Use of the MLVA allelic ladder as a calibration set

Staphylococcus aureus MLVA calibration protocol v0111

Standardizing typing techniques that are not sequence based has proven to be difficult. Although the separation of the PCR products obtained in MLVA is performed on a DNA sequencer, standardization may pose a problem for MLVA as well. The results of sizing of PCR products performed in one lab may differ from the results obtained in other labs. Such differences may have been caused by the use of different sequencers, different buffers, different capillaries etc. In order to eliminate such problems a calibration set may used which we may provide if you want to implement the MLVA in your lab. This calibration set is composed of a number of allelic ladders for each of the VNTR loci used in the MLVA. These allelic ladders contain each allele that we currently have identified. In figure 1 you can see the allelic ladder for the VNTR locus VNTR09_01 used in the *Staphylococcus aureus* MLVA.
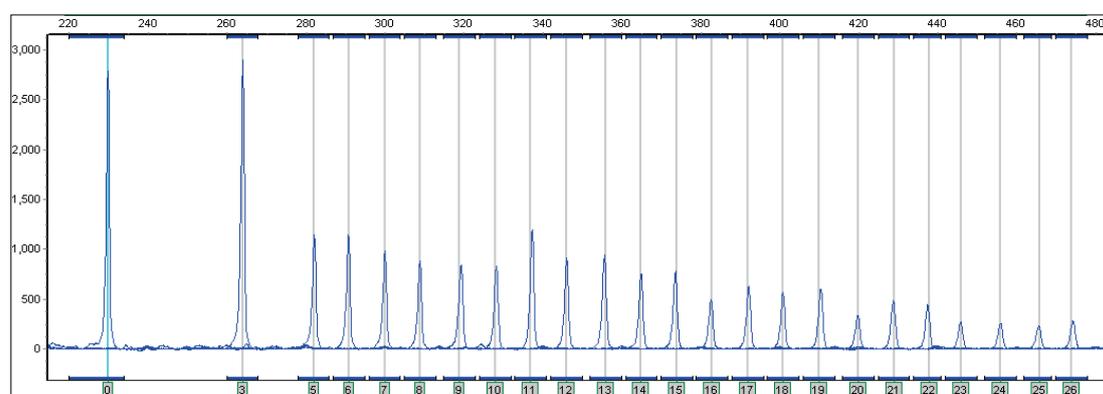

Figure 1. Peak profile created by the GeneMarker software for the VNTR locus VNTR09_01 used in the *S. aureus* MLVA.

Each peak represents an allele, starting on the left with allele '0' where the peak represents a PCR product obtained from an isolate that does not carry any repeat units in the VNTR09_01 locus. Nevertheless the regions flanking that normally flank the repeat units are present and consequently the PCR did yield a product. In the example of figure 1 alleles 1, 2 and 4 are lacking. Apparently when the allelic ladder was created no isolates with 1, 2 or 4 repeat units in the VNTR09_01 locus had been found yet. All other alleles, with up to 26 repeat units in the locus, are shown in the figure.

If you obtain the allelic ladders from us you can perform the MLVA PCRs and separate the PCR products on you sequencer. This will reveal the positions to which the alleles will migrate on you sequencer. Once you have established this, you can use this to define the bin positions for your own sequencing system. Although we do not recommend this, this would in principle even allow for the use of other fluorescent labels on the primers.

***Staphylococcus aureus* MLVA calibration set**

For the *S. aureus* MLVA the calibration set is composed of 9 vials. Each vial contains a mixture of PCR products comprising all currently identified alleles. To ensure amplification of all alleles the VNTR24_01 allelic ladder had to be split up into 2 vials.

Each allelic ladder should be used in a separate PCR. We do not recommend using a mixture of ladders because the large number of peaks and problems associated with spectral overlap of the fluorescent labels.

Protocol

Take **3 µl** of one of the 9 vials and amplify the DNA from this vial in the appropriate PCR mixture (e.g. for the VNTR09_01 use the Multiplex 1 PCR mixture) and using **22 cycles**. Perform the same experiment for each of the 8 other vials. After PCR, dilute the mixture according to protocol, add the size standard, denature this mixture and run the sample on your DNA sequencer. The apparent sizes you obtain on your sequencer can be translated into the allele numbers and used to adapt the GeneMarker panel file we supply on the website or to create your own panel file entirely. Alternatively you may use the data in any other type of software you utilize for the MLVA.

Remarks

- Not every possible allele has been found e.g. VNTR09_01 lacks the alleles with 1, 2 or 4 repeat units.
- The VNTR61_01 locus is heterogeneous in composition due to insertions and deletion in the regions flanking the repeats. The allelic ladder contains PCR products that are evenly spaced in the ladder. However, they will not be in the middle of the binning regions. Please check the GeneMarker panel file and the downloadable Excel file (Saureus_MLVA_allele_size_table.xls) to obtain the left en right binning sizes.
- You may notice that the peak height decreases with increasing allele size. This is caused by the fact that the PCR becomes less efficient for larger PCR products.
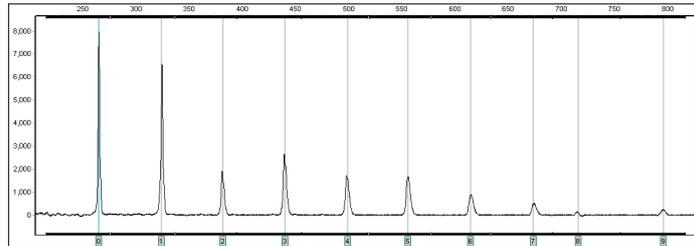
Composition of the *S. aureus* MLVA allelic ladders
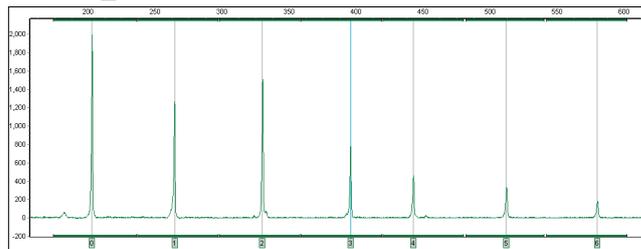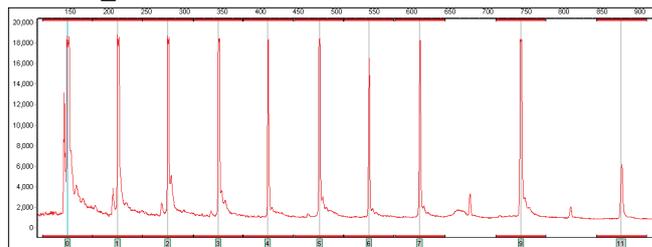
Use in multiplex 1 PCR
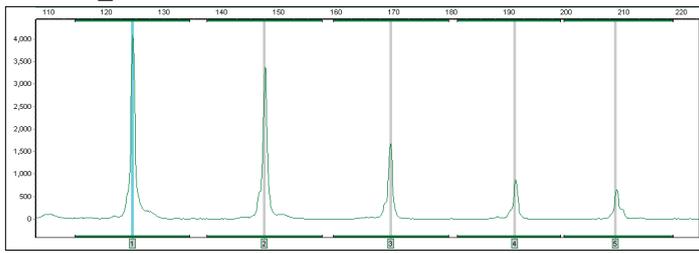### VNTR09_01
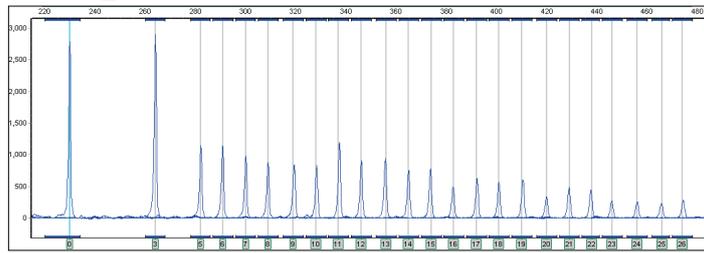
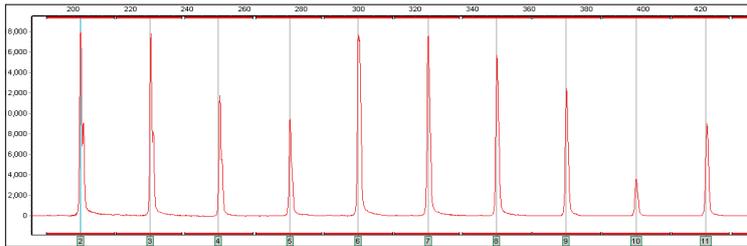### VNTR61_01

VNTR61_02

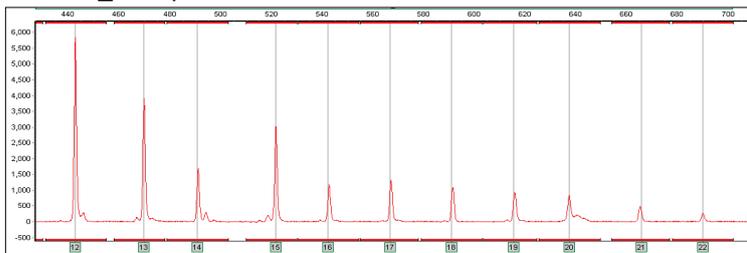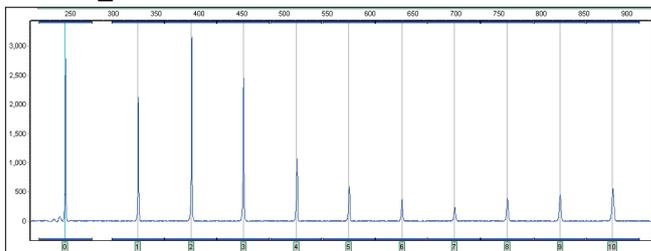

VNTR67_01

Use in multiplex 2 PCR

VNTR21_01

VNTR24_01 – pool 1

VNTR24_01 – pool 2

VNTR63_01

VNTR81_01