Lus10004601       | 88 | 571 | 0        | JGI |
|                             | Lus10023704       | 99  | 594 | 0       |                   |     |     |        | Lus10004545       | 95 | 439 | 2.1E-150 | JGI |
| <i>Manihot esculenta</i>    | Manes.03G156100.  | 100 | 688 | 0       | Manes.09G029500.  | 100 | 691 | 0      | Manes.17G065400.  | 92 | 575 | 0        | JGI |
|                             | 1                 |     |     |         | 1                 |     |     |        | 1                 |    |     |          |     |
| <i>Populus trichocarpa</i>  | Potri.008G173900. | 100 | 679 | 0       | Potri.006G055400. | 100 | 674 | 0      | Potri.011G078700. | 95 | 572 | 0        | JGI |
|                             | 4                 |     |     |         | 1                 |     |     |        | 1                 |    |     |          |     |
| <i>Ricinus communis</i>     | 29904.m003051     | 100 | 671 | 0       | 27810.m000652     | 85  | 445 | 2E-152 | 29693.m002070     | 88 | 556 | 0        | JGI |
| <i>Salix purpurea</i>       | SapurV1A.0021s02  | 100 | 683 | 0       | SapurV1A.0133s02  | 100 | 664 | 0      | SapurV1A.0031s02  | 95 | 560 | 0        | JGI |
|                             | 90.1              |     |     |         | 20.1              |     |     |        | 80.1              |    |     |          |     |
| <i>Citrus sinensis</i>      | orange1.1g009668  | 100 | 677 | 0       | orange1.1g047092  | 100 | 662 | 0      | orange1.1g012084  | 89 | 579 | 0        | JGI |
|                             | m                 |     |     |         | m                 |     |     |        | m                 |    |     |          |     |
| <i>Citrus clementina</i>    | Ciclev10004718m   | 100 | 677 | 0       | Ciclev10004757m   | 100 | 679 | 0      | Ciclev10018134m   | 89 | 557 | 0        | JGI |
| <i>Carica papaya</i>        | evm.model.superco | 100 | 681 | 0       | evm.model.superco | 100 | 693 | 0      | —                 | —  | —   | —        | JGI |
|                             | ntig_59.36        |     |     |         | ntig_85.132       |     |     |        |                   |    |     |          |     |
| <i>Gossypium raimondii</i>  | Gorai.006G253900. | 100 | 664 | 0       | Gorai.002G229800. | 100 | 687 | 0      | Gorai.009G157600. | 89 | 547 | 0        | JGI |
|                             | 1                 |     |     |         | 1                 |     |     |        | 3                 |    |     |          |     |
|                             | Gorai.001G131500. | 75  | 299 | 6.4E-99 |                   |     |     |        |                   |    |     |          | JGI |
|                             | 1                 |     |     |         |                   |     |     |        |                   |    |     |          |     |
| <i>Theobroma cacao</i>      | Thecc1EG021046t1  | 100 | 667 | 0       | Thecc1EG024797t1  | 100 | 680 | 0      | Thecc1EG029558t1  | 98 | 572 | 0        | JGI |
| <i>Arabidopsis thaliana</i> | AT4G17740.1       | —   | —   | —       | AT3G57680.1       | —   | —   | —      | AT5G46390.1       | —  | —   | —        | JGI |

|                             |                    |     |        |   |                    |     |        |   |                   |     |        |          |     |
|-----------------------------|--------------------|-----|--------|---|--------------------|-----|--------|---|-------------------|-----|--------|----------|-----|
| <i>Boechera stricta</i>     | Bostr.30275s0238.1 | 100 | 783    | 0 | Bostr.20903s0020.1 | 100 | 820    | 0 | Bostr.8819s0177.1 | 100 | 759    | 0        | JGI |
| <i>Brassica oleracea</i>    | Bol037109          | 100 | 759    | 0 | Bol045512          | 100 | 763    | 0 | Bol032116         | 95  | 676    | 0        | JGI |
| <i>capitata</i>             |                    |     |        |   |                    |     |        |   |                   |     |        |          |     |
| <i>Brassica rapa FPsc</i>   | Brara.K00491.1     | 100 | 757    | 0 | Brara.I03985.1     | 100 | 786    | 0 | Brara.I01932.1    | 100 | 714    | 0        | JGI |
| <i>Capsella grandiflora</i> | Cagra.3356s0066.1  | 100 | 779    | 0 | Cagra.0210s0005.1  | 100 | 817    | 0 | Cagra.0569s0015.1 | 95  | 737    | 0        | JGI |
| <i>Capsella rubella</i>     | Carubv10007107m    | 100 | 778    | 0 | Carubv10018381m    | 100 | 808    | 0 | Carubv10026297m   | 95  | 735    | 0        | JGI |
| <i>Eutrema salsugineum</i>  | Thhalv10024882m    | 100 | 764    | 0 | Thhalv10005897m    | 98  | 802    | 0 | Thhalv10001155m   | 100 | 727    | 0        | JGI |
| <i>Cucumis sativus</i>      | Cucs.a.383050.2    | 100 | 647    | 0 | Cucs.a.357250.2    | 100 | 683    | 0 | Cucs.a.212480.1   | 95  | 537    | 0        | JGI |
| <i>Fragaria vesca</i>       | mrna28308.1-v1.0-  | 97  | 659    | 0 | mma14365.1-v1.0-   | 100 | 687    | 0 | mrna24893.1-v1.0- | 93  | 553    | 0        | JGI |
|                             |                    |     | hybrid |   |                    |     | hybrid |   |                   |     | hybrid |          |     |
| <i>Glycine max Wm82</i>     | Glyma.20G199000.   | 100 | 652    | 0 | Glyma.10G094900.   | 100 | 681    | 0 | Glyma.05G165100.  | 94  | 557    | 0        | JGI |
|                             | 1                  |     |        |   | 1                  |     |        |   | 5                 |     |        |          |     |
| <i>Malus domestica</i>      | MDP0000219700      | 99  | 661    | 0 | MDP0000305189      | 100 | 649    | 0 | —                 | —   | —      | —        | JGI |
|                             | MDP0000251269      | 100 | 654    | 0 |                    |     |        |   |                   |     |        |          | JGI |
| <i>Medicago truncatula</i>  | Medtr1g073130.1    | 100 | 653    | 0 | Medtr1g052135.1    | 100 | 676    | 0 | Medtr8g088150.1   | 96  | 555    | 0        | JGI |
| <i>Phaseolus vulgaris</i>   | Phvul.L001972.1    | 99  | 644    | 0 | Phvul.007G255600.  | 100 | 684    | 0 | Phvul.002G245300. | 93  | 555    | 0        | JGI |
|                             |                    |     |        | 1 |                    |     |        | 1 |                   |     |        |          |     |
| <i>Prunus persica</i>       | Prupe.1G052500.2   | 100 | 664    | 0 | Prupe.7G068000.1   | 100 | 702    | 0 | Prupe.4G074500.1  | 94  | 561    | 0        | JGI |
| <i>Trifolium pratense</i>   | Tp57577_TGAC_v     | 100 | 663    | 0 | Tp57577_TGAC_v     | 100 | 664    | 0 | Tp57577_TGAC_v    | 94  | 503    | 4.7E-176 | JGI |
|                             | 2_mRNA19200        |     |        |   | 2_mRNA23564        |     |        |   | 2_mRNA6999        |     |        |          |     |

### Group III Chlorarachniophyta

#### (0) (Eukaryotic)

|                            |   |   |   |   |   |   |   |   |               |      |     |       |     |
|----------------------------|---|---|---|---|---|---|---|---|---------------|------|-----|-------|-----|
| <i>Bigelowiella natans</i> | — | — | — | — | — | — | — | — | Bignal 87480  | C-87 | 173 | 1E-45 | JGI |
|                            |   |   |   |   |   |   |   |   | Bignal 133319 | A-94 | 125 | 6E-37 | JGI |

### Dinophyta

#### (Eukaryotic)

|                        |                         |              |   |   |            |   |   |            |      |     |       |      |
|------------------------|-------------------------|--------------|---|---|------------|---|---|------------|------|-----|-------|------|
| <i>Symbiodinium</i>    | —                       | —            | — | — | —          | — | — | OLP86183.1 | A-89 | 236 | 3E-52 | NCBI |
| <i>microadriaticum</i> |                         |              |   |   |            |   |   |            |      |     |       |      |
| <b>Outgroup</b>        | <i>Escherichia coli</i> | WP_096246988 |   |   | MRF42352.1 |   |   |            |      |     |       | NCBI |

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

| <b>Names</b>            | <b>Primer sequences (5'-3')</b> | <b>Purposes</b>  |
|-------------------------|---------------------------------|--|
| A-F1                    | TTCCTTGATGGTGGTTCAAGTC          | <i>atctpa</i> genotyping                                       |
| A-R1                    | GCCTCGAGGAAGAGAAGATTG           | <i>atctpa</i> genotyping                                       |
| A-F2                    | AGAGTTGGAATTTCGGGGAA            | <i>AtCtpA</i> full length cDNA amplification                   |
| A-R2                    | TCATCTAGAAAAAGTAGGCCTTGATTG     | <i>AtCtpA</i> full length cDNA amplification                   |
| B-F1                    | ATGGAAAAAGTTGTAACCCTAAATCATG    | <i>AtCtpB</i> CDS amplification and <i>atctpb-1</i> genotyping |
| B-F2                    | TGATGGACGTTAGCGAAGAC            | <i>atctpb-2/-3</i> genotyping                                  |
| B-R                     | TCAAGAACGCTGTTCCATTAGATCGTC     | <i>AtCtpB</i> CDS amplification and <i>atctpb</i> genotyping   |
| C-F                     | ATGAGGCTTTGCTTCCATTTCCTCGC      | <i>AtCtpC</i> CDS amplification and <i>atctpc</i> genotyping   |
| C-R1                    | GAAACTGGGTCTGTGCATTA            | <i>atctpc</i> genotyping                                       |
| C-R2                    | TCATCAACTTGTGGAGAATACTGCAC      | <i>AtCtpC</i> CDS amplification                                |
| BP1                     | ATTTGCCGATTCGGAAC               | SALK line genotyping   |
| BP2                     | GCCTTTCAGAAATGGATAATAGCCTGCTTCC | SAIL line genotyping   |
| BP3                     | ATAATAACGCTCGGACATCTACATTTC     | GABI line genotyping   |
| pRI101-AN-F             | GATCAAAAGCAAGTCTTCACTG          | Transgene amplification  |
| pRI101-AN-R             | AAATTCGAGCTCGGTAGCAATTG         | Transgene amplification  |
| TP <sub>AtCtpA</sub> -R | ACCCCATGATGGTGGTGAATCAG         | Transgene amplification  |
| Actin-F                 | CTCTCCCGCTATGTATGTCGCCATCC      | <i>Actin</i> gene amplification                                |
| Actin-R                 | CTGTGAACGATTCTGGACCTGCCCTC      | <i>Actin</i> gene amplification                                |
| GAPDH-F                 | CCATGGCTTCGGTTACTTT             | qRT-PCR of internal control gene                               |
| GAPDH-R                 | CACCTTAAGCTTGGCCTCAG            | qRT-PCR of internal control gene                               |
| qPCR (CtpA)-F           | TTCTGTTCTGCTTGTCTCTTCC          | qRT-PCR of <i>AtCtpA</i>                                       |
| qPCR (CtpA)-R           | CTCTGTAACGAAACCAGCTTTG          | qRT-PCR of <i>AtCtpA</i>                                       |
| qPCR (CtpB)-F           | TCGTGAGGTTACTACAGTACAG          | qRT-PCR of <i>AtCtpB</i>                                       |
| qPCR (CtpB)-R           | AAGCAGCATCTGCTGATCTTAG          | qRT-PCR of <i>AtCtpB</i>                                       |
| qPCR (CtpC)-F           | GTAGTTCTCAAGGTGAAGCATG          | qRT-PCR of <i>AtCtpC</i>                                       |
| qPCR (CtpC)-R           | ATCTTTCTAGCCAGCGCATTG           | qRT-PCR of <i>AtCtpC</i>                                       |