

Table S1 List of SNPs in genome sequences from some wild types (GT-S, PCC-N/P and YF), Δhol and $\Delta holR$

Yellow, SNPs specific in $\Delta holR$; light green, SNPs specific in the wild type strain YF; pale orange, SNPs in the wild type strain YF common to PCC-P/N strains; gray, SNPs derived from sequence error reported (1); light blue, SNPs specific in Δhol

Genomic position ¹	Database ¹	Wild type			YF Δhol ⁴	YF $\Delta holR$ ⁴	Gene ⁵	Amino acid substitution
		GT-S ²	PCC-P/N ³	YF ⁴				
462452	C	C	C	C	C	G	<i>sll1512</i>	Asp->His
481709	C	C	C	T	T	T	<i>sll1502</i>	silent
488923	G	G	G	A	A	A	<i>slr1609</i>	Gly->Asp
831647	C	C	T	T	T	T	intergenic (<i>ssl3441-sll1815</i>)	
943495	G	A	A	A	A	A	<i>slr1834</i>	Val->Ile
1012958	G	T	T	T	T	T	intergenic (<i>ssl3177-sll1633</i>)	
1153565	C	C	C	C	C	A	<i>slr0887</i>	Pro->Thr
1204616	G	G	A	A	A	A	<i>slr1865</i>	Cys->Tyr
1364187	A	G	G	G	G	G	<i>sll0838</i>	silent
1403160	G	G	G	T	T	T	intergenic (<i>slr1257-slr1258</i>)	
1905889	G	G	G	G	T	G	<i>sll1434</i>	Thr->Asn
2198893	T	C	C	C	C	C	<i>sll0142</i>	silent
2301721	A	G	G	G	G	G	<i>slr0168</i>	Lys->Glu
2584302	T	T	T	T	C	T	<i>slr0060</i>	Ile->Thr
2614680	C	C	C	C	T	C	<i>slr0479</i>	Thr->Ile
3014665	T	T	C	C	C	C ⁷	<i>slr0302</i>	silent
3142651	A	G	G	G	G	G	<i>sll0045</i>	silent

¹These columns show the genomic position and the nucleotide in the database (<http://genome.kazusa.or.jp/cyanobase/Synechocystis>). ²Tajima, N., Sato, S.,

Maruyama, F., Kaneko, T., Sasaki, N. V., Kurokawa, K., Ohta, H., Kanesaki, Y., Yoshikawa, H., Tabata, S., Ikeuchi, M., and Sato, N. (2011) *DNA Res.* **18**, 393-399. ³Kanesaki, Y., Shiwa, Y., Tajima, N., Suzuki, M., Watanabe, S., Sato, N., Ikeuchi, M., and Yoshikawa, H. (2012) *DNA Res.* **19**, 67-79. ⁴The wild type used in this work and the derived mutants are shown in these columns. ⁵All SNPs are shown as the nucleotide difference from the database. Thus, SNPs of *sll* genes (coded by the complementary strand) are shown in the complementary nucleotides.

Table S2 Oligonucleotides used in this study

Primer name	Sequence ¹	Use (restriction site introduced)
pRTho1f	5'-GGCTTTGTCAAATGCTTCCT-3'	Forward primer of RT-PCR for <i>ho1</i>
pRTho1r	5'-GTCATTGGGTTTCATCCACAA-3'	Reverse primer of RT-PCR for <i>ho1</i>
pRTho2f	5'-AACTCCGCTACGGTACCCAA-3'	Forward primer of RT-PCR for <i>ho2</i>
pRTho2r	5'-ATGGGATGACCAGCGGTACT-3'	Reverse primer of RT-PCR for <i>ho2</i>
pRT1874f1	5'-AAACCATTTCGCCCCGGCATT-3'	Forward primer of RT-PCR for <i>chlA_{II}</i>
pRT1874r1	5'-TAGCGCTGGCACAGGCTTCT-3'	Reverse primer of RT-PCR for <i>chlA_{II}</i>
pRThemN1f	5'-CATCACCCAGCACAAACCAG-3'	Forward primer of RT-PCR for <i>hemN</i>
pRThemN1r	5'-TCGGGTTTGGCAAAATGGTC-3'	Reverse primer of RT-PCR for <i>hemN</i>
RT-slr1181-f	5'-AGTTCCAATGCCATTGGCTT-3'	Forward primer of RT-PCR for <i>psbA1</i>
RT-slr1181-r	5'-CGATGTTGTAGGTTTCTTCC-3'	Reverse primer of RT-PCR for <i>psbA1</i>
RT-sll1512-f	5'-AAGGTTTTCCCTCATACTGC-3'	Forward primer of RT-PCR for <i>sll1512</i>
RT-sll1512-r	5'-TCAGAAGACTGCATTCACAG-3'	Reverse primer of RT-PCR for <i>sll1512</i>
RT-slr0056-f	5'-CTCACCTGTATGTTGTTGTC-3'	Forward primer of RT-PCR for <i>chlG</i>
RT-slr0056-r	5'-TCATGATCACGCAGATCCAA-3'	Reverse primer of RT-PCR for <i>chlG</i>
pRTporf	5'-TGACAAAGGTTGGCACGTGA-3'	Forward primer of RT-PCR for <i>por</i>
pRTporr	5'-TCGCTTCCCTGTTCCGAAAG-3'	Reverse primer of RT-PCR for <i>por</i>
RT-slr0008-f	5'-AAACCCTGAGCTTAGACGAA-3'	Forward primer of RT-PCR for <i>ctpA</i>
RT-slr0008-r	5'-CTGATTATCCTGCAAAGCTC-3'	Reverse primer of RT-PCR for <i>ctpA</i>
RT-sll1220-f	5'-GTTTTAAGGTGTTAGACGCC-3'	Forward primer of RT-PCR for <i>hoxE</i>
RT-sll1220-r	5'-CTTAACCAAGGTTGTATCGC-3'	Reverse primer of RT-PCR for <i>hoxE</i>
RT-sll0217-f	5'-TACAATTGCTTGATGGGCTC-3'	Forward primer of RT-PCR for <i>flv4</i>
RT-sll0217-r	5'-TCGGTCTTGAGCAAAATTGG-3'	Reverse primer of RT-PCR for <i>flv4</i>
RT-sll0920-f	5'-TCATTCAGCGTAATCTGACC-3'	Forward primer of RT-PCR for <i>ppc</i>
RT-sll0920-r	5'-TTTGGTAATAATCCGGTGGC-3'	Reverse primer of RT-PCR for <i>ppc</i>
RT-slr0301-f	5'-AGATCACAGCCAAGTGTTAG-3'	Forward primer of RT-PCR for <i>ppsA</i>
RT-slr0301-r	5'-CATCAAATTTTCATCAGGGCG-3'	Reverse primer of RT-PCR for <i>ppsA</i>
RT-sll1471-f	5'-TATCGTCAGGTGTTCAACGA-3'	Forward primer of RT-PCR for <i>cpcG2</i>
RT-sll1471-r	5'-CTTCCCTGCTAGATAAACCC-3'	Reverse primer of RT-PCR for <i>cpcG2</i>
RT-sll1324-f	5'-ATACCTTGTTTCATCCTAGCG-3'	Forward primer of RT-PCR for <i>atpF</i>
RT-sll1324-r	5'-ATACTGCGTTCGATTAACCG-3'	Reverse primer of RT-PCR for <i>atpF</i>
pRRN16f1	5'-CCACACTGGGACTGAGACAC-3'	Forward primer of RT-PCR for <i>rrn16Sa</i>

pRRN16r1	5'-TTGCGGGACTTAACCCAACA-3'	Reverse primer of RT-PCR for <i>rrn16Sa</i>
Multiplex library P1 adaptors	5'-ATCACCGACTGCCCATAGAGAGGTT-3' 5'-CCTCTCTATGGGCAGTCGGTGAT-3'	short SOLiD P1 adaptors
Barcode 001 P2 adaptors	5'-CGCCTTGGCCGTACAGCAG-3' 5'-CTGCCCCGGGTTCCCTCATTCTCT GTGTAAGAGG CTGCTGTACGGCCAAGGCG-3'	short SOLiD P2 adaptors of <i>Δhol</i> genome
Barcode 002 P2 adaptors	5'-CGCCTTGGCCGTACAGCAG-3' 5'-CTGCCCCGGGTTCCCTCATTCTCT AGGGAGTGGT CTGCTGTACGGCCAAGGCG-3'	short SOLiD P2 adaptors of <i>ΔholR</i> genome
Multiplex Library PCR-1	5'-CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT-3'	Forward primer of nick-translation and PCR
Multiplex Library PCR-2	5'-CTGCCCCGGGTTCCCTCATTCT-3'	Reverse primer of nick-translation and PCR
sll1512-f	5'-GTAGATCTTCCATCACCTGT-3'	Forward primer of <i>sll1512</i> fragment
sll1512-r	5'-TACCTTTACCAATCCTTGC-3'	Reverse primer of <i>sll1512</i> fragment
slr0887-f	5'-TTACGTCCCTATAGGAGAAC-3'	Forward primer of <i>slr0887</i> fragment
slr0887-r	5'-CTAAATGCGGCTGAAACGT-3'	Reverse primer of <i>slr0887</i> fragment
sll1512-f2	5'-AT GGATCC ACCCGGCGTAGATCTT-3'	Forward primer for <i>Δsll1512</i> (BamHI)
sll1512-r2	5'-T GGGATCC CAACTCCAGTAG-3'	Reverse primer for <i>Δsll1512</i> (BamHI)
1214specf1	5'-CGAGCTCGAATTCTCATGTTATCCCCTGCTCGCGCAGGCT-3'	Forward primer for spectinomycin cartridge ³
1214specr1	5'-GTAACCGGCCTTTCATCGGGCTTAGTAAAGCCCTCGCTA-3'	Reverse primer for spectinomycin cartridge ³
pASK5-1512-f	5'-TAGTCAG GTCTC CGGCGCCGAAAAGGTTTTCCCTCATAC-3'	Forward primer for Sll1512 overexpression plasmid (BsaI)
pASK5-1512-r	5'-ATCGCG GGTCTC ATATCATTAGTTTGGTCCTTCGCCGG-3'	Reverse primer for Sll1512 overexpression plasmid (BsaI)
PchlAII-f2	5'-TCTAGGAATACCGGGGAAAT-3'	Forward primer for upper region of <i>chlAII</i>
PchlAII-r2	5'-CGTTAATTAACAGGAGAATC-3'	Reverse primer for upper region of <i>chlAII</i>
PpsbAI-f2	5'-CTTGATCTGGCATTACGAG-3'	Forward primer for upper region of <i>psbAI</i>
PpsbAI-r2	5'-AGCGAATAATTACGAAGTAA-3'	Reverse primer for upper region of <i>psbAI</i>

¹ Restriction sites introduced for subcloning are underlined.

² Particular sites for each P2 adapter are bold.

³ Minamizaki, K., Mizoguchi, T., Goto, T., Tamiaki, H., and Fujita, Y. (2008) *J. Biol. Chem.* **283**, 2684-2692.

Supplementary Figure Legends

Figure S1. RT-PCR analysis for the transcript levels of nine genes.

Total RNA was isolated from the wild-type (lanes 1 and 4), ΔhoI (lanes 2 and 5) and $\Delta hoIR$ (lanes 3 and 6) cells. ‘Aerobic’ samples (lanes 1, 2 and 3) were grown under low-oxygen conditions for 5 days and then transferred under aerobic conditions and grown for 2 days. ‘Low-oxygen’ samples (lanes 4, 5 and 6) were grown under low-oxygen conditions for 7 days. The housekeeping gene *rrn16Sa* was used as a control. Gene names and ORF IDs are shown in the left and right parts, respectively. Cycle numbers of RT-PCR were 6 and 25 for *rrn16Sa* and the others, respectively.

Figure S2. Two point mutation sites found in the strain $\Delta hoIR$

After mapping the short reads from strains ΔhoI (light green bars) and $\Delta hoIR$ (light blue bars) to the reference genome *Synechocystis* 6803, bam files created were visualized using IGV Genome browser in a 644-bp (A, B) and a 81-bp (C, D) scales (1). A G-to-C transversion at the *sll1512* gene region (A, C) and a C-to-A transversion at the *slr0887* gene region (B, D) were found in the strain $\Delta hoIR$ (each lower window) and not in the strain ΔhoI (each upper window). Respective annotated gene and the amino acid sequences are shown in the bottom line. The G-to-C transversion at the *sll1512* coding strand is corresponding to the C-to-G transversion in the database.

1. Robinson, J. T., *et al.* (2011) Integrative Genomics Viewer. *Nat. Biotech.* **29**, 24-26.

Figure S3. Multiple alignment of amino acid sequences of probable Sll1512-orthologs from 12 cyanobacteria, HxlR and OhrR from *Bacillus subtilis*, OhrR from *Xanthomonas campestris* and MarR from *E. coli*. The completely conserved residues in this alignment are shaded in dark blue. The conserved residues among more than 10 cyanobacteria are shaded in blue. Three additional Cys residues conserved only in the four cyanobacterial homologues are shaded in light blue. The helix-turn-helix motif in Sll1512 predicted by a motif prediction program (HELIX-TURN-HELIX MOTIF PREDICTION; 1, 2) is shaded in yellow. Asp35 of Sll1512 and the corresponding Asp26 in MarR from *E. coli* are shaded in light green. A MarR variant with the point mutation of Asp26 to Asn constitutively binds to the operator resulting in no expression of a reporter gene even in the presence of an inducer (3). 6803, *Synechocystis* 6803; 7002, *Synechococcus* 7002; Tri, *Trichodesmium erythraeum*; Aca, *Acaryochloris*

marina; 7120, *Anabaena* sp. PCC 7120; Ana, *Anabaena variabilis*; Nos, *Nostoc punctiforme*; 7822, *Cyanothece* sp. PCC 7822; 7424, *Cyanothece* sp. PCC 7424; 6301, *Synechococcus elongatus* PCC 6301; 7942, *Synechococcus elongatus* PCC 7942; 8102, *Synechococcus* sp. WH 8102; BsHxlR, HxlR from *Bacillus subtilis*; BsOhrR, OhrR from *B. subtilis*; XcOhrR, OhrR from *X. campestris*; and EcMarR, MarR from *E. coli*. The numbers on the right side indicate the position in the alignments at the end of each row. ‘*’ and ‘†’ indicate strains harboring a low-oxygen inducible *psbA* gene copy (4) and a *chlA_{II}-ho2-hemN* gene cluster, respectively.

1. Dodd, I. B, and Egan, J. B. (1990) Helix-turn-helix DNA-binding motifs prediction: Improved detection of helix-turn-helix DNA-binding motifs in protein sequences. *Nucleic Acids Res.* **18**, 5019-5026.
2. http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_hth.html
3. Alekshun, M. N., and Levy, S. B. (1999) Characterization of MarR supersuppressor mutants. *J Bacteriol* **181**, 3303-3306.
4. Summerfield, T. C., Toepel, J., and Sherman, L. A. (2008) Low-oxygen induction of normally cryptic *psbA* genes in cyanobacteria. *Biochemistry* **47**, 12939-12941

Figure S4. A. An SDS-PAGE profile of purified Sll1512 proteins. The wild-type Sll1512 (lane 2; 0.7 µg) and Sll1512-D35H variant (lane 4; 0.5 µg) were purified as Strep-tag fusion proteins from the soluble fractions of *E. coli* cells harboring pASK5-Sll1512 (lane 1; 1.7 µg) and pASK5-Sll1512D35H (lane 3; 1.4 µg). **B.** Gel mobility shift assays of the ³²P-labelled DNA fragments of *chlA_{II}* promoters by Sll1512 and Sll1512-D35H in polyacrylamide gel. Purified Sll1512 (lanes 1-3) and Sll1512-D35H (lanes 4-6) were added to the reaction mixtures (100 nM). In this reaction, a DNA fragment upstream of *chlA_{II}* (574 bp from the position -1 to -574 of *chlA_{II}*) was amplified by PCR from genomic DNA of *Synechocystis* 6803 (Table S2). Proteins were incubated with probes (0.1 nM) in 20 µl of binding buffer in the presence of dithiothreitol (10 mM; lanes 2 and 5) or dithionite (10 mM; lanes 3 and 6) or no additional reagents (lanes 1 and 4) for 20 min at 30°C. The mixtures were separated by a native 4% polyacrylamide gel. Gels were dried, and the signals were detected by autoradiography. Formed DNA-protein complexes are shown by the arrow, and the asterisk ‘*’ indicates free probes.

Figure S5. Consensus sequence in upstream sequences of the *chlA_{II}* and the *psbA1* genes.

A consensus sequence “TT(C/A)CC-N_{4/3}-GG(A/T)AA” was found in upstream sequences of *chlA_{II}* (6803chlAII) and *psbA1* (6803psbA1) from *Synechocystis* 6803 and *chlA_{II}* (*acsF*) from *Synechococcus* 7002 (7002chlAII). The sequences would be a recognition site for ChlR. The numbers indicate the location of the sequences from the initial codons of the target genes.

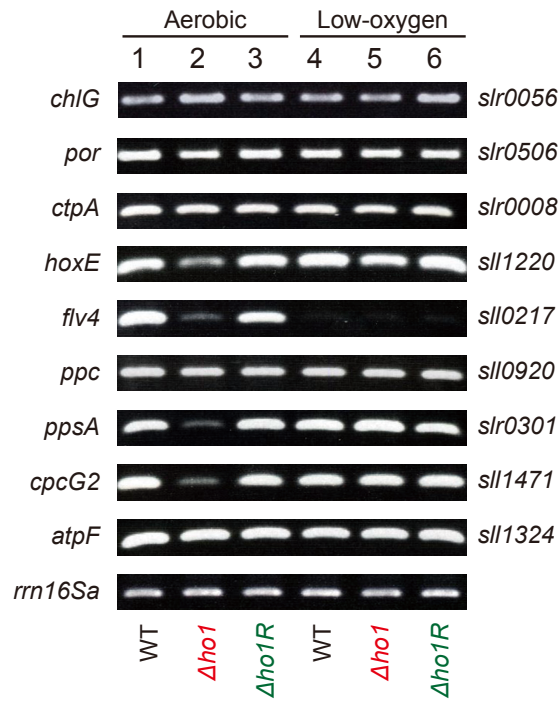


Figure S1. RT-PCR analysis for the transcript levels of nine genes.

Total RNA was isolated from the wild-type (lanes 1 and 4), Δ *ho1* (lanes 2 and 5) and Δ *ho1R* (lanes 3 and 6) cells. 'Aerobic' samples (lanes 1, 2 and 3) were grown under low-oxygen conditions for 5 days and then transferred under aerobic conditions and grown for 2 days. 'Low-oxygen' samples (lanes 4, 5 and 6) were grown under low-oxygen conditions for 7 days. The housekeeping gene *rrn16Sa* was used as a control. Gene names and ORF ID are shown in the left and right parts, respectively. Cycle numbers of RT-PCR were 6 and 25 for *rrn16Sa* and the others, respectively.

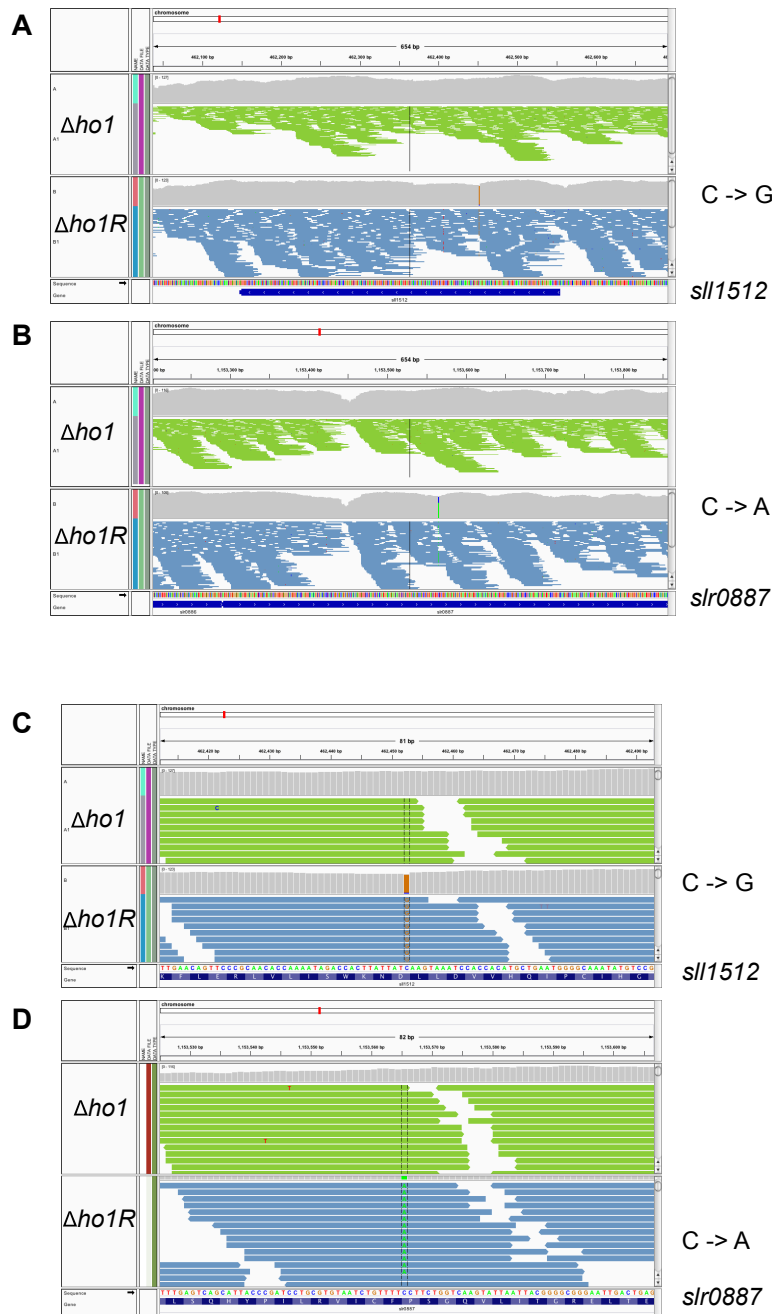


Figure S2. Two point mutation sites found in the strain *Δho1R*

After mapping the short reads from strains *Δho1* (light green bars) and *Δho1R* (light blue bars) to the reference genome *Synechocystis* 6803, bam files created were visualized using IGV Genome browser in a 644-bp (**A, B**) and a 81-bp (**C, D**) scales (1). A G-to-C transversion at the *slI1512* gene region (**A, C**) and a C-to-A transversion at the *slr0887* gene region (**B, D**) were found in the strain *Δho1R* (each lower window) and not in the strain *Δho1* (each upper window). Respective annotated gene and the amino acid sequences are shown in the bottom line. The G-to-C transversion at the *slI1512* coding strand is corresponding to the C-to-G transversion in the database.

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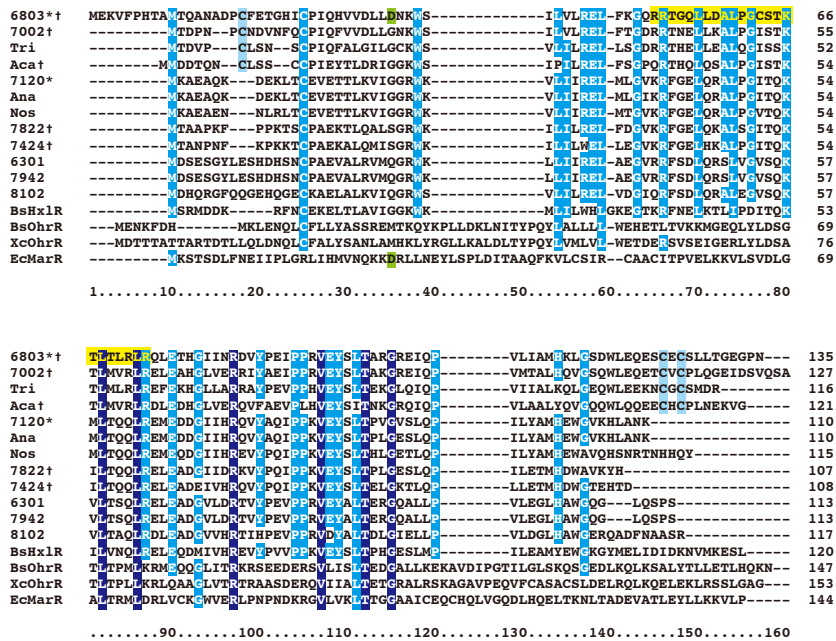


Figure S3. Multiple alignment of amino acid sequences of probable SII1512-orthologs from 12 cyanobacteria, HxlR and OhrR from *Bacillus subtilis*, OhrR from *Xanthomonas campestris* and MarR from *E. coli*.

The completely conserved residues in this alignment are shaded in dark blue. The conserved residues among more than 10 cyanobacteria are shaded in light blue. Three additional Cys residues conserved only in the four cyanobacterial homologues are shaded in light blue. The helix-turn-helix motif in SII1512 predicted by a motif prediction program (HELIX-TURN-HELIX MOTIF PREDICTION; 1,2) is shaded in yellow. Asp35 of SII1512 and the corresponding Asp26 in MarR from *E. coli* are shaded in light green. A MarR variant with the point mutation of Asp26 to Asn constitutively binds to the operator resulting in no expression of a reporter gene even in the presence of an inducer (3). 6803, *Synechocystis* 6803; 7002, *Synechococcus* 7002; Tri, *Trichodesmium erythraeum*; Aca, *Acarochloris marina*; 7120, *Anabaena* sp. PCC 7120; Ana, *Anabaena variabilis*; Nos, *Nostoc punctiforme*; 7822, *Cyanothece* sp. PCC 7822; 7424, *Cyanothece* sp. PCC 7424; 6301, *Synechococcus elongatus* PCC 6301; 7942, *Synechococcus elongatus* PCC 7942; 8102, *Synechococcus* sp. WH 8102; BsHxlR, HxlR from *Bacillus subtilis*; BsOhrR, OhrR from *B. subtilis*; XcOhrR, OhrR from *X. campestris*; and EcMarR, MarR from *E. coli*. The numbers on the right side indicate the position in the alignments at the end of each row. ‘*’ and ‘†’ indicate strains harboring a low-oxygen inducible *psbA* gene copy (4) and a *chlA_H-ho2-hemN* gene cluster, respectively.

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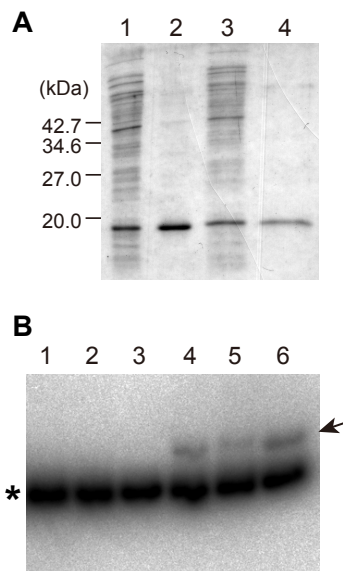


Figure S4. Purification of Sll1512 and gel shift assay in the presence of dithiothreitol and dithionite.

A. An SDS-PAGE profile of purified Sll1512 proteins. The wild-type Sll1512 (lane 2; 0.7 μ g) and Sll1512-D35H variant (lane 4; 0.5 μ g) were purified as Strep-tag fusion proteins from the soluble fractions of *E. coli* cells harboring pASK5-Sll1512 (lane 1; 1.7 μ g) and pASK5-Sll1512D35H (lane 3; 1.4 μ g). **B.** Gel mobility shift assays of the 32 P-labelled DNA fragments of *chlA_H* promoters by Sll1512 and Sll1512-D35H in polyacrylamide gel. Purified Sll1512 (lanes 1-3) and Sll1512-D35H (lanes 4-6) were added to the reaction mixtures (100 nM). In this reaction, a DNA fragment upstream of *chlA_H* (574 bp from the position -1 to -574 of *chlA_H*) was amplified by PCR from genomic DNA of *Synechocystis* 6803 (Table S2). Proteins were incubated with probes (0.1 nM) in 20 μ l of binding buffer in the presence of dithiothreitol (10 mM; lanes 2 and 5) or dithionite (10 mM; lanes 3 and 6) or no additional reagents (lanes 1 and 4) for 20 min at 30°C. The mixtures were separated by a native 4% polyacrylamide gel. Gels were dried, and the signals were detected by autoradiography. Formed DNA-protein complexes are shown by the arrow, and the asterisk '*' indicates free probes.

6803chlAII: -173 TTTT^ATTCCCTTTGGAACTATG -153
 6803psbA1 : -230 TTTT^ATACC-TTTGGAAACCAAC -210
 7002chlAII: -107 TTTT^ATACCCA-GG^ATAACTATA -91
 consensus : TT^A_CCC^{NNNN}_{NNN}GG^A_TAA

Figure S5. Consensus sequence in upstream sequences of the *chlA_{II}* and the *psbA1* genes.
 A consensus sequence TT(C/A)CC-N_{4/3}-GG(A/T)AA was found in upstream sequences of *chlA_{II}* (6803chlAII) and *psbA1* (6803psbA1) from *Synechocystis* 6803 and *chlA_{II}* (*acsF*) from *Synechococcus* 7002 (7002chlAII). The sequences would be a recognition site for ChlR. The numbers indicate the location of the sequences from the initial codons of the target genes.