Methods S1. Plasmid construction.

pOJ-\textit{gon}P1 and pOJ-\textit{gon}P8

An internal fragment of genes \textit{gon}P1 and \textit{gon}P8 was amplified with the primer pairs EcoRI-P1/HindIII-P1 and EcoRI-P8/HindIII-P8 (Table S1), respectively. The resulting PCR products were digested with EcoRI/HindIII and cloned into the same sites of plasmid pOJ260 to yield the gene disruption plasmids pOJ-\textit{gon}P1 and pOJ-\textit{gon}P8. The correct insertion of the \textit{gon}P1 (3,504 bp) and \textit{gon}P8 (2,238 bp) gene fragments into pOJ260 was confirmed by sequencing with primer M13r. Plasmids pOJ-\textit{gon}P1 and pOJ-\textit{gon}P8 were transferred to \textit{Streptomyces caniferus} GUA-06-05-006A by intergeneric conjugation to achieve \textit{gon}P1 and \textit{gon}P8 inactivation. Gene disruption in the resulting strains, \textit{gon}P1\textsuperscript{−} and \textit{gon}P8\textsuperscript{−}, was verified by PCR with the primer pairs cf\textit{P}1/M13r and cf\textit{P}8/M13r, respectively. Primers cf\textit{P}1 and cf\textit{P}8 anneal to sequences outside the internal fragment used for gene inactivation and primer M13r anneals to the pOJ260 backbone.

pD-\textit{gon}M4

The upstream (UP-\textit{M}4) and downstream (DW-\textit{M}4) sequences flanking gene \textit{gon}M4 were amplified with the primer pairs SpeI-\textit{M}4/NsiI-\textit{M}4 and NdeI-\textit{M}4/EcoRV-\textit{M}4 (Table S1), respectively. DW-\textit{M}4 (2,367 pb) and plasmid pEFBA-oriT were digested with NdeI/EcoRV and ligated to generate plasmid p\textit{M}4-NE. This plasmid and fragment UP-\textit{M}4 (2,444 pb) were then digested with SpeI/NsiI and ligated to afford plasmid p\textit{M}4-\text{NE}N. Insertion of UP-\textit{M}4 and DW-\textit{M}4 at both sides of the \textit{aac(3)IV} gene in p\textit{M}4-\text{NE}N was verified by PCR and sequencing with primers SpeI-\textit{M}4, NsiI-\textit{M}4, NdeI-\textit{M}4 and EcoRV-\textit{M}4. A 1.6-kbp fragment containing the hygromycin B resistance (\textit{Hyg}\textsuperscript{R}) gene marker, \textit{hyg}, was extracted from pLHyg by SpeI/NheI digestion and cloned into the XbaI site of p\textit{M}4-\text{NE}N to yield the gene-replacement plasmid pD-\textit{gon}M4, which was transferred to \textit{Streptomyces caniferus} GUA-06-05-006A by intergeneric conjugation to achieve \textit{gon}M4 deletion. Replacement of \textit{gon}M4 in the resulting \textit{Hyg}\textsuperscript{R} \textit{Amp}\textsuperscript{R} strain, \textit{Δgon}M4, was verified by PCR with primers cf\textit{M}4 (outside the deletion cassette) and Apra60 (internal to the Apm\textsuperscript{R} gene marker).
pD-gon\textit{MT}

The upstream (UP-\textit{MT}) and downstream (DW-\textit{MT}) sequences flanking gene gon\textit{MT} were amplified with the primer pairs Spel-\textit{MT}/NsiI-\textit{MT} and BglII-\textit{MT}/EcoRV-\textit{MT} (Table S1), respectively. DW-\textit{MT} (2,273 bp) and plasmid pEFBA-oriT were digested with BglII/EcoRV and BamHI/EcoRV, respectively, and ligated to generate plasmid p\textit{MT-BE}. This plasmid and fragment UP-\textit{MT} (2,294 bp) were then digested with SpeI/NsiI and ligated to afford plasmid p\textit{MT-BESN}. Insertion of UP-\textit{MT} and DW-\textit{MT} at both sides of the \textit{aac(3)IV} gene in p\textit{MT-BESN} was verified by PCR and sequencing with primers Spel-\textit{MT}, NsiI-\textit{MT}, BglII-\textit{MT} and EcoRV-\textit{MT}. A 1.6-kbp fragment containing the hygromycin B resistance (Hyg\textsuperscript{R}) gene marker, hyg, was extracted from pLHyg by Spel/NheI digestion and cloned into the XbaI site of p\textit{MT-BESN} to produce the gene replacement plasmid pD-gon\textit{MT}, which was transferred to \textit{Streptomyces caniferus} GUA-06-05-006A by intergeneric conjugation to achieve gon\textit{MT} deletion. Replacement of gon\textit{MT} in the resulting Hyg\textsuperscript{S} Amp\textsuperscript{R} strain, Δgon\textit{MT}, was verified by PCR with primers cf\textit{MT} (outside the deletion cassette) and ApraII (internal to the Apm\textsuperscript{R} gene marker).

pD-gon\textit{SL}

The upstream (UP-\textit{SL}) and downstream (DW-\textit{SL}) sequences flanking gene gon\textit{SL} were amplified with the primer pairs Spel-\textit{SL}/NsiI-\textit{SL} and NdeI-\textit{SL}/EcoRV-\textit{SL} (Table S1), respectively. UP-\textit{SL} (2,138 bp) and plasmid pEFBA-oriT were digested with SpeI/NsiI and ligated to generate plasmid p\textit{SL-SN}. This plasmid and fragment DW-\textit{SL} were digested with NdeI/EcoRV. Digestion of DW-\textit{SL} with NdeI generated two fragments, of 1.945 and 211 bp, respectively, the 211-bp fragment was cloned into p\textit{SL-SN} (NdeI/EcoRV sites) to yield plasmid p\textit{SL-SN-200}, which was then digested with NdeI and ligated to the 1.945-bp fragment to produce plasmid p\textit{SL-SNNE}. The correct cloning orientation of the 1.945-bp DW-\textit{SL} fragment was confirmed by PCR with primers NdeI-\textit{SL} and EcoRV-\textit{SL}. In addition, insertion of UP-\textit{SL} and DW-\textit{SL} at both sides of the \textit{aac(3)IV} gene in p\textit{SL-SNNE} was verified by sequencing with primers NdeI-\textit{SL}, EcoRV-\textit{SL}, Spel-\textit{SL} and NsiI-\textit{SL}. A 1.6-kbp fragment containing the hygromycin B resistance (Hyg\textsuperscript{R}) gene marker, hyg, was then extracted from pLHyg by Spel/NheI digestion and cloned into the XbaI site of p\textit{SL-NESN} to yield the gene replacement plasmid pD-gon\textit{SL}, which was transferred to \textit{Streptomyces}
*caniferus* GUA-06-05-006A by intergeneric conjugation to achieve gonSL deletion. Replacement of gonSL in the resulting Hyg⁺ Amp⁺ strain, ΔgonSL, was verified by PCR with primers cfSL (outside the deletion cassette) and Apra60 (internal to the Apm⁺ gene marker).

**pD-gonS1**

The upstream (UP-S1) and downstream (DW-S1) sequences flanking gene gonS1 were amplified with the primer pairs fNsiI-S1/rNsiI-S1 and BglII-S1/EcoRV-S1 (Table S1), respectively. DW-S1 (2,131 bp) and plasmid pEFBA-oriT were digested with BglII/EcoRV and BamHI/EcoRV, respectively, and ligated to generate plasmid pS1-BE. This plasmid and fragment UP-S1 (2,096 bp) were then digested with NsiI and ligated to afford plasmid pS1-BENN. Correct cloning orientation of the gonS1 upstream fragment was confirmed by PCR with primers fNsiI-S1 and apra60. In addition, insertion of UP-S1 and DW-S1 at both sides of the aac(3)IV gene in pS1-BENN was verified by sequencing with primers fNsiI-S1, rNsiI-S1, BglII-S1 and EcoRV-S1. A 1.6-kbp fragment containing the hygromycin B resistance (Hyg⁺) gene marker, hyg, was extracted from pLHyg by SpeI/Nhel digestion and cloned into the XbaI site of pS1-BENN to yield the gene replacement plasmid pD-gonS1, which was transferred to *Streptomyces caniferus* GUA-06-05-006A by intergeneric conjugation to achieve gonS1 deletion. Replacement of gonS1 in the resulting Hyg⁺ Amp⁺ strain, ΔgonS1, was verified by PCR with primers cfS1 (outside the deletion cassette) and Apra60 (internal to the Apm⁺ gene marker).

**pD-gonS2**

The upstream (UP-S2) and downstream (DW-S2) sequences flanking gene gonS2 were amplified with the primer pairs NsiI-S2/Spel-S2 and fNdeI-S2/rNdeI-S2 (Table S1), respectively. DW-S2 (2,434 bp) and plasmid pEFBA-oriT were digested with NdeI and ligated to generate plasmid pS2-NN. Insertion of the gonS2 downstream fragment in the correct orientation was confirmed by PCR with fNdeI-S2 and apraII. This plasmid and UP-S2 (2,208 bp) were then digested with NsiI/Spel and ligated to afford plasmid pS2-NNNS. Insertion of UP-S2 and DW-S2 at both sides of the aac(3)IV gene in pS2-NNNS was verified by sequencing with primers NsiI-S2, Spel-S2, fNdeI-S2 and rNdeI-S2. A 1.6-kbp
fragment containing the hygromycin B resistance (Hyg\textsuperscript{R}) gene marker, hyg, was extracted from pLHyg by SpeI/NheI digestion and cloned into the XbaI site of pSI-BENN to yield the gene replacement plasmid pD-gonS2, which was transferred to \textit{Streptomyces caniferus} GUA-06-05-006A by intergeneric conjugation to achieve gonS2 deletion. Replacement of gonS2 in the resulting Hyg\textsuperscript{S} Amp\textsuperscript{R} strain, ΔgonS2, was verified by PCR with primers cfS2 (outside the deletion cassette) and ApraII (internal to the Apm\textsuperscript{R} gene marker).

\textbf{pD-gonCP}

The upstream (UP-CP) and downstream (DW-CP) sequences flanking gene gonCP were amplified with the primer pairs SpeI-CP/NsiI-CP and BamHI-CP/EcoRV-CP (Table S1), respectively. DW-CP (2,548 bp) and plasmid pEFBA-oriT were digested with BamHI/EcoRV and ligated to generate plasmid pCP-BE. This plasmid and UP-CP (2,100 bp) were then digested with SpeI/NsiI and ligated to afford plasmid pCP-BESN. Insertion of UP-CP and DW-CP at both sides of the \textit{aac(3)IV} gene in pCP-BESN was verified by PCR and sequencing with primers SpeI-CP, NsiI-CP, BamHI-CP and EcoRV-CP. A 1.6-kbp fragment containing the hygromycin B resistance (Hyg\textsuperscript{R}) gene marker, hyg, was extracted from pLHyg by SpeI/NheI digestion and cloned into the XbaI site of pCP-BESN to yield the gene replacement plasmid pD-gonCP, which was transferred to \textit{Streptomyces caniferus} GUA-06-05-006A by intergeneric conjugation to achieve gonCP deletion. Replacement of gonLCP in the resulting Hyg\textsuperscript{S} Amp\textsuperscript{R} strain, ΔgonCP, was verified by PCR with primers cfCP (outside the deletion cassette) and Apra60 (internal to the Apm\textsuperscript{R} gene marker).

\textbf{pD-gonMR}

The upstream (UP-MR) and downstream (DW-MR) sequences flanking gene gonMR were amplified with the primer pairs SpeI-MR/NsiI-MR and BglII-MR/EcoRV-MR (Table S1), respectively. DW-MR (2,393 bp) and plasmid pEFBA-oriT were digested with BglII/EcoRV and BamHI/EcoRV, respectively, and ligated to generate plasmid pMR-BE. This plasmid and UP-MR (2,571 bp) were then digested with SpeI/NsiI and ligated to yield plasmid pMR-BESN. Insertion of UP-MR and DW-MR at both sides of the \textit{aac(3)IV} gene in pMR-BESN was verified by PCR and sequencing with primers SpeI-MR, NsiI-MR,
BglII-MR and EcoRV-MR. A 1.6-kbp fragment containing the hygromycin B resistance (Hyg\(^R\)) gene marker, hyg, was extracted from pLHyg by SpeI/NheI digestion and cloned into the XbaI site of pMR-BESN to yield the gene replacement plasmid pD-gonMR, which was transferred to *Streptomyces caniferus* GUA-06-05-006A by intergeneric conjugation to achieve gonMR deletion. Replacement of gonMR in the resulting Hyg\(^s\) Amp\(^R\) strain, ΔgonMR, was verified by PCR with primers cMR (outside the deletion cassette) and ApraII (internal to the Apm\(^R\) gene marker).

**pD-gonL1**
The upstream (UP-L1) and downstream (DW-L1) sequences flanking gene gonL1 were amplified with the primer pairs SpeI-L1/NsiI-L1 and BglII-L1/EcoRV-L1 (Table S1), respectively. DW-L1 (2,500 bp) and plasmid pEFBA-oriT were digested with BglII/EcoRV and BamHI/EcoRV, respectively, and ligated to generate plasmid pL1-BE. This plasmid and UP-L1 (2,732 bp) were then digested with SpeI/NsiI and ligated to afford plasmid pL1-BESN. Insertion of UP-L1 and DW-L1 at both sides of the aac(3)IV gene in pL1-BESN was verified by PCR and sequencing with primers SpeI-L1, NsiI-L1, BglII-L1 and EcoRV-L1. A 1.6-kbp fragment containing the hygromycin B resistance (Hyg\(^R\)) gene marker, hyg, was extracted from pLHyg by SpeI/NheI digestion and cloned into the XbaI site of pL1-BESN to yield the gene replacement plasmid pD-gonL1, which was transferred to *Streptomyces caniferus* GUA-06-05-006A by intergeneric conjugation to achieve gonL1 deletion. Replacement of gonL1 in the resulting Hyg\(^s\) Amp\(^R\) strain, ΔgonL1, was verified by PCR with primers cFL1 (outside the deletion cassette) and ApraII (internal to the Apm\(^R\) gene marker).

**pD-orf9**
The upstream (UP-orf9) and downstream (DW-orf9) sequences flanking gene orf9 were amplified with the primer pairs SpeI-orf9/NsiI-orf9 and BglII-orf9/EcoRV-orf9 (Table S1), respectively. DW-orf9 (2,434 bp) and plasmid pEFBA-oriT were digested with BglII/EcoRV and BamHI/EcoRV, respectively, and ligated to generate plasmid pGorf9-BE. This plasmid and UP-orf9 (2,496 bp) were then digested with SpeI/NsiI and ligated to afford plasmid pGorf9-BESN. Insertion of UP-orf9 and DW-orf9 at both sides of the
aac(3)IV gene in pGorf9-BESN was verified by PCR and sequencing with primers SpeI-orf9, NsiI-orf9, BglIII-orf9 and EcoRV-orf9. A 1.6-kbp fragment containing the hygromycin B resistance (Hyg\textsuperscript{R}) gene marker, hyg, was extracted from pLHyg by SpeI/NheI digestion and cloned into the Xbal site of pGorf10-BESN to produce the gene replacement plasmid pD-orf9, which was transferred to Streptomyces caniferus GUA-06-05-006A by intergeneric conjugation to achieve orf9 deletion. Replacement of orf9 in the resulting Hyg\textsuperscript{g} Amp\textsuperscript{R} strain, Δ5201, was verified by PCR with primers cf09 (outside the deletion cassette) and Apra60 (internal to the Apm\textsuperscript{R} gene marker).

pD-orf10
The upstream (UP-orf10) and downstream (DW-orf10) sequences flanking gene orf10 were amplified with the primer pairs SpeI-orf10/NsiI-orf10 and BglIII-orf10/EcoRV-orf10 (Table S1), respectively. DW-orf10 (2,380 bp) and plasmid pEFBA-oriT were digested with BglII/EcoRV and BamHI/EcoRV, respectively, and ligated to generate plasmid pGorf10-BE. This plasmid and UP-orf10 (2,667 bp) were then digested with SpeI/NsiI and ligated to afford plasmid pGorf10-BESN. Insertion of UP-orf10 and DW-orf10 at both sides of the aac(3)IV gene in pGorf10-BESN was verified by PCR and sequencing with primers SpeI-orf10, NsiI-orf10, BglIII-orf10 and EcoRV-orf10. A 1.6-kbp fragment containing the hygromycin B resistance (Hyg\textsuperscript{R}) gene marker, hyg, was extracted from pLHyg by SpeI/NheI digestion and cloned into the Xbal site of pGorf10-BESN to produce the gene replacement plasmid pD-orf10, which was transferred to Streptomyces caniferus GUA-06-05-006A by intergeneric conjugation to achieve orf10 deletion. Replacement of orf10 in the resulting Hyg\textsuperscript{g} Amp\textsuperscript{R} strain, Δ5257, was verified by PCR with primers cf10 (outside the deletion cassette) and ApraII (internal to the Apm\textsuperscript{R} gene marker).

pD-orf11
The upstream (UP-orf11) and downstream (DW-orf11) sequences flanking gene orf11 were amplified with the primer pairs SpeI-orf11/NsiI-orf11 and NdeI-orf11/EcoRV-orf11 (Table S1), respectively. UP-orf11 (2,195 bp) and plasmid pEFBA-oriT were digested with SpeI/NsiI and ligated to generate plasmid pGorf11-SN. This plasmid and DW-orf11 (2,136 bp) were then digested with NdeI/EcoRV and ligated to afford plasmid pGorf11-SNNE.
Insertion of UP-orf11 and DW-orf11 at both sides of the aac(3)IV gene in pGorf11-SNNE was verified by PCR and sequencing with primers SpeI-orf11, NsiI-orf11, NdeI-orf11 and EcoRV-orf11. A 1.6-kbp fragment containing the hygromycin B resistance (Hyg<sup>R</sup>) gene marker, hyg, was extracted from pLHyg by SpeI/NheI digestion and cloned into the XbaI site of pGorf11-SNNE to produce the gene replacement plasmid pD-orf11, which was transferred to Streptomyces caniferus GUA-06-05-006A by intergeneric conjugation to achieve orf11 deletion. Replacement of orf11 in the resulting Hyg<sup>S</sup> Amp<sup>R</sup> strain, Δ5259, was verified by PCR with primers cf11 (outside the deletion cassette) and ApraII (internal to the Apm<sup>R</sup> gene marker).

pD-orf13
The upstream (UP-orf13) and downstream (DW-orf13) sequences flanking gene orf13 were amplified with the primer pairs SpeI-orf13/PstII-orf13 and BamHI-orf13/EcoRV-orf13 (Table S1), respectively. DW-orf13 (2,347 bp) and plasmid pEFBA-oriT were digested with BamHI/EcoRV and ligated to generate plasmid pGorf13-BE. This plasmid and UP-orf13 (2,360 bp) were then digested with SpeI/PstI and ligated to afford plasmid pGorf13-BESP. Insertion of DW-orf13 and UP-orf13 at both sides of the aac(3)IV gene in pGorf13-BESP was verified by PCR and sequencing with primers SpeI-orf13, PstII-orf13, BamHI-orf13 and EcoRV-orf13. A 1.6-kbp fragment containing the hygromycin B resistance (Hyg<sup>R</sup>) gene marker, hyg, was extracted from pLHyg by SpeI/NheI digestion and cloned into the XbaI site of pGorf13-BESP to produce the gene replacement plasmid pD-orf13, which was transferred to Streptomyces caniferus GUA-06-05-006A by intergeneric conjugation to achieve orf13 deletion. Replacement of orf13 in the resulting Hyg<sup>S</sup> Amp<sup>R</sup> strain, Δ5261, was verified by PCR with primers cf13 (outside the deletion cassette) and Apra60 (internal to the Apm<sup>R</sup> gene marker).

pC-gonP8
A DNA fragment of 7,326 bp containing the entire ORF of gonP8 (nt -20 to +7,306 from start codon) was amplified with the primer pair cpP8-Xb/cpP8-ERV (Table S1). The resulting PCR fragment was digested with XbaI/EcoRV and cloned into the same sites of plasmid pSETHe (see methods in the article), under the control of the ermE*p promoter, to
yield the complementation plasmid pC-gonP8. Insertion of gonP8 into pSETHe was confirmed by PCR and sequencing with primers cpP8-Xb and cpP8-ERV. Plasmid pC-gonP8 was then transferred to the mutant strain gonP8 by intergeneric conjugation to produce the strain CPgonP8 in which gonP8 inactivation was complemented.

**pC-gonM4**

A DNA fragment of 998 bp containing the entire ORF of gonM4 (nt -26 to +972 from start codon) was amplified with the primer pair cpM4-Xb/cpM4-ERV (Table S1). The resulting PCR fragment was digested with XbaI/EcoRV and cloned into the same sites of plasmid pSETHe (see methods in the article), under the control of the ermE*p promoter, to yield the complementation plasmid pC-gonM4. Insertion of gonM4 into pSETHe was confirmed by PCR and sequencing with primers cpM4-Xb and cpM4-ERV. Plasmid pC-gonM4 was transferred to the mutant strain ΔgonM4 by intergeneric conjugation to produce the strain CPgonM4 in which gonM4 deletion was complemented.

**pC-gonMT**

A DNA fragment of 1,211 bp containing the entire ORF of gonMT (nt -65 to +1,146 from start codon) was amplified with the primer pair cpMT-Xb/cpMT-ERV (Table S1). The resulting PCR fragment was digested with XbaI/EcoRV and cloned into the same sites of plasmid pSETHe (see methods in the article), under the control of the ermE*p promoter, to yield the complementation plasmid pC-gonMT. Insertion of gonMT into pSETHe was confirmed by PCR and sequencing with primers cpMT-Xb and cpMT-ERV. Plasmid pC-gonMT was transferred to the mutant strain ΔgonMT by intergeneric conjugation to produce the strain CPgonMT in which gonMT deletion was complemented.

**pC-gonSL**

A DNA fragment of 2,514 bp containing the entire ORF of gonSL (nt -23 to +2,491 from start codon) was amplified with the primer pair cpSL-Xb/cpSL-ERV (Table S1). The resulting PCR fragment was digested with XbaI/EcoRV and cloned into the same sites of plasmid pSETHe (see methods in the article), under the control of the ermE*p promoter, to yield the complementation plasmid pC-gonSL. Insertion of gonSL into pSETHe was
confirmed by PCR and sequencing with primers cpSL-Xb and cpSL-ERV. Plasmid pC-gonSL was transferred to the mutant strain ΔgonSL by intergeneric conjugation to produce the strain CPgonSL in which gonSL deletion was complemented.

pC-gonS1
A DNA fragment of 1,135 bp containing the entire ORF of gonS1 (nt -24 to +1,111 from start codon) was amplified with the primer pair cpS1-Xb/cpS1-ERV (Table S1). The resulting PCR fragment was digested with XbaI/EcoRV and cloned into the same sites of plasmid pSETHe (see methods in the article), under the control of the ermE*p promoter, to yield the complementation plasmid pC-gonS1. Insertion of gonS1 into pSETHe was confirmed by PCR and sequencing with primers cpS1-Xb and cpS1-ERV. Plasmid pC-gonS1 was transferred to the mutant strain ΔgonS1 by intergeneric conjugation to produce the strain CPgonS1 in which gonS1 deletion was complemented.

pC-gonS2
A DNA fragment of 1,125 bp containing the entire ORF of gonS2 (nt -23 to +1,102 from start codon) was amplified with the primer pair cpS2-Xb/cpS2-ERV (Table S1). The resulting PCR fragment was digested with XbaI/EcoRV and cloned into the same sites of plasmid pSETHe (see methods in the article), under the control of the ermE*p promoter, to yield the complementation plasmid pC-gonS2. Insertion of gonS2 into pSETHe was confirmed by PCR and sequencing with primers cpS2-Xb and cpS2-ERV. Plasmid pC-gonS2 was transferred to the mutant strain ΔgonS2 by intergeneric conjugation to produce the strain CPgonS2 in which gonS2 deletion was complemented.

pC-gonCP
A DNA fragment of 1,361 bp containing the entire ORF of gonCP (nt -31 to +1,330 from start codon) was amplified with the primer pair cpCP-Xb/cpCP-ERV (Table S1). The resulting PCR fragment was digested with XbaI/EcoRV and cloned into the same sites of plasmid pSETHe (see methods in the article), under the control of the ermE*p promoter, to yield the complementation plasmid pC-gonCP. Insertion of gonCP into pSETHe was confirmed by PCR and sequencing with primers cpCP-Xb and cpCP-ERV. Plasmid pC-
gonCP was transferred to the mutant strain ΔgonCP by intergeneric conjugation to produce the strain CPgonCP in which gonCP deletion was complemented.

pC-gonMR
A DNA fragment of 600 bp containing the entire ORF of gonMR (nt -30 to +570 from start codon) was amplified with the primer pair cpMR-Xb/cpMR-ERV (Table S1). The resulting PCR fragment was digested with XbaI/EcoRV and cloned into the same sites of plasmid pSETHe (see methods in the article), under the control of the ermE*p promoter, to yield the complementation plasmid pC-gonMR. Insertion of gonMR into pSETHe was confirmed by PCR and sequencing with primers cpMR-Xb and cpMR-ERV. Plasmid pC-gonMR was transferred to the mutant strain ΔgonMR by intergeneric conjugation to produce the strain CPgonMR in which gonMR deletion was complemented.

pC-gonL1
A DNA fragment of 3,005 bp containing the entire ORF of gonL1 (nt -24 to +2,981 from start codon) was amplified with the primer pair cpL1-Xb/cpL1-ERV (Table S1). The resulting PCR fragment was digested with XbaI/EcoRV and cloned into the same sites of plasmid pSETHe (see methods in the article), under the control of the ermE*p promoter, to yield the complementation plasmid pC-gonL1. Insertion of gonL1 into pSETHe was confirmed by PCR and sequencing with primers cpL1-Xb and cpL1-ERV. Plasmid pC-gonL1 was transferred to the mutant strain ΔgonL1 by intergeneric conjugation to produce the strain CPgonL1 in which gonL1 deletion was complemented.
### Table S1. Primers used in this work

| Primer | Sequence (5'-3') | PCR product and purpose |
|--------|------------------|-------------------------|
| EcoRI-P1 | TATAGAATTCTCGCTCAAGGAGGTGCT | internal gene fragment for gonP1 inactivation |
| HindIII-P1 | TATAAAGCTTGGGCTCAACTCTTTAGTGTGC | |
| EcoRI-P8 | TATAGAATTCTGTAGTCCCCAGGAAGTAGGACAA | internal gene fragment for gonP8 inactivation |
| HindIII-P8 | TATAAAGCTTGGAGATGGTGAGAGGGTTACGTG | |
| SpeI-M4 | TATAACTAGTGACCATGCTGCTCCCCACTT | upstream flanking region for gonM4 deletion |
| NsiI-M4 | TATAATGCATTACTCTGCTCACACACCTACGG | |
| NdeI-M4 | TATAACATGCAAATCGGCTGTATGTGAAGA | downstream flanking region for gonM4 deletion |
| EcoRV-M4 | TATAGATACGTCTGAGTGGAGGACTACT | |
| SpeI-MT | TATAACTAGTGGTGAGAGGTTAAGATG | upstream flanking region for gonMT deletion |
| NsiI-MT | TATAATGCATCCATGAAGAGGAGGAAGTG | |
| BglII-MT | TATAAGATCGTGTACACTCAGCCAAAAGAGT | downstream flanking region for gonMT deletion |
| EcoRV-MT | TATAGATACGTTCTGGAATCTGTTGGT | |
| SpeI-SL | TATAACTAGTCTCAGAGGAGGCTCTGTACCTT | upstream flanking region for gonSL deletion |
| NsiI-SL | TATAATGCATTACGAGCAGAACACTCCGACAGA | |
| NdeI-SL | TATAACATGCAAATCGGCTGTATGTGAAGA | downstream flanking region for gonSL deletion |
| EcoRV-SL | TATAGATACACTGCAAATGACAGTAGCA | |
| fNsiI-S1 | TATAATGCATTACGAGCAGAACACTCCGACAGA | upstream flanking region for gonS1 deletion |
| rNsiI-S1 | TATAATGCATTACGAGCAGAACACTCCGACAGA | |
| BglII-S1 | TATAAGATCGTGTACACTCAGCCAAAAGAGT | downstream flanking region for gonS1 deletion |
| ERV-S1 | TATAGATACGTCTGGAATCTGTTGGT | |
| fNdeI-S2 | TATAACATGCAAATCGGCTGTATGTGAAGA | downstream flanking region for gonS2 deletion |
| rNdeI-S2 | TATAACATGCAAATCGGCTGTATGTGAAGA | |
| NsiI-S2 | TATAATGCATTACGAGCAGAACACTCCGACAGA | |
| SpeI-S2 | TATAACTAGTCTGGAAGAAGTGGGTACGG | upstream flanking region for gonS2 deletion |
| Restriction Enzyme | Sequence | Function |
|------------------|----------|----------|
| EcoRV-CP         | TATAGATATCGCACAGACCTTCTCCTCCAG | downstream flanking region for gonCP deletion |
| BamHI-CP         | TATAGGATCTACGAACTGGCTTGACCTG | |
| NsiI-CP          | TATAATGCATGTCGGGAGAGGTCGCTACTA | upstream flanking region for gonCP deletion |
| Spel-CP          | TATAACTAGTGTTGATATCTCCGTCTGAGT | |
| EcoRV-MR         | TATAGATATCCGATGAAGAACCCGTTCAC | downstream flanking region for gonMR deletion |
| BglII-MR         | TATAAGATCTGGCTGTGACCTG | |
| NsiI-MR          | TATAATGCATCCTCCAGCGGTAGGTCG | upstream flanking region for gonMR deletion |
| Spel-MR          | TATAACTAGTTACCTTCTGGCTTGCTCTCC | |
| EcoRV-L1         | TATAGATATCGCACCCCTGCTCTACTGCTG | downstream flanking region for gonL1 deletion |
| BglII-L1         | TATAAGATCTGGCAGCAACATCTGACATCC | |
| NsiI-L1          | TATAATGCATGTCGGGAGAGGTCGCTACTCAG | upstream flanking region for gonL1 deletion |
| Spel-orf9        | TATAACTAGTGCTCCGAGGTGCTGAC | upstream flanking region for orf9 deletion |
| NsiI-orf9        | TATAATGCATGTCGTCGTACGGGAACGG | |
| BglII-orf9       | TATAAGATCTCCAGCAGGAGAACCCTCTACC | downstream flanking region for orf9 deletion |
| EcoRV-orf9       | TATAGATATCCGATCTGGCAGCACTGTC | |
| BglII-orf10      | TATAAGATCTGGTCGTACGGGCTCTCAGCAG | downstream flanking region for orf10 deletion |
| NsiI-orf10       | TATAATGCATACTTGCTCCGAGGTGTCAG | upstream flanking region for orf10 deletion |
| Spel-orf10       | TATAACTAGTTATCGGAGATCCCTGAGCAAC | |
| EcoRV-orf11      | TATAGATATCGGCTCCTACGCTGGATCCTT | downstream flanking region for orf11 deletion |
| Ndel-orf11       | TATAGATCGGACTGGACGGACACAGTACCT | |
| NsiI-orf11       | TATAATGCATGTCGCTTGATGCGCTGAACT | upstream flanking region for orf11 deletion |
| Spel-orf11       | TATAACTAGTGCTCCGAGGTGCTGTC | |
| Spel-orf13       | TATAACTAGTTACCTTGTCCCTGTCAGGTTCAG | upstream flanking region for orf13 deletion |
| Pstl-orf13       | TATAGTCACTCGCTGATGAAAGTTGAGCAG | |
| BamHI-orf13      | TATAGGATCTGGTCGATGAAAGTTGAGCAG | downstream flanking region for orf13 deletion |
| EcoRV-orf13      | TATAGATATCGGAGAACCCTGAGG | |
| cpP8-ERV  | TATAGATATCGACTCCACGGGAGTTGATGT | gonP8 complementation |
| cpP8-Xb  | TATATCTAGATGATGATGGAATCCGAGAAGC |
| cpM4-Xb  | TATATCTAGAGCTCGACGAGCCCCCGAG |
| cpM4-ERV | TATATGATCCATGTGGAACCTGTGCGATG |
| cpMT-Xb  | TATATCTAGACCCTCCTCCTTCATGG | ΔgonMT complementation |
| cpMT-ERV | TATATATCTCTGGACGCTTATGACACAA |
| cpSL-Xb  | TATATCTAGAAGCCCTGAGATGAGTC |
| cpSL-ERV | TATAGATATCGAGCTCGACGAGCGCGT |
| cpS1-Xb  | TATATGATACACAGAAGATGAGATT | ΔgonS1 complementation |
| cpS1-ERV | TATATGATACCTCTTGACCTGGATGTC |
| cpS2-Xb  | TATATCTAGAGGAGCGGAGAACCCAG |
| cpS2-ERV | TATAGATATCCTGATTCTCGGATA |
| cpCP-ERV | TATAGATATCCCAATGGGAATTCACTGATAA | ΔgonCP complementation |
| cpCP-Xb  | TATATCTAGACGCAGGAGAGACCCAATCC |
| cpMR-ERV | TATAGATATCCTGACCCTGCGGCTCCAG |
| cpMR-Xb  | TATATCTAGAGGAGCGGAGAGACCCAATCC | ΔgonMR complementation |
| cpL1-ERV | TATAGATATCGACCCGGGATAGTATA | ΔgonL1 complementation |
| cpL1-Xb  | TATATCTAGAAGGTGCGTGACAGGAGACT |
| cfM4     | CTGGACGGATATGGAACTGT | used with Apra60 for ΔgonM4 confirmation |
| cfMT     | CACACCAGCATGAACGACT | used with ApraII for ΔgonMT confirmation |
| cfSL     | TCTTCTGCTAGCTGTGGAGAC | used with Apra60 for ΔgonSL confirmation |
| cfS1     | GCGTGGATGCTCTACACCTC | used with Apra60 for ΔgonS1 confirmation |
| cfS2     | GGCCGAGGACTCCTCAG | used with ApraII for ΔgonS2 confirmation |
| cfCP     | TCGACTTCGAGACCAATGTG | used with Apra60 for ΔgonCP confirmation |
| cfMR     | CTCACCTGCTGCTCTCA | used with ApraII for ΔgonMR confirmation |
| cfL1     | CACCGGCTACCTCCTTCC | used with ApraII for ΔgonL1 confirmation |
| cf09     | CTTCATCCACGAGACAAAGG | used with Apra60 for Δ5201 confirmation |
| cf10     | AGCACCAGATCAGTTCCTTC | used with ApraII for Δ5257 confirmation |
|  | Sequence | Description |
|---|---|---|
| cf11 | GAAGACCTCCACCAGGTCCA | used with ApraII for Δ5259 confirmation |
| cf13 | CGACGTTGTGACGAGTACG | used with Apra60 for Δ5261 confirmation |
| Apra60 | CCAAGGTGGAAGCTGACC | reverse primer annealing to aac(3)IV |
| ApraII | CTTCAGGATGGCAAGTTGGT | forward primer annealing to aac(3)IV |
| cfP1 | GAAGCGACCAGTCCGTAAC | used with M13r for gonP1 confirmation |
| cfP8 | GTGATTGGAATCCGAGAAGC | used with M13r for gonP8 confirmation |
| M13r | CAGGAAACACGCTATGAC | M13 reverse primer |