DNA Preparation

To obtain a DNA preparation, a loopful of *Staphylococcus aureus* cells are washed with distilled water and incubated with 200 µl of 6% InstaGene matrix solution (BIO-RAD, München, Germany) for 20 min at 56°C. The suspension is vortexed and heated for 8 min at 100°C and centrifuged at 8,000 x *g* for 2-3 min. Twenty microliters of the supernatant are used for PCR amplification. Alternatively, several loops of bacterial cells are washed with distilled water and incubated in 500 µl TE buffer (Tris-HCl, 10 mM; EDTA, 0.1mM; pH 7.5) for 10 min at 100°C for bacterial inactivation. The 500 µl cell suspension plus 150 µl of acid-washed glass beads (#G 4649; Sigma, Taufkirchen, Germany) are then lysed mechanically using a Mixer Mill MM 200 (Retsch GmbH, Haan, Germany) at maximum speed for 7 min. The lysate is centrifuged at 12,000 x g for 10 min to precipitate cellular debris and 200 µl of the supernatant is transferred to a new sterile tube and cleaned further with a QIAamp DNA Blood Mini Kit (Blood and Body Fluid Spin Protocol; Qiagen, Hilden, Germany). Five microliters of the supernatant are used for PCR amplification. The lysates is then stored at -20°C prior to carrying out the PCR.

DNA Amplification

For amplification of the *Staphylococcus* protein A (*spa*) repeat region, a PCR is performed in a total volume of 50 µl containing cleaned DNA, 200 µM deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), 10 pmol of each primer, 5 µl of 10-fold concentrated PCR Buffer II (Applied Biosystems), $MgCl_2$ 1.5 mM, and 1.25 U of AmpliTaq DNA polymerase (Applied Biosystems). Thermal cycling reactions consist of an initial denaturation (5 min at 80°C) followed by 35 cycles of denaturation (45 s at 94°C), annealing (45 s at 60°C), and extension (90 s at 72°C), with a single final extension (10 min at 72° C). HPLC-cleaned primers spa-1113f (5'- TAA AGA CGA TCC TTC GGT GAG C -3') and spa-1514r (5'- CAG CAG TAG TGC CGT TTG CTT -3') are used for amplification. The primers are numbered from the 3' end of the primer on the forward strand of *S. aureus* (GenBank accession no. J01786; spa-1113f [1092-1113] & spa-1514r [1534-1514]).

DNA Sequencing

The PCR product is purified by an enzymatic method using exonuclease I (New England Biolabs GmbH, Frankfurt-Hoechst, Germany) and shrimp alkaline phosphatase (Amersham Pharmacia Biotech). Briefly, 5 µl of the PCR product is incubated with 1 U of each enzyme at 37°C for 30 minutes. Then the enzymes are inactivated at 80°C for 15 minutes and the PCR products finally stored at 4°C. The amplicons are sequenced using the ABI Prism BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems). The sequencing reaction requires 1.0 µl of premix from the kit, 1.5 µl TrisHCl/$MgCl_2$ buffer (400mM Tris-HCl; 10mM $MgCl_2$), 10 pmol of sequencing primer, and 2 µl of the cleaned PCR product in a total volume of 10 µl. The same primers used in the PCR are used for sequencing with an annealing temperature of 60°C. All sequencing reactions are performed using a T1 Thermocycler (Whatman Biometra, Göttingen, Germany) with 25 cycles of denaturation (96°C, 10 s), and extension (60°C, 4 min). The sequencing products are purified using the Centri-Sep Spin Columns (Princeton Separations, Adelphia, NJ) and are prepared for running on the ABI 3100 Avant Genetic Analyzer in accordance with the instructions of the manufacturer (Applied Biosystems). Alternatively, larger batches of sequencing products are purified with MultiScreen HV plates (Millipore, Billerica, Massachusetts, USA) loaded with Sephadex G50 Superfine columns (Amersham Biosciences, Freiburg, Germany) according to the instructions of the manufacturer (Millipore Tech Note TN053; i.e. including a 150 µl pre-rinse step necessary for capillary sequencers).

Spa Sequence Analysis

The software Ridom StaphType™ (Ridom GmbH, Würzburg, Germany) is used for *spa* sequence analysis.

Ridom GmbH gives no guarantee that the protocols described in this document will be successful in every laboratory. Ridom GmbH cannot therefore, be held liable for any problems arising during their implementation.