Supplementary Information

A library of tunable, portable and inducer-free promoters derived from cyanobacteria

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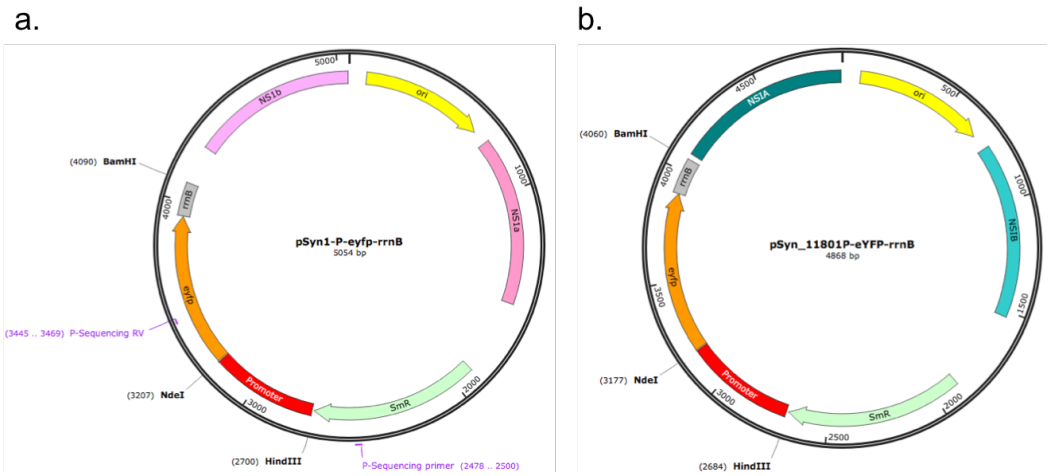


Figure S1: The plasmids maps used in the study obtained using SnapGene. (a) The vector used for integration of the *eyfp* construct in *S. elongatus* PCC 7942. (b) The pSyn_1 vector was modified to pSyn_11801 which has the changed neutral site of *S. elongatus* PCC 11801 for recombination in both *S. elongatus* PCC 11801 and PCC 11802.

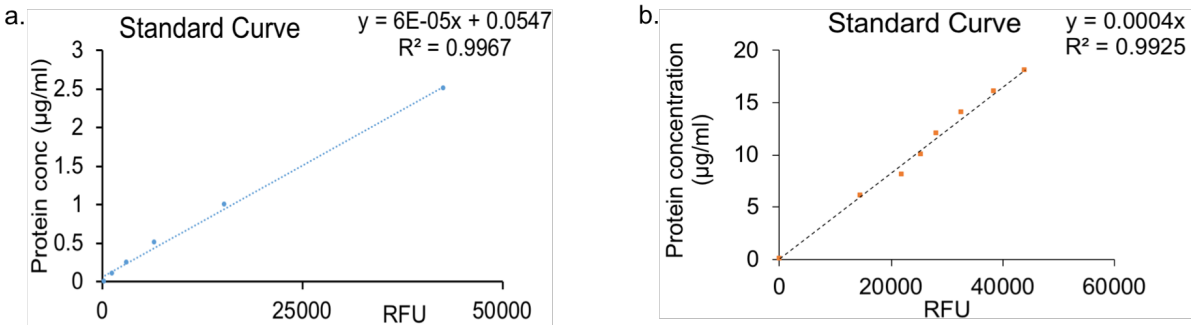


Figure S2: The standard curve for eYFP. Protein concentration used for the standard curved were (a) 0.1-2.5 µg/ml, which was used for estimating eYFP protein concentration from cyanobacterial cells and (b) 0-18 µg/ml in case of *E. coli* strains.

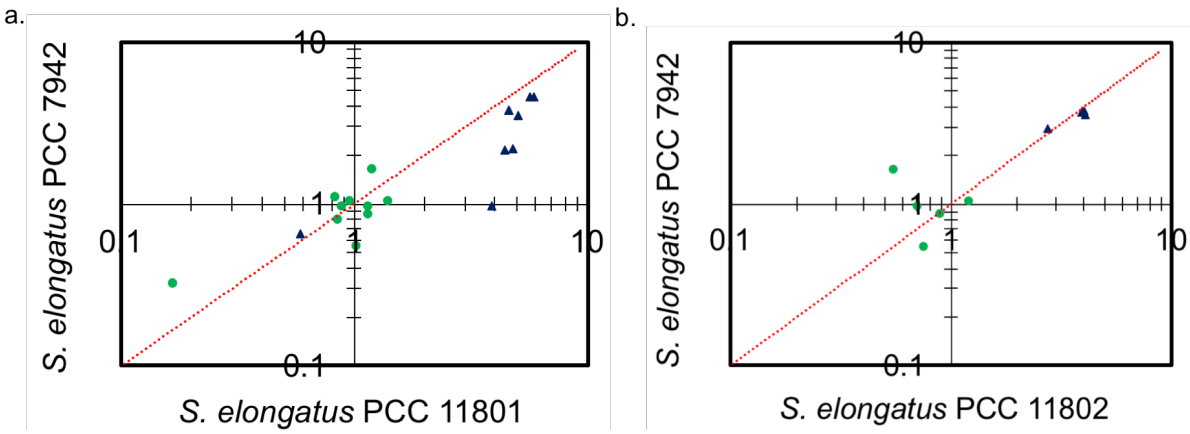


Figure S3: The promoter strength comparison between related strains: (a) Between *S. elongatus* PCC 7942 and PCC 11801, (b) Between *S. elongatus* PCC 7942 and PCC 11802 under high light and elevated CO_2 levels. While the cpcB promoters are represented by blue triangles, rbcL promoters are represented by green circles.

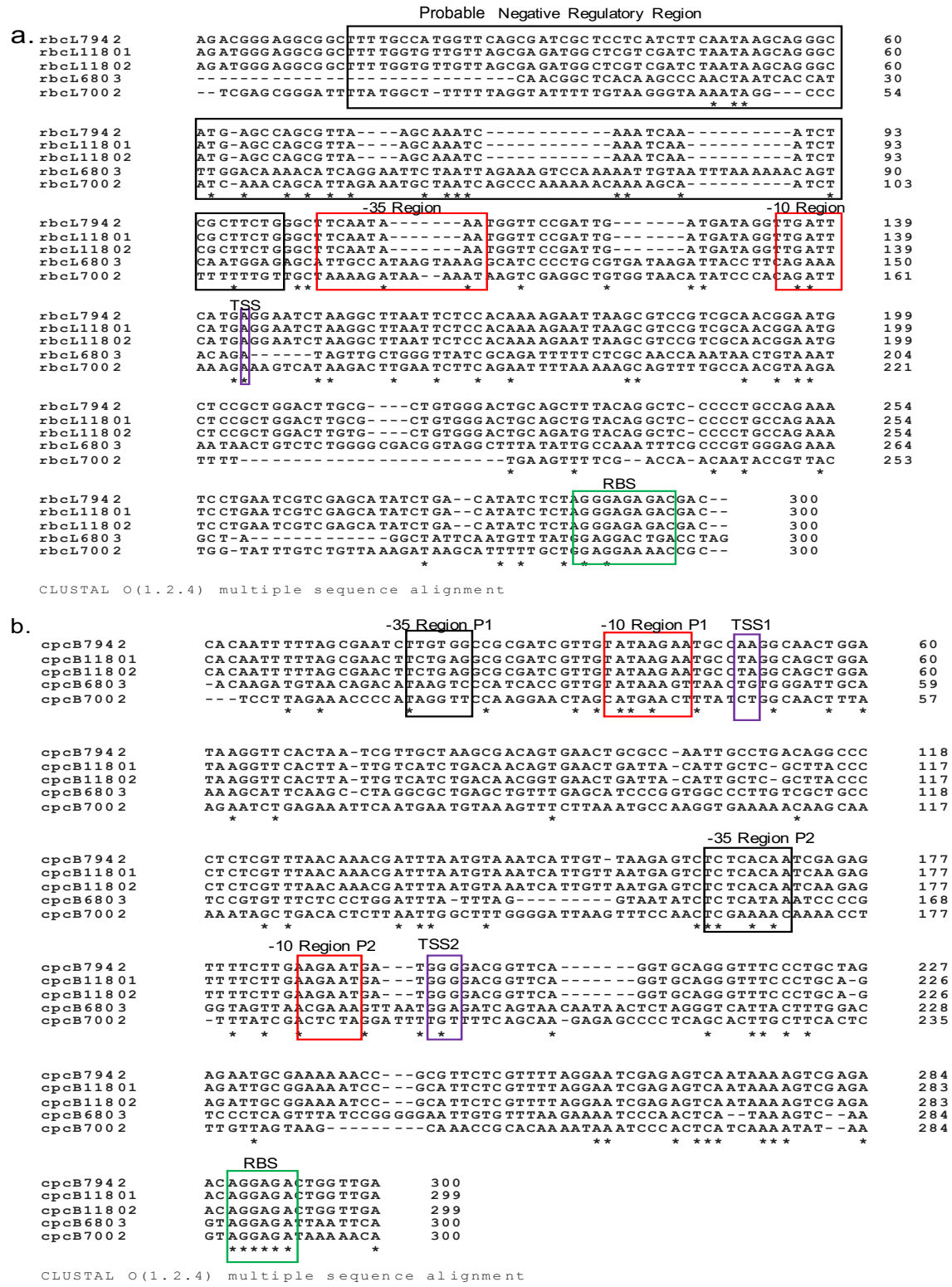
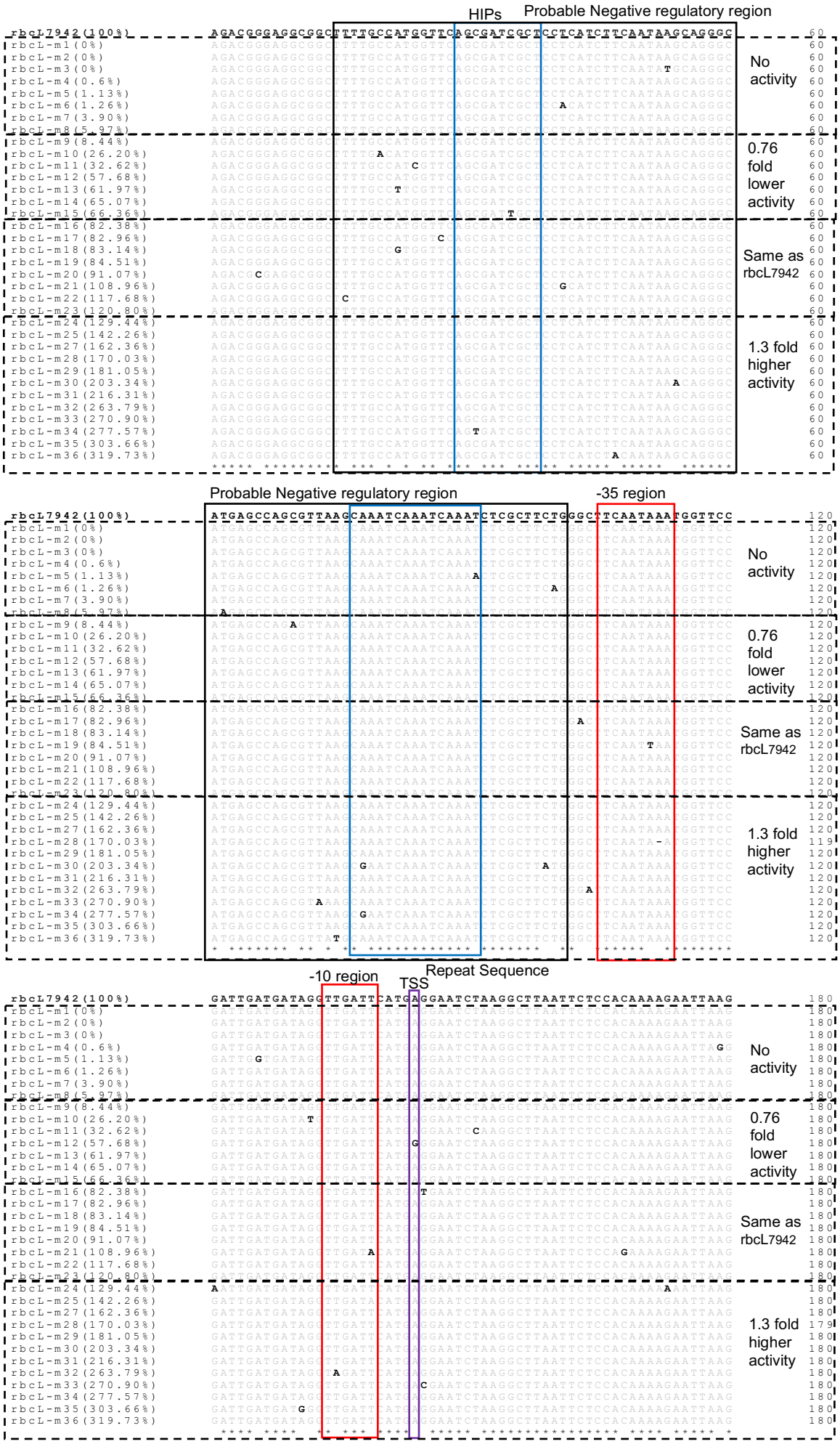


Figure S4: Sequence comparison of the promoters rbcL(a) and cpcB(b) among five strains obtained using CLUSTALΩ. The important regions are highlighted using different boxes.


HIPs
Probable Negative regulatory region
No activity
0.76 fold lower activity
Same as rbcL7942
1.3 fold higher activity
Probable Negative regulatory region
-35 region
No activity
0.76 fold lower activity
Same as rbcL7942
1.3 fold higher activity
-10 region
TSS
Repeat Sequence
No activity
0.76 fold lower activity
Same as rbcL7942
1.3 fold higher activity

```
rbcL7942(100%)      CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m1(0%)         CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m2(0%)         CGTCCGTCGCAACGGAATGCTCCGCTGGACCTGCGCTGTAGGACTGCAGCTTTACGGCT 240
rbcL-m3(0%)         CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m4(0.6%)       CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m5(1.13%)      CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAACTTTACAGTCT 241
rbcL-m6(1.26%)      CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m7(3.90%)      CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m8(5.97%)      CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m9(8.44%)      CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m10(26.20%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m11(32.62%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m12(57.68%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m13(61.97%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m14(65.07%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m15(66.36%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m16(82.38%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m17(82.96%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m18(83.14%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m19(84.51%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m20(91.07%)    CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m21(108.96%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCCTTACAGGCT 240
rbcL-m22(117.68%)   CGTCCGTCGTAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m23(120.80%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m24(129.44%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m25(142.26%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m27(162.36%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTGCAGGCT 240
rbcL-m28(170.03%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 239
rbcL-m29(181.05%)   CATCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m30(203.34%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m31(216.31%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m32(263.79%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m33(270.90%)   CGTCCGTCGCAACGGAATGCTCCACTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m34(277.57%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m35(303.66%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
rbcL-m36(319.73%)   CGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGTGGGACTGCAGCTTTACAGGCT 240
                    * ******* ************** ****** ******** ******** * ** * * **
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No activity

0.76 fold lower activity

Same as rbcL7942

1.3 fold higher activity

RBS

```
rbcL7942(100%)      CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m1(0%)         CCCCCTGCCAGAAATCATGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m2(0%)         CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m3(0%)         CCCCCTGCCAGAAATCCTGAATCGTCTAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m4(0.6%)       CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m5(1.13%)      CCCCCTGCCAGAAATCCTGAATCGTCAAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m6(1.26%)      CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m7(3.90%)      CC-CCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 299
rbcL-m8(5.97%)      CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m9(8.44%)      CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m10(26.20%)    CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m11(32.62%)    CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m12(57.68%)    CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m13(61.97%)    CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m14(65.07%)    CCCCCTGCCAGAAATCCTGAATCGTTGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m15(66.36%)    CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m16(82.38%)    CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 279
rbcL-m17(82.96%)    CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m18(83.14%)    CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGA---------------------- 300
rbcL-m19(84.51%)    CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m20(91.07%)    CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m21(108.96%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m22(117.68%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m23(120.80%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m24(129.44%)   CCCCCTGCCAAAAATCCTGAATCGTCAAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m25(142.26%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m27(162.36%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGAATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m28(170.03%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGAAGAGACGAC 299
rbcL-m29(181.05%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m30(203.34%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m31(216.31%)   CTCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m32(263.79%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m33(270.90%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m34(277.57%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m35(303.66%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
rbcL-m36(319.73%)   CCCCCTGCCAGAAATCCTGAATCGTCGAGCATATCTGACATATCTCTAGGGAGAGACGAC 300
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No activity

0.76 fold lower activity

Same as rbcL7942

1.3 fold higher activity

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CLUSTAL O(1.2.4) multiple sequence alignment
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Figure S5: Sequence alignment of the synthetic promoters derived from the $P_{rbcL7942}$ promoter (except for the highly mutated $P_{rbcL\text{-}m26}$) using CLUSTALΩ. While the mutations are shown in bold black, the sequence same as wild type are shown in light grey. The important regions are highlighted using different boxes. The activity of the promoters were calculated with respect to wild type $P_{rbcL7942}$ under high light and 0.04% CO_2 and provided in parenthesis. The $P_{rbcL7942}$ is considered as 100%. The promoters are categorized with respect to the promoter activity under ambient condition.

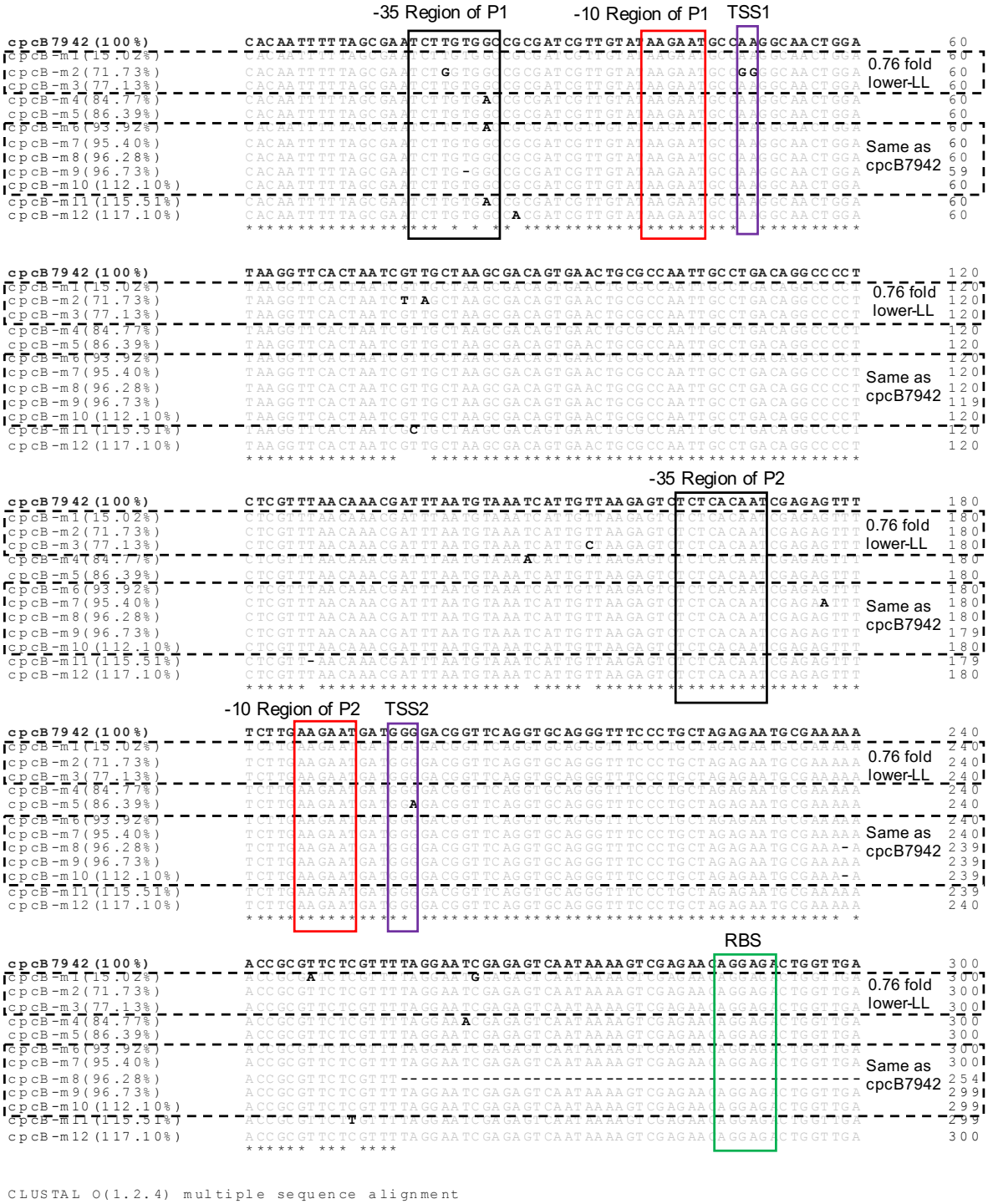

Figure S6: Sequence alignment of all synthetic promoters derived from the $P_{cpcB7942}$ promoter aligned using CLUSTALΩ. While the mutations are shown in bold black, the sequence same as wild type are shown in light grey. The important regions are highlighted using different boxes. The activity of the promoters were calculated with respect to wild type $cpcB_{7942}$ under low light and high CO_2 conditions (HC-LL) and are provided in parenthesis. The $cpcB_{7942}$ is considered as 100%. The promoters are categorized with respect to the promoter activity under respective conditions.

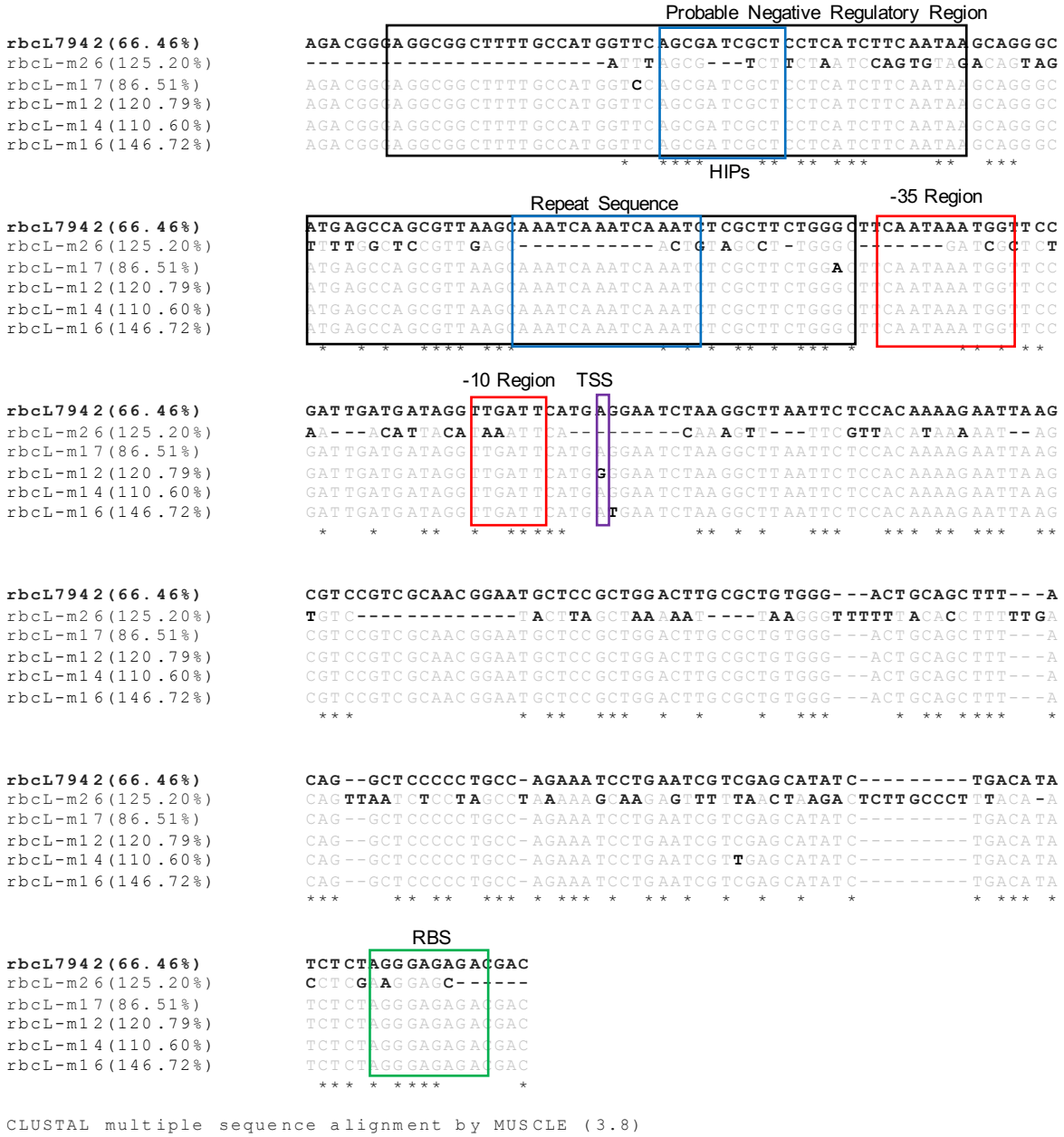

Figure S7: Alignment of mutant rbcL promoters that showed non-repressed phenotype under elevated CO_2 conditions (HC-HL) obtained using CLUSTALΩ. While the mutations are shown in bold black, the sequence same as wild type are shown in light grey. The parenthesis includes the % change in activity under 1% CO_2 as compared to its growth under 0.04% CO_2. The important regions are highlighted using different boxes.

```
                                        -35 Region of P1          -10 Region of P1  TSS1
cpcB7942(100%)      CACAATTTTTAGCGAATCTTGTGGCCGCGATCGTTGTATAAGAATGCCAAGGCAACTGGA   60
cpcB-m6(131.59%)    CACAATTTTTAGCGAATCTTGTGACCGCGATCGTTGTATAAGAATGCCAAGGCAACTGGA   60
cpcB-m9(130.24%)    CACAATTTTTAGCGAATCTTG-GGCCGCGATCGTTGTATAAGAATGCCAAGGCAACTGGA   59
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cpcB7942(100%)      TAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  120
cpcB-m6(131.59%)    TAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  120
cpcB-m9(130.24%)    TAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  119
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                                                                  -35 Region of P2
cpcB7942(100%)      CTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTCACAATCGAGAGTTT  180
cpcB-m6(131.59%)    CTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTCACAATCGAGAGTTT  180
cpcB-m9(130.24%)    CTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTCACAATCGAGAGTTT  179
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                    -10 Region of P2  TSS2
cpcB7942(100%)      TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAAA  240
cpcB-m6(131.59%)    TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAAA  240
cpcB-m9(130.24%)    TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAAA  239
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                                                                          RBS
cpcB7942(100%)      ACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  300
cpcB-m6(131.59%)    ACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  300
cpcB-m9(130.24%)    ACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  299
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CLUSTAL O(1.2.4) multiple sequence alignment
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Figure S8: Sequence alignment of mutant cpcB promoters that showed 1.3 fold higher activity under high light intensities (HC-HL) obtained CLUSTALΩ . The relative activity is provided in parenthesis with respect to wild type promoter. While the mutations are shown in bold black, the sequence same as wild type are shown in light grey. The important regions are highlighted using different boxes.

```
                                        -35 Region P1             -10 Region P1    TSS1
cpcB7942(100%)      CACAATTTTTAGCGAATCTTGTGGCCGCGATCGTTGTATAAGAATGCCAAGGCAACTGGA   60
cpcB-m1(18.69%)     CACAATTTTTAGCGAATCTTGTGGCCGCGATCGTTGTATAAGAATGCCAAGGCAACTGGA   60
cpcB-m3(27.47%)     CACAATTTTTAGCGAATCTTGTGGCCGCGATCGTTGTATAAGAATGCCAAGGCAACTGGA   60
cpcB-m4(61.66%)     CACAATTTTTAGCGAATCTTGTGACCGCGATCGTTGTATAAGAATGCCAAGGCAACTGGA   60
cpcB-m7(55.63%)     CACAATTTTTAGCGAATCTTGTGGCCGCGATCGTTGTATAAGAATGCCAAGGCAACTGGA   60
cpcB-m10(62.07%)    CACAATTTTTAGCGAATCTTGTGGCCGCGATCGTTGTATAAGAATGCCAAGGCAACTGGA   60
cpcB-m11(75.14%)    CACAATTTTTAGCGAATCTTGTGACCGCGATCGTTGTATAAGAATGCCAAGGCAACTGGA   60
cpcB-m12(68.40%)    CACAATTTTTAGCGAATCTTGTGGCCACGATCGTTGTATAAGAATGCCAAGGCAACTGGA   60
                    ******************** ***** ** ************************** ********

cpcB7942(100%)      TAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  120
cpcB-m1(18.69%)     TAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  120
cpcB-m3(27.47%)     TAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  120
cpcB-m4(61.66%)     TAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  120
cpcB-m7(55.63%)     TAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  120
cpcB-m10(62.07%)    TAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  120
cpcB-m11(75.14%)    TAAGGTTCACTAATCCCTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  120
cpcB-m12(68.40%)    TAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGACAGGCCCCT  120
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                                                                  -35 Region P2
cpcB7942(100%)      CTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTCACAATCGAGAGTTT  180
cpcB-m1(18.69%)     CTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTCACAATCGAGAGTTT  180
cpcB-m3(27.47%)     CTCGTTTAACAAACGATTTAATGTAAATCATTGCTAAGAGTCTCTCACAATCGAGAGTTT  180
cpcB-m4(61.66%)     CTCGTTTAACAAACGATTTAATGTAAAACATTGTTAAGAGTCTCTCACAATCGAGAGTTT  180
cpcB-m7(55.63%)     CTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTCACAATCGAGAATTT  180
cpcB-m10(62.07%)    CTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTCACAATCGAGAGTTT  180
cpcB-m11(75.14%)    CTCGTT-AACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTCACAATCGAGAGTTT  179
cpcB-m12(68.40%)    CTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTCACAATCGAGAGTTT  180
                    ****** ******************** ***** ********************** *** ***

                    -10 Region P2  TSS2
cpcB7942(100%)      TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAAA  240
cpcB-m1(18.69%)     TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAAA  240
cpcB-m3(27.47%)     TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAAA  240
cpcB-m4(61.66%)     TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAAA  240
cpcB-m7(55.63%)     TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAAA  240
cpcB-m10(62.07%)    TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAA-  239
cpcB-m11(75.14%)    TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAAA  239
cpcB-m12(68.40%)    TCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCCTGCTAGAGAATGCGAAAAA  240
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                                                                          RBS
cpcB7942(100%)      ACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  300
cpcB-m1(18.69%)     ACCGCGATCTCGTTTTAGGAATGGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  300
cpcB-m3(27.47%)     ACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  300
cpcB-m4(61.66%)     ACCGCGTTCTCGTTTTAGGAAACGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  300
cpcB-m7(55.63%)     ACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  300
cpcB-m10(62.07%)    ACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  299
cpcB-m11(75.14%)    ACCGCGTTCTTGTTTTAGGAATCGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  299
cpcB-m12(68.40%)    ACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATAAAAGTCGAGAACAGGAGACTGGTTGA  300
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CLUSTAL O(1.2.4) multiple sequence alignment
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Figure S9: Sequence alignment of mutant cpcB promoters that showed 0.76 fold lower activity under high light intensities (HC-HL) and the relative activity is provided in

parenthesis with respect to wild type promoter. The alignment was obtained using CLUSTALΩ. The important regions are highlighted using different boxes. While the mutations are shown in bold black, the sequence same as wild type are shown in light grey.

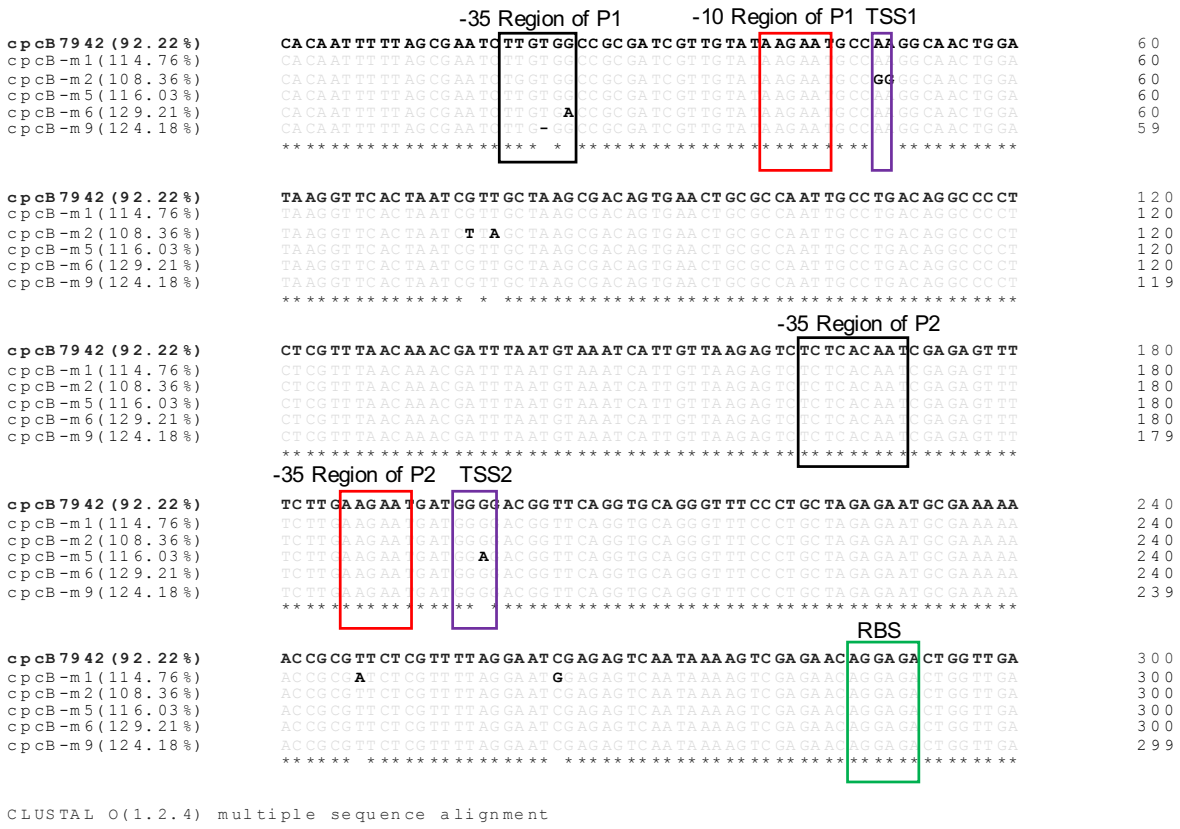

Figure S10: Alignment of cpcB mutant promoters that showed no repression under high light intensities (HL) as compared to low light intensities (LL) was obtained using CLUSTALΩ. The important regions are highlighted using different boxes. While the mutations are shown in bold black, the sequence same as wild type are shown in light grey.

Table S1: The sequences of native and mutated promoters used in this study

> *S. elongatus* PCC 7942 rbcL$_{7942}$
AGACGGGAGGCGGCTTTTGCCATGGTTCAGCGATCGCTCCTCATCTTCAATAAGC
AGGGCATGAGCCAGCGTTAAGCAAATCAAATCAAATCTCGCTTCTGGGCTTCAAT
AAATGGTTCCGATTGATGATAGGTTGATTCATGAGGAATCTAAGGCTTAATTCTC
CACAAAAGAATTAAGCGTCCGTCGCAACGGAATGCTCCGCTGGACTTGCGCTGT
GGGACTGCAGCTTTACAGGCTCCCCCTGCCAGAAATCCTGAATCGTCGAGCATAT
CTGACATATCTCTAGGGAGAGACGAC

> *S. elongatus* PCC 7942 rbcL-m1
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AGGGCATGAGCCAGCGTTAAGCAAATCAAATCAAATCTCGCTTCTGGGCTTCAAT
AAATGGTTCCGATTGATGATAGGTTGATTCATGAGGAATCTAAGGCTTAATTCTC
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CTGACATATCTCTAGGGAGAGACGAC

> *S. elongatus* PCC 7942 rbcL-m2
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CTGACATATCTCTAGGGAGAGACGAC

> *S. elongatus* PCC 7942 rbcL-m3
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AAATGGTTCCGATTGATGATAGGTTGATTCATGAGGAATCTAAGGCTTAATTCTC
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CTGACATATCTCTAGGGAGAGACGAC

> *S. elongatus* PCC 7942 rbcL-m4
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CTGACATATCTCTAGGGAGAGACGAC

> *S. elongatus* PCC 7942 rbcL-m5
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TCTGACATATCTCTAGGGAGAGACGAC

> *S. elongatus* PCC 7942 rbcL-m6
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> *S. elongatus* PCC 7942 rbcL-m7

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> *S. elongatus* PCC 7942 rbcL-m8

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> *S. elongatus* PCC 7942 rbcL-m9

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> *S. elongatus* PCC 7942 rbcL-m10

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> *S. elongatus* PCC 7942 rbcL-m11

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> *S. elongatus* PCC 7942 rbcL-m12

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> *S. elongatus* PCC 7942 rbcL-m13

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> *S. elongatus* PCC 7942 rbcL-m14

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> *S. elongatus* PCC 7942 rbcL-m15
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> *S. elongatus* PCC 7942 rbcL-m16
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> *S. elongatus* PCC 7942 rbcL-m19
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> *S. elongatus* PCC 7942 rbcL-m20
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> *S. elongatus* PCC 7942 rbcL-m21
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> *S. elongatus* PCC 7942 rbcL-m22

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> *S. elongatus* PCC 7942 rbcL-m23

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> *S. elongatus* PCC 7942 rbcL-m24

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> *S. elongatus* PCC 7942 rbcL-m25

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> *S. elongatus* PCC 7942 rbcL-m26

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> *S. elongatus* PCC 7942 rbcL-m27

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> *S. elongatus* PCC 7942 rbcL-m28

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> *S. elongatus* PCC 7942 rbcL-m29

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> *S. elongatus* PCC 7942 rbcL-m30
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> *S. elongatus* PCC 7942 rbcL-m31
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> *S. elongatus* PCC 7942 rbcL-m32
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> *S. elongatus* PCC 7942 rbcL-m33
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> *S. elongatus* PCC 7942 rbcL-m34
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> *S. elongatus* PCC 7942 rbcL-m35
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> *S. elongatus* PCC 7942 rbcL-m36
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> *S. elongatus* PCC 11801 $rbcL_{11801}$

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> *S. elongatus* PCC 11802 $rbcL_{11802}$

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> *S. elongatus* PCC 7942 $cpcB_{7942}$

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> *S. elongatus* PCC 7942 cpcB-m1

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> *S. elongatus* PCC 7942 cpcB-m2

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> *S. elongatus* PCC 7942 cpcB-m3

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> *S. elongatus* PCC 7942 cpcB-m4

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AAAGTCGAGAACAGGAGACTGGTTGA

> *S. elongatus* PCC 7942 cpcB-m5

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> *S. elongatus* PCC 7942 cpcB-m6

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> *S. elongatus* PCC 7942 cpcB-m7

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CACAATCGAGAATTTTCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCC
CTGCTAGAGAATGCGAAAAAACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATA
AAAGTCGAGAACAGGAGACTGGTTGA

> *S. elongatus* PCC 7942 cpcB-m8

CACAATTTTTAGCGAATCTTGTGGCCGCGATCGTTGTATAAGAATGCCAAGGCAA
CTGGATAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTG
ACAGGCCCCTCTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCT
CACAATCGAGAGTTTTCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCC
CTGCTAGAGAATGCGAAAAACCGCGTTCTCGTTT

> *S. elongatus* PCC 7942 cpcB-m9

CACAATTTTTAGCGAATCTTGGGCCGCGATCGTTGTATAAGAATGCCAAGGCAAC
TGGATAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTGA
CAGGCCCCTCTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTC
ACAATCGAGAGTTTTCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCC
TGCTAGAGAATGCGAAAAAACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATAA
AAGTCGAGAACAGGAGACTGGTTGA

> *S. elongatus* PCC 7942 cpcB-m10

CACAATTTTTAGCGAATCTTGTGGCCGCGATCGTTGTATAAGAATGCCAAGGCAA
CTGGATAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTG
ACAGGCCCCTCTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCT
CACAATCGAGAGTTTTCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCC
CTGCTAGAGAATGCGAAAAACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATAA
AAGTCGAGAACAGGAGACTGGTTGA

> *S. elongatus* PCC 7942 cpcB-m11

CACAATTTTTAGCGAATCTTGTGACCGCGATCGTTGTATAAGAATGCCAAGGCAA
CTGGATAAGGTTCACTAATCGCTGCTAAGCGACAGTGAACTGCGCCAATTGCCTG
ACAGGCCCCTCTCGTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCTC
ACAATCGAGAGTTTTCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCC
TGCTAGAGAATGCGAAAAAACCGCGTTCTTGTTTTAGGAATCGAGAGTCAATAA
AAGTCGAGAACAGGAGACTGGTTGA

> *S. elongatus* PCC 7942 cpcB-m12

CACAATTTTTAGCGAATCTTGTGGCCACGATCGTTGTATAAGAATGCCAAGGCAA
CTGGATAAGGTTCACTAATCGTTGCTAAGCGACAGTGAACTGCGCCAATTGCCTG
ACAGGCCCCTCTCGTTTAACAAACGATTTAATGTAAATCATTGTTAAGAGTCTCT
CACAATCGAGAGTTTTCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCC
CTGCTAGAGAATGCGAAAAAACCGCGTTCTCGTTTTAGGAATCGAGAGTCAATA
AAAGTCGAGAACAGGAGACTGGTTGA

> *S. elongatus* PCC 11801 $cpcB_{11801}$

CACAATTTTTAGCGAACTTCTGAGGCGCGATCGTTGTATAAGAATGCCTAGGCAG
CTGGATAAGGTTCACTTATTGTCATCTGACAACAGTGAACTGATTACATTGCTCG
CTTACCCCTCTCGTTTAACAAACGATTTAATGTAAATCATTGTTAATGAGTCTCTC
ACAATCAAGAGTTTTCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCC
TGCAGAGATTGCGGAAAATCCGCATTCTCGTTTTAGGAATCGAGAGTCAATAAA
AGTCGAGAACAGGAGACTGGTTGA

> *S. elongatus* PCC 11802 $cpcB_{11802}$

CACAATTTTTAGCGAACTTCTGAGGCGCGATCGTTGTATAAGAATGCCTAGGCAG
CTGGATAAGGTTCACTTATTGTCATCTGACAACGGTGAACTGATTACATTGCTCG
CTTACCCCTCTCGTTTAACAAACGATTTAATGTAAATCATTGTTAATGAGTCTCTC
ACAATCAAGAGTTTTCTTGAAGAATGATGGGGACGGTTCAGGTGCAGGGTTTCCC
TGCAGAGATTGCGGAAAATCCGCATTCTCGTTTTAGGAATCGAGAGTCAATAAA
AGTCGAGAACAGGAGACTGGTTGA

Table S2: List of Primers employed for the construction of the vectors and strains used in the study.

Primer description	Primer sequence
Eyfp_rrnB_op_rv	gaccaccgcgctactgccgccaggcactactatttgtacagttcatccatg
Eyfp_rrnB_op_fw	catggatgaactgtacaaatagtagtgcctggcggcagtagcgcggtggtc
BamHI_rrnB_rv	gactggatccaaggcccagtctttcgactgagcctttc
HindIII_cpcB_7942_fw	gatcaagcttcacaatttttagcgaatcttgtg
NdeI_cpcB_7942_rv	gatccatatgtcaaccagtctcctgttctcgac
NdeI_eyfp_fw	atacatatggtgagcaaaggcgaagaactg
NdeI_rbcL _7942_rv	gatccatatggtcgtctctccctagagatatgtcag
HindIII_rbcL_7942_fw	ctaagcttagacgggaggcggcttttg
HindIII_cpcB_11801_fw	gatcaagcttcacaatttttagcgaacttc
NdeI_cpcB_11801_rv	gatccatatgtcaaccagtctcctgttctcg
HindIII_rbcL_11801_fw	gatcaagcttcggaaaaagcgccgtaattacac
NdeI_rbcL_11801_rv	gatccatatggtcgtctctccctagagatatgtcag
7942-NSI_F	cgaagatggaaaagctcaagcggaagggacccaac
7942-NS1_R	gcgatttgggtagcgctgccttccccttc
P-sequencing_F	catttggtacagcgcagtaaccg
P-sequencing_R	gcttttgaaaaaatcgtgcagtttc
11801/2-NSI-fw	caacgcctattccaagggcg
11801/2-NSI-rv	ctctctgaggggatgcagtgct