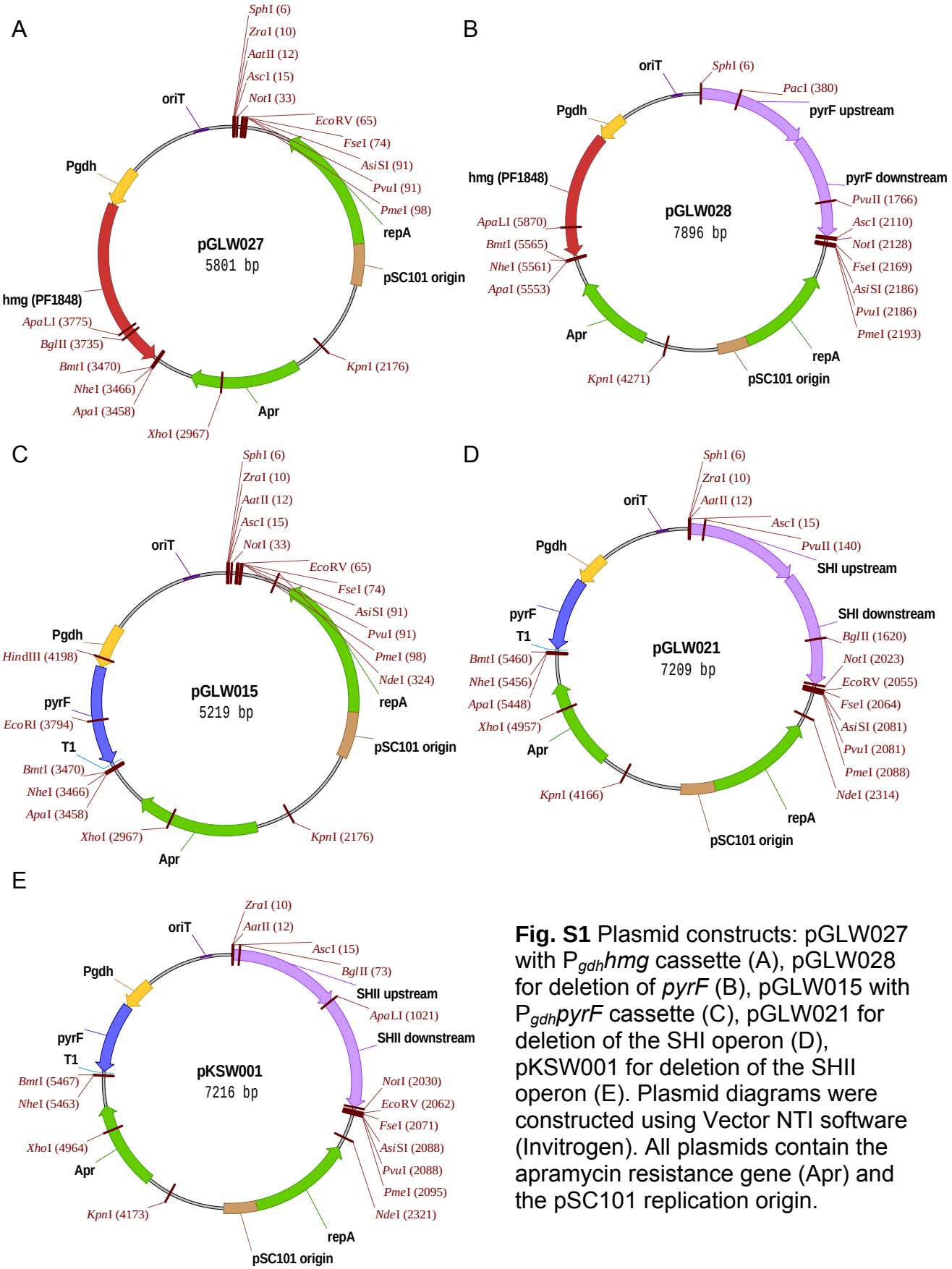

Fig. S1 Plasmid constructs: pGLW027 with P_{gdh}*hmg* cassette (A), pGLW028 for deletion of *pyrF* (B), pGLW015 with P_{gdh}*pyrF* cassette (C), pGLW021 for deletion of the SHI operon (D), pKSW001 for deletion of the SHII operon (E). Plasmid diagrams were constructed using Vector NTI software (Invitrogen). All plasmids contain the apramycin resistance gene (Apr) and the pSC101 replication origin.

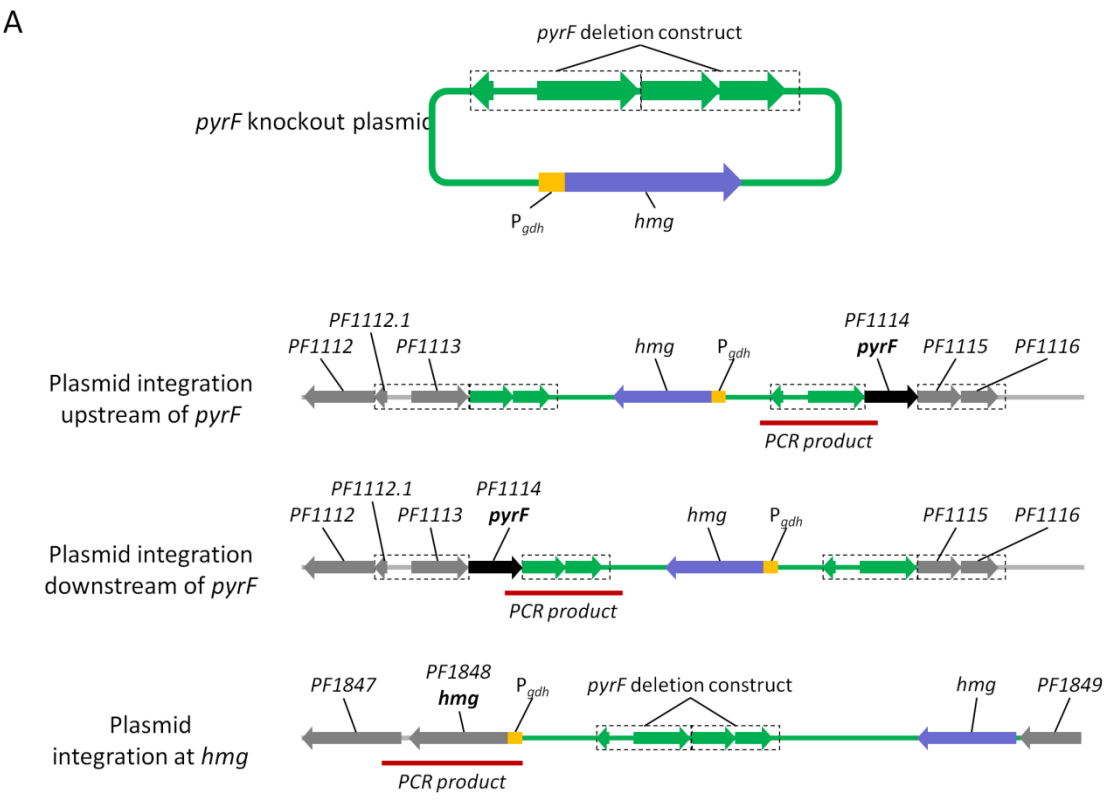

Fig. S2 Simvastatin resistant isolates 1 and 2 contain plasmid integration at three locations. A. Diagrams of plasmid integration at homologous region upstream of *pyrF*, (top) downstream of *pyrF* (middle), and at *hmg* (bottom). The PCR products used to verify integration events are shown with a bold red line. B. Gel showing PCR products indicated in *A* for simvastatin isolates 1 and 2 compared to wild type, demonstrating plasmid integration upstream of *pyrF*, downstream of *pyrF*, and at *hmg*. A PCR product can only be obtained in the event of an integration since primer pairs used contain one primer inside the plasmid and one primer within the genome and not in the plasmid.

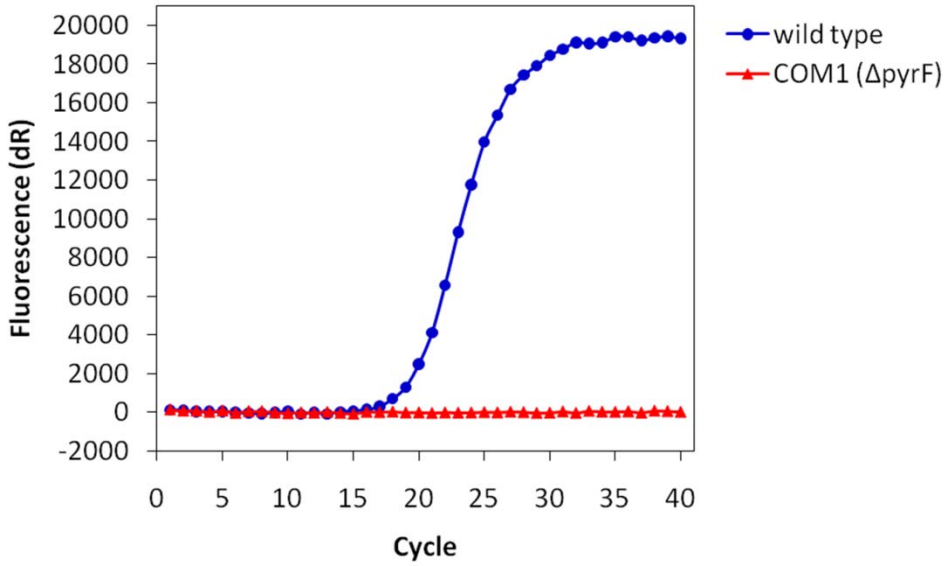

Fig. S3 Amplification plots from quantitative reverse transcription PCR (RT-qPCR) with primers specific to the *pyrF* gene, amplified from cDNA generated from RNA extracted from cultures of wild type and COM1.

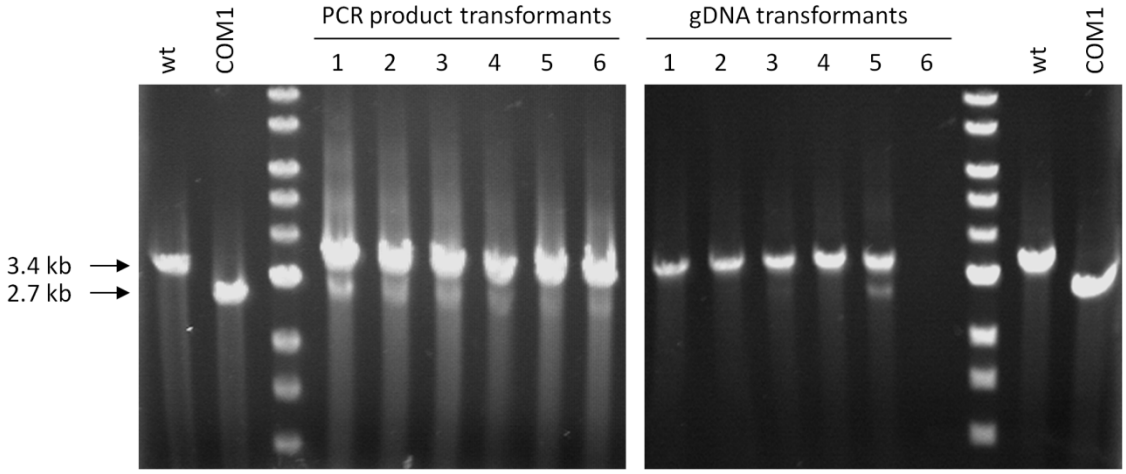

Fig. S4 PCR screen of transformants obtained using either a PCR product of the wild type *pyrF* region (left panel) or intact genomic DNA (gDNA) containing the wild type *pyrF* allele (right panel). Genomic DNA from the wild type (wt) and COM1 strains were included as controls. PCR was performed with primers targeting the region of the *pyrF* locus. All transformants have the *pyrF* locus restored to wild type (3.4 kb), and some still contain mixtures with the *pyrF* deletion (2.7 kb).

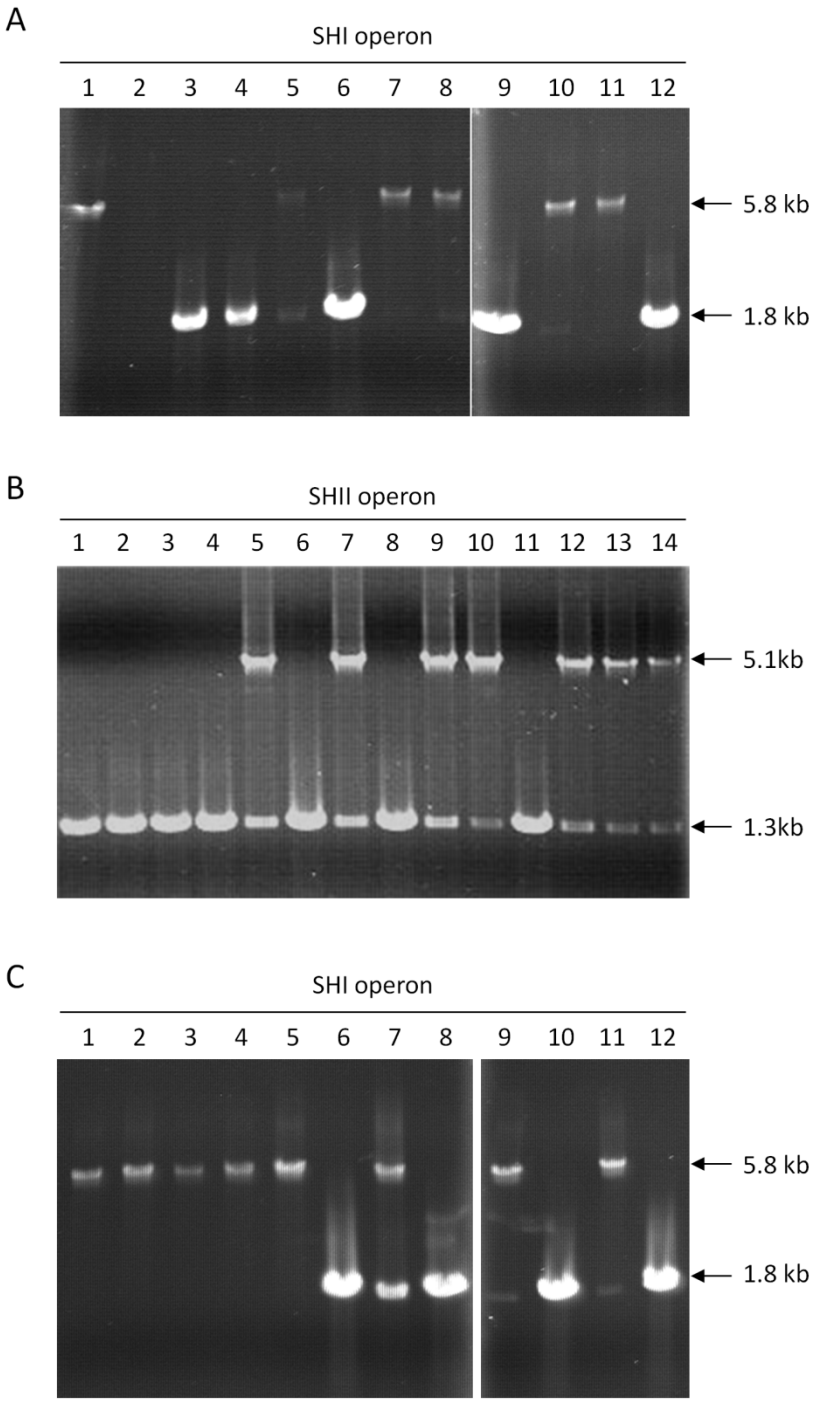

Fig. S5 PCR screen for SHI and SHII operon deletions. PCR products of SHI or SHII operon genome region in isolates screened to find deletions of the SHI operon in the COM1 strain (A), the SHII operon in the COM1 strain (B), the SHI operon in the ΔSHII strain (C). PCR was performed with primer sets in which at least one primer in the set was outside the homologous regions on the transforming plasmid DNA.

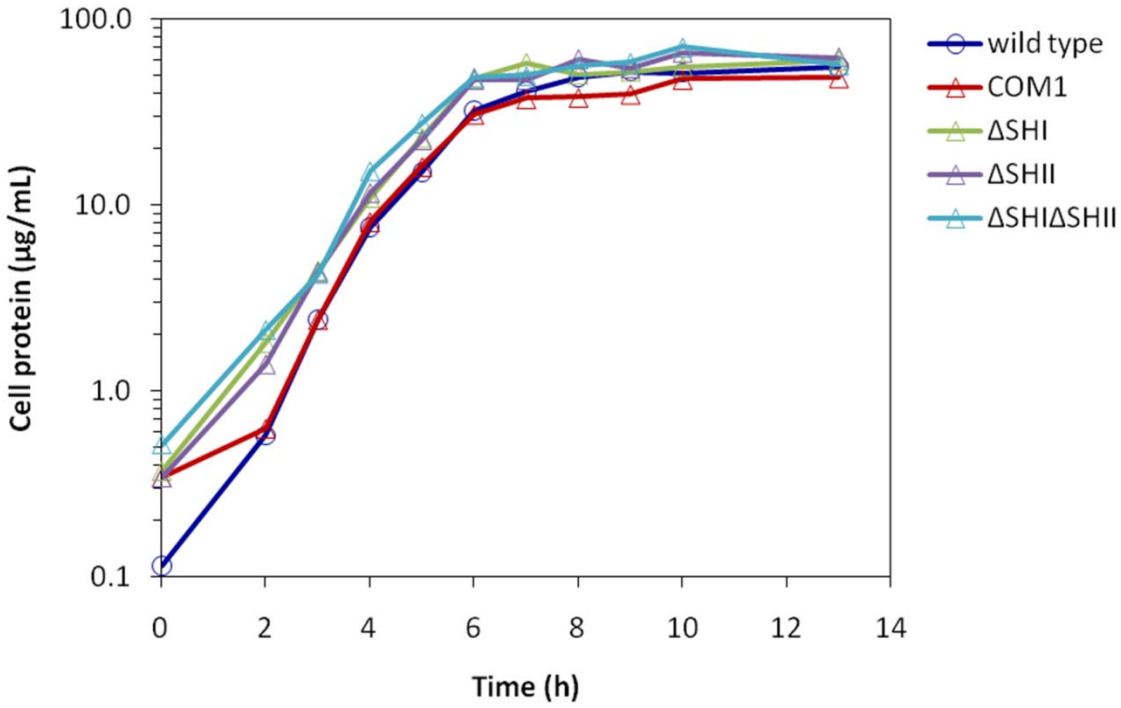

Fig. S6 Growth of ΔSHI, ΔSHII, and ΔSHIΔSHII mutants compared to COM1 and wild type strains on YEM complex medium. Uracil was added to the medium at a concentration of 20 µM.

Table S1. Primers used in the study.

Primer sequence (5'-3')[a]	Purpose
pyrF knockout vector and deletion confirmation	
*agagagaggcatgc*cacctacctcctatattgttccatg	primer for *pyrF* ~1-kb upstream flanking region, with SphI site plus 8 extra bases
*ttgagctccat*tcagccacctccaatatttcc	primer for *pyrF* ~1-kb upstream flanking region, with overlap to 5' end of 1-kb downstream flanking region
*aggtggctga*atggagctcaagataaagcacc	primer for *pyrF* ~1-kb downstream flanking region, with overlap to 3' end of 1-kb upstream flanking region
*agagagagggcgcgcc*gtcaagaggatgattaggtagagc	primer for *pyrF* ~1-kb downstream flanking region, with AscI site plus 8 extra bases
ctcaactgtgatgtttgtcttgc	confirmation/sequencing primer for *pyrF* deletion
cgttggcaaacaacttcctg	confirmation/sequencing primer for *pyrF* deletion
gaaataactccaagaccacttcc	confirmation primer for plasmid insertion at *pyrF* genome region
gaaagctggagcagattacatc	confirmation primer for plasmid insertion at *pyrF* genome region
Vector primers	
ccaaggaaagtctacacgaacc	primer upstream from MCS on pJHW008 derivative vectors
ctgagacaacttgttacagcttc	primer downstream from MCS on pJHW008 derivative vectors
hmg genome region	
cattccatctccaatgaactttgc	confirmation/sequencing primer for plasmid insertion at *hmg*
tgctcaacaaggttagagaagc	confirmation/sequencing primer for plasmid insertion at *hmg*
gaacaaatggaacgtcttcacc	confirmation/sequencing primer for plasmid insertion at *hmg*
gacgtgttggaggatctcaag	confirmation/sequencing primer for plasmid insertion at *hmg*
P_{gdh}*pyrF* cassette	
gattgaaaatggagtgagctgag	primes from 5' end of *gdh* promoter region
gttcatccctccaaattaggtg	primes from 3' end of the *gdh* promoter region
*aatcacctaatttggagggatgaac*atgattgtactagcgttggacg	primes from 5' end of *pyrF*, with overlap to 3' end of the *gdh* promoter
*ctaaaaaagatt*ttatcttgagctccattcttt cacc	primes from 3' end of *pyrF*, with putative termination sequence T1 from *hpyA1*
SHI operon knockout vector and deletion confirmation	
*agagagagggcgcgcc*tgagtatgaagctagggagaac	primer for SHI operon upstream ~1-kb flanking region, with AscI site plus 8 extra bp
*caacaaaaatagataaaaaggattaa*acaaaccacctcccaatgag	primer for SHI operon upstream ~1-kb flanking region, with overlap to 5' end of SHI downstream 1-kb flanking region
ttaatccttttatctatttttgttgag	primer for SHI operon downstream ~1-kb flanking region
*agagagaggcggccgc*taggatttcttgtagctctagtac	primer for SHI operon downstream ~1-kb flanking region, with NotI site plus 8 extra bases
cagtgaatggctttggaacc	confirmation/sequencing primer for SHI operon deletion
gaaagggagtatttagggacac	confirmation/sequencing primer for SHI operon deletion
agaagagggacttcaaggcg	sequencing primer for SHI operon deletion
SHII operon knock-out vector and deletion confirmation	
*taaggcgcgcc*atttagaccatcctccttt	primer for SHII operon upstream ~1-kb flanking region, with AscI site plus 8 extra bp
*acgaagtgca*caacttttctcacctccttt	primer for SHII operon upstream ~1-kb flanking region, with overlap to 5' end of SHI downstream ~1-kb flanking region
*agaaaagttg*tgcacttcgtcaagctttaa	primer for SHII operon downstream ~1-kb flanking region with overlap to 3' end of SHI upstream ~1-kb flanking region

*tccttagagcggccgc*ggtagatgctttaa	primer for SHII operon downstream 1-kb flanking region, with NotI site and 8 extra bases
cattatgcacatcaccctacaaga	sequencing primer for SHII operon deletion
agaaatccaagggaagtccttgaa	sequencing primer for SHII operon deletion
ctccctcacagccttactaggatt	confirmation/sequencing primer for SHII operon deletion
aagcagttacggcaatccacgata	confirmation/sequencing primer for SHII operon deletion
RT-qPCR	
cgttgttgttgtgctagatcc	forward primer for qPCR of PF0971 (*por*)
gatggcttcctctatgctctc	reverse primer for qPCR of PF0971 (*por*)
tcaaaaccagaatacagggagg	forward primer for qPCR of PF0891 (*shIβ*)
ccttctctctcctcaccttg	reverse primer for qPCR of PF0891 (*shIβ*)
cagtttgtccagctgacgat	forward primer for qPCR of PF0892 (*shIγ*)
caggctttagtctatggacaac	reverse primer for qPCR of PF0892 (*shIγ*)
ggaagcgtttcaactgagga	forward primer for qPCR of PF0893 (*shIδ*)
cttccagagctcttctaatggc	reverse primer for qPCR of PF0893 (*shIδ*)
tgagcagtacagcgaagttg	forward primer for qPCR of PF0894 (*shIα*)
ccgtataggaggtcagcattg	reverse primer for qPCR of PF0894 (*shIα*)
cataaggccaagggatgctatg	forward primer for qPCR of PF1329 (*shIIβ*)
ctccttctttcgtagtatgggtc	reverse primer for qPCR of PF1329 (*shIIβ*)
ccaatacagctttgcatcagaag	forward primer for qPCR of PF1330 (*shIIγ*)
taacggagccattccaagtc	reverse primer for qPCR of PF1330 (*shIIγ*)
ctggaactgtatcgacacagag	forward primer for qPCR of PF1331 (*shIIδ*)
ctcttctgtaagcctctttcgag	reverse primer for qPCR of PF1331 (*shIIδ*)
acggtgaggttaaggatgctag	forward primer for qPCR of PF1332 (*shIIα*)
caaggaggtagaggtggagtg	reverse primer for qPCR of PF1332 (*shIIα*)
gaaatactcgagcttggagagg	forward primer for qPCR of PF1113
caacggtaacctcaataggttcc	reverse primer for qPCR of PF1113
gatctaaagctggcagacatc	forward primer for qPCR of PF1114 (*pyrF*)
ccaggatggctcatctcaac	reverse primer for qPCR of PF1114 (*pyrF*)
gttgcggagttcgataagacc	forward primer for qPCR of PF1115
cctcatccacaactactctcttg	reverse primer for qPCR of PF1115
ttgaagatggctaaggagttgg	forward primer for qPCR of PF1116
cggttctccaatcacaacatc	reverse primer for qPCR of PF1116

[a] Primer tails are indicated in italics.