Methylotrophy in mycobacteria: Dissection of the methanol metabolism pathway in *Mycobacterium smegmatis*

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Running head: Methanol metabolism in *Mycobacterium smegmatis*

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURES S1, S2, S3, and S4

SUPPLEMENTARY TABLE S1

Figure S1

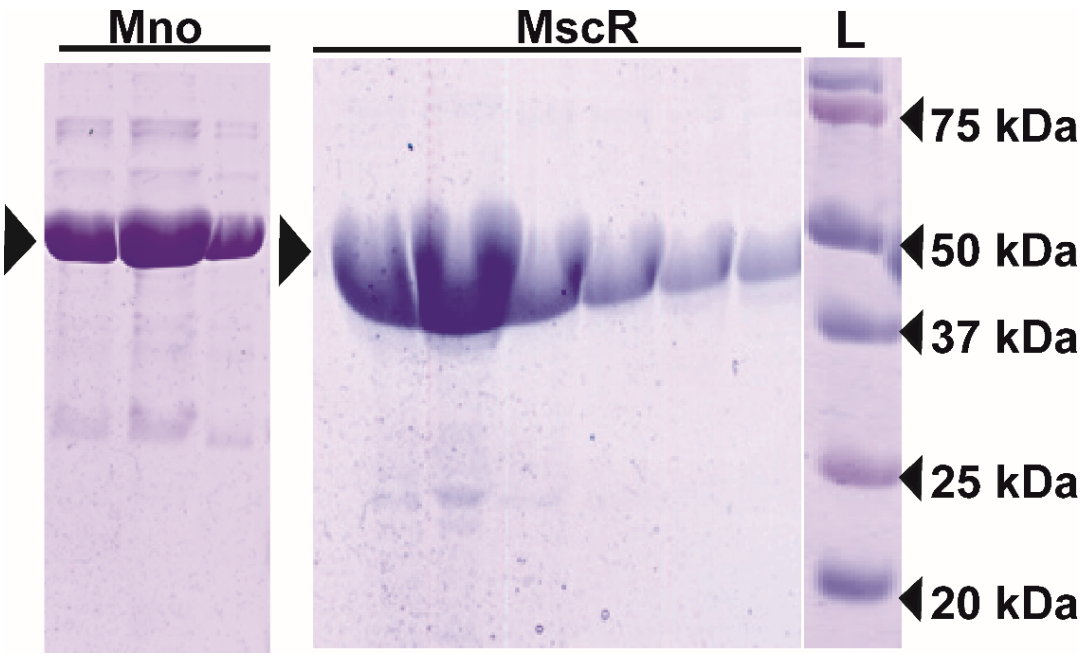

Figure. S1: SDS-PAGE gel demonstrating the purity of Mno and MscR.

Mno and MscR were purified on Ni-NTA affinity chromatography. The eluted proteins were loaded on an SDS-PAGE gel to assess the purity. Only the eluted proteins are shown; purified protein bands are marked with black triangle. Protein molecular weight ladder ('L') was used to compare the mobility of the both the proteins. A few of the ladder bands are marked with their molecular weights mentioned on the right.

Figure. S2

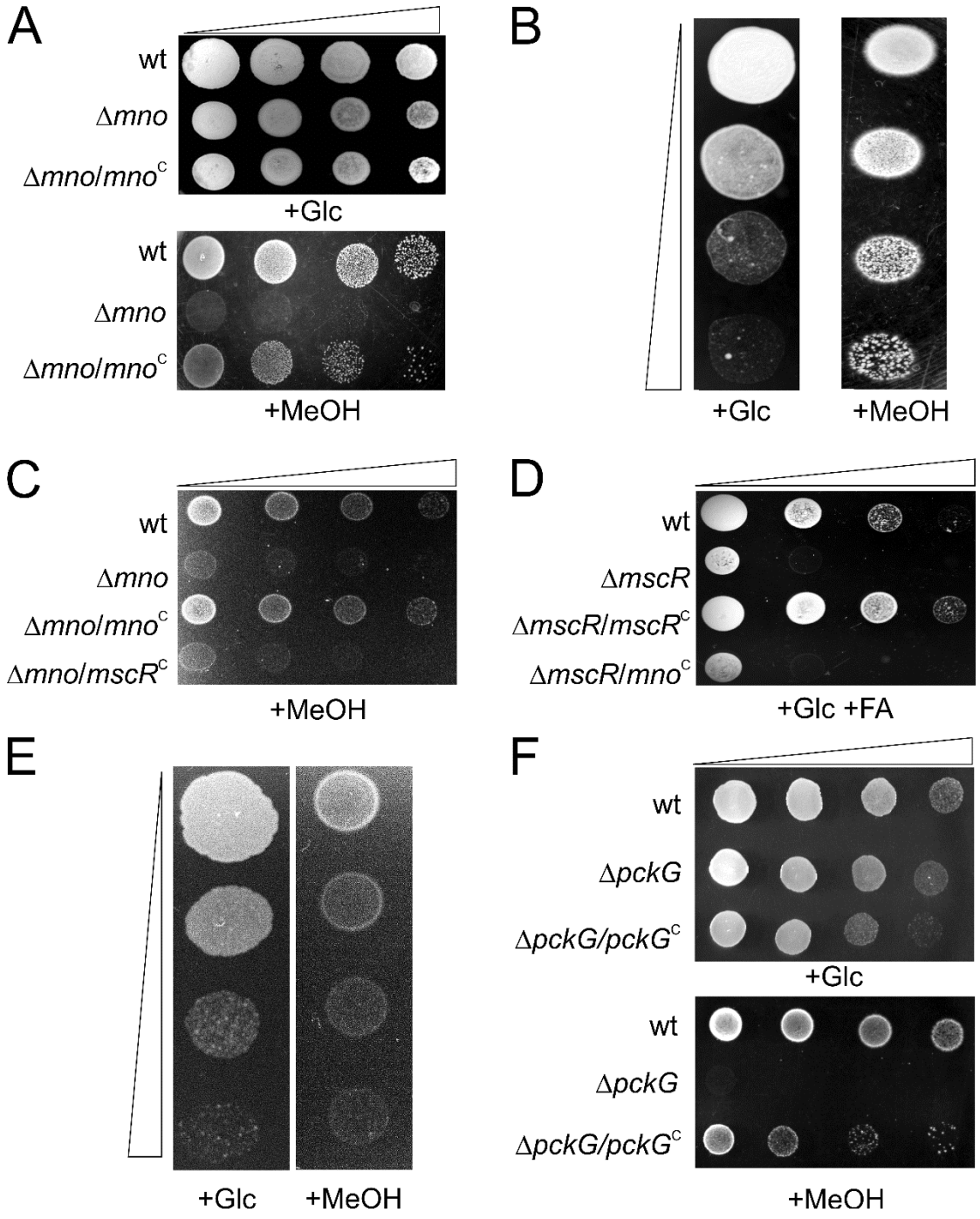

Figure. S2: Spot assays of *Msm* wild-type and mutants on appropriate carbon sources.

In addition to the growth curves, spot assays were performed for the wild-type and the mutants which are involved in the methylotrophic metabolism in *Msm*. The assays were performed on

either glucose (+Glc), methanol (+MeOH), or in the presence of both glucose and formaldehyde (+Glc +FA) as the carbon sources. (A) Physiological role of *mno* was confirmed by growing bacteria on either methanol or glucose. The top panel shows the bacterial growth in the presence of 2% glucose. While *M. smegmatis* Δ*mno* is unable to utilize methanol as sole carbon source (bottom panel), Δ*mno* complemented using pADhpMno shows growth on 2% methanol. Both wild-type *M. smegmatis* (wt) and Δ*mno* carry pSS1 vector. (B) Spot assay was carried out with *Msm*Δ*mscR* on glucose and methanol as the sole carbon sources. Data show that *mscR* knockout can utilize methanol as the sole carbon source. (C) Spot assays were carried out to assess the complementation of the loss of Mno by MscR since the latter possesses methanol dehydrogenase activity in vitro. Panel shows that the wild-type *Msm* (wt) and the Δ*mno* complemented with pADhpMno (Δ*mno*/*mno*^{C}) are able to grow on methanol, whereas *Msm*Δ*mno* and Δ*mno*/*mscR*^{C} (Δ*mno* complemented with pSWhpMscR) cannot. The data thus show that MscR cannot complement the loss of Mno in vivo. (D) Similarly, Mno which possesses formaldehyde dehydrogenase activity is unable to complement the loss of MscR in vivo, when the bacteria are grown in the presence of glucose and formaldehyde. Complementation could be successfully accomplished only by using pSWhpMscR in the Δ*mscR* strain. Δ*mscR*/*mno*^{C} denotes Δ*mscR* complemented with pADhpMno. In both panels C and D, the wt and the knock out strains (Δ*mno* and Δ*mscR*) were transformed with empty plasmid (pSS1). (E) Spot assay carried out with the *MSMEG_3103* knockout shows that the cells are able to grow on methanol as the sole carbon source. (F) Spot assays were performed with Δ*pckG* to decipher the role of Pck in methanol metabolism. In contrast to glucose (top panel), Δ*pckG* does not show growth on methanol as the sole carbon source (bottom panel). Δ*pckG* complemented with pADhpPck (Δ*pckG/pckG*^{C}) successfully restores growth on methanol. In each panel, the triangle represents the dilutions of

the spotted culture, which are 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}, in that order. Experiments in each case were repeated at least three times; only one representative image is shown here in each panel.

Figure. S3

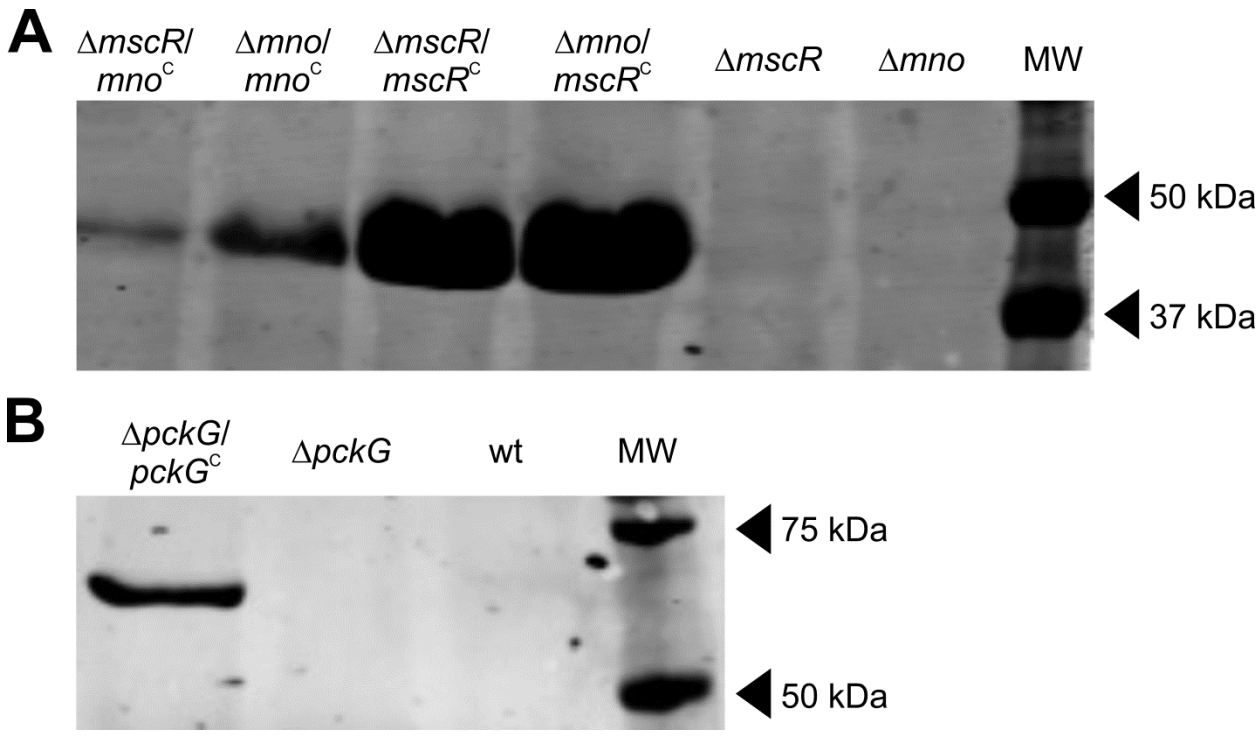

Figure S3. Confirmation of the expression of recombinant proteins by Western blotting

The expressed protein in each of the complemented strain (represented with superscript 'C') was confirmed by probing the blot with anti-His antibodies. Panel A shows the expression of Mno and MscR (first four lanes). The knockout cells that were transformed with empty vector do not show any expression. Panel B shows the expression of Pck; here, the wild-type (wt) and the knockout cells were transformed with empty vector and do not show any expression. In both the panels, MW represents the molecular weight marker with two bands marked.

Figure. S4

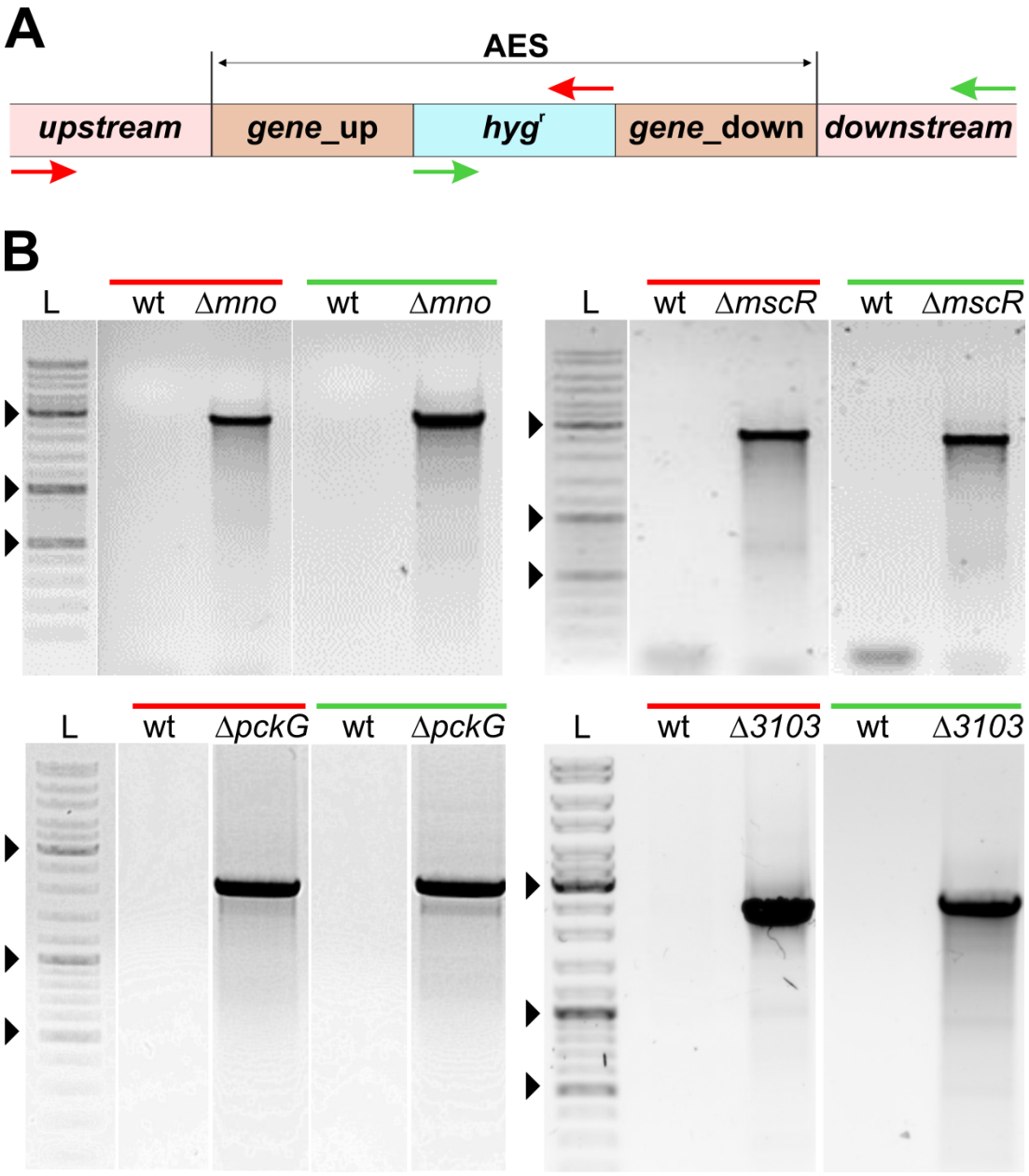

Figure S4. Genetic knockout confirmation by PCR

Panel A shows the strategy for the knockout confirmation by PCR using genomic DNA as template. One set of primers was designed specific for the hygromycin cassette (*hyg*^{r}) and

another set at ~ 500 bp flanking regions of the allelic exchange substrate (AES; marked by arrow). Two such PCR reactions were performed – one amplifying the upstream region + *hyg*^{r} (red) and the other amplifying the downstream region + *hyg*^{r} (green). B) Ethidium bromide-stained agarose gel images showing the amplified DNA bands in the knockouts for four genes *viz*. *mno*, *mscR*, *pckG*, and *MSMEG_3103* (*3103*) are shown; 'wt' represents the wild-type bacterium. Amplification between 2 – 3 kb (depending upon the knockout) is a confirmation of the knockout; 'wt' does not show this amplified band. Further, the amplified region was verified by sequencing. L indicates the DNA ladder with the three marked bands corresponding to 0.5, 1.0, and 3.0 kb in that order, in each panel. The horizontal bar colour (either red or green) matches with the set of primers used in panel A.

Table S1: List of oligos used in the present study. Sequence of each oligo in 5' to 3' is given. The purpose of each oligo in the present study is also mentioned for easy reference.

Oligo	**Sequence (5'-3')**	**Purpose**
mnopMSFor	ATGGCCATTGAGCTGAACCAGATTTGG	Cloning of *mno* in pMS-QS-CHS and pSS1
mnopMSRev	GTAGGACTCGTTGATCGCGTGGTTC	
mnoRTFor	TCTGCTTGTTGGTGGACTTG	Real Time PCR for *mno* expression
mnoRTRev	GTCGAACCCCAAGGACTACA	
mnoUpFor	GCATGTCCGACGCAGCCCGCCCACCGC	Amplification *mno* upstream fragment
mnoUpRev	CGCCACGTACATCACCACCTGGTTCAGCTCAATGGCC	
mnoHygFor	CATTGAGCTGAACCAGGTGGTGATGTACGTGGCG	Amplification of *hyg*^{r} casette
mnoHygRev	GATCGCGTGGTTCACCGATCCGGGGGGCGTC	
mnoDownFor	CCTGACGCCCCCCGGATCGGTGAACCACGCGATC	Amplification *mno* downstream fragment
mnoDownRev	GAGGTCAACACCACCCACGGCACCG	
mnoLongFor	GCGTTGAACGTCGGCGCTGCCGGTGG	PCR confirmation of Δ*mno*
mnoLongRev	GTGCGGGTTGAACCCCTTGTCGGCGTCC	
mscRpMSFor	ATGCCTCAGACTGTGCGCGGTGTGATTTCTCG	Cloning of *mscR* in pMS-QS-CHS and pSS1
mscRpMSRev	TCGGTCCAACACCACCACGGAGCGCAGC	
mscRRTFor	CTGTGCGCGGTGTGATTTCTCG	Real Time PCR for *mscR* expression
mscRRTRev	CAACAGGAACGGGAACTCGTCG	
mscRUpFor	CCAACTCCTCAGGGTCCTGTTCGG	Amplification *mscR* upstream fragment
mscRUpRev	CGTACATCACCACGCGCACAGTCTGAGGCATGG	
mscRHygFor	GACTGTGCGCGTGGTGATGTACGTGGCGAACTCC	Amplification of *hyg*^{r} cassette
mscRHygRev	CGGTCCAACACCAGATCCGGGGGGCGTCAGGC	
mscRDownFor	CCCCGGATCTGGTGTTGGACCGATGAGCG	Amplification *mscR* downstream fragment
mscRDownRev	GACCACGGTTTCGGCGGGCAGC	
mscRLongFor	GCTGCACGGCCAGTTGCTCGTAGATGTTCTCG	PCR confirmation of Δ*mscR*
mscRLongRev	CCACCTCTTCGGGCGTGCTGCTCTGC	
rpoBRTFor	TCGATGTCACTGTCCTTCTCGGATC	Real Time PCR for *rpoB* expression
rpoBRTRev	GACCGTCTGGCTCTTGATCTC	

fdhDRTFor	GGAGATCCGGGTCAACGGCACG	Real Time PCR for *fdhD* expression
fdhDRTRev	GTCCAGCACGTTGTAGGTGTTGACC	
fdhFRTFor	GATTATGACGCCGATTACGACGATCACG	Real Time PCR for *fdhF* expression
fdhFRTRev	CGAAGCCGTGACGCTGGTTCAGG	
3103RTFor	CGACGATTGGACCGATCTCGACACG	Real Time PCR for *MSMEG_3103* expression
3103RTRev	GCTGGAACAGCGTGTACGCGAGC	
3103UpFor	CTTCATGACCTTGTCGATGTCGGCATCG	Amplification *MSMEG_3103* upstream fragment
3103UpRev	CGTACATCACCACTGATCTCTTCGGCCGTGGTCACG	
3130HygFor	CCACGGCCGAAGAGATCAGTGGTGATGTACGTGGCG	Amplification of *hyg*^{r} casette
3103HygRev	CGAGCGATCGCTCGATCCGGGGGGCGTCAGG	
3103DownFor	CCGGATCGAGCGATCGCTCGAGAACTGATCG	Amplification *MSMEG_3103* downstream fragment
3103DownRev	CTTGATCAGCAGGTTGGGCCGGTCG	
3103LongFor	CGAGCACCAGCGTGGTGAGCACGG	PCR confirmation of Δ*MSMEG_3103*
3103LongRev	GTCGAGGCCCACAGCGGACGCTGCACG	
pckGpMSFor	ATGACCTCAGCGACCATTCCGGGTTTGG	Cloning of *pckG* in pMS-QS-CHS and pSS1
pckGpMSRev	GCCCTCTTCGGACAGGCGGTGCTTGAGC	
pckGRTFor	CACCGCACCGACTAAACATCAGG	Real Time PCR for *pckG* expression
pckGRTRev	GGATTGAGCTTCTGGAAGGTGC	
pckGUpFor	GCTGGTCGGGTTGCTGCTGGCGGTGTGG	Amplification *pckG* upstream fragment
pckGUpRev	GTACATCACCACAAACCCGGAATGGTCGCTGAGG	
pckGHygFor	CCATTCCGGGTTTGTGGTGATGTACGTGGCGAAC	Amplification of *hyg*^{r} casette
pckGHygRev	CCCTCTTCGGACAGGGATCCGGGGGGCGTCAGG	
pckGDownFor	GACGCCCCCCGGATCCCTGTCCGAAGAGGGCTAAC	Amplification *pckG* downstream fragment
pckGDownRev	CCAGTGCCTCGCTCCGCCGGAGGTGACG	
pckGLongFor	CGTCGGCTTCGGACTCGGGGTCGCGCTGG	PCR confirmation of Δ*pckG*
pckGLongRev	CTCTTCCCACCCCATGTCGAGCATG	