

Supporting Information

Novel tools for genomic modification and heterologous gene expression in the phylum *Planctomycetota*

Tom Haufschild¹, Jonathan Hammer¹, Nico Rabold¹, Veronika Plut¹, Christian Jogler^{1,2,*} and Nicolai Kallscheuer^{1,*}

1 - Department of Microbial Interactions, Institute for Microbiology, Friedrich Schiller University, 07743 Jena, Germany

2 - Cluster of Excellence Balance of the Microverse, Friedrich Schiller University, Jena, Germany

*Corresponding author:

Christian Jogler, e-mail: christian.jogler@uni-jena.de

Nicolai Kallscheuer, e-mail: nicolai.kallscheuer@uni-jena.de

Running Title: Novel genetic tools for planctomycetes

Keywords:

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Supporting Tables

The following supporting tables are provided as a separate Excel file:

Table S1. Strains and plasmids used in the study.

Table S2. Oligonucleotides used in the study.

Supporting Figures

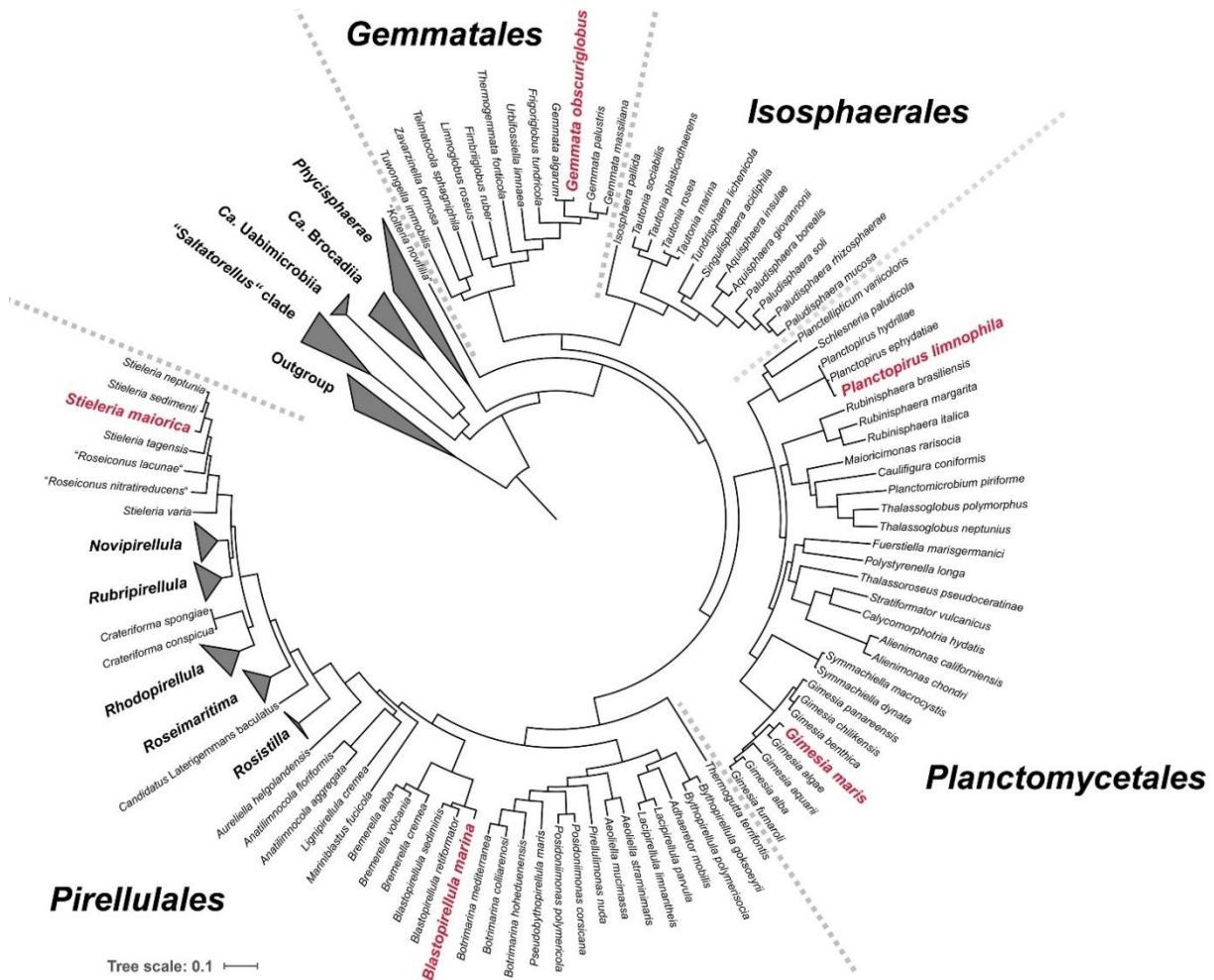


Figure S1. Phylogenetic tree highlighting the hitherto genetically modified planctomycetotal species. Multi-locus sequence analysis-based maximum likelihood phylogenetic tree based on all current type strains in the phylum *Planctomycetota*. Dashed lines delineate different orders. For simplicity, only the species names are shown on the branches while the names of the respective type strains were omitted. Species that have been genetically modified in this and previous studies are highlighted in red. The outgroup consists of three genomes of strains outside of the phylum *Planctomycetota*, but part of the *Planctomycetota*-*Verrucomicrobiota*-*Chlamydia* (PVC) superphylum.

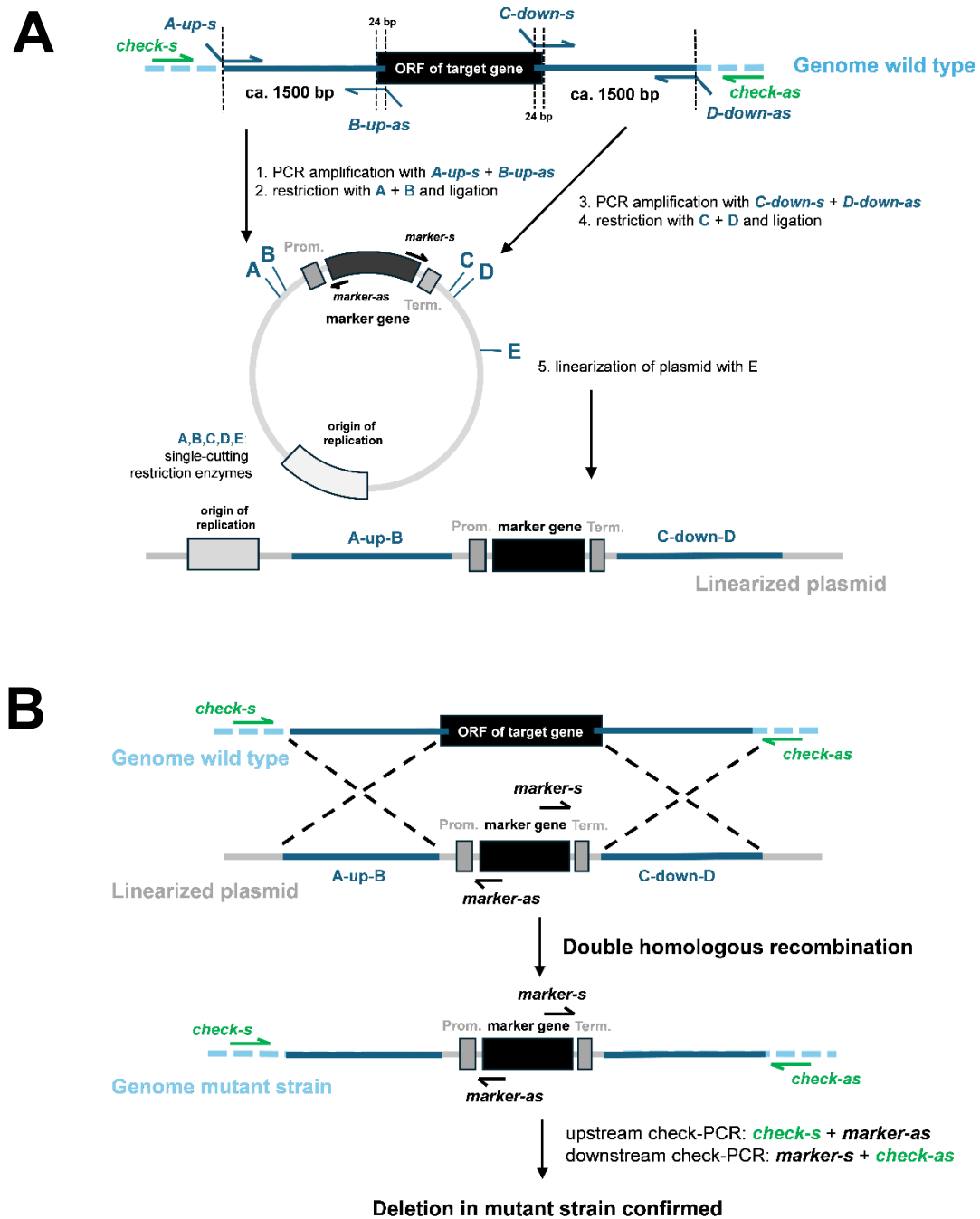


Figure S2. Gene deletion strategy via double homologous recombination. A) amplification and cloning steps: ca. 1500 bp flanking regions (including the first and last 24 bp of the open reading frame of the targeted gene) were amplified and cloned into the multiple cloning sites of a plasmid harboring the desired resistance marker gene that is later inserted into the chromosome in the deletion mutant. B) enforced double homologous recombination after transformation of the planctomycetotal strain.

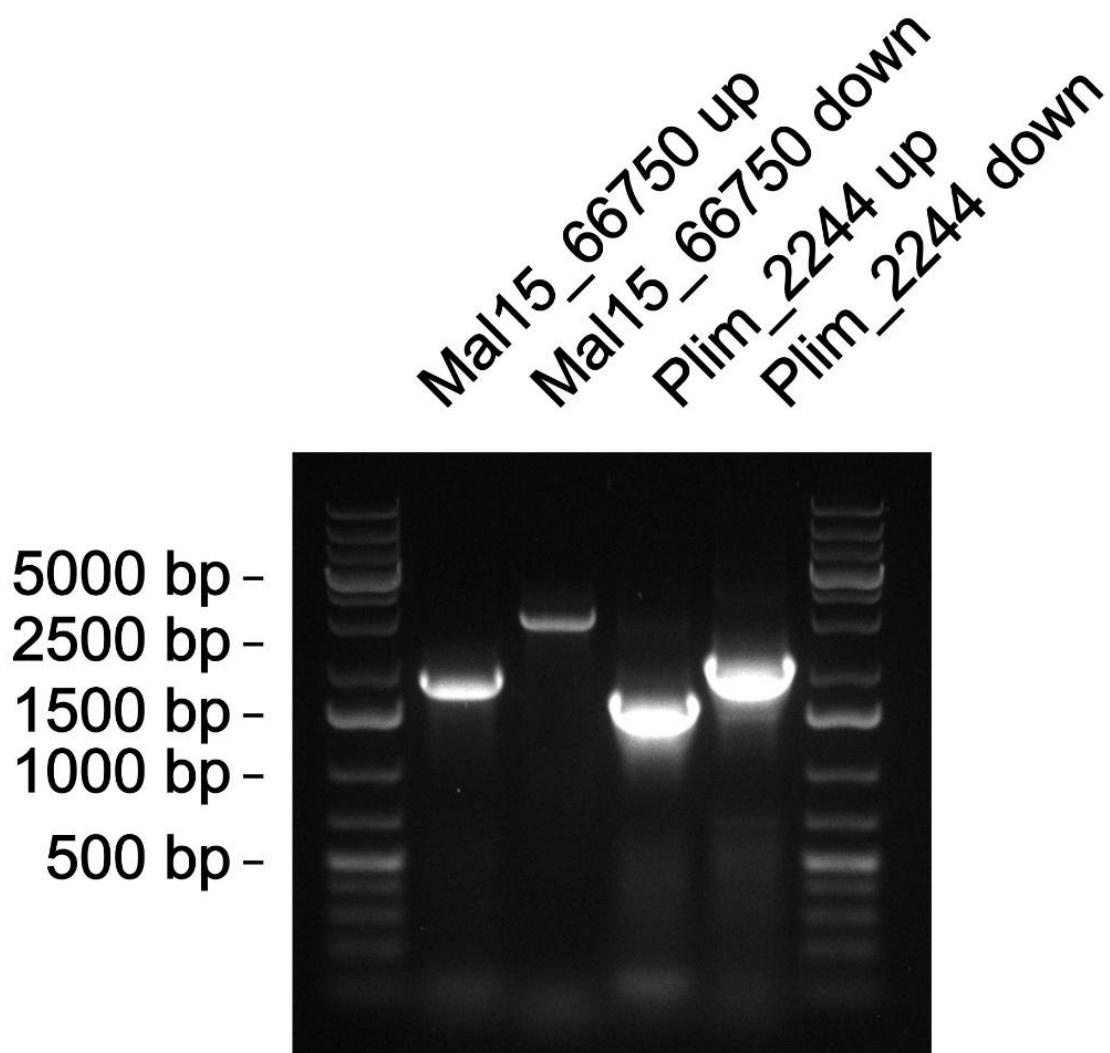


Figure S3. PCR-based verification of the *hpnD* deletion in *S. maiorica* and *P. limnophila*. PCR products of up- and downstream flanks of the *hpnD* deletion mutant strains with primers as shown in Fig. S2B confirmed the successful exchange of the native gene with the resistance cassette. Depending on the primers used, expected product sizes ranged between 1.7 and 3.2 kilobases.

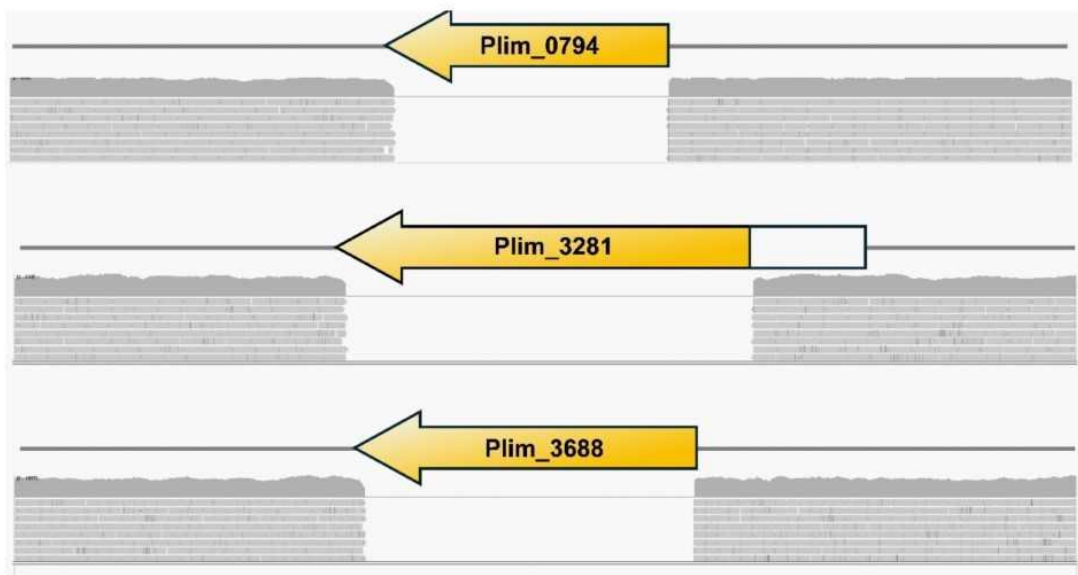
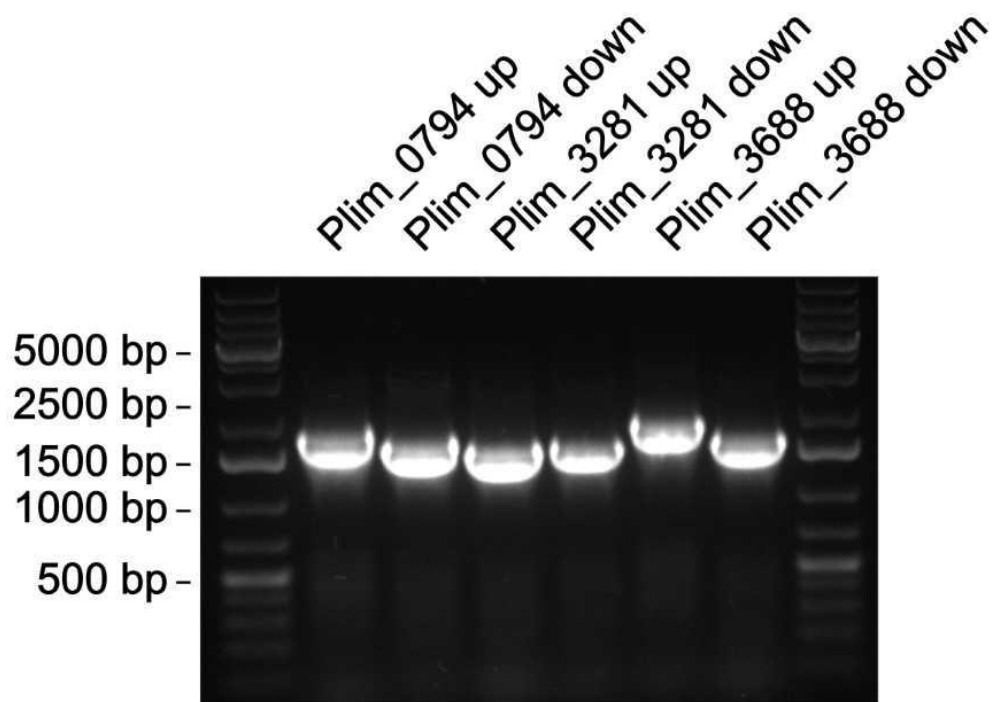
A**B**

Figure S4. Verification of the *pilQ* deletions in *P. limnophila*. A) Obtained short reads of Illumina-sequenced genomic DNA of the *pilQ* single gene deletion mutants were mapped against the reference sequence (wild-type strain). The absence of reads in the coding region of the putative *pilQ* genes Plim_0794, Plim_3281 and Plim_3688 confirmed the expected single gene deletions. The non-filled white box indicates an alternative start codon in the automated RefSeq vs. GenBank-annotated genome of *P. limnophila*. B) PCR-based verification of up- and downstream flanks of the three genomic loci modified in the triple deletion mutant. Depending on the primers used (Fig. S2B), expected product sizes ranged between 1.7 and 1.9 kilobases.

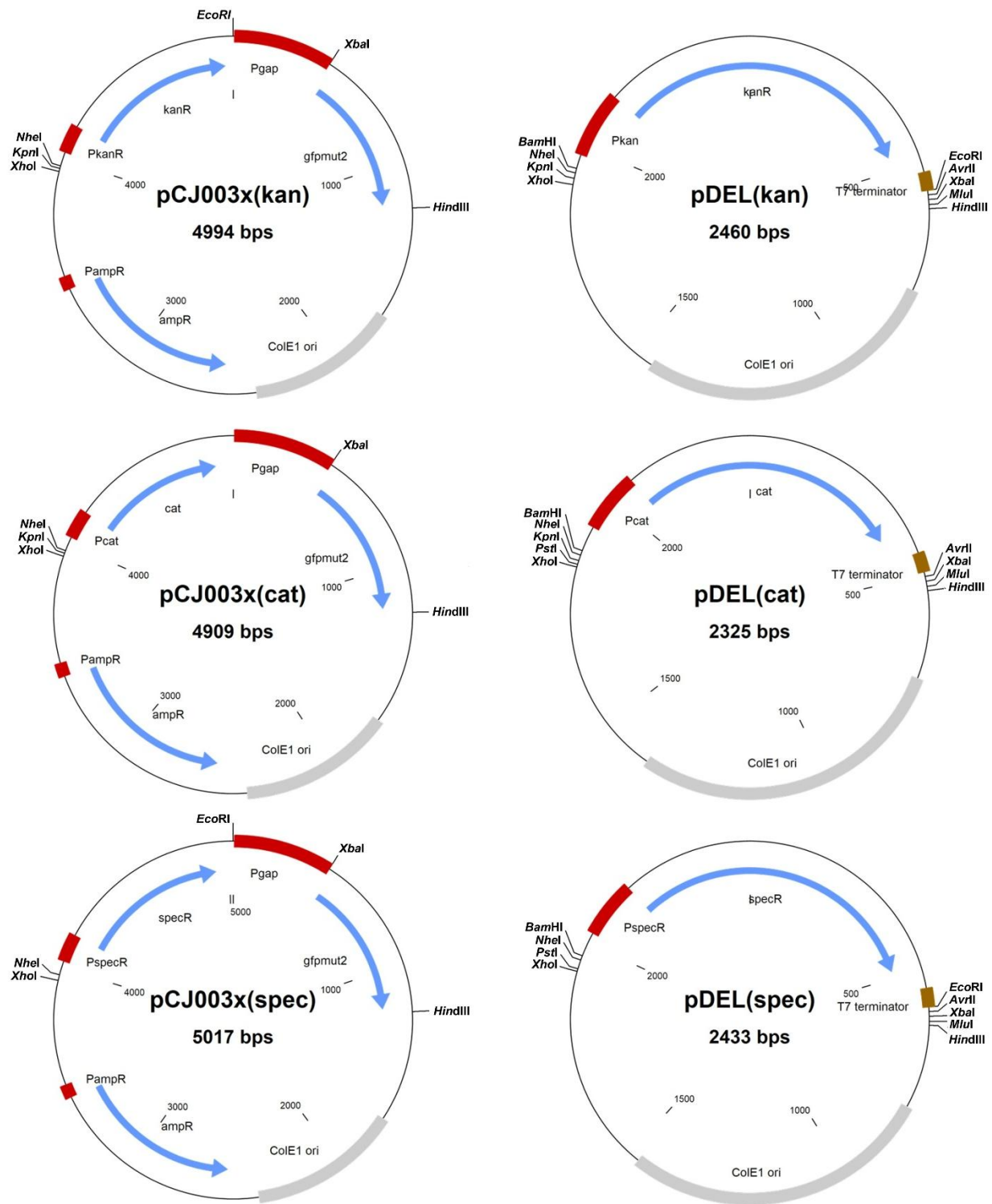


Figure S5. Plasmid maps of the pCJ003x and pDEL plasmids with different resistance marker genes. Unique sites for restriction enzymes available for cloning are shown. In the pCJ003x plasmids the *P_{gap}*-*gfpmut2* region was excised during cloning of the downstream homology region. kanR: kanamycin resistance gene, cat: chloramphenicol acetyltransferase (chloramphenicol resistance gene), specR: spectinomycin resistance gene, ampR: ampicillin resistance gene.

Nucleotide sequences used in the study

a) Gene sequences

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b) Promoter sequences

Predicted -35 and -10 regions are underlined.

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