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Supplemental Information

Uncovering the substrate of olefin synthase loading domains in cyanobacteria *Picosynechococcus* sp. strain PCC 7002

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Figure S1. Fatty acid methyl ester analysis for AYG042 and AYG043. A) Total fatty acyl analysis from strain AYG042 (Δ*olsWT*::lox72; *glpK*::cLac143-*ols04*-*lacI*-*aaC1*). B) Total fatty acyl analysis from strain AYG043 (Δ*olsWT*::lox72; *glpK*::cLac143-*ols08*-*lacI*-*aaC1*).

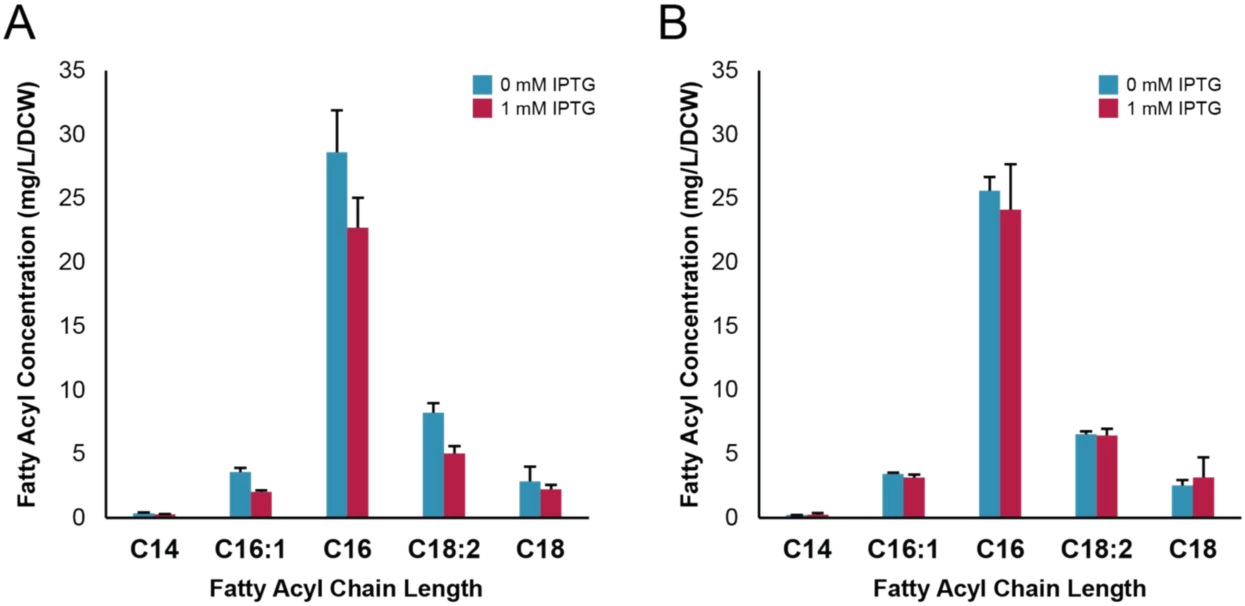

Figure S2. Fatty acid methyl ester analysis for AYG042 and AYG046. A) Total fatty acyl analysis from strain AYG042 (Δ*olsWT*::lox72; *glpK*::cLac143-*ols04*-*lacI*-*aaC1*) induced with 1 mM IPTG (red bars) or induced with 1 mM IPTG and supplemented with 0.5 mM tridecanoic acid. The second panel shows odd-chain species. B) Total fatty acyl analysis from strain AYG046 (Δ*olsWT*::lox72; *glpK*::cLac143-*ols04*-*lacI*-*aaC1*; Δ*aas*::*aphA1*) induced with 1 mM IPTG (red bars) or induced with 1 mM IPTG and supplemented with 0.5 mM tridecanoic acid. The second panel shows odd-chain species.

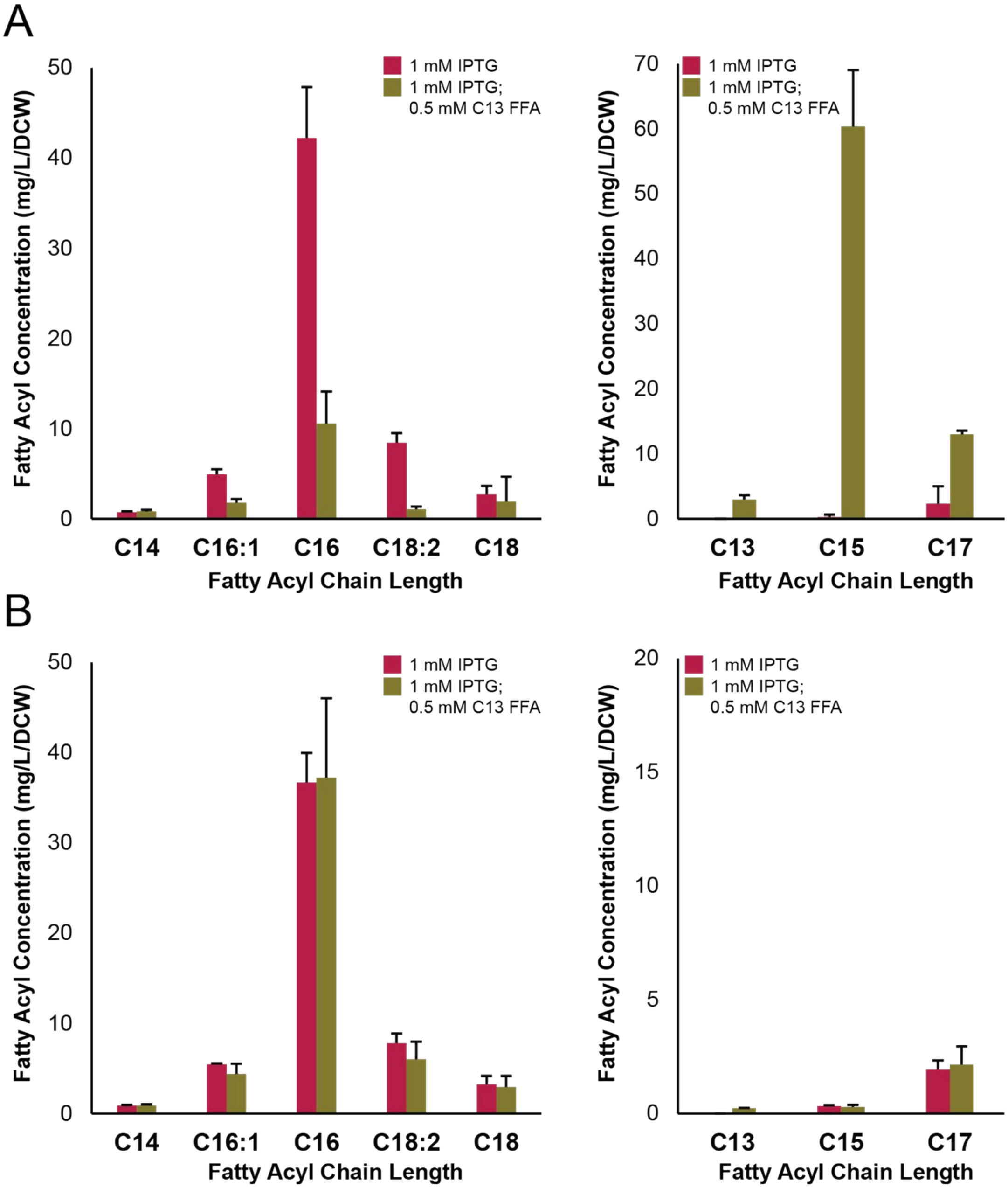

Figure S3. Fatty acid methyl ester analysis for AYG043 and AYG047. A) Total fatty acyl analysis from strain AYG043 (Δ*olsWT*::lox72; *glpK*::cLac143-*ols08*-*lacI*-*aaC1*) induced with 1 mM IPTG (red bars) or induced with 1 mM IPTG and supplemented with 0.1 mM pentadecanoic acid. The second panel shows odd-chain species. B) Total fatty acyl analysis from strain AYG047 (Δ*olsWT*::lox72; *glpK*::cLac143-*ols08*-*lacI*-*aaC1*; Δ*aas*::*aphA1*) induced with 1 mM IPTG (red bars) or induced with 1 mM IPTG and supplemented with 0.1 mM pentadecanoic acid. The second panel shows odd-chain species.

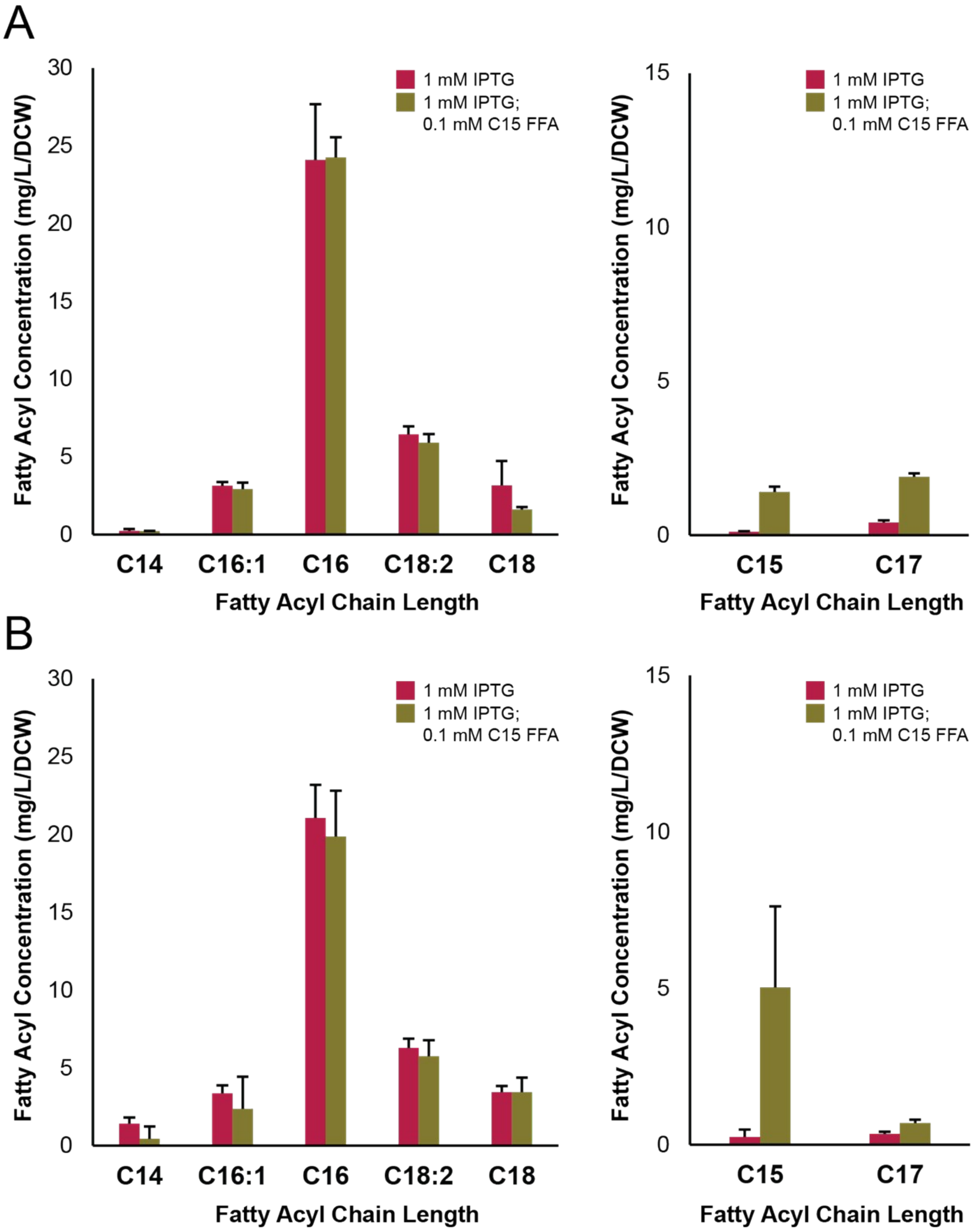

Figure S4. *In vitro* CAS assay for OlsWT. A) SDS-PAGE gel of AKTA fractions for purification of OlsWT FAAL-ACP didomain. B) *In vitro* FAAL activation for Ols LD-ACP di-domain against long-chain fatty alcohols. Octadecanoic acid is used as the positive control for activation. All data represent the mean ± s.d. of biological triplicates. *P* values were analysed based on student two-tailed *t* test assuming equal variances. * $P < 0.0001$, ** $P < 0.003$. C) *In vitro* FAAL activation for Ols LD-ACP di-domain against long-chain fatty methyl esters. Octadecanoic acid is used as the positive control for activation. All data represent the mean ± s.d. of biological triplicates. *P* values were analysed based on student two-tailed *t* test assuming equal variances. * $P < 0.00001$, ** $P < 0.003$.

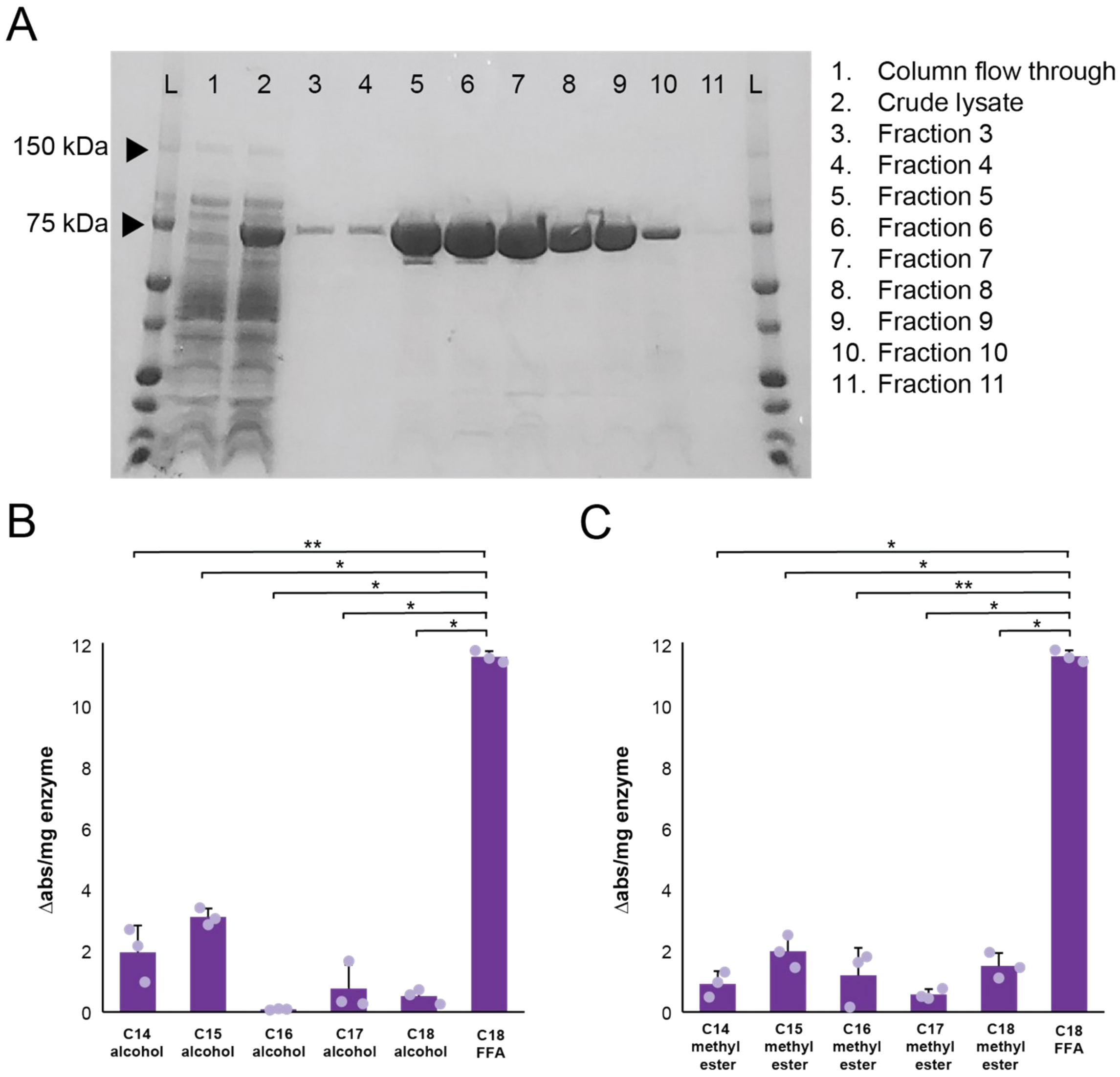

Figure S5. α-olefin analysis for PCC 7002 wildtype and AYG058. A) Production of α-olefins in PCC 7002 wildtype strain and AYG058 (Δ*a1174*::lox66-*aaC1*-lox71).

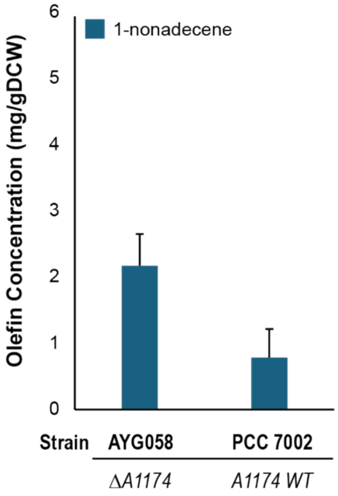

Table S1. Thioesterases in Picosynechococcus sp. strain PCC 7002. Thioesterases identified by ThYme: Thioester-active EnzYme database from *Picosynechococcus* sp. strain PCC 7002.

Family	Protein name	Protein Identifier	Protein Evidence & Literature	Gene Name	Gene and Pathway Identifiers
TE12	1,4-dihydroxy-2-naphthoyl-CoA hydrolase	UniProt: DNCH_SYNP2, B1XL26 GenBank: CP000951, ACA99281.1 RefSeq: WP_012306904.1	Inferred from Homology Literature: B1XL26	SYNPCC7002_A1284	STRING: 32049.SYNPCC7002_A1284 KEGG: SYP:SYNPCC7002_A1284 eggNOG: COG0824 HOGENOM: CLU_101141_5_3_3
TE23	Zn-dependent hydrolase	UniProt: B1XNC4_SYNP2, B1XNC4 GenBank: CP000951, ACB00834.1, ACB00834 RefSeq: WP_012308451.1	Predicted	SYNPCC7002_A2866	STRING: 32049.SYNPCC7002_A2866 KEGG: SYP:SYNPCC7002_A2866 eggNOG: COG0491 HOGENOM: CLU_030571_7_0_3
TE23	Hydroxyacylglutathione hydrolase	UniProt: GLO2_SYNP2, B1XQ70 GenBank: CP000951, ACA99852.1 RefSeq: WP_012307475.1	Inferred from Homology Literature: B1XQ70	gloB	STRING: 32049.SYNPCC7002_A1864 KEGG: SYP:SYNPCC7002_A1864 eggNOG: COG0491 HOGENOM: CLU_030571_4_1_3
TE21	Serine esterase	UniProt: Q8KX36_SYNP2, Q8KX36 GenBank: AF381041, CP000951, AAN03557.1, ACB00517.1, AAN03557, ACB00517 RefSeq: WP_012308135.1	Predicted	sll1284	STRING: 32049.SYNPCC7002_A2540 KEGG: SYP:SYNPCC7002_A2540 eggNOG: COG0400 HOGENOM: CLU_049413_5_1_3
TE22	S-formylglutathione hydrolase	UniProt: B1XIK2_SYNP2, B1XIK2 GenBank: CP000951, ACA98873.1, ACA98873 RefSeq: WP_012306497.1	Inferred from Homology	SYNPCC7002_A0869	STRING: 32049.SYNPCC7002_A0869 KEGG: SYP:SYNPCC7002_A0869 eggNOG: COG0627 HOGENOM: CLU_056472_0_0_3

Table S2. Nucleotide and amino acid sequences of OlsWT FAAL-ACP. Sequences for the OlsWT FAAL-ACP coding region and C-terminal 6xHis tag for heterologous expression and purification in *E. coli*.

Nucleotide sequence of OlsWT FAAL-ACP-6xHis
ATGGTTGGTCAATTTGCAAATTTCGTCGATCTGCTCCAGTACAGAGCTAAACTTCAGGCGCGGAAAACCGTGTTTAGTTTTCTGGCTGATGGCGAAGCGGAATCTGCGGCCCTGACCTACGGAGAATTAGACCAAAAAGCCCAGGCGATCGCCGCTTTTTTGCAAGCTAACCAGGCTCAAGGGCAACGGGCATTATTACTTTATCCACCGGGTTTAGAGTTTATCGGTGCCTTTTTGGGATGTTTGTATGCTGGTGTTGTTGCGGTGCCAGCTTACCCACCACGGCCGAATAAATCCTTTGACCGCCTCCATAGCATTATCCAAGATGCCCAGGCAAAATTTGCCCTCACCACAACAGAACTTAAAGATAAAATTGCCGATCGCCTCGAAGCTTTAGAAGGTACGGATTTTCATTGTTTGGCTACAGATCAAGTTGAATTAATTTCAGGAAAAAATTGGCAAAAACCGAACATTTCCGGCACAGATCTCGCTTTTTTGCAATACACCAGTGGCTCCACGGGCGATCCTAAAGGAGTGATGGTTTCCCACCACAATTTGATCCACAACTCCGGCTTGATTAACCAAGGATTCCAGGATACAGAGGCGAGTATGGGCGTTTCCTGGTTGCCGCCCTACCATGATATGGGCTTGATCGGTGGGATTTTACAGCCCATCTATGTGGGAGCAACGCAAATTTTAATGCCTCCCGTGGCCTTTTTGCAGCGACCTTTTCGGTGGCTAAAGGCGATCAACGATTATCGGGTTTCCACCAGCGGTGCGCCGAATTTTGCCTATGATCTCTGTGCCAGCCAAATTACCCCGGAACAAATCAGAGAACTCGATTTGAGCTGTTGGCGACTGGCTTTTTCCGGGGCCGAACCGATCCGCGCTGTGACCCTCGAAAATTTTGCGAAAACCTTCGCTACAGCAGGCTTTCAAAAATCAGCATTTTATCCCTGTTATGGTATGGCTGAAACCACCCTGATCGTTTCCGGTGGTAATGGTCGTGCCCAGCTTCCCCAGGAAATTATCGTCAGCAAACAGGGCATCGAAGCAAACCAAGTTCGCCCTGCCCAAGGGACAGAAACAACGGTGACCTTGGTCGGCAGTGGTGAAGTGATTGGCGACCAAATTGTCAAAATTGTTGACCCCCAGGCTTTAACAGAATGTACCGTCGGTGAAATTGGCGAAGTATGGGTTAAGGGCGAAAGTGTTGCCCAGGGCTATTGGCAAAAGCCAGACCTCACCCAGCAACAATTCCAGGGAAACGTCGGTGCAGAAACGGGCTTTTTACGCACGGGCGATCTGGGTTTTTTGCAAGGTGGCGAACTGTATATTACGGGTCGTTTAAAGGATCTCCTGATTATCCGGGGGCGCAACCACTATCCCCAGGACATTGAATTAACCGTCGAAGTGGCCCATCCCGCTTTACGACAGGGGGCCGGAGCCGCTGTATCAGTAGACGTTAACGGGGAAGAACAGTTAGTCATTGTCCAGGAAGTTGAGCGTAAATATGCCCGCAAATTAAATGTCGCGGCAGTAGCCCAAGCTATTCGTGGGGCGATCGCCGCCGAACATCAACTGCAACCCCAGGCCATTTGTTTTATTAAACCCGGTAGCATTCCCAAAACATCCAGCGGGAAGATTCGTCGCCATGCCTGCAAAGCTGGTTTTCTAGACGGAAGCTTGGCTGTGGTTGGGGAGTGGCAACCCAGCCACCAAAAAGAAGGAAAAGGAATTGGGACACAAGCCGTTACCCCTTCTACGACAACATCAACGAATTTTCCCCTGCCTGACCAGCACCAACAGCAAATTGAAGCCTGGCTTAAGGATAATATTGCCCATCGCCTCGGCATTACGCCCCAACAATTAGACGAAACGGAACCCTTTGCAAGTTATGGGCTGGATTCAGTGCAAGCAGTACAGGTCACAGCCGACTTAGAGGATTGGCTAGGTCGAAAATTAGACCCCACTCTGGCCTACGATTATCCGACCATTCGCACCCTGGCTCAGTTTTTGGTCCAGGGTAATCAAGCGCTAGAGAAAATACCACAGGTGCCGAAAATTCAGGGCACTAGTCATCATCACCACCATCATTAG
Amino acid sequence of OlsWT FAAL-ACP-6xHis (76.18 kDa)
MVGQFANFVDLLQYRAKLQARKTVFSFLADGEAESAALTYGELDQKAQAIAAFLQANQAQGQRALLLYPPGLEFIGAFLGCLYAGVVAVPAYPPRPNKSFDRLHSIIQDAQAKFALTTTELKDKIADRLEALEGTDFHCLATDQVELISGKNWQKPNISGTDLAFLQYTSGSTGDPKGVMVSHHNLIHNSGLINQGFQDTEASMGVSWLPPYHDMGLIGGILQPIYVGATQILMPPVAFLQRPFRWLKAINDYRVSTSGAPNFAYDLCASQITPEQIRELDLSCWRLAFSGAEPIRAVTLENFAKTFATAGFQKSAFYPCYGMAETTLIVSGGNGRAQLPQEIIVSKQGIEANQVRPAQGTETTVTLVGSGEVIGDQIVKIVDPQALTECTVGEIGEVWVKGESVAQGYWQKPDLTQQQFQGNVGAETGFLRTGDLGFLQGGELYITGRLKDLLIIRGRNHYPQDIELTVEVAHPALRQGAGAAVSVDVNGEEQLVIVQEVERKYARKLNVAAVAQAIRGAIAAEHQLQPQAICFIKPGSIPKTSSGKIRRHACKAGFLDGSLAVVGEWQPSHQKEGKGIGTQAVTPSTTTSTNFPLPDQHQQQIEAWLKDNIAHRLGITPQQLDETEPFASYGLDSVQAVQVTADLEDWLGRKLDPTLAYDYPTIRTLAQFLVQGNQALEKIPQVPKIQGTSHHHHHH*

Table S3. Fused Ols homologs. Cyanobacterial strains and GenBank accession numbers of Ols homologs with single ORF architecture.

Organism	Protein	NCBI accession no.
Picosynechococcus sp. PCC 7002	Ols	WP_012306795.1
Geminocystis herdmanii	Ols	WP_017293948.1
Geminocystis sp. NIES-3709	Ols	WP_060833030.1
Cyanobacterium sp. IPPAS B-1200	Ols	WP_071891741.1
filamentous cyanobacterium ESFC-1	Ols	WP_018399776.1
Leptolyngbya sp. PCC 7376	Ols	WP_015132477.1
Limnothrix rosea IAM M-220	Ols	OKH15930.1
Picosynechococcus sp. NKBG15041c	Ols	WP_024544961.1
Picosynechococcus sp. PCC 7003	Ols	WP_065713741.1
Picosynechococcus sp. NKBG042902	Ols	WP_030006270.1
Picosynechococcus sp. PCC 73109	Ols	WP_062433466.1
Picosynechococcus sp. PCC 7117	Ols	WP_065710482.1
Picosynechococcus sp. PCC 8807	Ols	WP_065716260.1
Prochloron didemni P1-Palau	Ols	AEH57210.1
Pleurocapsa sp. PCC 7327	Ols	AFY79044.1
Gloeothece citriformis	Ols	WP_012599249.1
Gloeothece verrucosa	Ols	WP_013321938.1
Myxosarcina sp. GI1	Ols	WP_072013783.1
Stanieria cyanosphaera PCC 7437	Ols	AFZ37598.1
Stanieria sp. NIES-3757	Ols	BAU65096.1
Chroococcidiopsis sp. PCC 6712	Ols	2505786305
Xenococcus sp. PCC 7305	Ols	WP_006509673.1
Pleurocapsa sp. PCC 7319	Ols	WP_019509581.1
Synechococcus sp. BDU 130192	Ols	WP_099240974.1
Picosynechococcus sp. PCC 11901	Ols	WP_138073101.1
Synechococcus sp. NIES-970	Ols	BAW95865.1
Limnothrix sp. RL_2_0	Ols	NJN72921.1
Stanieria cyanosphaera	Ols	WP_322785848.1
Oscillatoria sp. FACHB-1406	Ols	WP_190722065.1
Xenococcaceae cyanobacterium MO_188.B32	Ols	MDJ0687119.1
Pannus brasiliensis	Ols	WP_332864208.1
Chondrocystis sp. NIES-4102	Ols	BAZ45335.1
Xenococcaceae cyanobacterium MO_167.B27	Ols	MDJ0744409.1
Pleurocapsales cyanobacterium LEGE 10410	Ols	MBE9045458.1
Hydrococcus sp. Prado102	Ols	MCU0532977.1
Xenococcaceae cyanobacterium MO_207.B15	Ols	MDJ0534008.1
Chlorogloea purpurea SAG 13.99	Ols	MBR8830412.1
Jaaginema sp. PMC 1080.18	Ols	MEC4805692.1
Waterburya agarophytonicola	Ols	WP_263858202.1
Waterburya agarophytonicola KI4	Ols	MCC0177306.1

Hydrococcus sp. CRU_1_1	Ols	NJP18749.1
Xenococcaceae cyanobacterium MO_188.B29	Ols	MDJ0634241.1
Xenococcus sp. MO_188.B8	Ols	MDJ0898498.1
Synechococcales cyanobacterium	Ols	MEB3358629.1
Prochloraceae cyanobacterium	Ols	MDJ0714931.1
Cyanobacteria bacterium J06638_38	Ols	MEO1004272.1
Xenococcus sp. PCC 7305	Ols	WP_040897601.1
Pleurocapsa sp. SU_5_0	Ols	NJK56232.1
Aphanothece hegewaldii	Ols	WP_106454891.1
Pleurocapsa sp. FMAR1	Ols	WP_319420823.1
Pleurocapsa minor HA4230-MV1	Ols	MBW4533747.1
Cyanobacteria bacterium J06629_2	Ols	MEO1799315.1
Cyanobacteria bacterium J06631_2	Ols	MEO1669330.1
Acaryochloris thomasi	Ols	WP_110985684.1
Cyanobacteria bacterium J06631_6	Ols	MEO1690211.1
Pleurocapsa sp. PCC 7319	Ols	WP_036801169.1
Symploca sp. SIO2C1	Ols	NEP08925.1
Hyella patelloides	Ols	WP_144871376.1
Prochloraceae cyanobacterium	Ols	MDJ0724562.1
Symploca sp. SIO1A3	Ols	NER47858.1
Lyngbya confervoides	Ols	WP_166275387.1
Symploca sp. SIO1C2	Ols	NER22839.1
Symploca sp. SIO2D2	Ols	NEQ65432.1
Cyanobacteria bacterium J06633_2	Ols	MEO1519424.1
Cyanobacteriota bacterium	Ols	MEB3300721.1
Geminocystis sp. GBBB08	Ols	WP_308253890.1
Pleurocapsales cyanobacterium LEGE 06147	Ols	MBE9169519.1
Cyanobacterium sp. HL-69	Ols	AUC60343.1
Synechococcus sp. RSCCF101	Ols	QEY31360.1
Synechococcaceae cyanobacterium	Ols	MEB3322462.1
Candidatus hydrogenedentota bacterium	Ols	MCC6694529.1

Table S4. Discrete Ols homologs. Cyanobacterial strains and GenBank accession numbers of Ols homologs with two contiguous ORFs architecture.

Organism	Protein	NCBI accession no.
Moorena producens 3L	FAAL-ACP	EGJ35087.1
Moorena producens 3L	Ols	EGJ35088.1
Moorena producens JHB	FAAL-ACP	AHH34188.1
Moorena producens JHB	Ols	AHH34189.1
Moorena bouillonii PNG	FAAL-ACP	AHH34187.1
Moorena bouillonii PNG	Ols	AHH34186.1
Moorena producens PAL-8-15-08-1	FAAL-ACP	AOW99347.1
Moorena producens PAL-8-15-08-1	Ols	AOW99348.1
Leptolyngbya sp. PCC 6406	FAAL-ACP	WP_008319225.1
Leptolyngbya sp. PCC 6406	Ols	WP_008319224.1
Cyanobacterium stanieri PCC 7202	FAAL	AFZ46264.1
Cyanobacterium stanieri PCC 7202	Ols	AFZ46265.1
Moorena sp. SIO1G6	FAAL-ACP	WP_287311417.1
Moorena sp. SIO1G6	Ols	WP_287311419.1
Moorena sp. SIO3A2	FAAL-ACP	WP_287265499.1
Moorena sp. SIO3A2	Ols	WP_287265498.1
Moorena sp. SIO1F2	FAAL-ACP	WP_293039362.1
Moorena sp. SIO1F2	Ols	WP_293039359.1
Moorena sp. SIO4A1	FAAL-ACP	WP_293083652.1
Moorena sp. SIO4A1	Ols	WP_293083655.1
Moorena sp. SIO3I6	FAAL-ACP	WP_293101409.1
Moorena sp. SIO3I6	Ols	WP_293101411.1
Moorena sp. SIO2B7	FAAL-ACP	NES84493.1
Moorena sp. SIO2B7	Ols	NES84492.1
Nodosilinea sp. WJT8-NPBG4	FAAL-ACP	MBW4460282.1
Nodosilinea sp. WJT8-NPBG4	Ols	MBW4460283.1
Nodosilinea sp. LEGE 06152	FAAL-ACP	WP_194056451.1
Nodosilinea sp. LEGE 06152	Ols	WP_194056449.1
Cyanobacteria bacterium REEB459	FAAL-ACP	MBU6228941.1
Cyanobacteria bacterium REEB459	Ols	MBU6228942.1
Cyanobacteria bacterium J06633_1	FAAL-ACP	MEO1464984.1
Cyanobacteria bacterium J06633_1	Ols	MEO1465987.1
aff. *Roholtiella* sp. LEGE 12411	FAAL-ACP	WP_193999149.1
aff. *Roholtiella* sp. LEGE 12411	Ols	WP_194000438.1
Cyanothece sp. SIO1E1	FAAL-ACP	NET35007.1
Cyanothece sp. SIO1E1	Ols	NET35008.1
Moorena sp. SIO3H5	FAAL-ACP	WP_293079527.1
Moorena sp. SIO3H5	Ols	WP_293079525.1
Moorena sp. SIOASIH	FAAL-ACP	WP_293090731.1
Moorena sp. SIOASIH	Ols	WP_293090732.1

Moorena sp. SIO3I8	FAAL-ACP	WP_293024902.1
Moorena sp. SIO3I8	Ols	WP_293024900.1
Nostoc indistinguendum CM1-VF10	FAAL-ACP	MBW4455971.1
Nostoc indistinguendum CM1-VF10	Ols	MBW4457030.1
Moorena sp. SIO3C2	FAAL-ACP	NEP47791.1
Moorena sp. SIO3C2	Ols	NEP50038.1
Moorena sp. SIO3B2	FAAL-ACP	WP_287355871.1
Moorena sp. SIO3B2	Ols	WP_287356230.1
Leptolyngbya sp. KIOST-1	FAAL-ACP	WP_156119730.1
Leptolyngbya sp. KIOST-1	Ols	WP_052050152.1
Halomicronema hongdechloris	FAAL-ACP	WP_088431332.1
Halomicronema hongdechloris	Ols	WP_080805125.1
Anabaena sp. UHCC 0451	FAAL-ACP	WP_323359471.1
Anabaena sp. UHCC 0451	Ols	WP_323359089.1
Moorena bouillonii	FAAL-ACP	WP_081431241.1
Moorena bouillonii	Ols	WP_081431360.1

Table S5. Strains list. Strains used in this study.

Strain	Genotype or relevant characteristics	Source
Escherichia coli		
DH5α	F- Φ80*lacZ*ΔM15 Δ(*lacZYA-argF*) U169 *recA1 endA1 hsdR*17 (rk-,mk+) *phoA supE*44 *thi*-1 *gyrA*96 *relA*1 λ-	Invitrogen
BAP1	BL21(DE3) Δ*prp*RBCD::T7prom-*sfp*,T7prom-*prpE*	
Synechococcus sp. strain PCC 7002	Wild type	Pasteur Culture Collection
AYG032	Δ*olsWT*::lox66-*aaC1*-lox71	This work
AYG035	Δ*olsWT*::lox72	This work
AYG042	Δ*olsWT*::lox72; *glpK*::cLac143-*ols04-lacI-aaC1*	This work
AYG043	Δ*olsWT*::lox72; *glpK*::cLac143-*ols08-lacI-aaC1*	This work
AYG046	Δ*olsWT*::lox72; *glpK*:: cLac143-*ols04-lacI-aaC1*; Δ*aas*::*aphA1*	This work
AYG047	Δ*olsWT*::lox72; *glpK*:: cLac143-*ols08-lacI-aaC1*; Δ*aas*::*aphA1*	This work
AYG058	Δ*a1174*::lox66-*aaC1*-lox71	This work

Table S6. Plasmids list. Plasmids used in this study.

Plasmid	Relevant characteristics	Source
pAYG009	Empty integration vector for *olsWT* locus	This work
pAYG025	Vector for integration of lox66−72 flanked gentamycin resistance cassette into *olsWT* locus	This work
pAYG027	Vector for insertion of Ols08 and gentamycin resistance cassette downstream of *glpK* locus	This work
pAYG029	Vector for insertion of Ols04 and gentamycin resistance cassette downstream of *glpK* locus	This work
pAYG035	Vector for integration of lox66−72 flanked gentamycin resistance cassette into *a1174* locus	This work
pAYG061	Vector for insertion of kanamycin resistance cassette into *aas* locus	This work
pLIC-LD-ACP1	pLIC-KM with LD and ACP1 as target gene for OlsWT expression	Mendez-Perez *et al.*
pCJ111	rbcLXS7002 integrative plasmid expresses CRE recombinase as a translational fusion to rbcL	Jones *et al.*
pCJ162	lox66−72 flanked gentamycin resistance cassette integrates into aquI integration site	Jones *et al.*

Table S7. Primers list. Primers used in this study.

Primer	Description	Sequence (5'-3')
rTAC41	lox66 For	GACCTTGTGACGACTAATACCG
rTAC42	lox71 Rev	TCGGGTTTGATTTTGTAAAGTACCG
rQY286	ols04 insertion cPCR Rev	GCAATGACATGATTAACCCAG
rQY294	ols08 insertion cPCR Rev	ACCCATCACGATTGAAGGTT
rQY314	ols08 insertion cPCR For	GGTGAGACATTTCCGCTTA
rQY322	ols04 insertion cPCR For	ATCCTTAATCTGGTTCAGAC
rQY323	ols04 insertion cPCR For	TGCTAACTACCGCCAAAGCA
rJA002	pTwistAmp_Uni9F_Gibson-Rev	AGGTCAGGCGGAATGGCACT
rJA005	pTwistAmp_Uni9R_Gibson-For	AGGCTAGGTGGAGGCTCAGTG
rJA114	glpK cPCR For	CTGAAAATCTGTGGTGCCAG
rJA115	glpK cPCR Rev	TCCGATCACCGCCATCTTTG
rAYG010	olsWT deletion cPCR For	GATTGAGTTTATTCCCCTCG
rAYG011	olsWT deletion cPCR Rev	GCAATCTCTTCCAACTGACG
rAYG021	Ols04 insertion cPCR Rev	CCCATGCTCCCCAGTT
rAYG028	olsWT homology arms cPCR For	AAATATTTAAAGCCTAATTCTTCG
rAYG029	olsWT homology arms cPCR Rev	ACATTTGTTCCTTATTTCGG
rAYG034	olsWT internal cPCR Rev	TCTTACTCATCATGCCC
rAYG035	olsWT internal cPCR For	CAGACCTATCTAGTGACAGG
rAYG040	For glpK_cLac143	CGAGGCCTGCGGTGCTTTGG
rAYG041	Rev glpK_cLac143	CATGGAATTAATCTCCTACTTGACTTTATGAGTTGGTGTGTG
grAYG013	Ols homology upstream_rev_1	GACTTGAGACGGCCCAAACTGCGTCTCGCCTTGTATGCCTTAGCAACTCCTGT
grAYG014	Ols homology upstream_for_1	AGTGCCATTCCGCCTGACCTTGCAGGCGGCTGTGTCCTAT
grAYG015	Ols homology downstream_rev_1	ACTGAGCCTCCACCTAGCCTTATTGAGGTATCGCGAGATGGCTCC
grAYG016	Ols homology downstream_for_1	CGAGACGCAGTTTGGGCCGTCTCAAGTCAAAATATTGCAAAGTGCAGTCCGAA
grAYG043	Ols08_for_1	CAAGTAGGAGATTAATTCCATGGGATATGTCAAACACTCT
grAYG049	For_Ols homology arm plasmid GmR_lox	CAAAATCAAACCCGAAAAATATTGCAAAGTGCAGTCCG
grAYG050	Rev_Ols homology arm plasmid GmR_lox	TAGTCGTCACAAGGTCGTATGCCTTAGCAACTCCTGTGAAT
grAYG052	Ols08_rev_3	CCAAAGCACCGCAGGCCTCGTTAGAATTCGTTGCTCAGAATCG
grAYG054	Ols04_rev_3	CCAAAGCACCGCAGGCCTCGTTAGAATTCCGGGATCAGCTTAGATG
grAYG055	Ols04_for_3	CAAGTAGGAGATTAATTCCATGGCCACTGAACGATCT

Table S8. Nucleotide sequence encoding OlsWT region. Nucleotide sequence for the OlsWT coding region (blue region; SYNPCC7002_A1173) and the relevant upstream (gold region; SYNPCC7002_A1174) and downstream (green region: SYNPCC7002_A1172) coding regions.

Nucleotide sequence of A1172, A1173 (OlsWT), A1174
ATGACCATTACTTCCCCCGCTCATCCCCATACCGATTACAGCTGGCAATGGCACGGCTTCAATATTAACTATCGTCAGTGGGGCACCCAGGGGCTGCCCGTTCTTTTCGTCCATGGCTTTGGGGCCTCGGCCGGTCATTGGCGCAAAAATCTTCCGGTTTTAGGGGAACATTACCGCTGCTATGCCATCGACTTACTGGGCTTTGGGAAATCGGCAAAACCCCAACCGGAGGTTGAAGCGGACTACACTTTTGAAACTTGGGCCACCCAGATTAAGGCGTTCTGTGCTGAAATCATTGGTGAACCGGCTTTTCTAGTTGGTAATTCCATTGGTTGTGTCGTTGTCATGCAGGCGGCTGTGTCCTATCCCCACTGGGTGCGGGGGGTTGTGGCACTCAATTTTTCCCTGCGGCTGTTCCATGAGCGCAATCTTTTAAAAGCACCTTTTTATCAACGCTGGGGCGTTCCCCTCTTCCAAAAACTCTTGACCCAAACCCCCCTCGGTTCCTTGTTCTTTAAGCAATTGGCCCAGCCGAAAACAATCCGCAAAATTTTAGCCCAGGCCTACCGAGACAAAACAGCGATTACCGATGAGTTGGTGGAGCTGATCCTGACCCCCGCCCAGGACCCAGGGGCGGCAGCGGTTTTCCTGGCCTTTACGAGTTATTCCCAGGGGCCACTCCCGGACGACCTGCTGCCCCAGTTGCATTGCCCCACGGCAGTTTTGTGGGGAACAGCGGATCCGTGGGAACCAGTTGATCTGGGCCGTGCCCTTGTCGCCCAATATCCTCAGATTGAGTTTATTCCCCTCGATAATGTCGGCCATTGTCCCCAGGATGAAGCTCCGGCATTAGTCAACGGCTATTTACTCGATTGGTTAGGGCGACAACAGTCAGCGTAGCCTAGGGCTTTTACACTCAAATCAGTTGAAACCAGGCCCTCCATAATCGAAGGAACGGCGGATTTTGGGAGGATTTACCGTGGGGAGGGAACTCACGGTTTAATATGGTTAACTAGGCTATAGCCCGTGGGTATTCGTAAAAATTGCTAGGATACGGAGTCCAGAATTTCCATTCACAGGAGTTGCTAAGGCATACATGGTTGGTCAATTTGCAAATTTCGTCGATCTGCTCCAGTACAGAGCTAAACTTCAGGCGCGGAAAACCGTGTTTAGTTTTCTGGCTGATGGCGAAGCGGAATCTGCGGCCCTGACCTACGGAGAATTAGACCAAAAAGCCCAGGCGATCGCCGCTTTTTTGCAAGCTAACCAGGCTCAAGGGCAACGGGCATTATTACTTTATCCACCGGGTTTAGAGTTTATCGGTGCCTTTTTGGGATGTTTGTATGCTGGTGTTGTTGCGGTGCCAGCTTACCCACCACGGCCGAATAAATCCTTTGACCGCCTCCATAGCATTATCCAAGATGCCCAGGCAAAATTTGCCCTCACCACAACAGAACTTAAAGATAAAATTGCCGATCGCCTCGAAGCTTTAGAAGGTACGGATTTTCATTGTTTGGCTACAGATCAAGTTGAATTAATTTCAGGAAAAAATTGGCAAAAACCGAACATTTCCGGCACAGATCTCGCTTTTTTGCAATACACCAGTGGCTCCACGGGCGATCCTAAAGGAGTGATGGTTTCCCACCACAATTTGATCCACAACTCCGGCTTGATTAACCAAGGATTCCAGGATACAGAGGCGAGTATGGGCGTTTCCTGGTTGCCGCCCTACCATGATATGGGCTTGATCGGTGGGATTTTACAGCCCATCTATGTGGGAGCAACGCAAATTTTAATGCCTCCCGTGGCCTTTTTGCAGCGACCTTTTCGGTGGCTAAAGGCGATCAACGATTATCGGGTTTCCACCAGCGGTGCGCCGAATTTTGCCTATGATCTCTGTGCCAGCCAAATTACCCCGGAACAAATCAGAGAACTCGATTTGAGCTGTTGGCGACTGGCTTTTTCCGGGGCCGAACCGATCCGCGCTGTGACCCTCGAAAATTTTGCGAAAACCTTCGCTACAGCAGGCTTTCAAAAATCAGCATTTTATCCCTGTTATGGTATGGCTGAAACCACCCTGATCGTTTCCGGTGGTAATGGTCGTGCCCAGCTTCCCCAGGAAATTATCGTCAGCAAACAGGGCATCGAAGCAAACCAAGTTCGCCCTGCCCAAGGGACAGAAACAACGGTGACCTTGGTCGGCAGTGGTGAAGTGATTGGCGACCAAATTGTCAAAATTGTTGACCCCCAGGCTTTAACAGAATGTACCGTCGGTGAAATTGGCGAAGTATGGGTTAAGGGCGAAAGTGTTGCCCAGGGCTATTGGCAAAAGCCAGACCTCACCCAGCAACAATTCCAGGGAAACGTCGGTGCAGAAACGGGCTTTTTACGCACGGGCGATCTGGGTTTTTTGCAAGGTGGCGAACTGTATATTACGGGTCGTTTAAAGGATCTCCTGATTATCCGGGGGCGCAACCACTATCCCCAGGACATTGAATTAACCGTCGAAGTGGCCCATCCCGCTTTACGACAGGGGGCCGGAGCCGCTGTATCAGTAGACGTTAACGGGGAAGAACAGTTAGTCATTGTCCAGGAAGTTGAGCGTAAATATGCCCGCAAATTAAATGTCGCGGCAGTAGCCCAAGCTATTCGTGGGGCGATCGCCGCCGAACATCAACTGCAACCCCAGGCCATTTGTTTTATTAAACCCGGTAGCATTCCCAAAACATCCAGCGGGAAGATTCGTCGCCATGCCTGCAAAGCTGGTTTTCTAGACGGAAGCTTGGCTGTGGTTGGGGAGTGGCAACCCAGCCACCAAAAAGAAGGAAAAGGAATTGGGACACAAGCCGTTACCCCTTCTACGACAACATCAACGAATTTTCCCCTGCCTGACCAGCACCAACAGCAAATTGAAGCCTGGCTTAAGGATAATATTGCCCATCGCCTCGGCATTACGCCCCAACAATTAGACGAAACGGAACCCTTTGCAAGTTATGGGCTGGATTCAGTGCAAGCAGTACAGGTCACAGCCGACTTAGAGGATTGGCTAGGTCGAAAATTAGACCCCACTCTGGCCTACGATTATCCGACCATTCGCACCCTGGCTCAGTTTTTGGTCCAGGGTAATCAAGCGCTAGAGAAAATACCACAGGTGCCGAAAATTCAGGGCAAAGAAATTGCCGTGGTGGGTCTCAGTTGTCGTTTTCCCCAAGCTGACAACCCCGAAGCTTTTTGGGAATTATTACGTAATGGTAAAGATGGAGTTCGCCCCCTTAAAACTCGCTGGGCCACGGGAGAATGGGGTGGTTTTTTAGAAGATATTGACCAGTTTGAGCCGCAATTTTTTGGCATTTCCCCCCGGGAAGCGGAACAAATGGATCCCCAGCAACGCTTACTGTTAGAAGTAACCTGGGAAGCCTTGGAACGGGCAAATATTCCGGCAGAAAGTTTACGCCATTCCCAAACGGGGGTTTTTGTCGGCATTAGTAATAGTGATTATGCCCAGTTGCAGGTGCGGGAAAACAATCCGATCAATCCCTACATGGGGACGGGCAACGCCCACAGTATTGCTGCGAATCGTCTGTCTTATTTCCTCGATCTCCGGGGCGTTTCTCTGAGCATCGATACGGCCTGTTCCTCTTCTCTGGTGGCGGTACATCTGGCCTGTCAAAGTTTAATCAACGGCGAATCGGAGTTGGCGATCGCCGCCGGGGTGAATTTGATTTTGACCCCCGATGTGACCCAGACTTTTACCCAGGCGGGCATGATGAGTAAGACGGGCCGTTGCCAGACCTTTGATGCCGAGGCTGATGGCTATGTGCGGGGCGAAGGTTGTGGGGTCGTTCTCCTCAAACCCCTGGCCCAGGCAGAACGGGACGGGGATAATATTCTCGCGGTGATCCACGGTTCGGCGGTGAATCAAGATGGACGCAGTAACGGTTTGACGGCTCCCAACGGGCGATCGCAACAGGCCGTTATTCGCCAAGCCCTGGCCCAAGCCGGCATTACCGCCGCCGATTTAGCTTACCTAGAGGCCCACGGCACCGGCACGCCCCTGGGTGATCCCATTGAAATTAATTCCCTGAAGGCGGTTTTACAAACGGCGCAGCGGGAACAGCCCTGTGTGGTGGGTTCTGTGAAAACAAACATTGGTCACCTCGAGGCAGCGGCGGGCATCGCGGGCTTAATCAAGGTGATTTTGTCCCTAGAGCATGGAATGATTCCCCAACATTTGCATTTTAAGCAGCTCAATCCCCGCATTGATCTAGACGGTTTAGTGACCATTGCGAGCAAAGATCAGCCTTGGTCAGGCGGGTCACAAAAACGGTTTGCTGGGGTAAGTTCCTTTGGGTTTGGTGGCACCAATGCCCACGTGATTGTCGGGGACTATGCTCAACAAAAATCTCCCCTTGCTCCTCCGGCTACCCAAGACCGCCCTTGGCATTTGCTGACCCTTTCTGCTAAAAATGCCCAGGCCTTAAATGCCCTGCAAAAAAGCTATGGAGACTATCTGGCCCAACATCCCAGCGTTGACCCACGCGATCTCTGTTTGTCTGCCAATACCGGGCGATCGCCCCTCAAAGAACGTCGTTTTTTTGTCTTTAAACAAGTCGCCGATTTACAACAAACTCTCAATCAAGATTTTCTGGCCCAACCACGCCTCAGTTCCCCCGCAAAAATTGCCTTTTTGTTTACGGGGCAAGGTTCCCAATACTACGGCATGGGGCAACAACTGTACCAAACCAGCCCAGTATTTCGGCAAGTGCTGGATGAGTGCGATCGCCTCTGGCAGACCTATTCCCCCGAAGCCCCTGCCCTCACCGACCTGCTGTACGGTAACCATAACCCTGACCTCGTCCACGAAACTGTCTATACCCAGCCCCTCCTCTTTGCTGTTGAATATGCGATCGCCCAACTATGGTTAAGCTGGGGCGTGACGCCAGACTTTTGCATGGGCCATAGCGTCGGCGAATATGTCGCGGCTTGTCTGGCGGGGGTAT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GGCTCTTGGCAAACTGTTGAGACCGAATCTGTGAACCCAGGCCCTGACGATCTCAATGATTGGTTGTATCAGGTGGCGTGGACGCCC
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AGCATGCTTGATTTTTTGGCTGACGTAGATGCCCTTGCCAAACAATTAGGCGATCGCCCCTTTACCTTGGTGGGCCACTCCATGGGTT
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CGACGCTGAAACCGGTAATCACCTGACGACCCATCTCGATTACCTCGCCGCGCCCCCCCAACACCCGATCTTCCCCAGCCTAGAAGT
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GGATCATGCAACTCCAGCGGAATCAAAAGCGGCCCACGGGTTGACCCCAGCCCAGATGGCCGAATCCATTCGGGCGGCATTTTTCCA
GAAAGACGCCGCCAAGACAACCACCACCGTTTAA
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Table S9. Nucleotide sequence of OlsWT region of AYG032. Nucleotide sequence for the Δ*olsWT*::lox66-*aaC1*-lox71 region (blue region; gentamicin resistance *aaC1*) and the relevant upstream (gold region; SYNPCC7002_A1174) and downstream (green region: SYNPCC7002_A1172) coding regions.

Nucleotide sequence of AYG032 (Δ*olsWT*::lox66-*aaC1*-lox71)
ATGACCATTACTTCCCCCGCTCATCCCCATACCGATTACAGCTGGCAATGGCACGGCTTCAATATTAACTATCGTCAGTGGGGCACCC AGGGGCTGCCCGTTCTTTTCGTCCATGGCTTTGGGGCCTCGGCCGGTCATTGGCGCAAAAATCTTCCGGTTTTAGGGGAACATTACCG CTGCTATGCCATCGACTTACTGGGCTTTGGGAAATCGGCAAAACCCCAACCGGAGGTTGAAGCGGACTACACTTTTGAAACTTGGGCC ACCCAGATTAAGGCGTTCTGTGCTGAAATCATTGGTGAACCGGCTTTTCTAGTTGGTAATTCCATTGGTTGTGTCGTTGTCATGCAGGC GGCTGTGTCCTATCCCCACTGGGTGCGGGGGGTTGTGGCACTCAATTTTTCCCTGCGGCTGTTCCATGAGCGCAATCTTTTAAAAGCA CCTTTTTATCAACGCTGGGGCGTTCCCCTCTTCCAAAAACTCTTGACCCAAACCCCCCTCGGTTCCTTGTTCTTTAAGCAATTGGCCCA GCCGAAAACAATCCGCAAAATTTTAGCCCAGGCCTACCGAGACAAAACAGCGATTACCGATGAGTTGGTGGAGCTGATCCTGACCCCC GCCCAGGACCCAGGGGCGGCAGCGGTTTTCCTGGCCTTTACGAGTTATTCCCAGGGGCCACTCCCGGACGACCTGCTGCCCCAGTT GCATTGCCCCACGGCAGTTTTGTGGGGAACAGCGGATCCGTGGGAACCAGTTGATCTGGGCCGTGCCCTTGTCGCCCAATATCCTCA GATTGAGTTTATTCCCCTCGATAATGTCGGCCATTGTCCCCAGGATGAAGCTCCGGCATTAGTCAACGGCTATTTACTCGATTGGTTAG GGCGACAACAGTCAGCGTAGCCTAGGGCTTTTACACTCAAATCAGTTGAAACCAGGCCCTCCATAATCGAAGGAACGGCGGATTTTGG GAGGATTTACCGTGGGGAGGGAACTCACGGTTTAATATGGTTAACTAGGCTATAGCCCGTGGGTATTCGTAAAAATTGCTAGGATACG GAGTCCAGAATTTCCATTCACAGGAGTTGCTAAGGCATACGACCTTGTGACGACTAATACCGTTCGTATAATGTATGCTATACGAAGTT ATCTGCAGCGGCCGCTACTAGTACAACAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCAT GAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGTTACGCAACAGCAACGATGTTACGCAGCAGGGCAGTCG CCCTAAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATTCGCACATGTAGGCTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCT CTTGATCTTTTCGGTCGTGAGTTCGGAGACGTAGCCACCTACTCCCAACATCAGCCGGACTCCGATTACCTCGGGAACTTGCTCCGTA GTAAGACATTCATCGCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGCTTACGTTCTGCCCAAGTTTGAGCAGCC GCGTAGTGAGATCTATATCTATGATCTCGCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAATCTCCTCAA GCATGAGGCCAACGCGCTTGGTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGATCCCGCAGTGGCTCTCTATACAAAGTTG GGCATACGGGAAGAAGTGATGCACTTTGATATCGACCCAAGTACCGCCACCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGA GCCTCTAGTATATAAACGCAGAAAGGCCCACCCGAAGGTGAGCCAGTGTGACTCTAGTAGAGAGCGTTCACCGACAAACAACAGATAA AACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCTCTAGTATAACTTCGTATAATGTATGCTATACGAACGG TACTTTACAAAATCAAACCCGAAAAATATTGCAAAGTGCAGTCCGAAAACCCGTAAATATTAGATCCTAAACATCTGTGGAACCTTGACC AAGATTCCCACACAACCCCATGGGATCATCCGGACTAACCGGGATCTTCTGATTAAAATTGTTAGACCTGATATCCTGAGAACTAGACC CCTTTATAAGATCGCTCTCTAGAAACACCCATGCACCTGAGTGAAATTACCCATCCGAACCAACTCCACGGCTTGACCGTCCGTCAGTT GGAAGAGATTGCCCGGCAAATTCGCGAAAAACACCTCCAGACGATTGCCGCAAGTGGTGGCCACCTCGGCCCTGGTTTAGGGGTGGT TGAACTAACCCTCGCCCTCTACCAAACCCTTGATCTCGATCGCGACAAGGTGGTGTGGGATGTCGGTCATCAGGCTTATCCCCATAAA TTGATTACCGGACGCTACAACGAATTCCACACCCTCCGGCAAAAGGATGGGGTAGCTGGCTACCTTAAGCGCAGTGAAAATGTGTTTG ATCATTTTGGTGCTGGCCATGCCTCGACCAGTATTTCCGCAGCGCTAGGGATGGCATTGGCACGGGATGCCAAGGGAGAAGAGTTTA AATGTGTGGCAGTCATTGGTGATGGTGCCTTGACCGGCGGCATGGCCCTCGAAGCCATCAACCATGCGGGTCATTTACCCGATACGA ATTTAATGGTGGTTCTCAATGACAATGAAATGTCTATTTCCCCGAATGTGGGAGCCATCTCGCGATACCTCAATAAAGTTCGCCTCAGT GATCCCGTTCAGTTCTTGACCGATAACCTCGAAGAACAGGTTAAGCACCTCCCGTTCCTTGGGGACTCCCTCACCCCAGAGATGGAAC GGCTTAAGGACAGCATGAAACGCTTGGCTGTCTCAAAAGTTGGCGCCGTCATCGAAGAATTAGGCTTTAAATATTTTGGCCCCGTGGA TGGCCACAACCTCGAAGAGCTGATCCGCACCTTTAAACAGGCCCATAAAGCCAAAGGCCCGACCCTCGTCCACGTCGCCACAGTTAA AGGCAAAGGTTATGCGATCGCCGAAAAAGATCAAGTAGGCTACCATGCCCAGAAACCCTTTGACTTGGCCACAGGCAAGGCTTTCCCC TCGAAGAAACCAACCCCACCGAGCTATTCCAAGGTCTTTGCCCATGCCCTGACCACCCTCGCAGAAAACAACCCCAAAATTGTCGGGA TTACAGCGGCGATGGCCACTGGCACCGGGTTAGATAAGCTCCAACAGAGATTACCAAAACAGTACATCGACGTTGGTATCGCCGAACA ACATGCCGTTACCCTAGCAGCGGGCCTCGCCTGTGAAGGAATGCGGCCAGTGGTGGCGATTTATTCGACCTTCCTGCAGCGGGCCTA CGACCAAATCATCCATGATGTTTGTATTCAAAAATTACCAGTCTTTTTCTGCCTTGACCGGGCGGGTATCGTCGGTGCCGACGGGCCG ACCCACCAGGGGATGTATGACATTGCCTATCTCCGCCTGATTCCCAATATTGTGTTGATGGCACCCAAGGATGAAGCTGAACTGCAAC GGATGCTCGTCACGGGGATTGAATACACGGACGGGGCGATCGCCATGCGTTATCCCAGGGGGAGTGGCATTGGCGCACCGTTAATG GAAGACGGCTGGGAACCCCTGCCCATCGGTAAAGGAGAAATCCTGCGCAATGGTGATGACATTCTCCTGATCGGCTATGGTGCCATG GTGCACTCGACCCTCCAGGTGGCCGAAATTCTCAGTGAACACGGCATTAGCGCCACGGTGATCAACGCCCGTTTTGTGAAACCCCTG GATTCTGAACTCATTGCGCCCCTCGCCAAGCAAATTGGTAAAGTGGCCACCTTTGAAGAGGGTTGCTTAATGGGGGGTTTTGGGTCTG CGGTCTGTGAAGCGCTCCAGGATCATGATGTGCTTGTTCCCGTAAAACGCTTTGGCATTGGGGATGTGCTTGTGGATCATGCAACTCC AGCGGAATCAAAAGCGGCCCACGGGTTGACCCCAGCCCAGATGGCCGAATCCATTCGGGCGGCATTTTTCCAGAAAGACGCCGCCAA GACAACCACCACCGTTTAA

Table S10. Nucleotide sequence of OlsWT region of AYG035. Nucleotide sequence for the Δ*olsWT*::lox72 region the relevant upstream (gold region; SYNPCC7002_A1174) and downstream (green region: SYNPCC7002_A1172) coding regions.

Nucleotide sequence of AYG035 (Δ*olsWT*::lox72)
ATGACCATTACTTCCCCCGCTCATCCCCATACCGATTACAGCTGGCAATGGCACGGCTTCAATATTAACTATCGTCAGTGGGGCACCCAGGGGCTGCCCGTTCTTTTCGTCCATGGCTTTGGGGCCTCGGCCGGTCATTGGCGCAAAAATCTTCCGGTTTTAGGGGAACATTACCGCTGCTATGCCATCGACTTACTGGGCTTTGGGAAATCGGCAAAACCCCAACCGGAGGTTGAAGCGGACTACACTTTTGAAACTTGGGCCACCCAGATTAAGGCGTTCTGTGCTGAAATCATTGGTGAACCGGCTTTTCTAGTTGGTAATTCCATTGGTTGTGTCGTTGTCATGCAGGCGGCTGTGTCCTATCCCCACTGGGTGCGGGGGGTTGTGGCACTCAATTTTTCCCTGCGGCTGTTCCATGAGCGCAATCTTTTAAAAGCACCTTTTTATCAACGCTGGGGCGTTCCCCTCTTCCAAAAACTCTTGACCCAAACCCCCCTCGGTTCCTTGTTCTTTAAGCAATTGGCCCAGCCGAAAACAATCCGCAAAATTTTAGCCCAGGCCTACCGAGACAAAACAGCGATTACCGATGAGTTGGTGGAGCTGATCCTGACCCCCGCCCAGGACCCAGGGGCGGCAGCGGTTTTCCTGGCCTTTACGAGTTATTCCCAGGGGCCACTCCCGGACGACCTGCTGCCCCAGTTGCATTGCCCCACGGCAGTTTTGTGGGGAACAGCGGATCCGTGGGAACCAGTTGATCTGGGCCGTGCCCTTGTCGCCCAATATCCTCAGATTGAGTTTATTCCCCTCGATAATGTCGGCCATTGTCCCCAGGATGAAGCTCCGGCATTAGTCAACGGCTATTTACTCGATTGGTTAGGGCGACAACAGTCAGCGTAGCCTAGGGCTTTTACACTCAAATCAGTTGAAACCAGGCCCTCCATAATCGAAGGAACGGCGGATTTTGGGAGGATTTACCGTGGGGAGGGAACTCACGGTTTAATATGGTTAACTAGGCTATAGCCCGTGGGTATTCGTAAAAATTGCTAGGATACGGAGTCCAGAATTTCCATTCACAGGAGTTGCTAAGGCATACGACCTTGTGACGACTAATACCGTTCGTATAATGTATGCTATACGAAGTTATTAACTTCGTATAATGTATGCTATACGAACGGTACTTTACAAAATCAAACCCGAAAAATATTGCAAAGTGCAGTCCGAAAACCCGTAAATATTAGATCCTAAACATCTGTGGAACCTTGACCAAGATTCCCACACAACCCCATGGGATCATCCGGACTAACCGGGATCTTCTGATTAAAATTGTTAGACCTGATATCCTGAGAACTAGACCCCTTTATAAGATCGCTCTCTAGAAACACCCATGCACCTGAGTGAAATTACCCATCCGAACCAACTCCACGGCTTGACCGTCCGTCAGTTGGAAGAGATTGCCCGGCAAATTCGCGAAAAACACCTCCAGACGATTGCCGCAAGTGGTGGCCACCTCGGCCCTGGTTTAGGGGTGGTTGAACTAACCCTCGCCCTCTACCAAACCCTTGATCTCGATCGCGACAAGGTGGTGTGGGATGTCGGTCATCAGGCTTATCCCCATAAATTGATTACCGGACGCTACAACGAATTCCACACCCTCCGGCAAAAGGATGGGGTAGCTGGCTACCTTAAGCGCAGTGAAAATGTGTTTGATCATTTTGGTGCTGGCCATGCCTCGACCAGTATTTCCGCAGCGCTAGGGATGGCATTGGCACGGGATGCCAAGGGAGAAGAGTTTAAATGTGTGGCAGTCATTGGTGATGGTGCCTTGACCGGCGGCATGGCCCTCGAAGCCATCAACCATGCGGGTCATTTACCCGATACGAATTTAATGGTGGTTCTCAATGACAATGAAATGTCTATTTCCCCGAATGTGGGAGCCATCTCGCGATACCTCAATAAAGTTCGCCTCAGTGATCCCGTTCAGTTCTTGACCGATAACCTCGAAGAACAGGTTAAGCACCTCCCGTTCCTTGGGGACTCCCTCACCCCAGAGATGGAACGGCTTAAGGACAGCATGAAACGCTTGGCTGTCTCAAAAGTTGGCGCCGTCATCGAAGAATTAGGCTTTAAATATTTTGGCCCCGTGGATGGCCACAACCTCGAAGAGCTGATCCGCACCTTTAAACAGGCCCATAAAGCCAAAGGCCCGACCCTCGTCCACGTCGCCACAGTTAAAGGCAAAGGTTATGCGATCGCCGAAAAAGATCAAGTAGGCTACCATGCCCAGAAACCCTTTGACTTGGCCACAGGCAAGGCTTTCCCCTCGAAGAAACCAACCCCACCGAGCTATTCCAAGGTCTTTGCCCATGCCCTGACCACCCTCGCAGAAAACAACCCCAAAATTGTCGGGATTACAGCGGCGATGGCCACTGGCACCGGGTTAGATAAGCTCCAACAGAGATTACCAAAACAGTACATCGACGTTGGTATCGCCGAACAACATGCCGTTACCCTAGCAGCGGGCCTCGCCTGTGAAGGAATGCGGCCAGTGGTGGCGATTTATTCGACCTTCCTGCAGCGGGCCTACGACCAAATCATCCATGATGTTTGTATTCAAAAATTACCAGTCTTTTTCTGCCTTGACCGGGCGGGTATCGTCGGTGCCGACGGGCCGACCCACCAGGGGATGTATGACATTGCCTATCTCCGCCTGATTCCCAATATTGTGTTGATGGCACCCAAGGATGAAGCTGAACTGCAACGGATGCTCGTCACGGGGATTGAATACACGGACGGGGCGATCGCCATGCGTTATCCCAGGGGGAGTGGCATTGGCGCACCGTTAATGGAAGACGGCTGGGAACCCCTGCCCATCGGTAAAGGAGAAATCCTGCGCAATGGTGATGACATTCTCCTGATCGGCTATGGTGCCATGGTGCACTCGACCCTCCAGGTGGCCGAAATTCTCAGTGAACACGGCATTAGCGCCACGGTGATCAACGCCCGTTTTGTGAAACCCCTGGATTCTGAACTCATTGCGCCCCTCGCCAAGCAAATTGGTAAAGTGGCCACCTTTGAAGAGGGTTGCTTAATGGGGGGTTTTGGGTCTGCGGTCTGTGAAGCGCTCCAGGATCATGATGTGCTTGTTCCCGTAAAACGCTTTGGCATTGGGGATGTGCTTGTGGATCATGCAACTCCAGCGGAATCAAAAGCGGCCCACGGGTTGACCCCAGCCCAGATGGCCGAATCCATTCGGGCGGCATTTTTCCAGAAAGACGCCGCCAAGACAACCACCACCGTTTAA

Table S11. Nucleotide sequence encoding GlpK wildtype region. Nucleotide sequence encoding GlpK (orange region; SYNPCC7002_A2842) and downstream homology arm (gray region).

Nucleotide sequence of wildtype *glpK*
ATGACCATGGCCCATCAAAAATACATTCTTGCGTTAGACCTCGGTACCACTGGTAATCGTGCCCTTTTGTTTAACCACAAAGGCGATATTGTTGCCCAAGCCTATAAAGAATTAACCCAATATTATCCCCAGCCGGGCTGGGTCGAACATGATGCGACGGAAATTTGGAACGACACCAAAGCCGTCATGCAACAGGTCGTCAACAATAGCAGCATTGAAACCCAGGATATCGCGGCGATCGGCCTGACGGTGCAGCGGGAAACCTGTGTCCTCTGGGATAAAACCACTGGCAAACCACTTCATAAAGCAATTGTCTGGCAGGATCGGCGGACGGCTCCCCTCTGTCAAACCCTCAGTGCGGCAGGCAAAGCGGCAGAAATTTACGATAAAACGGGCTTGGTGTTGGATGCTTATTTTTCGGCGACGAAACTGAATTGGTTATTAAGCTGGGCTAAGGAAAATAGTGCCATTAACCCCAATAATGTCCTCGCTGGAACCATTGACACCTGGGCCTTGTGGAACCTCACGGGAGGCAAAGTCCACGCCACAGATCACAGTAATGCCAGCCGGACAATGTTGCTCAATCTCAACCAAAAAGATTGGGATCCGGATTTGTTGGATTTATTCGATATTCCCCGCCAGATGATGCCAACGGTGCAGTCGAGCCTAGGGGAATTTGGCAAAACCGATCCAAGTTTATTGGGGGCGGCAATTCCGATCACCGCCATCTTTGGGGATCAACAGGCGGCCCTCTATGCCCATGGTTGCGATCGCCCCGGCTTATTAAAATGCACCTATGGCACAGGGGCGTTTTTGGTGGCGCACACGGGCGACGAGATTAAACGCTCCAAACATAAGCTCCTAACGACCATCGCGCAACCATTGGATACAAGCCCATCACGATGGCACAAATCTCGATATTGGCTATGCCCTCGAAGGGAGTATGTTCACCGCTGGGGCTTGTATCCAATGGTTGCGCGATGGCCTACAAATTATCGAAACGGCGGCGGAAACGAATGATCTTGCCCAAGGGGTGAATGATAATGGCGGCGCTTATTTTGTTCCGGCTTTAAGTGGTCTGGGAGCGCCCCACTGGGATATGAGTGCCAGGGGCGCATTTCTGGGTTTAACGAGAGGCGTCAAAAAAGAACACATGGTACGGGCTGTCCTAGAGGCGATCGCCTACCAAGCCAAAGAAGTAGTCGAAGCGATTAACCAAGATTCCGGTACGCCGATCCAAGAATTAAAAGTCGATGGCGGCGCGTGCAATAACGACTTTCTGATGCAATTCCAAGCTGACGTGTTGGGTATTCCCGTGGAACGTCCCGCTGTCCTCGATGCCACGGCCCAGGGGGCAGCCTTTGCCGCTGGATTAGCTGTCGGTTTTTGGGATGATTACCAAACCCTGGTGCAAAACCGCAAAATTGATTACGTCTTTAAGCCCAGTGCCAACGCCTCCCAAGCCCAAGCCCATTTCAAAGTCTGGCAAAAAGCCGTTGAACGAGCGAAAAACTGGGCCTAACCCCCTCTTGCCTACAGCATCTCCCCCAGGGGAGAATTCTTCCTGTTTCAACTCCCTCTAACGTAAACCCATTGAATTTAAAAAAGACTTTATGACTGCTTTACTGCTCCATGACCAACATTATTCCCTTGATCATGAAGCCTTTCTCTCAACCCTCAGCAACACAGAAAATTTACTCATTATTCAAGATCTAGATGGCGTTTGCATGGGGTTAGTCAAAGACCCCTTAACCCGCAAAATTGATCCTGACTATATCCGCGCCACACGCAAGTTTAGAGACCACTTCTTTGTCCTCACCAACGGTGAACATGAAGGCAGAAGGGGAGTAAATCGCATCGTTGAACGGGCATTTCGCAATGTTGAAGCCAAAGAGGAAACAAGCTATTTACCTGGTTTAGCAGCAGGGGGTGTGCAATGGCAGACAGATAATGGCCAAATTTCCCATCCCGGTGTTAGCCAAGCAGAACTCGATTTCCTTGCCACAGTGCCAGATTTAATTGGTCAAAGTTTAGGACAATTTTTTACTAAATATGTTGATATTTTTCCCGCTGAGCTTCAACCTGAGCTGATCCATGCTTCTGTTTTAGATAATCTCGTTTCACCGACGGCAAATTTAAACGTCCTGGCCGAATATTTAGGCGATCGCCTTGAGATTTACCAAGACCTCCAGCGCACCATGGAAACCCTGATGAATGATTTGCTAGAAAAAGCTGGCCAACAGGGTTTAGACAATAGTTTTTTCGTGCACTATGCGCCCAATTTAGGCAGAGATAATGTGGGGAAAGAATTGTCCGCTTTGCCACAG

Table S12. Nucleotide sequence of GlpK region of AYG042. Nucleotide sequence encoding GlpK (orange region; SYNPCC7002_A2842) and the integrated *ols04-lacI-aaC1* cassette downstream (gold region; *ols04*) (blue region; gentamicin resistance *aaC1*). Downstream homology arm (gray region) is also shown.

Nucleotide sequence of AYG042 (*glpK*::cLac143-*ols04-lacI-aaC1*)
ATGACCATGGCCCATCAAAAATACATTCTTGCGTTAGACCTCGGTACCACTGGTAATCGTGCCCTTTTGTTTAACCACAAAGGCGATAT TGTTGCCCAAGCCTATAAAGAATTAACCCAATATTATCCCCAGCCGGGCTGGGTCGAACATGATGCGACGGAAATTTGGAACGACACC AAAGCCGTCATGCAACAGGTCGTCAACAATAGCAGCATTGAAACCCAGGATATCGCGGCGATCGGCCTGACGGTGCAGCGGGAAACC TGTGTCCTCTGGGATAAAACCACTGGCAAACCACTTCATAAAGCAATTGTCTGGCAGGATCGGCGGACGGCTCCCCTCTGTCAAACCC TCAGTGCGGCAGGCAAAGCGGCAGAAATTTACGATAAAACGGGCTTGGTGTTGGATGCTTATTTTTCGGCGACGAAACTGAATTGGTT ATTAAGCTGGGCTAAGGAAAATAGTGCCATTAACCCCAATAATGTCCTCGCTGGAACCATTGACACCTGGGCCTTGTGGAACCTCACG GGAGGCAAAGTCCACGCCACAGATCACAGTAATGCCAGCCGGACAATGTTGCTCAATCTCAACCAAAAAGATTGGGATCCGGATTTGT TGGATTTATTCGATATTCCCCGCCAGATGATGCCAACGGTGCAGTCGAGCCTAGGGGAATTTGGCAAAACCGATCCAAGTTTATTGGG GGCGGCAATTCCGATCACCGCCATCTTTGGGGATCAACAGGCGGCCCTCTATGCCCATGGTTGCGATCGCCCCGGCTTATTAAAATG CACCTATGGCACAGGGGCGTTTTTGGTGGCGCACACGGGCGACGAGATTAAACGCTCCAAACATAAGCTCCTAACGACCATCGCGCA ACCATTGGATACAAGCCCATCACGATGGCACAAATCTCGATATTGGCTATGCCCTCGAAGGGAGTATGTTCACCGCTGGGGCTTGTAT CCAATGGTTGCGCGATGGCCTACAAATTATCGAAACGGCGGCGGAAACGAATGATCTTGCCCAAGGGGTGAATGATAATGGCGGCGC TTATTTTGTTCCGGCTTTAAGTGGTCTGGGAGCGCCCCACTGGGATATGAGTGCCAGGGGCGCATTTCTGGGTTTAACGAGAGGCGTC AAAAAAGAACACATGGTACGGGCTGTCCTAGAGGCGATCGCCTACCAAGCCAAAGAAGTAGTCGAAGCGATTAACCAAGATTCCGGTA CGCCGATCCAAGAATTAAAAGTCGATGGCGGCGCGTGCAATAACGACTTTCTGATGCAATTCCAAGCTGACGTGTTGGGTATTCCCGT GGAACGTCCCGCTGTCCTCGATGCCACGGCCCAGGGGGCAGCCTTTGCCGCTGGATTAGCTGTCGGTTTTTGGGATGATTACCAAAC CCTGGTGCAAAACCGCAAAATTGATTACGTCTTTAAGCCCAGTGCCAACGCCTCCCAAGCCCAAGCCCATTTCAAAGTCTGGCAAAAA GCCGTTGAACGAGCGAAAAACTGGGCCTAACCCCCTCTTGCCTACAGCATCTCCCCCAGGGGAGAATTCTTCCTGTTTCAACTCCCAA GGCATCTCCAAACGAATTGTGAGCGCTCACAATTCGGAATTCTTAACAAAAAAGCAGGAATAAAATTAACAAGATGTAATTGACATAAGT CCCATCACCGTTGTATAAATGTGTGGAATTGTGAGCGGATAACAATTTCACACACCAACTCATAAAGTCAAGTAGGAGATTAATTCCATG GCCACTGAACGATCTTTGCTGGATAAGTATCAAACCTTCATTGATCGTCTGGCCGAACGCGTGGCATCTCGTCCGGAAAAAACCGCCT TTACTTTTTTGCAGGATGGAGAGACAGAGTCCTCATCTCTGTCGTACCAAGAGCTTGAGGCCAAAGCTACCGCCATTGCGACGGTCCT 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Table S13. Nucleotide sequence of GlpK region of AYG043. Nucleotide sequence encoding GlpK (orange region; SYNPCC7002_A2842) and the integrated *ols08-lacI-aaC1* cassette downstream (gold region; *ols08*) (blue region; gentamicin resistance *aaC1*). Downstream homology arm (gray region) is also shown.

Nucleotide sequence of AYG043 (*glpK*::cLac143-*ols08-lacI-aaC1*)

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Table S14. Nucleotide sequence encoding Aas wildtype region. Nucleotide sequence encoding Aas (green region; SYNPCC7002_A0675) and upstream/downstream homology arms (gray regions).

Nucleotide sequence of wildtype *aas*
TTTGGGGGTAGCGATGCGAATATTCATGCCGACTAACGCGCCACCGAGGAGGATCGAGTGGGCGACATTATTGCCATCACCCACATAGGTCATGGTCAGGCCTTCCAGTTGGCCGAAGGTTTCCTGCATCGTTTGCAGATCCGCCAACATCTGACAGGGGTGTTCTAAATCTGTAAGGGCGTTGATAATCGGCATTTCACAGTAATCAGCGAAGGTTTCGAGATCCTGTTGATCAAAGGTACGAATCGCCAAAATATCGAGGTAGCGGTCTAAAACCCTGGCGGTGTCCTCGATGGGCTCGCCGCGACCCACTTGGGTGCGGTTTGGGTTCAGGTCAATGACATTGCCACCCAGTTGATACATAGCCACGCTAAAGGAGACTCTGGTGCGGGTAGAGGCTTTGTAGAACAACAGGCCGAGGGTTTTGGGGCAGGTGGGGGCGATTTTTCCCTGTTTGAGATCTGCCGCCAGGTTTAGCAGGGTAATAATTTCTGTGCTGCTGAGATCTGCAATGCTCAGAAGATCCCGACCCTTGAGTGCATTCATCCTGTTTTCGGCTCCTTACCGTGGCAAAAAAATCATTATAAGCTCCCCTCCAGATTCCACAAAAGCTTGCAAGGGCGATCGCCACTGGTCTTCGTCAGGAAATGTTGATGGCGATCCCAAGGGGGTGACACTTGAAAAACTGTCTACTTTTGGGGAGATGGGTGAAATCTGGTTTTAAAGTAGGATTGTAGCCATGATTTCGGCTTGTTTTTTGCGCTTTTAAATGGAATTGCCATGAGCACTGCTCCCTACGACCAGATCCAATCTTTATCAAAAATTTGGGCGATCGCCGCCGAGCGTTATGCCGACATTGTTGCCCTCTATGATCCCCACCAAAAACCAGAATTCAAACTGACCTATCATCAGCTCTGGCAAGGGATTCAGCAATTTGGGGCTGGCCTCCAAGCCCTCGGTCTTCAACCCCAGGAAAAAGTCGCCCTGATTGCGGATAATAGCCCCCGTTGGTTCATCGCCGATCAAGGTAGTATTGCGGCGGGGGCAGTGAATGCGGTGCGCTCATCCCAGGCAGAAAAACAAGAACTGCTCTACATCTACACTGACAGCGAAAGTCGATTTCTCATCGTCGAAGACCGCAAAACCTTTGAACGCCTCAATCCAGAAATTACAAATCTCCAACCCAGGGCCGTCATTTTCCTTACCGACGAAGAAGTGCCAGAAACGGAACTCCCGATTTTCAACTTTTCCCAACTGATGACCCTCGGCAGCCAAGAACCCCTGACGATGCCTGGGCGATCGCCCCAAGATTTGATGACCTTGATCTACACTTCTGGGACGACGGGCAAACCCAAAGGGGTGATGCTTTCCCACGGCAATGTCCTCTACCAAGTGCGCAACCTCGACCAGGTGATTCAACCGAATCCTGGCGATGTCACCCTCAGCATTCTCCCCACCTGGCATTCCTATGAACGGGCTGCTGAATATTTCCTCCTGTCCCAGGGCTGCACCCAAATTTATACGAATATCCGCAACATCAAAAAAGATCTCCAAACCTACAAGCCCGGTTACATGGTTGCGGTGCCGCGCATTTGGGAATCGATTTACGAAGGAGTGCAGAAAAATCTCCGGGAACAACCCGCCAAAAAACAAAAGCTGGTGAACTTTTTCCTCACCTGCTCCCAAAATTATGTCCTGGCCCAGCGTATCGCCAACAACCTCAGCCTGGAGCATTTCCATGTTTCTTCCCTGACGCGGTTAATGGCTAGAGCAAAGGCGATCGCCCTTTATCCTTTTCACCTCCTCGGCAACAAACTCGTTTACGAAAAAATTCGGGGGGCCACTGGCGGCAAAATTCGCTACGTAATCAGCGGCGGCGGCTCCCTCGCCCAACACATCGACACCTTCTTTGAAATCGTGGGGATCAATATCCTTGTGGGTTATGGCCTCACCGAAACTTCTCCTGTGACCAATGCCCGGCGGCCCGAACGCAATGTGTGTGGTTCTGCTGGCCCCGCCCTCAAGGGCACCGAAATTCGCATTGTTGATCCTGAAACGCGACAAACCCTTTCCCAAGGCCAAAAGGGACTGGTTTTAATCCGTGGCCCCCAGGTAATGCAGGGCTACTACCGCAAACCCGAAGCCACCGCCAAGGCCATTGATCCCGAAGGTTGGTTTGACAGCGGCGACCTCGGTTGGGTGACGCCCCAGGGGGATCTCGTTTTAACGGGCCGGGCAAAGGATACTATTGTCCTCACCAACGGCGAAAACATCGAACCCCAGCCCATCGAAGATGCCTGCGCCCGCAGTCCCTACATCGATCAAATTATGCTGGTGGGCCAGGATCAAAAATCCCTTGGTGCTTTAATTGTGCCGAACCTCGAAGCCCTCGGTCAGTGGATGTCCCAGGGTGGCCTCGGTTTGCAATTGCCAGATCCTGACATTCCTTCAGAACAACTGCCCCAAACGGATTTGTACAGTAAGCCAGTCTTGAATTTATTTAAACAGGAGCTGAATCGAGAAATTAAAAATCGACCTGGCTATCGGGTGGATGACCGCATTAGTGATTTTGCGTTGATTTTGGAGCCTTTTTCCATTGAGAACGGCCTGATGACCCAGACGCTAAAGGTAAAGCGGCCAGTGGTGGCAGAGAAATATGCGGCCTTAATTGCTTCCCTTTACGACAAAGATTAAGCGATCCGAATGGCGGAATCTTCGGGTATTGATGCTACCCTGAAAATATTTTGTAGATAGGGCCCCTTTAGCCCACTGATTTAACAAAACCGAGTTACATTTCTCTAGGAAAACATTCACATGGACGGAAACCAAGTGCCTACTGTTACCCTCAAACGCCCAATTAATCTGAAGGTCATCGTGACCCCCCGCTGGAAAGAGGAAGTACAGCAGCAACTCCAGGGACAAATAGCGACTTTTGATGGTCAATTACAACAACTGGATGTCCAAGGAAATCGCACCATTAGTGAACTTCAACAACAGGGCAATACCCCCCAGGTCGCTCAACAAATGGAAAATATCCAACTCCAGGTTAACCAAAAAAAGCGGGAACTCCTTGAGAAGAAAAACCAAGTGCTCCAGCAGCTCCAACAGGTACAAAACTTAGAATTAGATCAAGAGTTTCTCCAGGGTCAAATGGAGAGTTTCTTTGAAGTGAAAGTGGGCGATAATCTGGTGCGCAAGCTGAATGTTGAACTTGTGGTCCGGGATGGTGTTGTTGAAGAAATTCGCGGCGACCTCTAATTTTTTTTGTGATTGCTGTCGAGTGACAGTGTTCGGCTCCCCCGATGACACTTGCGGTGCGGGGGATTTTTAATGGGGAAAAAATTCATGAAAATTGGCATTGTCGGGCTGGGTTTAATCGGTGGATCTTTGGCGATCGCCTTTCGGGAAAAAGGCTTAGAAGTGTTGGGAGTTTCTCGGAAAAAT

Table S15. Nucleotide sequence of Aas region of strains AYG046/AYG047. Nucleotide sequence of Δ*aas*::*aphA1* (blue region; kanamycin resistance *aphA1*) and upstream/downstream homology arms (gray regions).

Nucleotide sequence of AYG046/47 (Δ*aas::aphA1*)
TTTGGGGGTAGCGATGCGAATATTCATGCCGACTAACGCGCCACCGAGGAGGATCGAGTGGGCGACATTATTGCCATCACCCACATAGGTCATGGTCAGGCCTTCCAGTTGGCCGAAGGTTTCCTGCATCGTTTGCAGATCCGCCAACATCTGACAGGGGTGTTCTAAATCTGTAAGGGCGTTGATAATCGGCATTTCACAGTAATCAGCGAAGGTTTCGAGATCCTGTTGATCAAAGGTACGAATCGCCAAAATATCGAGGTAGCGGTCTAAAACCCTGGCGGTGTCCTCGATGGGCTCGCCGCGACCCACTTGGGTGCGGTTTGGGTTCAGGTCAATGACATTGCCACCCAGTTGATACATAGCCACGCTAAAGGAGACTCTGGTGCGGGTAGAGGCTTTGTAGAACAACAGGCCGAGGGTTTTGGGGCAGGTGGGGGCGATTTTTCCCTGTTTGAGATCTGCCGCCAGGTTTAGCAGGGTAATAATTTCTGTGCTGCTGAGATCTGCAATGCTCAGAAGATCCCGACCCTTGAGTGCATTCATCCTGTTTTCGGCTCCTTACCGTGGCAAAAAAATCATTATAAGCTCCCCTCCAGATTCCACAAAAGCTTGCAAGGGCGATCGCCACTGGTCTTCGTCAGGAAATGTTGATGGCGATCCCAAGGGGGTGACACTTGAAAAACTGTCTACTTTTGGGGAGATGGGTGAAATCTGGTTTTAAAGTAGGATTGTAGCCATGATTTCGGCAAGGAAAGGAATTCTTAACAAAAAAGCAGGAATAAAATTAACAAGATGTAACAGACATAAGTCCCATCACCGTTGTATAAAGTTAACTGTGGGATTGCAAAAGGAGCTCCCAACTCATAAAGTCAAGTAGGAGATTAATTCCATGAGCCATATTCAGCGTGAAACGAGCTGTAGCCGTCCGCGTCTGAACAGCAACATGGATGCGGATCTGTATGGCTATAAATGGGCGCGTGATAACGTGGGTCAGAGCGGCGCGACCATTTATCGTCTGTATGGCAAACCGGATGCGCCGGAACTGTTTCTGAAACATGGCAAAGGCAGCGTGGCGAACGATGTGACCGATGAAATGGTGCGTCTGAACTGGCTGACCGAATTTATGCCGCTGCCGACCATTAAACATTTTATTCGCACCCCGGATGATGCGTGGCTGCTGACCACCGCGATTCCGGGCAAAACCGCGTTTCAGGTGCTGGAAGAATATCCGGATAGCGGCGAAAACATTGTGGATGCGCTGGCCGTGTTTCTGCGTCGTCTGCATAGCATTCCGGTGTGCAACTGCCCGTTTAACAGCGATCGTGTGTTTCGTCTGGCCCAGGCGCAGAGCCGTATGAACAACGGCCTGGTGGATGCGAGCGATTTTGATGATGAACGTAACGGCTGGCCGGTGGAACAGGTGTGGAAAGAAATGCATAAACTGCTGCCGTTTAGCCCGGATAGCGTGGTGACCCACGGCGATTTTAGCCTGGATAACCTGATTTTCGATGAAGGCAAACTGATTGGCTGCATTGATGTGGGCCGTGTGGGCATTGCGGATCGTTATCAGGATCTGGCCATTCTGTGGAACTGCCTGGGCGAATTTAGCCCGAGCCTGCAAAAACGTCTGTTTCAGAAATATGGCATTGATAATCCGGATATGAACAAACTGCAATTTCATCTGATGCTGGATGAATTTTTCTAACCGAGGGCGGTGCTTTGGCAGGATCCGGCTGTCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGACGGGTTTTTTGTCTAGATCAACGGCCTCAACCTACTACTGGGCTGCTTCCTAATGCAGGAGTCGCGATCCGAATGGCGGAATCTTCGGGTATTGATGCTACCCTGAAAATATTTTGTAGATAGGGCCCCTTTAGCCCACTGATTTAACAAAACCGAGTTACATTTCTCTAGGAAAACATTCACATGGACGGAAACCAAGTGCCTACTGTTACCCTCAAACGCCCAATTAATCTGAAGGTCATCGTGACCCCCCGCTGGAAAGAGGAAGTACAGCAGCAACTCCAGGGACAAATAGCGACTTTTGATGGTCAATTACAACAACTGGATGTCCAAGGAAATCGCACCATTAGTGAACTTCAACAACAGGGCAATACCCCCCAGGTCGCTCAACAAATGGAAAATATCCAACTCCAGGTTAACCAAAAAAAGCGGGAACTCCTTGAGAAGAAAAACCAAGTGCTCCAGCAGCTCCAACAGGTACAAAACTTAGAATTAGATCAAGAGTTTCTCCAGGGTCAAATGGAGAGTTTCTTTGAAGTGAAAGTGGGCGATAATCTGGTGCGCAAGCTGAATGTTGAACTTGTGGTCCGGGATGGTGTTGTTGAAGAAATTCGCGGCGACCTCTAATTTTTTTTGTGATTGCTGTCGAGTGACAGTGTTCGGCTCCCCCGATGACACTTGCGGTGCGGGGGATTTTTAATGGGGAAAAAATTCATGAAAATTGGCATTGTCGGGCTGGGTTTAATCGGTGGATCTTTGGCGATCGCCTTTCGGGAAAAAGGCTTAGAAGTGTTGGGAGTTTCTCGGAAAAAT

Table S16. Nucleotide sequence encoding hydrolase A1174 wildtype region. Nucleotide sequence encoding putative hydrolase A1174 (orange region; SYNPCC7002_A1174) and upstream/downstream homology arm (gray regions).

Nucleotide sequence of wildtype *a1174*
CACCAAAGCCCAATTCCGCGCCCAAATCGCCGTGGCCCTGGGGGGACGCGCCGCCGAGGATATTGTCTTTGGCTACGATGAAATCACTTCTGGGGCCTCCCAGGATATCCAAATGCTCACGAACATTGCCCGTCAAATGGTGACAAAATTTGGGATGTCAGAACTGGGTCACTTTGCCCTAGAAACCAATCGGGGTGAAGTTTTCCTCAGAAATGATTGGTTTGGGGAGCGGCCTGAATATTCCGAGGCGATCGCCCAGCGCATTGACCTCAAGGTGCGCGAAATTATCAACGAGTGCTACGAAACTGCCAAGCAAATTATTCGGGACAATCGTCAACTTGTAGATCGTCTGGTGGATCGTCTCATCGAAGAAGAAACCATCGAAGGCGAAGACTTTAGTCGTCTCGTCAACGAAGCCCTTGGTCAAACCATCGACCTCCAAAAGACTGCTTCCGTTGCCTAAATACCATGAAAAGCTTAATCCTCTGGAGCATCAAAGGCTACCGTCGCTGGATTTCCCCCCTATTCCCCCCCAGTTGTCGCTTTCAGCCCACCTGTTCCCAATATGCCCTCGAAGCCGTGGAACGCTTTGGTGTACTCCGGGGCAGTTGGCTCGCCCTAAAGCGCCTTGGCCGCTGTCACCCGTTTCACCCTGGGGGTTATGATCCGGTGCCCCATCTTTGCCATCACGATTTAGACAACACGCCCTAGGACATTTGTTCCTTATTTCGGTTTTATCCATCGCTCACCATGACCATTACTTCCCCCGCTCATCCCCATACCGATTACAGCTGGCAATGGCACGGCTTCAATATTAACTATCGTCAGTGGGGCACCCAGGGGCTGCCCGTTCTTTTCGTCCATGGCTTTGGGGCCTCGGCCGGTCATTGGCGCAAAAATCTTCCGGTTTTAGGGGAACATTACCGCTGCTATGCCATCGACTTACTGGGCTTTGGGAAATCGGCAAAACCCCAACCGGAGGTTGAAGCGGACTACACTTTTGAAACTTGGGCCACCCAGATTAAGGCGTTCTGTGCTGAAATCATTGGTGAACCGGCTTTTCTAGTTGGTAATTCCATTGGTTGTGTCGTTGTCATGCAGGCGGCTGTGTCCTATCCCCACTGGGTGCGGGGGGTTGTGGCACTCAATTTTTCCCTGCGGCTGTTCCATGAGCGCAATCTTTTAAAAGCACCTTTTTATCAACGCTGGGGCGTTCCCCTCTTCCAAAAACTCTTGACCCAAACCCCCCTCGGTTCCTTGTTCTTTAAGCAATTGGCCCAGCCGAAAACAATCCGCAAAATTTTAGCCCAGGCCTACCGAGACAAAACAGCGATTACCGATGAGTTGGTGGAGCTGATCCTGACCCCCGCCCAGGACCCAGGGGCGGCAGCGGTTTTCCTGGCCTTTACGAGTTATTCCCAGGGGCCACTCCCGGACGACCTGCTGCCCCAGTTGCATTGCCCCACGGCAGTTTTGTGGGGAACAGCGGATCCGTGGGAACCAGTTGATCTGGGCCGTGCCCTTGTCGCCCAATATCCTCAGATTGAGTTTATTCCCCTCGATAATGTCGGCCATTGTCCCCAGGATGAAGCTCCGGCATTAGTCAACGGCTATTTACTCGATTGGTTAGGGCGACAACAGTCAGCGTAGCCTAGGGCTTTTACACTCAAATCAGTTGAAACCAGGCCCTCCATAATCGAAGGAACGGCGGATTTTGGGAGGATTTACCGTGGGGAGGGAACTCACGGTTTAATATGGTTAACTAGGCTATAGCCCGTGGGTATTCGTAAAAATTGCTAGGATACGGAGTCCAGAATTTCCATTCACAGGAGTTGCTAAGGCATACATGGTTGGTCAATTTGCAAATTTCGTCGATCTGCTCCAGTACAGAGCTAAACTTCAGGCGCGGAAAACCGTGTTTAGTTTTCTGGCTGATGGCGAAGCGGAATCTGCGGCCCTGACCTACGGAGAATTAGACCAAAAAGCCCAGGCGATCGCCGCTTTTTTGCAAGCTAACCAGGCTCAAGGGCAACGGGCATTATTACTTTATCCACCGGGTTTAGAGTTTATCGGTGCCTTTTTGGGATGTTTGTATGCTGGTGTTGTTGCGGTGCCAGCTTACCCACCACGGCCGAATAAATCCTTTGACCGCCTCCATAGCATTATCCAAGATGCCCAGGCAAAATTTGCCCTCACCACAACAGAACTTAAAGATAAAATTGCCGATCGCCTCGAAGCTTTAGAAGGTACGGATTTTCATTGTTTGGCTACAGATCAAGTTGAATTAATTTCAGGAAAAAATTGGCAAAAACCGAACATTTCCGGCACAGATCTCGCTTTTTTGCAATACACCAGTGGCTCCACGGGCGATCCTAAAGGAGTGATGGTTTCCCACCACAATTTGATCCACAACTCCGGCTTGATTAACCAAGGATTCCAGGATACAGAGGCGAGTATGGGCGTTTCCTGGTTGCCGCCCTACCATGATATGGGCTTGATCGGTGGGATTTTACAGCCCATCTATGTGGGAGCAACGCAA

Table S17. Nucleotide sequence of A1174 region for strain AYG058. Nucleotide sequence for the Δ*a1174*::lox66-*aaC1*-lox71 region (blue region; gentamicin resistance *aaC1*) and the relevant upstream and downstream (gray regions) coding regions.

Nucleotide sequence of AYG058 (Δ*a1174*::lox66-*aaC1*-lox71)
CACCAAAGCCCAATTCCGCGCCCAAATCGCCGTGGCCCTGGGGGGACGCGCCGCCGAGGATATTGTCTTTGGCTACGATGAAATCACTTCTGGGGCCTCCCAGGATATCCAAATGCTCACGAACATTGCCCGTCAAATGGTGACAAAATTTGGGATGTCAGAACTGGGTCACTTTGCCCTAGAAACCAATCGGGGTGAAGTTTTCCTCAGAAATGATTGGTTTGGGGAGCGGCCTGAATATTCCGAGGCGATCGCCCAGCGCATTGACCTCAAGGTGCGCGAAATTATCAACGAGTGCTACGAAACTGCCAAGCAAATTATTCGGGACAATCGTCAACTTGTAGATCGTCTGGTGGATCGTCTCATCGAAGAAGAAACCATCGAAGGCGAAGACTTTAGTCGTCTCGTCAACGAAGCCCTTGGTCAAACCATCGACCTCCAAAAGACTGCTTCCGTTGCCTAAATACCATGAAAAGCTTAATCCTCTGGAGCATCAAAGGCTACCGTCGCTGGATTTCCCCCCTATTCCCCCCCAGTTGTCGCTTTCAGCCCACCTGTTCCCAATATGCCCTCGAAGCCGTGGAACGCTTTGGTGTACTCCGGGGCAGTTGGCTCGCCCTAAAGCGCCTTGGCCGCTGTCACCCGTTTCACCCTGGGGGTTATGATCCGGTGCCCCATCTTTGCCATCACGATTTAGACAACACGCCCTAGGACATTTGTTCCTTATTTCGGTTTTATCCATCGCTCACCGACCTTGTGACGACTAATACCGTTCGTATAATGTATGCTATACGAAGTTATCTGCAGCGGCCGCTACTAGTACAACAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGTTACGCAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATTCGCACATGTAGGCTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATCTTTTCGGTCGTGAGTTCGGAGACGTAGCCACCTACTCCCAACATCAGCCGGACTCCGATTACCTCGGGAACTTGCTCCGTAGTAAGACATTCATCGCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGCTTACGTTCTGCCCAGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTCGCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAATCTCCTCAAGCATGAGGCCAACGCGCTTGGTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGATCCCGCAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACTTTGATATCGACCCAAGTACCGCCACCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCCTCTAGTATATAAACGCAGAAAGGCCCACCCGAAGGTGAGCCAGTGTGACTCTAGTAGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCTCTAGTATAACTTCGTATAATGTATGCTATACGAACGGTACTTTACAAAATCAAACCCGACCTAGGGCTTTTACACTCAAATCAGTTGAAACCAGGCCCTCCATAATCGAAGGAACGGCGGATTTTGGGAGGATTTACCGTGGGGAGGGAACTCACGGTTTAATATGGTTAACTAGGCTATAGCCCGTGGGTATTCGTAAAAATTGCTAGGATACGGAGTCCAGAATTTCCATTCACAGGAGTTGCTAAGGCATACATGGTTGGTCAATTTGCAAATTTCGTCGATCTGCTCCAGTACAGAGCTAAACTTCAGGCGCGGAAAACCGTGTTTAGTTTTCTGGCTGATGGCGAAGCGGAATCTGCGGCCCTGACCTACGGAGAATTAGACCAAAAAGCCCAGGCGATCGCCGCTTTTTTGCAAGCTAACCAGGCTCAAGGGCAACGGGCATTATTACTTTATCCACCGGGTTTAGAGTTTATCGGTGCCTTTTTGGGATGTTTGTATGCTGGTGTTGTTGCGGTGCCAGCTTACCCACCACGGCCGAATAAATCCTTTGACCGCCTCCATAGCATTATCCAAGATGCCCAGGCAAAATTTGCCCTCACCACAACAGAACTTAAAGATAAAATTGCCGATCGCCTCGAAGCTTTAGAAGGTACGGATTTTCATTGTTTGGCTACAGATCAAGTTGAATTAATTTCAGGAAAAAATTGGCAAAAACCGAACATTTCCGGCACAGATCTCGCTTTTTTGCAATACACCAGTGGCTCCACGGGCGATCCTAAAGGAGTGATGGTTTCCCACCACAATTTGATCCACAACTCCGGCTTGATTAACCAAGGATTCCAGGATACAGAGGCGAGTATGGGCGTTTCCTGGTTGCCGCCCTACCATGATATGGGCTTGATCGGTGGGATTTTACAGCCCATCTATGTGGGAGCAACGCAA