Identification of novel *mazEF/pemIK* family toxin-antitoxin loci and their distribution in the *Staphylococcus* genus

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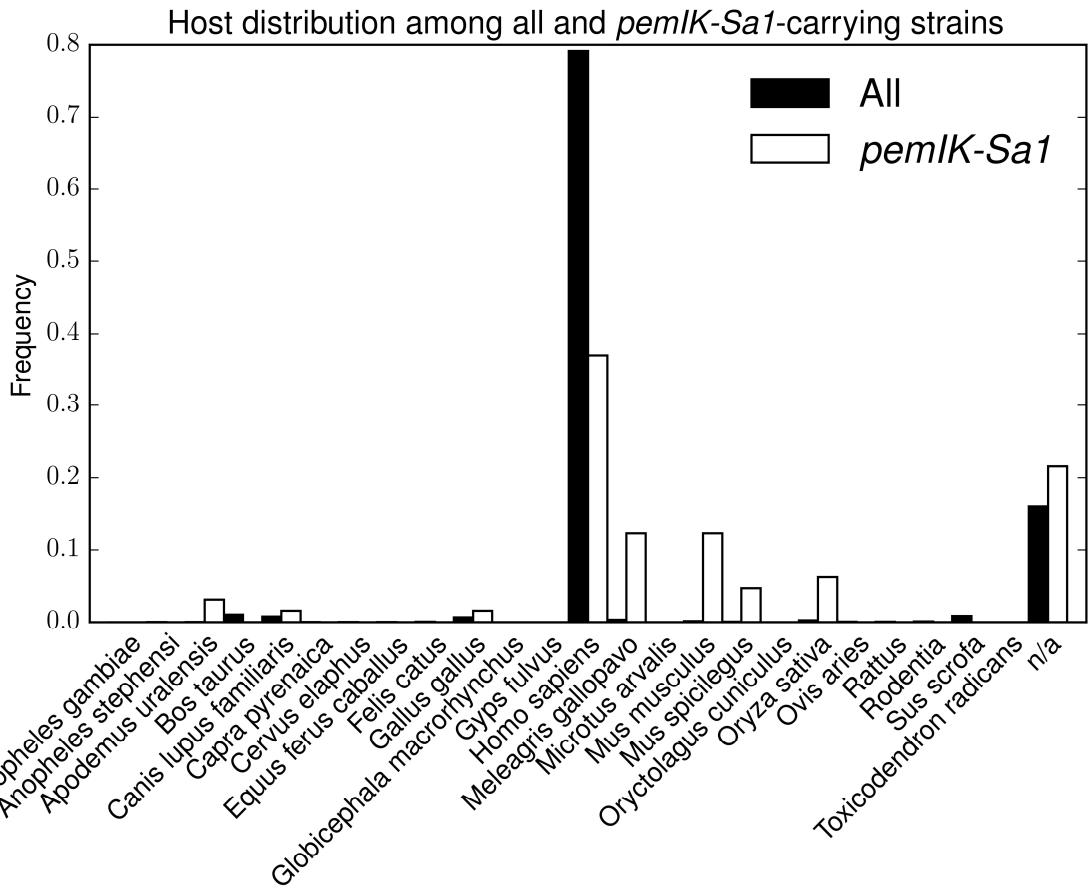
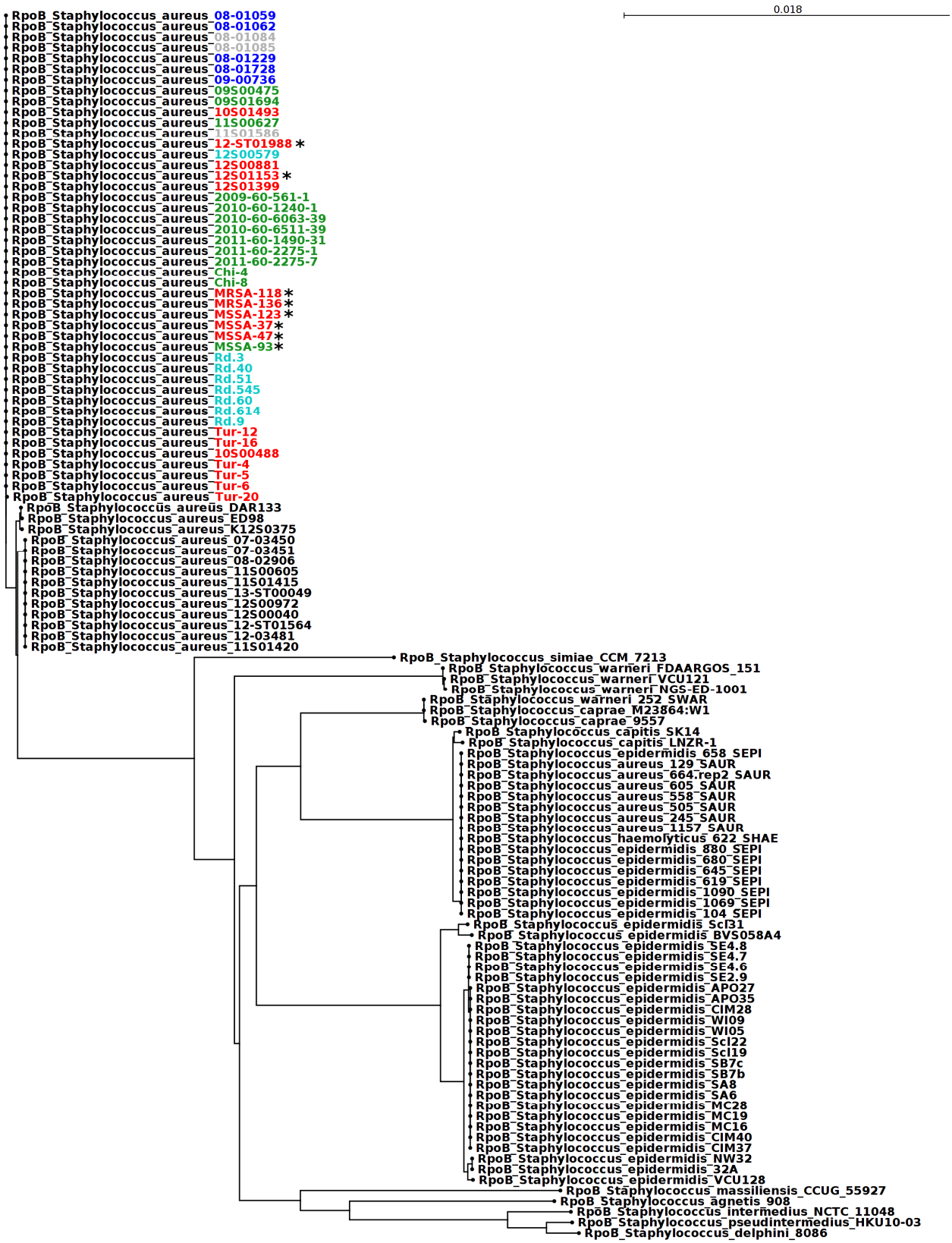
SUPPLEMENTARY DATA

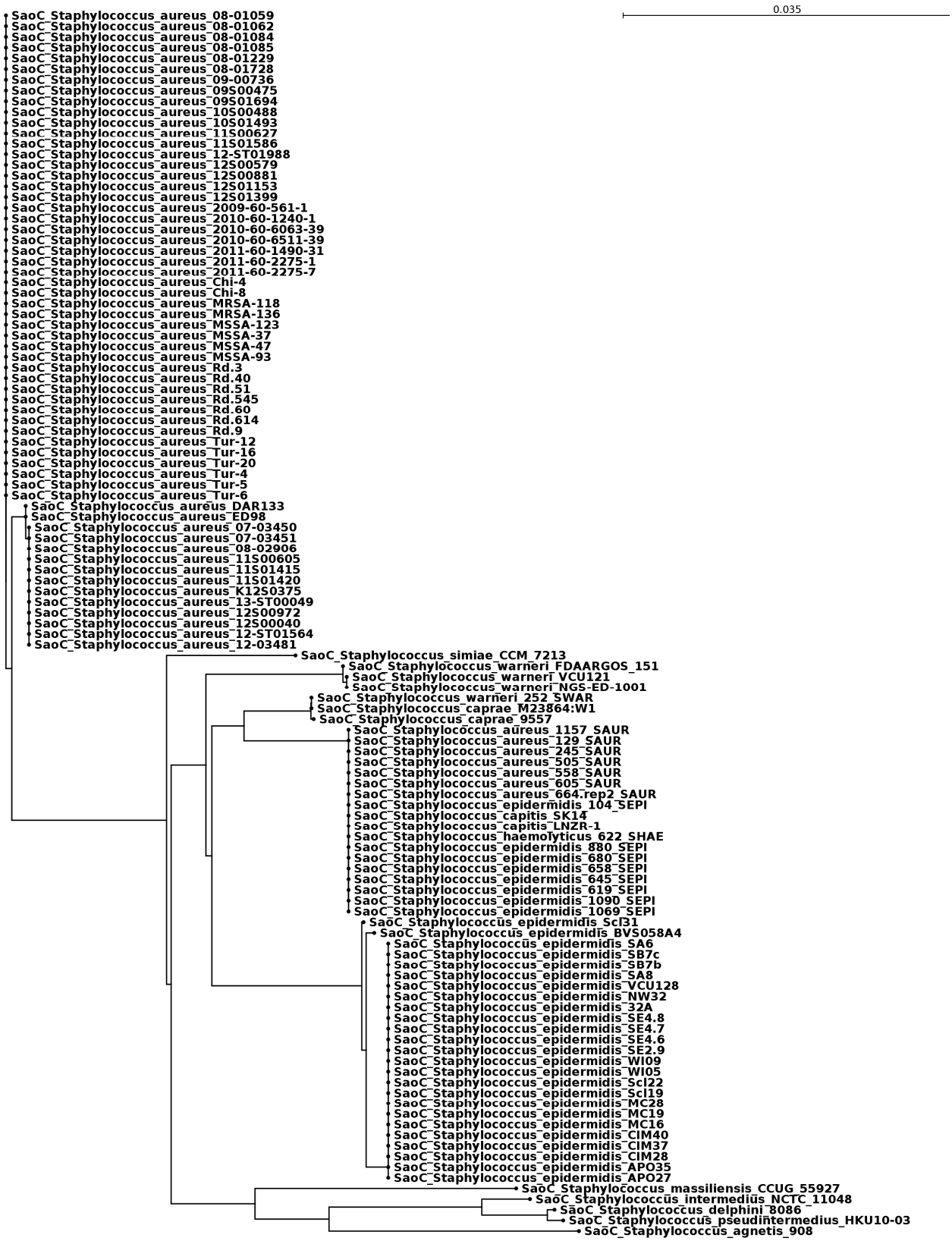
Supplementary note on construction of phylogenetic trees. The following parameters were used for multiple sequence alignments of DNA sequences; gap open cost: 20.0; gap extension cost: 1.0; end gap cost: free; alignment mode: very accurate; and the following ones for protein sequences; gap open cost: 10.0; gap extension cost: 1.0; end gap cost: free; alignment mode: very accurate. For DNA sequences the model choice and parameters were as following; construction method: neighbour joining [1]; nucleotide substitution model: general time reversible, GTR [2]; transition/transversion ratio: 2.0; include rate variation: yes; number of substitution rate categories: 8; gamma distribution parameter: 1.0; estimate substitution rate parameters, topology and gamma distribution parameter: yes; bootstrap analysis with 100 replicates; and for protein sequences as following; construction method: neighbour joining [1]; protein substitution model: WAG [3]; transition/transversion ratio: 2.0; include rate variation: yes; number of substitution rate categories: 8; gamma distribution parameter: 1.0; estimate substitution rate parameters, topology and gamma distribution parameter: yes; bootstrap analysis with 100 replicates. Prototypical protein sequences of all MazF/PemK toxins were aligned with the following parameters: gap open cost: 20.0; gap extension cost: 1.0; end gap cost: as any other; alignment mode: very accurate; and visualised using CLC Main Workbench.



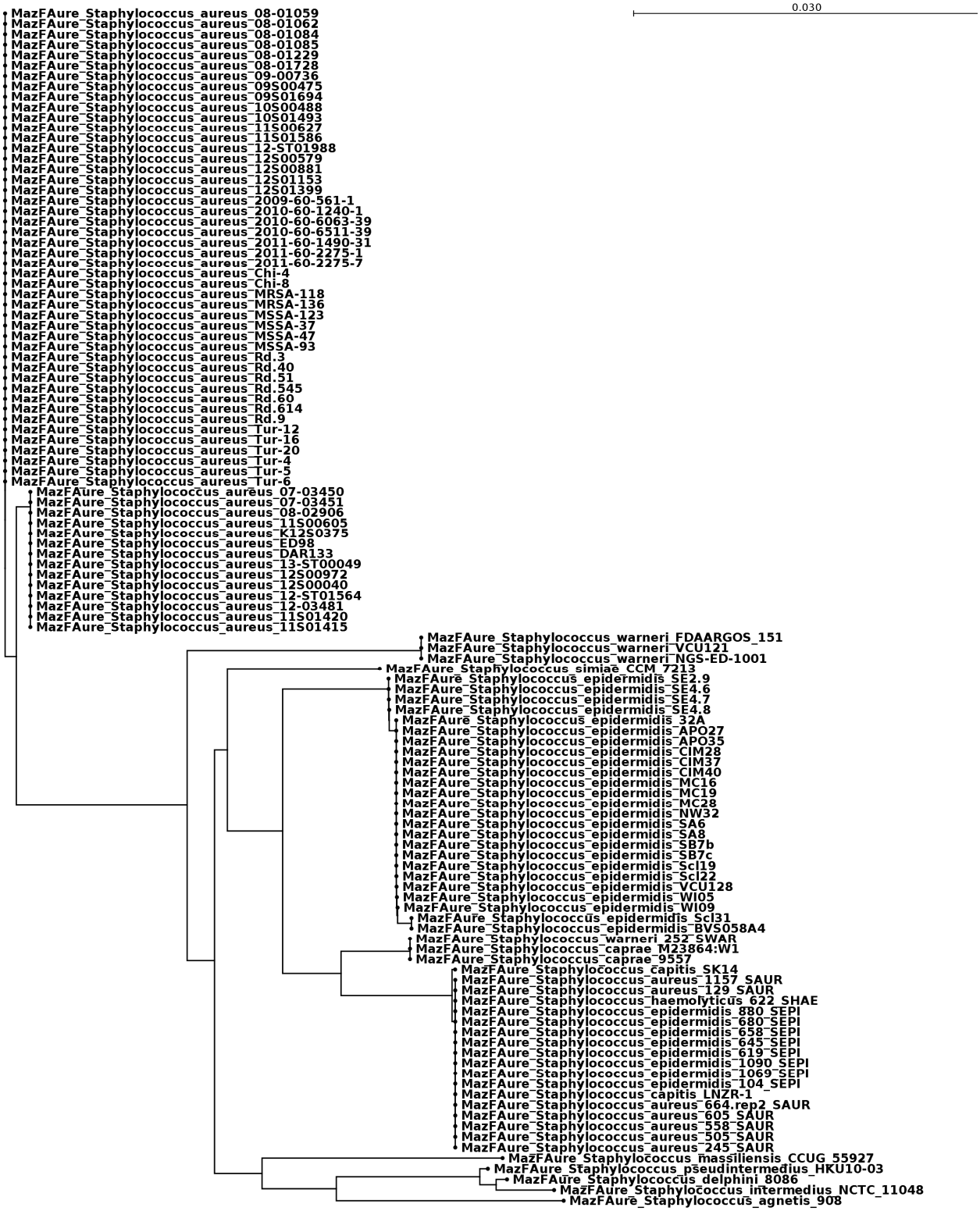
Suppl. fig. 1. Host distribution of *pemIK-Sa1* loci.



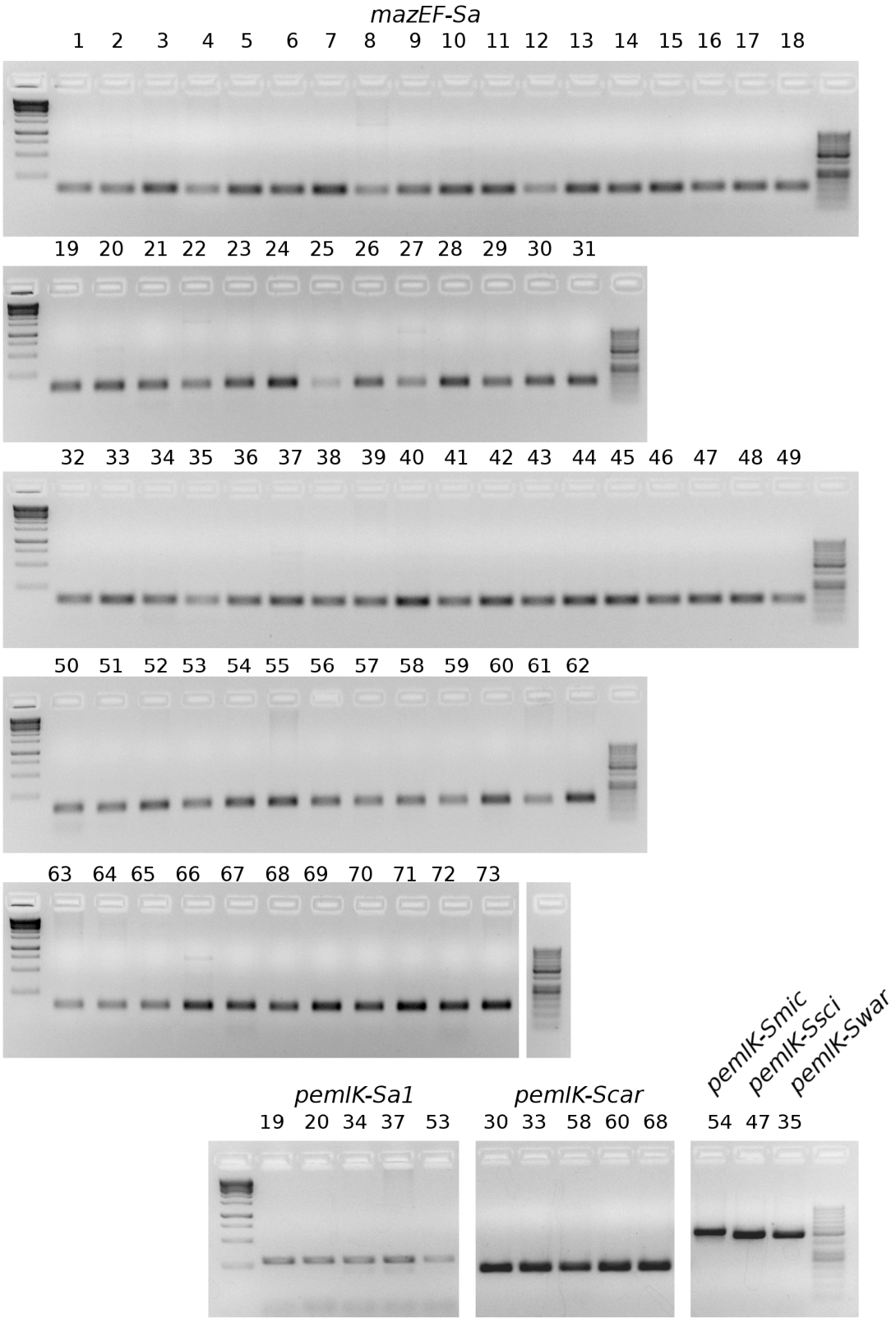
Suppl. fig. 2. Phylogenetic tree based on gapless alignment of *rpoB* gene sequences derived from strains isolated in Germany and all strains possessing *pemIK-Sa1* locus. Clolours of signatures of closely related strainsdenote the host:: unknown, light grey; *Bos taurus*, skyblue; *Gallus gallus*, green; *Homo sapiens*, blue; *Meleagris gallopavo*, red. The *pemIK-Sa1*-carrying strains are marked with asterisk.



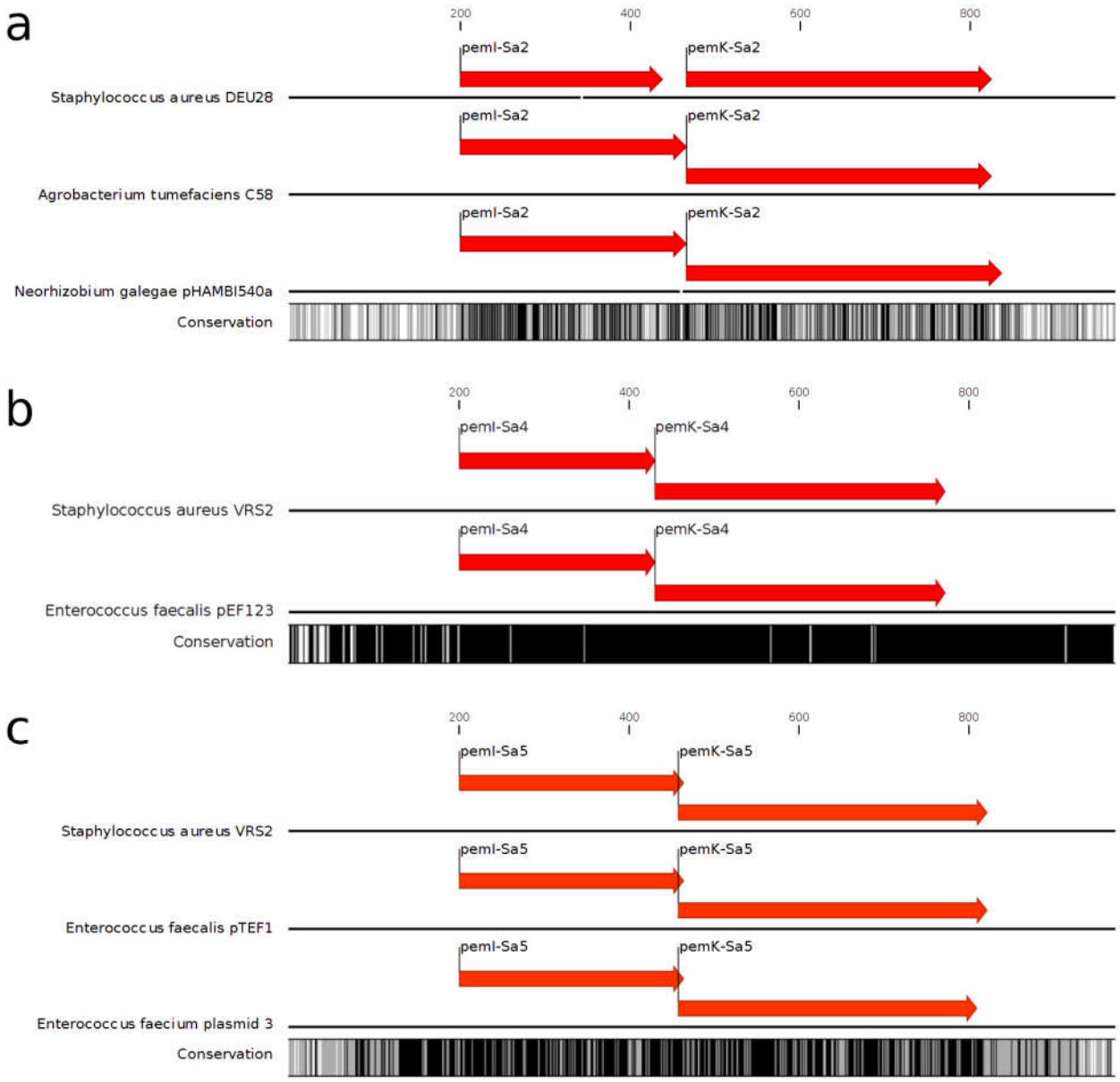
Suppl. fig. 3. Phylogenetic tree based on gapless alignment of *saoC* gene sequences derived from strains isolated in Germany and all strains possessing *pemIK-Sa1* locus.



Suppl. fig. 4. Phylogenetic tree based on gapless alignment of *mazF* gene sequences derived from strains isolated in Germany and all strains possessing *pemIK-Sa1* locus.



Suppl. fig. 5. Whole-lane images used for preparation of fig. 10: PCR screen for *mazEF/pemIK* loci. The numbers correspond to the number in fig. 10. The first and the last lane: length standards, respectively Gene Ruler 1 kb and Gene Ruler 50 bp (Thermocientific). White gaps: irrelevant lanes were removed. All fragments in one row are fragments of the same gel image.



Suppl. fig. 6. Conservation of nucleotide sequences of different *pemIK* loci occurring in *S. aureus* and other species. The conservation scale ranges from 50% to 100% (white to black). Each fragment is ~ 1 kb long, the bp ruler is placed above each alignment. In all cases higher conservation is observed within the coding sequences, which when translated are more than 80% similar. The adjacent non-coding sequences are more diverse, likely due to variability in species-specific promoter and other regulatory sequences. (**a**) *pemIK-Sa2* loci are the most divergent, still much higher conservation of coding sequences is clearly visible. (**b**) On the other hand, although *pemIK-Sa4* sequences are highly conserved, there are clear differences in 5'-UTRs. (**c**) *pemIK-Sa5* loci are more diverse than co-occurring with them *pemIK-Sa4* ones and as in other cases higher variability in non-conding sequences is observed.

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