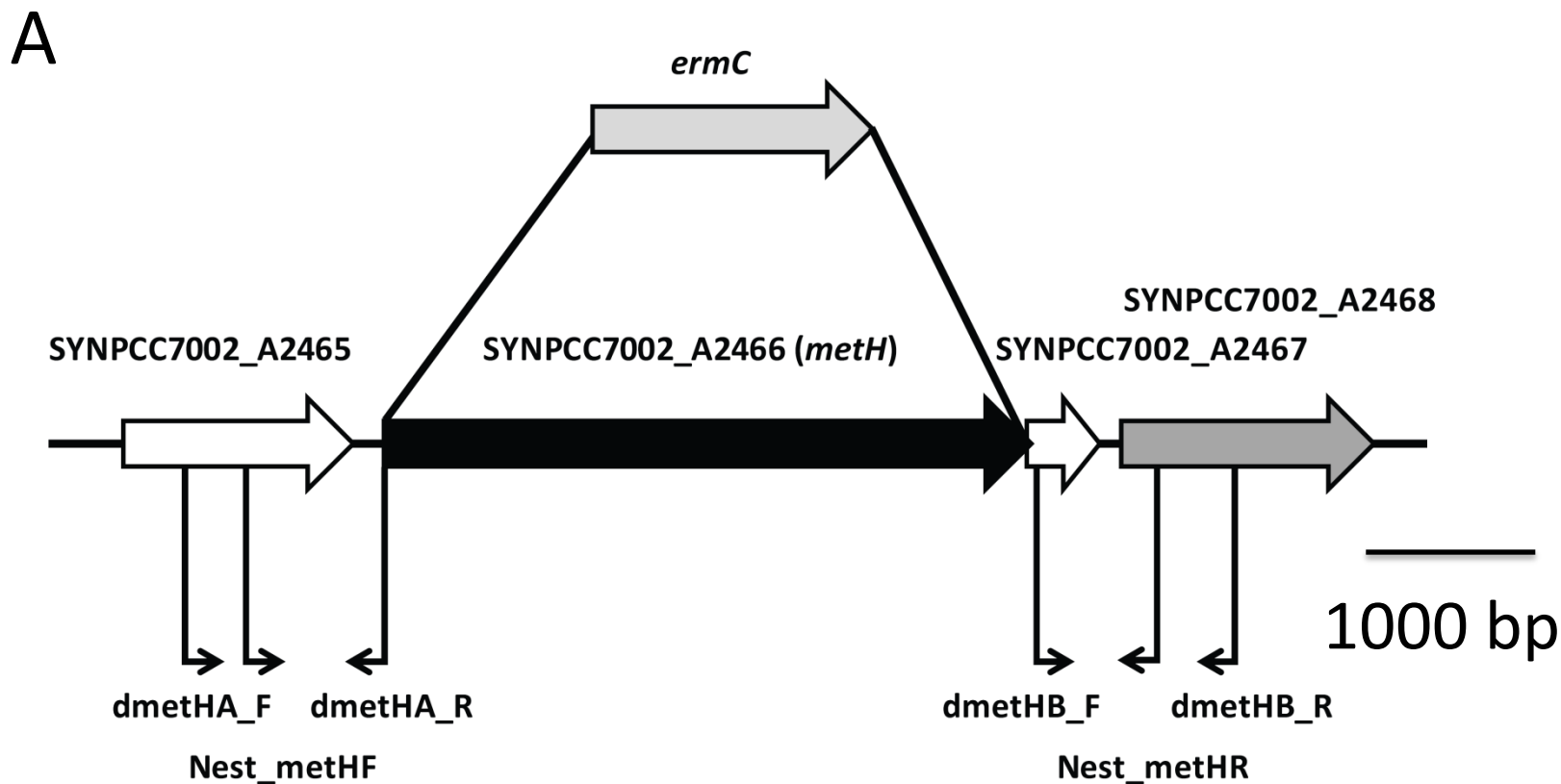


Figure S1

```
73109_MetE      MTMFQTTTLGYARMGKRRELKKALETFWGGTSSADDLLALRQDLEVKTWQAQLTAGIDRI
CYA_0167        -MAIETQTLGYARMGKRRELKKALEAFWGGTLEAEALLATLQDLESQAWRTQRQANLDHI
                :* *****:***** .*: ***  **** ::*:* * :*:
73109_MetE      AVGDQTLYDHVLDWTVRLGLIPRRFQGLSGLDRYFAMARGRDALPALEMTKWFDTNYHYL
CYA_0167        AVGDQTLYDHVLDWATWLGIPSRFQSLGSLERYFAMARGQEGLPALEMTKWFDTNYHYL
                *****:.* ***** **.******:*****:*****:*****:*****
73109_MetE      VPEIEDKMQPLGNFGEFLAMVQRSQAVLGDRAVPVLSVPTLLALSRHNGDLMAWLDQLL
CYA_0167        VPEIEADAIPOAHFDDFLATVRRRQGLGNRTSPVLLSPVTTLLALSQRSGELRGDLEKLL
                ***** . * ..* :*** *:***:.*:***: **:******:.*:* . *:*:
73109_MetE      PLYVDLLGQKALGVKEVQLHEPILVTSQAADLKTAVQNTYHQLASVGLPIQLVITYFDDL
CYA_0167        PLYRELLQELQRLGIREVQVHEPILVTSEGKSLQEAVEQTYRWLASVGIPLHLVITYFDDL
                *** :** :*: **:*:***:*****:.* .*: **:*:***: *****:.*:*****
73109_MetE      GVNYGWTQLPVAGISLDFTRGHNLDLLISQGF PADKILGAGVVDGRNIWQIHPDVILAM
CYA_0167        GETYPWAVELPVAGLSLDFTRGHNLVRAHGFPADKILGAGVVDGRNIWQIQPKAVLAT
                * .* *..:*****:*****:.*: .:*****:*****:.*:.*:
73109_MetE      LKKLQAIAPNLRVQPSASLQFVPHDATLETQLSEPLRNVL SFAEQKLEVVFLARMLNSE
CYA_0167        LQELQALAPNLRVQPSCSLQFVPHDAALETHLPEPLRNVL SFAEQKLAEVVLLARTLNGE
                *:*:***:*****.* *****:***:* *****:*****.*:*:*** **.*
73109_MetE      DTAAQQAEHQWQAFQFNPNPDVRQALASLTVEDFERSLSYSLRLDKQVKLPHLPTT
CYA_0167        DTAAQQQELEQQWQAFQDFNPNSTVRQALANLTAQDFQRS LPYEQRIGRQVKLPLPTT
                ***** *::*****:***** *****.*:***:*** * .*: :***** ****
73109_MetE      TIGSFPTKEVRQLRVKYKKGDISQAEYQRNIDT NIAECIKIQEEVGLDVLVHGEFERTD
CYA_0167        TIGSFPTPEVRQWRVKYKKEISQAEYEA AIDAEIAKCIRIQEEIGLDVLVHGEFERTD
                ***** **** *****:*****: **:*:***:***:*****:*****
73109_MetE      MVEYFGQQLGFAFTVNGWVQSYGSRCVRPPIIYGDVSRPQPM TVREFQVAQALTQKPKV
CYA_0167        MVEYFAQQLQGFAFTEHGWWVQSYGSRCVRPPIIYGDVSRPRPMTVREFKVAQSLTQKPKV
                *****.*:*:***** .*****:*****:*****:*****:***:*****
73109_MetE      GMLTAPITILNWSFSRADI SRRDQAFQIALALRQEVADLEAAGA QIIQVDEPALREGLPL
CYA_0167        GMLTGPVTMLNWSFPRVDVSRREQALQIALALRAEVADLEAAGAAMVQVDEPALREGLPL
                *****.*:*:***** *.*:***:***:***** ***** :*:*****
73109_MetE      KVQRWSDYLSWAVDAFRLATAIAQPQTQIHTHMCYCEFGDIIQDIERLDADVISIENSRS
CYA_0167        KKERWPEYLSWAVEAFRLTTAGAKPETQIHTHMCYSEFGDII EHIERLDADVLSIENSRS
                * :** :*****:***:*** *.*:***** .*****:*****:*****
73109_MetE      GNRTLQEVTEGGYSHQIGNGVYDIHSPVVPTEKQLVQQLKAGLANLPTQQIWNPNDCGLK
CYA_0167        SNKTL LQIAQAGYRHQVNGVYDVHSPAVPDTEQILRQLRLGVAHLPLEQTWVNPDCGLK
                .*:** :*:.* **.******:***.* :*:.*: **.* * * *****
73109_MetE      TRRWEEVPSLKHMAATQKLREEIDATTR
```

Figure S1. Sequence comparison of MetE from *Synechococcus* sp. strain PCC 73109 and *Synechococcus* sp. strain JA-3-3Ab. The gene encoding cobalamin-independent methionine synthase (73109_MetE) in *Synechococcus* sp. strain PCC 73109 was identified by its high amino acid sequence identity (73%) and similarity (85%) to the *metE-2* gene product of *Synechococcus* sp. strain JA-3-3Ab (CyanoBase: CYA_0167).



B

5.0 —

3.0 —

2.0 —

1.0 —

1 2

kbp

Figure S2. A. Scheme showing the construction of the $\Delta methH::ermC$ mutant by homologous recombination using primers in Table S1. Flanking regions are approximately 1.1 kb in length. The erythromycin (*ermC*) antibiotic resistance cassette was ligated into appropriate restriction sites at the ends of each flanking region (see further details in Materials and Methods). The resulting amplicon was used to transform strain AAP001 to generate strain AAP002. **B.** Electrophoresis of PCR amplicons using primers Nest_methHF and Nest_methHR (Table S1) shows the full segregation of the $\Delta methH::ermC$ allele in strain AAP002 (lane 2) grown in the absence of cobalamin. The same PCR primers were used to amplify the native *methH* gene in a WT strain (lane 1). The identities of amplicons were also verified by DNA sequencing.

Table S1

Table S1. Primers utilized in this study.

Name	Sequence
metEF	5'-AGG ACA ATA <u>CCA TAT GAC</u> GAT GTT TCA AAC AAC GAC-3' NdeI
metER	5'-CGA CTA CCC GTT GAT GTT <u>TGG ATC CGC</u> CGC GA-3' BamHI
dmetHA_F	5'-CCA ACG GGA CGT ACA TTA CT-3'
dmetHA_R	5'-AAG GCA <u>GAA TTC</u> ATG GAG AAA TGT TTC GGC GG-3' EcoRI
dmetHB_F	5'-ATA CTT <u>GGA TCC</u> TTG TCA AGC AAA GCC AAT TAT TT-3' BamHI
dmetHB_R	5'-CCC AAA CGT ATA AAC ATC TT TG-3'
Nest_metHF	5'-GGG AAC TGC GCA ATA ACA AT-3'
Nest_metHR	5'-GCC AGA TTG CCA TCG ATA AT-3'
PmetE73109F	5'-AAA AAA <u>CCG CGG</u> TAG CAC TGA GAA TAT GAA TTC AAT G -3' SacII
PmetE73109R	5'-AAA AAA <u>CCA TGG</u> TAT GTA TTG TCC TAA ATG AAT TTG A -3' NcoI
cpcBA6803_fusF	5'-GGT CAG GAT ATC GGT CAA GTA T-3'
cpcBA6803_fusR	5'-CGC CTA GGC <u>TGC TAG CCT</u> TTT GCA ATC CCA CAG TTA AC-3' NheI
metE73109_fusF	5'-GTT TGA AAA ATG <u>CTA GCC</u> TTT AGC ACT GAG AAT ATG AAT TC-3' NheI
metE73109_fusR	5'-AAA AAA <u>CCA TGG</u> TAT GTA TTG TCC TAA ATG AAT TTG A-3' NcoI
psbA26803_F	5'-GTT TAC <u>GAA TTC</u> AAA AAA CGA CAA TTA CAA GAA AGT A-3' EcoRI
psbA26803_R	5'-TCG TGG <u>CCA TGG</u> GGT TAT AAT TCC TTA TGT AT-3' NcoI
RT_metE_F	5'-GAT GGC TTG GTT GGA TCA GT-3'
RT_metE_R	5'-AGC ACA TTC CGT AAC GGT TC-3'
RT_16S_F	5'-TGG TCA TGC AAG TCT GCT GT-3'
RT_16S_R	5'-CCA ACA TCT CAC GAC ACG AG-3'