## **Excluded primers**

nuoL1\_Amplicon:245-727\_Forward:225-244

5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACCGAAACATTACCGCGAAC 3' nuoL1\_Amplicon:245-727\_Reverse:747-728

5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATAGCCGCGTTTTGCTCAAT 3' nuoL1\_ Amplicon:245-727\_Reverse:744-728 (shorter version)

5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATAGCCGCGTTTTGCTC 3' nuoL1 Amplicon:245-727 Forward:225-244 (including generic bases)

5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACCYAAACYTTACCGCGAAC 3' nuoL1\_Amplicon:245-727\_Reverse:747-728 (including generic bases)

5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATAGCCGCGTTTWGCTCAAT 3' nuoL2 Amplicon:714-1138 Forward:693-713

5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGTACGTCCTTAACACTTAC 3' nuoL2 Amplicon:714-1138 Reverse:1158-1139

5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTTCTATGTTCATTACTGG 3' weeC1 Amplicon:287-762 Forward:267-286

 $5 `TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCCAGTACACTGTAAAGCG 3' we cC1\_Amplicon: 287-762\_Reverse: 782\_763$ 

5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCGATTTGTTACTGGCGG 3' wecC1\_ Amplicon:287-762\_Reverse:782\_763 (including generic bases)

5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCGATTTGTTACTGGMGG 3' weeC2\_Amplicon:287-772\_Forward:267-286

5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCCAGTACACTGTAAAGCG 3' weeC2\_Amplicon:287-772\_Reverse:792-773

 $5°GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCATGCCTAGTTGTCGATTTG~3° group\_998\_Amplicon: 255-774\_Forward: 235-254$ 

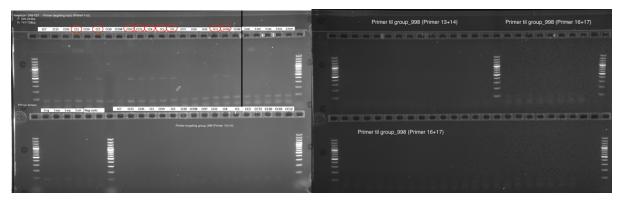
5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCACCAGCACTTTACCTCT 3' group 998 Amplicon:255-774 Reverse:794-775

5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCCGTTAAAAAACTGAAC 3' group\_998\_Amplicon:255-774\_Reverse:794-775 (including generic bases)

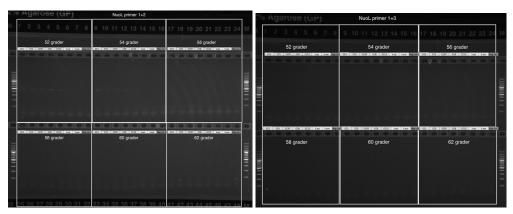
5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCYGTTAAAAAACTGAAC 3' group\_Amplicon:998\_576-977\_Forward:556-575

5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTACAAACGTAAACTTGGCAC 3' group 998\_Amplicon:576-977\_Reverse:997-978

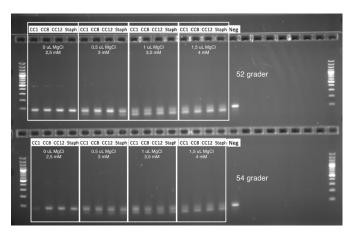
 $5 \\ \hbox{'} GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCCTAATGGTTTAAGTCCT 3 \\ \hbox{'}$ 



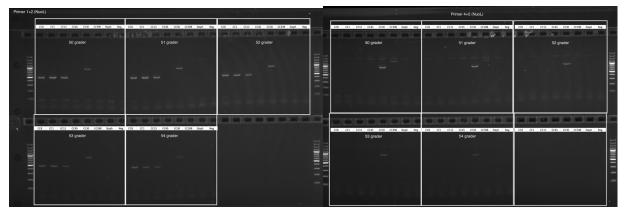
**Test of three random primer pairs (**nuoL primer, two g998 primer pairs). Test on a selection of CC isolates. Abbreviation: CC: clonal complex. *Epi: S. epidermidis; War=S. warneri; Hae:S. haemolyticu;, Hom: S. hominis; Lug: S. lugdunesis; Sap: S. saprophyticu;, Arg; S. argenteus; Ach: S. schweitzeri.* 



**Temperature gradient test** (nuoL1 primers). Test on a selection of CC isolates. Abbreviation: CC: clonal complex. *Epi: S. epidermidis; War=S. warneri* 



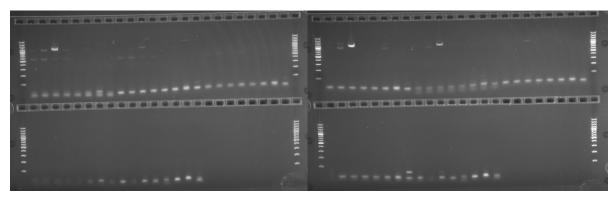
**Temperature gradient** + **change of MgCl**<sup>2+</sup> **concentration** (nuoL1 primers). Test of nuoL primers on CC1, CC8, and CC12 and a *Staphylococcus* control. Test of MgCl<sup>2+</sup> gradient (2.5 mM, 3 mM, 3.5 mM, and 4 mM). Abbreviation: CC: clonal complex; MgCl: magnesium chloride.



Change of elongation time and temperature gradient (nuoL1 primers). Change of elongration from 45 sec. to 1 min. PCR program: 3 min. at 95°C followed by 25 cycles of 20 sec at 98 °C, 15 sec at 50-54 °C, 1 min. at 72 °C, and 5 min. at 72 °C. Temperature gradient from 50-54 °C. Abbreviation: CC: clonal complex; Staph: Staphylococcus



Test of touchdown PCR (Tested all clonal complex isolates on nuoL primers). PCR program: (-0,5% touchdown 10 cycles,  $55^{\circ}\text{C} \rightarrow 50^{\circ}\text{C}$ ), annealing temperature  $52^{\circ}\text{C}$ 



**Test of new polymerases** + **longer elongation time.** Test of Phusion Hot Start II High-fidelity PCR master mix (left) and AQ97 High-Fidelity DNA Polymerase 2x Master Mix (right). Changed elongation time from 45 sec. to 1 min.