Table S6. Primers and plasmids used in this work

Primer	Sequence (5'-3')	PCR product and purpose	Plasmid
Gene deletion			
NsiI-*CM*	TATA**ATGCAT**AGTTGTCCCCGTACATCAGC	2,167-bp upstream flanking region for *gonCM* deletion	pD*gonCM*
SpeI-*CM*	TATA**ACTAGT**TAGGTCTCGACGGCCTTGTA		
EcoRV-*CM*	TATA**GATATC**CCTTTCTTGGTCGTGAAGGT	2,099-bp downstream flanking region for *gonCM* deletion	
NdeI-*CM*	TATA**CATATG**CCCGAACTCTCCATCGTCT		
NsiI-*D3*	TATA**ATGCAT**GACGTGCTCCTGGTGGTACT	2,072-bp upstream flanking region for *gonD3*deletion	pD*gonD3*
SpeI-*D3*	TATA**ACTAGT**GAGGACACCGTTCGTGGTT		
EcoRV-*D3*	TATA**GATATC**CACGAGTACGCCCCACAG	2,106-bp downstream flanking region for *gonD3*deletion	
BamHI-*D3*	TATA**GGATCC**TCACCGAGTTCGTTCTCTCC		
NsiI-*E*	TATA**ATGCAT**TCCTGCAATGGTGAGAAGAA	2,270-bp upstream flanking region for *gonE* deletion	pD*gonE*
SpeI-*E*	TATA**ACTAGT**CGCTGTCTGACGATGGAGTA		
EcoRV-*E*	TATA**GATATC**TCACACCATTCTGCTTCTCG	2,255-bp downstream flanking region for *gonE* deletion	
BamHI-*E*	TATA**GGATCC**CTCCGTGACTGATGAGGACA		
SpeI-*R1*	TATA**ACTAGT**GTGACCTACCTTCCGCAGAG	2,147-bp upstream flanking region for *gonR1* deletion	pD*gonR1*
NsiI-*R1*	TATA**ATGCAT**GTCAGGTCCATCGGGAAGA		
BamHI-*R1*	TATA**GGATCC**CCGCAGCATCGACAGTTC	2,021-bp downstream flanking region for *gonR1* deletion	
EcoRV-*R1*	TATA**GATATC**TACATCTTCTCGCACGTTGG		
NsiI-*R2*	TATA**ATGCAT**ACTGCACGTTGGTGTAGTGC	1,981-bp upstream flanking region for *gonR2* deletion	pD*gonR2*
SpeI-*R2*	TATA**ACTAGT**GCTGATGTACGGGGACAACT		
EcoRV-*R2*	TATA**GATATC**ACAGCCTTTAGGCGTGTTGT	2,277-bp downstream flanking region for *gonR2* deletion	
BamHI- *R2*	TATA**GGATCC**GGGTGAGGTCCACAGCAG		
SpeI-*G1*	TATA**ACTAGT**CTAACCGCCGTCGTACATCT	2,219-bp upstream flanking region for *gonG1* deletion	pD*gonG1*
NsiI-*G1*	TATA**ATGCAT**GTCTCATCCTGTGGTCTGTGG		
BamHI-*G1*	TATA**GGATCC**GGGATCTACACCCACCACAG	2,016-bp downstream flanking region for *gonG1* deletion	
EcoRV-*G1*	TATA**GATATC**AGGAGATGGGTGACGAACAG		

Table S1. Primers and plasmids used in this work (continued)

Primer	Sequence (5'-3')	PCR product and purpose	Plasmid
		Gene deletion	
SpeI-*G2*	TATA**ACTAGT**TGTCGACCGTTCCATACTCA	2,033-bp upstream flanking region for *gonG2* deletion	pD*gonG2*
NsiI-*G2*	TATA**ATGCAT**TGTAGTGGTGGTCGACGAAG		
BglII-*G2*	TATA**AGATCT**GGCCTGTTGGAGCAGTGG	2,079-bp downstream flanking region for *gonG2* deletion	
EcoRV-*G2*	TATA**GATATC**AGAGAGCCCTCCCTCATCTC		
XbaI-*G3*	TATA**TCTAGA**CCACACGGAATGAGGAGATT	2,110-bp upstream flanking region for *gonG3* deletion	pD*gonG3*
NsiI-*G3*	TATA**ATGCAT**ACTGCACCATGACCAGTGAC		
NdeI-*G3*	TATA**CATATG**CGGCGAACATACTGGAATCT	2,385-bp downstream flanking region for *gonG3* deletion	
EcoRV-*G3*	TATA**GATATC**ACGAGATCACCTCGCATCTT		
SpeI-*G4*	TATA**ACTAGT**GGGCACCTTGAAATAGGACA	2,000-bp upstream flanking region for *gonG4* deletion	pD*gonG4*
NsiI-*G4*	TATA**ATGCAT**CATGTGAAGGTATCGGCTCA		
BamHI-*G4*	TATA**GGATCC**ACAGCCTTTAGGCGTGTTGT	2,331-bp downstream flanking region for *gonG4* deletion	
EcoRV-*G4*	TATA**GATATC**CCTCACAGATGGTCCTCGAC		
		Mutant complementation (over-expression)	
EcoRV-c*CM*	TATA**GATATC**GCTGTGTCTGCCTCAGTATGG	1,426-bp fragment containing *gonCM* (-109 bp from start codon) for mutant complementation	pC*gonCM*
BamHI-c*CM*	TATA**GGATCC**TAAGGTCGCCGGCAGATAC		
EcoRV-c*D3*	TATA**GATATC**GGCCTGTTGGAGCAGTGG	1,466-bp fragment containing *gonD3* (-32 bp from start codon) for mutant complementation	pC*gonD3*
BamHI-c*D3*	TATA**GGATCC**GGCAGGACACTCACAGGAG		
EcoRV-c*E*	TATA**GATATC**GATCGCGAGGACTTCGTTC	875-bp fragment containing *gonE* (-106 bp from start codon) for mutant complementation	pC*gonE*
BamHI-c*E*	TATA**GGATCC**CGAAAACGTGAGTGACATGAG		
EcoRV-c*R2*	TATA**GATATC**CCTTGCGGATGGGTATCTC	1,083-bp fragment containing *gonR2* (-88 bp from start codon) for over-expression	pC*gonR2*
BamHI-c*R2*	TATA**GGATCC**CTCCGTGACTGATGAGGACA		

Table S1. Primers and plasmids used in this work (continued)

Primer	Sequence (5'-3')	PCR product and purpose	Plasmid
		Mutant complementation (over-expression)	
BglII-c*G1*	TATA**AGATCT**CCACAGACCACAGGATGAGA	1,436-bp fragment containing *gonG1* (-100 bp from start codon) for mutant complementation	p*CgonG1*
EcoRV-c*G1*	TATA**GATATC**CACAAGAGCCTTCACGGTTT		
BamHI-c*G2*	TATA**GGATCC**CGAGTGAAGGTGTCGATGTG	1,357-bp fragment containing *gonG2* (-107 bp from start codon) for mutant complementation	p*CgonG2*
EcoRV-c*G2*	TATA**GATATC**TCACCGAGTTCGTTCTCTCC		
BglII-c*G3*	TATA**AGATCT**TAGTCATTCAGCACCGTCGT	1,429-bp fragment containing *gonG3* (-92 bp from start codon) for mutant complementation	p*CgonG3*
EcoRV-c*G3*	TATA**GATATC**ACCTGACGCCACCCTATTC		
BamHI-c*G4*	TATA**GGATCC**GGTGAAAGAGCTTGGAGTGC	1,209-bp fragment containing *gonG4* (-24 bp from start codon) for over-expression	p*CgonG4*
EcoRV-c*G4*	TATA**GATATC**CTAGGGCGAACACGACCTCT		
NcoI-dHG^R	TATA**CCATGG**CATCAGCAAAAGGGGATGAT	1,635-bp fragment containing hygR gene from pDR4	pSETH
NcoI-rvHG^R	TATA**CCATGG**ATCGAAGAGAAGCAGGACGA		
		Mutant confirmation	
cf*CM*	CATCGTGGTGGTCTTACCG	used with ApraII for confirmation of *gonCM* deletion	
cf*D3*	GCCGTATGGCTGTGTTCTC	used with ApraII for confirmation of *gonD3*deletion	
cf*E*	AACTTCACCACATCGCACAG	used with ApraII for confirmation of *gonE* deletion	
cf*R1*	CAAGCCGATCCTCTTCTACG	used with Apra60 for confirmation of *gonR1*deletion	
cf*G1*	GTACGGCACCTCCTGCTG	used with Apra60 for confirmation of *gonG1*deletion	
cf*G2*	ACTACGTCCGTACGCTCCTG	used with Apra60 for confirmation of *gonG2* deletion	

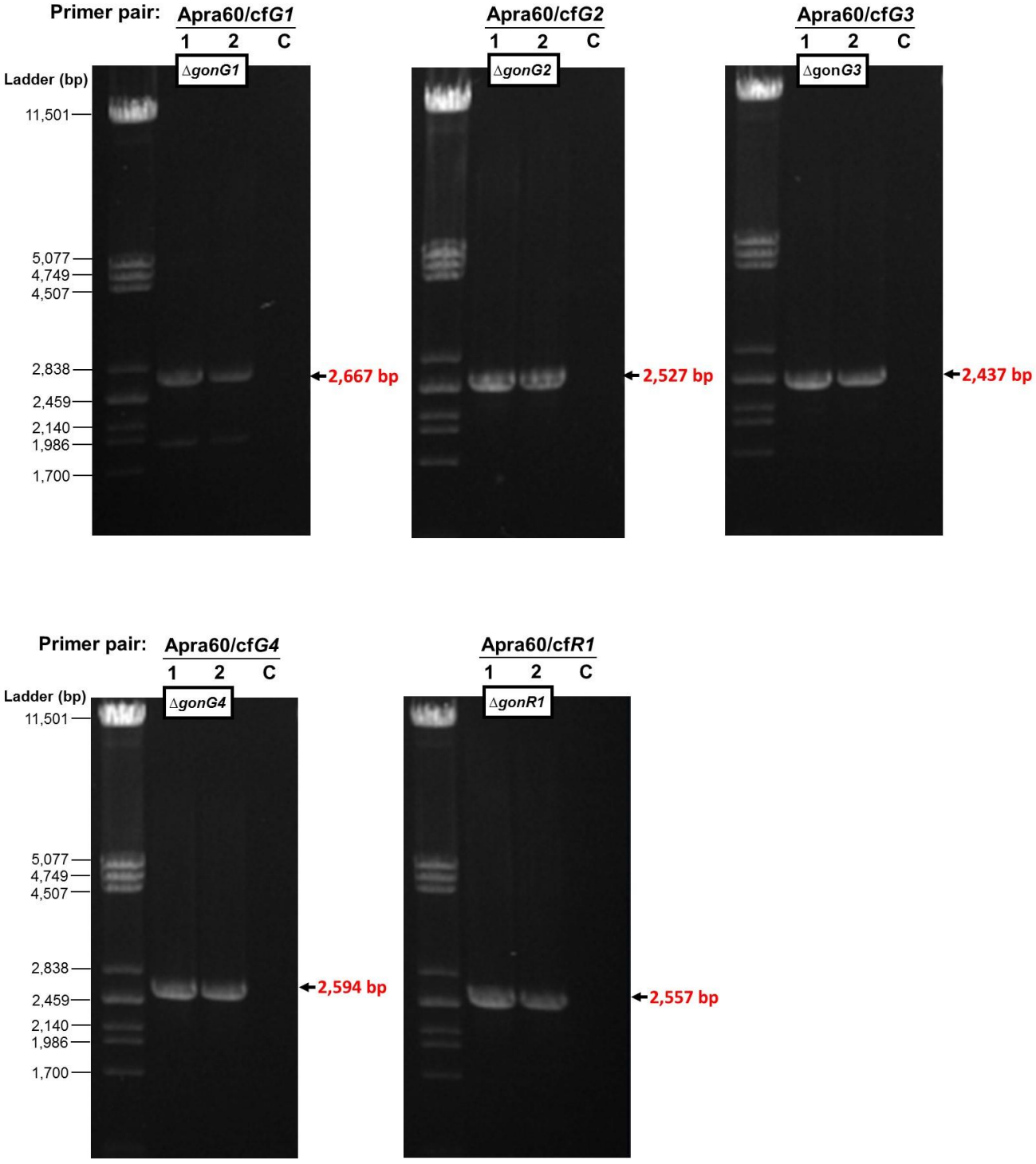
Table S1. Primers and plasmids used in this work (continued)

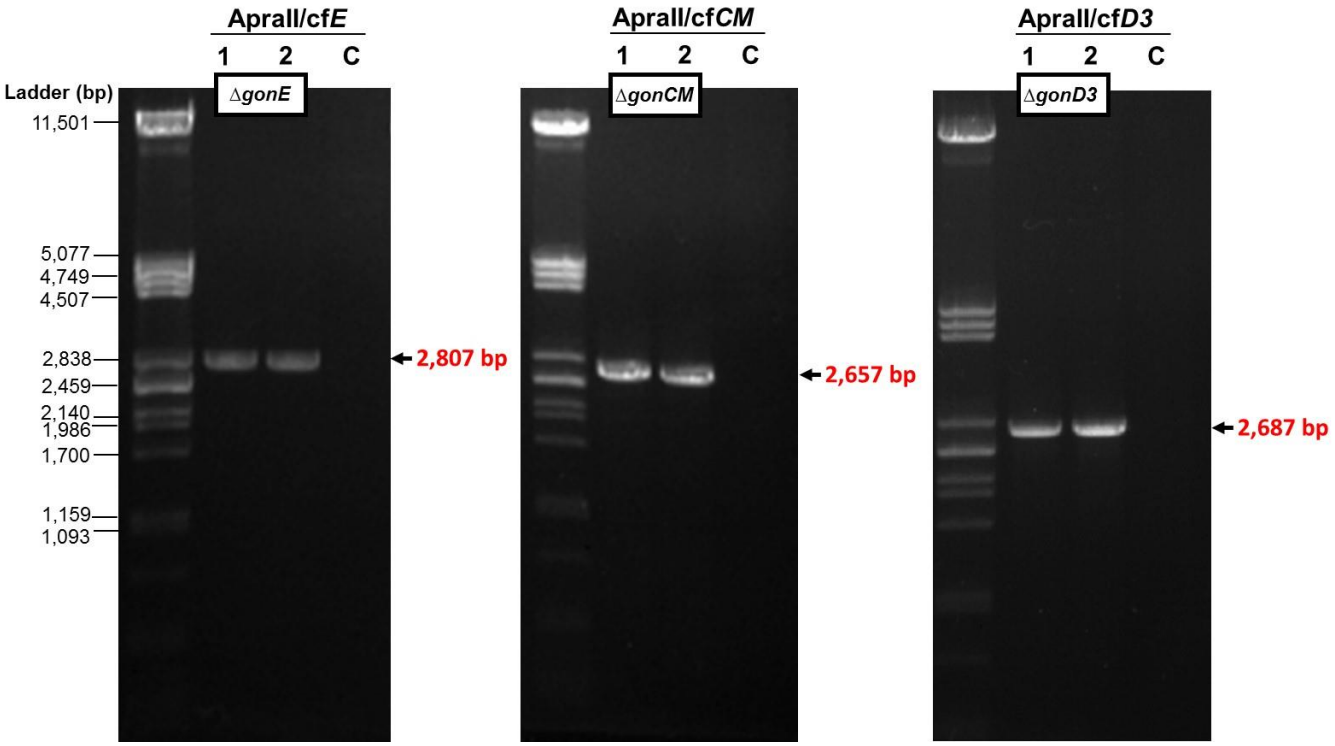
Primer	Sequence (5'-3')	PCR product and purpose	Plasmid
		Mutant confirmation	
cfG3	TGCAGCACCTCAAGAGAGAA	used with Apra60 for confirmation of gonR3deletion	
cf*G4*	GTGAAACCGAGTTGATGCAG	used with Apra60 for confirmation of gonG4deletion	
Apra60	CCAAGGTTGAGAAGCTGACC	reverse primer annealing *aac(3)IV*	
ApraII	CTTCAGGATGGCAAGTTGGT	forward primer annealing *aac(3)IV*	

Mutant confirmation by PCR

Gene deletion in *S. caniferus* GUA-06-05-006A was confirmed by colony PCR by using the procedure described in [1] with the following modifications. Colonies of *S. caniferus* GUA-06-05-006A mutants were suspended in 50 µl of 0.2M TES buffer, pH 7.5, with 1 µl lysozyme (50 mg/ml) and incubated for 40 min at 30ºC. The mix was centrifuged (10000 x g, 2 min) and the pellet thoroughly suspended in 10 µl DMSO. The resulting suspension (2 µl) was used as PCR template with the primer pairs described in Table S6. DNA bands obtained from PCR amplification were analyzed in 0.7% agarose gels. Fig. S5 shows the analysis of two colonies (1 and 2) and a control template (C) consisting in 30 ng of wild type *S. caniferus* GUA-06-05-006A genomic DNA from a pure extract. Band sizes were compared with 0.1 µg of lambda/PstI DNA marker. The expect band size is indicated in red.

Fig. S5. Gene deletion confirmation by PCR in mutant strains Δ*gonG1*, Δ*gonG2*, Δ*gonG3*, Δ*gonG4*, Δ*gonR1*, Δ*gonE*, Δ*gonCM* and Δ*gonD3*.





References

[1] Van Dessel W, Van Mellaert L, Geukens N, Anné J: **Improved PCR-based method for the direct screening of Streptomyces transformants.** *J Microbiol Methods* 2003 53:401-3.