

DSLST protocol for *Staphylococcus aureus*

A detailed protocol can be requested at contact@dlst.org.

The DLST is based on the analysis of 508 bp and 501 bp of two highly variable regions of the *clfB* and *spa* genes, respectively, of *Staphylococcus aureus*.

Chromosomal DNA extraction of *S. aureus*

The DNA extraction protocol is based on a filtration process in a 96-wells plate covered with a silica membrane (detailed references available upon request). This reduces time and cost per DNA extraction. However, traditional DNA extraction methods for *S. aureus* are also fully appropriate for DLST.

PCR amplification of the repetitive elements of the *clfB* and *spa* regions

This PCR protocol is used to amplify highly polymorphic repetitive regions of the *clfB* and *spa* genes. The priming regions are localized on highly conserved regions outside of the polymorphic regions. The size of the amplification products are between 800pb – 1.3kb for *clfB* and 1 – 1.5 kb for *spa*. The forward and reverse primers are:

SA_ <i>clfB</i> _1586.forward (5'-3')	TCGGTTGGAATAATGAGAATGTTG
SA_ <i>clfB</i> _2658.reverse (5'-3')	TTTGTGTTTTTCGCTCTTATCTCCT
SA_ <i>spa</i> _729.forward (5'-3')	ACTAGGTGTAGGTATTGCATCTGT
SA_ <i>spa</i> _1984.reverse (5'-3')	TCCAGCTAATAACGCTGCACCTAA

The two loci can be combined in a single multiplexed PCR with the following conditions: 0.05mM dNTPs, 0.05µM of each of the four primers, 1x Taq PCR buffer with MgCl₂ (1.5mM), 1.25U Taq polymerase (GenAmp high fidelity, Applied biosystem). PCR amplification was performed in a 25µl final volume using the following profile: 2min of initial denaturation at 94°C followed by 33 cycles of 15sec denaturation at 94°, 30 sec of annealing at 50°C, 1min of extension at 72°C with a 5 sec elongation time increase at each cycle from cycle 11 to 33; and a final extension of 7min at 72°C. Some optimization may be required according to the material used.

Single amplification of the *clfB* locus can be obtained with the following conditions: 0.05mM dNTPs, 0.05µM of each of the two *clfB* primers, 1x Taq PCR buffer with MgCl₂ (1.5mM), 1.25U Taq polymerase (GenAmp high fidelity, Applied biosystem). PCR amplification was performed in a 25µl final volume using the following profile: 2min of initial denaturation at 94°C followed by 33 cycles of 15sec denaturation at 94°, 30 sec of annealing at 58°C, 1min of extension at 72°C with a 5 sec elongation time increase at each cycle from cycle 11 to 33; and a final extension of 7min at 72°C.

Single amplification of the *spa* locus can be obtained with the following conditions: 0.1mM dNTPs, 0.25µM of each of the two *clfB* primers, 1.5mM of MgCl₂, 1x Taq PCR buffer without MgCl₂, 0.5U Taq polymerase (recombinant, Invitrogen). PCR amplification was performed in a 25µl final volume using the following profile: 3min of initial denaturation at 94°C followed by 34 cycles of 30sec denaturation at 94°, 30 sec of annealing at 54°C, 2min of extension at 72°C; and a final extension of 5min at 72°C.

Sequencing of the reverse fragment

DNA fragments of 508bp for *clfB* and 501bp for *spa* have been selected for the *S. aureus* DLST typing method and the protocol has been adapted for the 3' sequencing (i.e. using the reverse primer) of these fragments. Annealing temperature for the sequencing reaction is 58 °C for the *clfB* and 54°C for the *spa*.

Trimming patterns

Both sequences must be trimmed after a start signature that can be recognized on the beginning of the sequence:

<i>Locus</i>	<i>start signature</i>	<i>length</i>
<i>clfB</i>	CTTTAGGATTTGAT	508bp
<i>spa</i>	GTTAGCATCTGCATG	501bp

For shorter alleles (sequence repeatedly shorter than 508bp or 501bp), please [contact](#) the curator of the website.

Allele and DLST type assignment

Once you obtained a sequence for both *clfB* and *spa*, it is possible to obtain the allelic number corresponding to your sequence using the following options:

Submission of trace files.

A trace file (.abi) can be submitted for the *clfB* and *spa* loci. For both loci, the software will look for start signatures and will trim the sequences to their expected length (508 and 501bp). The sequences are then compared to the *clfB* and *spa* database of alleles. If alleles that are already known are found, the corresponding alleles are given. If one or two of the sequences are not known, the quality of the trace file is checked. A new allele number is given only if the quality of the trace file is good enough and if you have requested the permission to add new alleles using the [contact](#) form. If the quality is not good enough, a message indicate that a good quality trace file should be entered.

Submission of fasta files.

Fasta files with record named *clfB* and *spa* for one isolate at a time can be submitted. The fasta file must be in the following form:

```
>clfB  
Sequence of 508bp  
>spa  
Sequence of 501bp
```

It will soon be possible to submit fasta files with batches of isolates.

Submission of plain text sequences.

Plain text sequences can be entered for the *clfB* and *spa* loci. These sequences must start with the corresponding start signature and must be trimmed at the correct length.