A

```
N1                                                                         gtctattttttttcgagccaaa     22
P1         M  R  L  D  R  Q  P  L  L  H  V  E  K  R  M  P  T  S  L  W  L  V  V  G  A  V  I
          atgcgattggaccgtcagccactgctgcatgttgagaagcgaatgccaacatccctgtggttggttgttggtgcagtgattg    104
            A  A  V  C  V  V  F  A  A  S  H  N  G  T  H  L  S  A  T  S  P  P  I  L  T  T  T
        105 ctgctgtttgtgttgtttttgctgcttcccacaatggaacacacctgtcagcaacttcaccaccaatccttacaaccacc    184
             S  T  V  R  I  P  V  V  Q  N  F  E  P  G  L  T  A  S  S  N  H  L  S  N  G  I  P
        185 tcaactgtgcggattccagtggtacagaatttgaaccaggcctgacagcctcaagcaaccacttatcaaatggaattcc    264
              P  L  G  D  S  A  G  T  E  S  A  S  R  S  F  V  A  S  A  I  L  F  P  L  C  G
        265 tcccttgggtgacagtgctgggacagagagtgcatcccgcagttttgttgcgagtgcaatcctgtttcccctttgtggac    344
            L  L  A  T  V  A  F  I  M  A  K  K  N  P  Q  T  T  S  L  L  S  I  A  S  K  K  D
        345 ttcttgcaaccgtggcttttataatggcaaagaagaatccacaaacaacatctctcctctccatcgcgtccaagaaggat    424
```

```
P1           M  E  V  W  S  P  I  N  N  R  K  F  E  T  F  S  F  L  P  P  M  T  D  E  Q  I  S
PX           .  .  .  .  N  R  S  I  E  .  T  S  K  .  L  T  -  -  -  -  -  P  E  .  R  F  T
P8           .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
N1      425 atggaggtgtggagccccatcaacaacaggaagttcgagaccttctcctttctgcctcccatgactgatgagcagatttc    504
N2      845 ................................................................................    924
N3     1265 ................................................................................   1344
NX        x .............a..gttc..t.g.g..a.cc.caa.a..t..ga..---------------c.c..a....g.t..a.   x+64
NX+1  x+387 ................................................................................  x+466
N8    y-401 ................................................................................  y-322
```

```
P1            K  E  V  D  M  I  I  N  K  G  Y  S  P  F  L  E  F  A  A  P  E  N  S  S  I  S
PX            R  S  L  E  R  .  V  K  E  .  L  F  .  G  V  .  Y  .  P  .  R  .  C  F  R  A
P8            .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
N1      505 caaggaggtggacatgatcatcaacaaggggtattcccctttcctggagtttgctgcccccgagaacagcagcatttcca    584
N2      925 ................................................................................   1004
N3     1345 ................................................................................   1424
NX     x+65 ..g.tc.c.t..g.ga..tg.a..gg....actc.t....ggtg.t..a.ac..cc....tcg...tt..tt.cgcg...  x+129
NX+1  x+467 ................................................................................  x+546
N8    y-321 ................................................................................  y-242
```

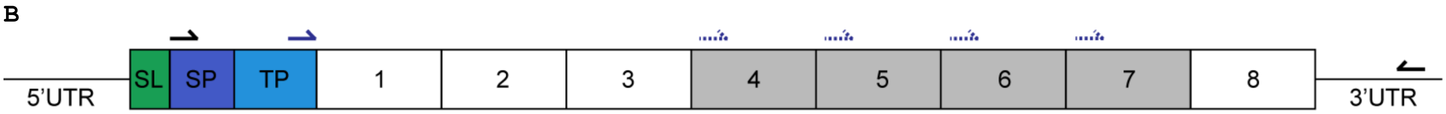
```
P1          S  E  S  T  T  R  F  S  G  T  T  S  G  Y  Y  D  N  R  Y  W  T  M  W  K  L  P  M
PX          K  S  C  .  A  N  .  A  D  P  -  -  -  -  -  .  S  .  D  .  .  .  .  .  .  .  .
P8          .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
N1      585 gtgagagcaccacccgcttctctggcaccacctctggctactacgacaaccggtactggacgatgtggaagctgcccatg    664
N2     1005 ................................................................................   1084
N3     1425 .........................g..g..t................................................   1504
NX    x+130 agagct....ag.gaa....g...a.c..---------------....g....g..........................  x+209
NX+1  x+547 ................................................................................  x+626
N8    y-241 ................................................................................  y-162
```

```
P1           F  G  C  T  D  P  S  Q  V  L  K  E  I  D  E  C  C  K  T  F  P  Q  C  Y  V  R  L
PX           .  .  .  .  .  .  N  .  .  .  .  .  V  I  .  .  .  .  I  Y  .  .  .  F  .  .  .
P8           .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
N1      665 tttggctgcactgatcccagccaggtgctgaaagaaattgatgagtgctgcaagaccttccctcagtgctatgtccgcct    744
N2     1085 ................................................................................   1164
N3     1505 ............................................------------------------------------   1548
NX    x+210 ...................a...............gg.cat............a.t..a...........t.........  x+289
NX+1  x+627 ................................................................................  x+706
N8    y-161 ................................................................................   y-82
```

```
P1            A  A  F  D  S  I  K  Q  V  Q  V  I  S  F  L  V  Q  R  P  P  S  N  V  N  M  A
PX            .  .  .  .  P  .  A  .  .  .  A  .  .  .  .  .  .  .  .  D  A  -  .  S  .  .
P8            .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  S  G  S  T  R
N1      745 ggcagccttcgactccatcaagcaggtgcaggtgatctcgttcttggtgcagcgcccccccagcaatgtgaacatggctg    824
N2     1165 ................................................................................   1244
N3          --------------------------------------------------------------------------------
NX    x+290 .............c.....gca..........ca.................a.....ggatgct---....g........  x+366
NX+1  x+707 .....................................................................c......----  x+782
N8     y-81 .................................................................g..gc.g..cccg.t    y-2
```

```
P1          A  M  T  G  E  K  D
PX          .  .  .  .  .  .  .
P8          *  -  -  -  -  -  -
N1      825 ccatgaccggtgagaaggat    844
N2     1245 ....................   1264
N3          --------------------
NX    x+367 ....................  x+386
NX+1        --------------------
N8      y-1 ga------------------ y>4000
```

```
N8      y+1   ctgctgctgtaatttttatttcgcatcgagcttgtggttgacatttgtgcaccgcaatgtgacacaccaaaggcggac   y+78
       y+79 acacctgggaaggagtacaggtttccattcagctggtagaaacccgcattccactgcttcaccaatgctatccagtgctc  y+158
      y+159 acgcagctgcattcacactcaccaagggaggataggatttgtacaacagtcaacactgcaacattcgtgccgcatctttt  y+238
      y+239 ttggtgaagtgcagaagattgcccattgaacaatgcccaagtgtgttgttgtgcaaat                         y+296
```



C

```
Euglena longa divergent          MEVWNRSIERTSKTLT-----PEERFTRSLERIVKEGLFPGVEYAPPRNC
Euglena longa canonical          MEVWSPINNRKFETFSFLPPMTDEQISKEVDMIINKGYSPFLEFAAPENS
Euglena gracilis                 MKVWNPVNNKKFETFSYLPPLSDAQIAKQVDMIIAKGLSPCLEFAAPENS
Eutreptiella gymnastica NIES-381 MKVWNPVNNKKFETFSYLPPLSNAEIARQVDFIIRNGLAPCLEFAPEECS
Chlamydomonas reinhardtii        MMVWTPVNNKMFETFSYLPPLTDEQIAAQVDYIVANGWIPCLEFAEADKA
Haematococcus pluvialis          MMVWQPINNKQYETFSYLPPLTSDQIARQVDYVVGNGWIPCLEFADASQA
Auxenochlorella protothecoides   MLVWTPTNNKMFETFSFLPPLSDDQISRQVDYIISNKWIPCLEFIDDPFP

Euglena longa divergent          FRAKSCTANFADP-----DSRDWTMWKLPMFGCTDPNQVLKEVIECCKIY
Euglena longa canonical          SISSESTTRFSGTTSGYYDNRYWTMWKLPMFGCTDPSQVLKEIDECCKTF
Euglena gracilis                 FIANDNTVRFSGTAAGYYDNRYWTMWKLPMFGCTDASQVLREISECRRAY
Eutreptiella gymnastica NIES-381 FVDDVATVRFQGSTAGYYDNRYWTMWKLPMFGCSSAQEVLREVQECTRHY
Chlamydomonas reinhardtii        YVSNESAIRFGSVSCLYYDNRYWTMWKLPMFGCRDPMQVLREIVACTKAF
Haematococcus pluvialis          YVSNASTVRFGSVSACYYDNRYWTLWKLPMFGCTDPSAVLTEISRASKAF
Auxenochlorella protothecoides   YVAQENTIRMGAVASNYYDNRYWTMWKLPMFGCNDGSQVLTEVGNCRKAF

Euglena longa divergent          PQCFVRLAAFDPIAQVQAISFLVQRP----DA-VSMAAMTGEKD
Euglena longa canonical          PQCYVRLAAFDSIKQVQVISFLVQRP----PSNVNMAAMTGEKD
Euglena gracilis                 PQCYVRLAAFDSVKQVQVISFVVQRPSGSSSSSWGMAAMTGEKD
Eutreptiella gymnastica NIES-381 PGCYVRLAAFDSIKQVQVVSFIVHQP----GQ-TAAWATTGEKD
Chlamydomonas reinhardtii        PDAYVRLVAFDNQKQVQIMGFLVQRP------------------
Haematococcus pluvialis          PQAYIRMVAFDNVRQVQIMSFLVQRP------------------
Auxenochlorella protothecoides   PDAYIRLVAFDNVRQVQIGGFLVHRP------------------
```

Figure S1. RuBisCO small subunit in *E. longa*. 1A**.** The partially reconstructed sequence of the *RbcS* mRNA and the corresponding RBCS precursor polyprotein in *Euglena longa*. The mRNA starts with a spliced leader sequence (in green), as is typical for mRNA molecules in Euglenozoa. Downstream is the rest of the 5'-UTR followed by the first part of the sequence encoding a predicted tripartite plastid-targeting sequence (amino acid residues in blue: dark blue represents a signal and light blue a transit peptide). The middle part of the figure (shaded in darker grey) shows an alignment of determined complete or partial sequences of repeats encoding the RBCS monomer (amino acid residues on top in black) followed by the linker decapeptide (amino acid residues on top in red). Sequences of the first two repeats (N1 and N2) are complete, the third repeat (N3) lacks the 3'-end of the sequence. NX (nucleotide sequence) and PX (protein sequence) correspond to the divergent repeat that is a part of the molecule, but whose exact position with respect to other repeats could not be determined. It is followed by a canonical repeat (NX+1; note that only a part of the linker sequence following this repeat was determined). The terminal repeat is denoted N8, assuming that the total number of repeats is eight (see the text). This last repeat terminates with a stop codon (TGA, in bold and italics) and is followed by a long 3'-UTR. Only the sequence of the first repeat is shown in full, in the other repeats dots represent the same nucleotides (amino acid residues) as in the first repeat, letters indicate substitutions as compared to the first repeat, dashes in black represent deletions, dashes in gray correspond to missing data. Position coordinates are indicated on the left and on the right of each line. The actual position of the beginning of the divergent repeat (NX) is unknown, so it is indicated as "x" and the positions downstream of it are counted accordingly. Likewise, the exact coordinates of the 3'-region of the mRNA molecule are unknown, so the last nucleotide of the stop codon was arbitrarily indicated as "y" and positions upstream and downstream are counted accordingly. Regions corresponding to primers used for PCR amplification are indicated by black boxes, regions corresponding to internal primers used for sequencing are indicated by dark blue boxes. 1B. Schematic representation of the *RbcS* transcript. SL, splice leader sequence; SP, signal peptide sequence; TP, transit peptide sequence. The grey box represents a divergent subunit, although its exact position was not determined. Arrows indicate primers used for PCR and sequencing as in 1A. The dashed arrow represents a primer that anneals to the divergent subunit. 1C. Alignment of RBCS sequences from euglenophytes and selected green algae. Only the region corresponding to the mature processed RBCS protein is shown, for euglenophytes also the linker decapeptide is included at the end of the sequence. Both the canonical and the divergent forms of RBCS are shown for *E. longa*.