Figure S1

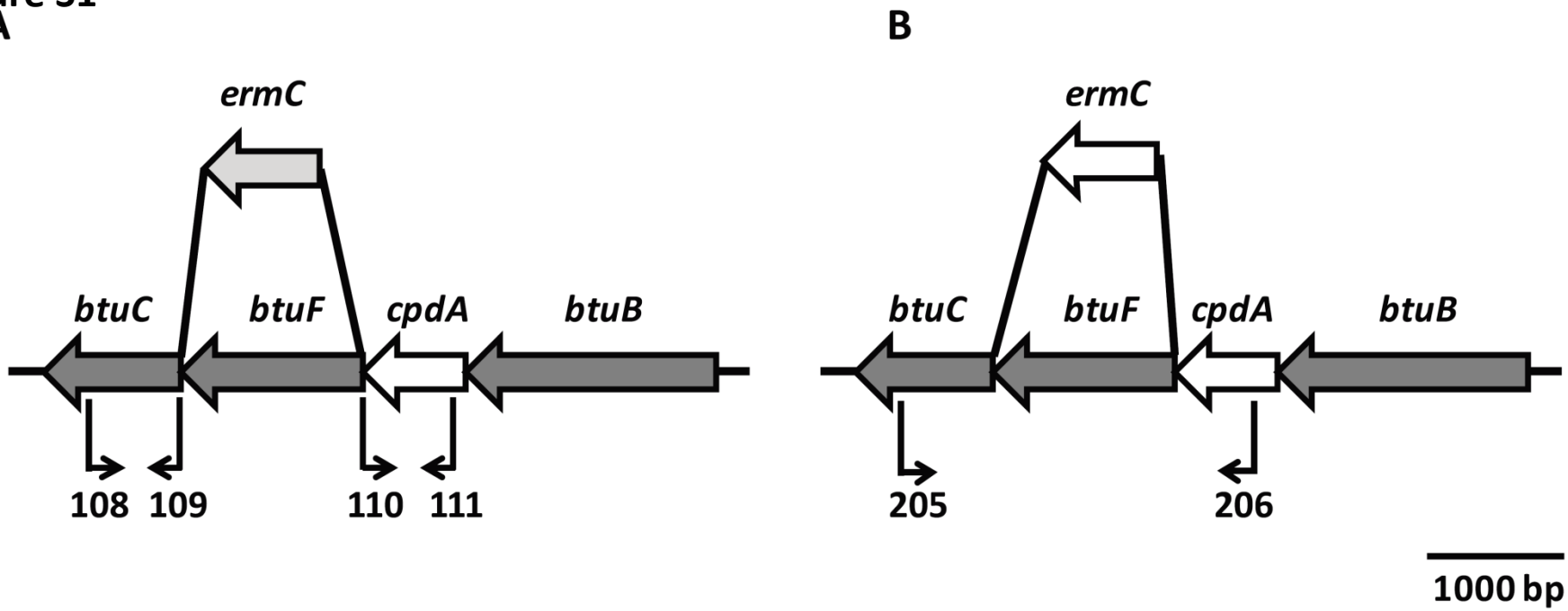


Figure S1. Scheme showing the deletion of the individual *btu* genes (*btuF* is the example shown) from *Synechococcus* sp. strain PCC 7002. A. Primers 108 and 109 were used to amplify a 659 bp upstream flanking region and primers 110 and 111 were used to amplify a 683 bp downstream flanking region of the putative *btuF* gene. The *ermC* erythromycin resistance cassette was ligated in a single ligation reaction to the upstream and downstream flanking regions after digestion. The *ΔbtuF::ermC* was amplified for transformation with primers 108 and 109 using the ligation mixture as template. B. The *ΔbtuF::ermC* construct contains upstream and downstream homologous regions of the *btuF* locus of *Synechococcus* sp. strain PCC 7002. The *ermC* erythromycin cassette was fused to the homologous flanking regions via the introduced NcoI and BamHI sites. Primers 205 and 206 were used for screening transformants. *Synechococcus* sp. strain PCC 7002 is naturally transformable and can insert recombinant DNA via homologous recombination. The scheme used to construct the *ΔbtuF::ermC* mutant was similarly used to delete *btuD, btuB, btuC* and SYNPCC7002_A0636, the putative 3',5'-cyclic-nucleotide phosphodiesterase gene.

Figure S2

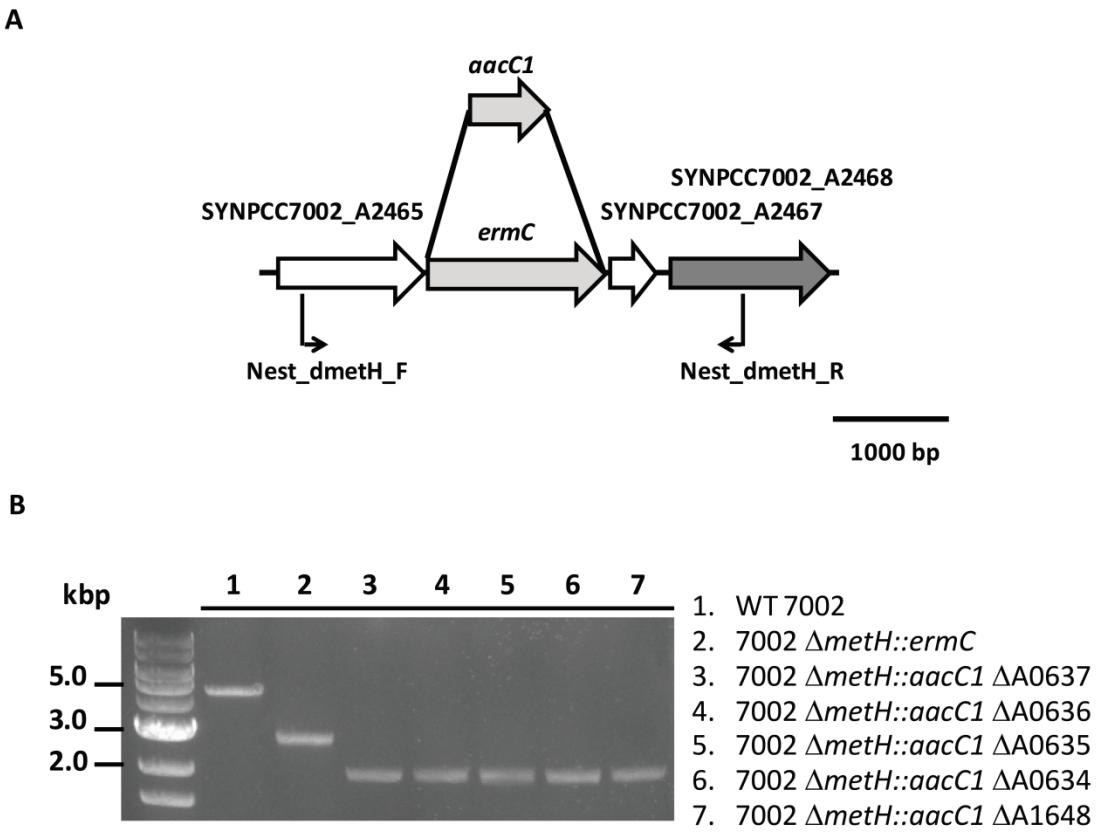
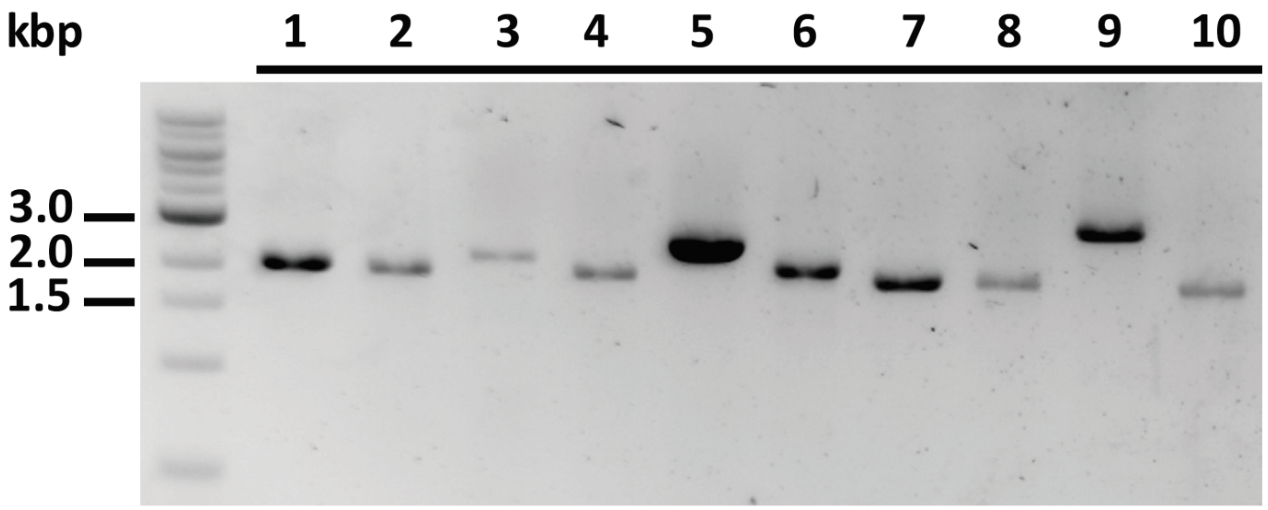


Figure S2. A. Scheme showing deletion of the *metH* gene by replacing the *ermC* gene of the original mutant strain with the *aacC1* gene encoding gentamicin resistance. **B.** Agarose gel electrophoresis of amplicons from the indicated strains. Deletion of the native cobalamin-dependent methionine synthase gene (*metH*) in the AAP005 *ΔbtuX*::*ermC* mutants was confirmed by PCR screening. A1648: *btuD,* putative ATPase subunit of ABC transporter for cobalamin; A0634: *btuC*, permease subunit of ABC transporter for cobalamin; A0635: *btuF*, periplasmic binding protein for ABC transporter for cobalamin; A0636: 3',5'-cyclic-nucleotide phosphodiesterase (CpdA); and A0637: *btuB*, TonB-dependent cobalamin outer membrane transporter for cobalamin uptake.

Figure S3



1. WT 7002 A1648
2. 7002 ΔA1648::Em^R
3. WT 7002 A0634
4. 7002 ΔA0634::Em^R
5. WT 7002 A0635
6. 7002 ΔA0635::Em^R
7. WT 7002 A0636
8. 7002 ΔA0636::Em^R
9. WT 7002 A0637
10. 7002 ΔA0637::Em^R

Figure S3. Agarose gel electrophoresis showing PCR amplicons confirming the construction of the deletion mutant strains indicated. PCR analyses using primers flanking the indicated genes show complete segregation of wild-type and mutant alleles in all cases. Identities of the amplicons were also verified by DNA sequencing. The *btu* genes from the wild type and the AAP005 *ΔbtuX::ermC* deletion mutants were amplified using the same primer pairs. A1648: *btuD* predicted ABC transporter ATPase component; A0634: *btuC,* permease subunit of cobalamin ABC transporter; A0635: *btuF,* periplasmic cobalamin binding protein of ABC transporter; A0636: 3',5'-cyclic-nucleotide phosphodiesterase (CpdA); and A0637: TonB-dependent outer membrane transporter for cobalamin.

Figure S4

The *btuB* gene upstream region in *Synechococcus* sp. PCC 7002 (SYNPCC7002_A0637) and *Synechococcus* sp. JA-3-3A (CYA_1108)

Figure S4. Alignment of the *btuB* gene upstream regions from *Synechococcus* sp. strain PCC 7002 (SYNPCC7002_A0637) and *Synechococcus* sp. strain JA-3-3A (CYA_1108) for identification of conserved RNA secondary structures of the B_{12} riboswitch. The arrows in the upper line show the complementary stems of the RNA secondary structure. Base-paired positions are highlighted in matching colors. Conserved B_{12}-box elements are in red. Secondary structures of putative terminators/ribosome sequesters are shown in blue, and poly-T tracts in terminators are shown in green. Start codons are shown in red and are underlined.

Table S1

Table S1. Primers utilized for amplifying antibiotic markers and *metH* locus.

Name	Sequence
ermC_F	5'-ATA TAA CCA TGG TAA AGA GGG TTA TAA TGA ACG AG-3' (NcoI)
ermC_R	5'-GTA CCC GGA TCC TCT AGA G-3' (BamHI)
aacC1_F	5'-AAA AAA GAA TTC CTA GAC CGA ACG CAG CGG T-3' (EcoRI)
aacC1_R	5'-AAA AAA GGA TCC CGT CGG CCG GGA AGC CG-3' (BamHI)
Nest_dmetH_F	5'-GGG AAC TGC GCA ATA ACA AT-3'
Nest_dmetH_R	5'-GCC AGA TTG CCA TCG ATA AT-3'

Table S2

A. Primers to amplify upstream and downstream flanking sites for individual *btu* gene knock-out construction

ID	Name	Sequence
100	A0637AF	5′-GCG ATC GCC TGG ATT ATT TA-3′
101	A0637AR	5′-ATG ACG CCA TGG (NcoI) AAG AAT ATT TTG GGC TAA TTT TTA ATG-3′
102	A0637BF	5′-TCC GTT GGA TCC (BamHI) CTC CGT TTC GTC CCC TAT T-3′
103	A0637BR	5′-TGT ATT CCA GGT GGA CGT GA-3′
104	A0636AF	5′-CTA TTT TCC GGG GTT TAG CAA T-3′
105	A0636AR	5′-TGA GAA CCA TGG (NcoI) AAA ATC GTG G-3′
106	A0636BF	5′-TGC GTA GGA TCC (BamHI) AAA ACG ATG CGT TTC CCC TTC-3′
107	A0636BR	5′-CTA ACA CCA CCG TTA ACC CC-3′
108	A0635AF	5′-AAC CAG ATC GGC TGA TTT TGA-3′
109	A0635AR	5′-GGA AAC CCA TGG (NcoI) TTT TTT CTC CTA CGC AAA CCA C-3′
110	A0635BF	5′-AAA AAA GGA TCC (BamHI) CCG TGA AAA TTC CCT ACA-3′
111	A0635BR	5′-CTG AAA AAA AGC CAG CAG TCC-3′
112	A0634AF	5′-CAA TAC CAG CGC CTT AGA TTC-3′
113	A0634AR	5′-AAA AAA CCA TGG (NcoI) TTT TCA CGG GGA ATC TTG CAA G-3′
114	A0634BF	5′-TCG TTA GGA TCC (BamHI) CAC TAA AAA TCC AGA AAT ATT CC-3′
115	A0634BR	5′-CTA TCG AGA TTA ATT GTT TTG GC-3′
116	A1648AF	5′-CCG ATG GTG ACC TGA ATG TTA G-3′
117	A1648AR	5′-TAC CGT CCA TGG (NcoI) CGA TCG CCT CGA TTA ATG GAG-3′
118	A1648BF	5′-GGG TTT GGA TCC (BamHI) GTA CAG ACC CCC TGG CCA TT-3′
119	A1648BR	5′-GGT GCG GGA ATT TAA TTT AGC-3′
120	PbtuB_F	5′-AAA AAA CCG CGG (SacII) ATC TAT TTA CAT TGG GGG CGA T-3′
121	PbtuB_R	5′-AAA AAA CCA TGG (NcoI) AAG AAT ATT TTG GGC TA-3′

B. Screening primers for sequencing the individual *btu* deletion constructs using colony PCR

ID	Name	Sequence
201	A0637F	5′-GAC CAA CCA CGC CCA GAT TA-3′
202	A0637R	5′-TCG CCA CCG GAG AAT TTT GA-3′
203	A0636F	5′-ATT TAG TGC CGG AAC TGG GG-3′
204	A0636R	5′-GGC ATC GAG GCG ATA GTT CA-3′
205	A0635F	5′-CGG CGG TGC AAC AGT TTA AA-3′
206	A0635R	5′-ACT GTT CCC GCT GGA TTT CC-3′
207	A0634F	5′-TTC CTT GGG GTT AAC GGT GG-3′
208	A0634R	5′-TCG GTG GAA ACT GGA ATC GA-3′
209	A1648F	5′-GCC GAT GGT GAC CTG AAT GT-3′

Table S2. A. Primers to amplify 600-1000 bp of upstream and downstream flanking regions for individual *btu* gene knock-out construction. Four primers were designed for each construct; two primers for the upstream flank (denoted by the letter A) and two primers for the downstream flank (denoted by the letter B). **B.** Screening primers for sequencing the individual *btu* deletion constructs using colony PCR. Primers were designed in the upstream and downstream flanking regions of the individual *btu* genes.