Use of the MLVA allelic ladder as a calibration set

Streptococcus pneumoniae MLVA calibration protocol v01

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 BOX 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 BOX locus BOX_04 used in the *Streptococcus pneumoniae* MLVA.

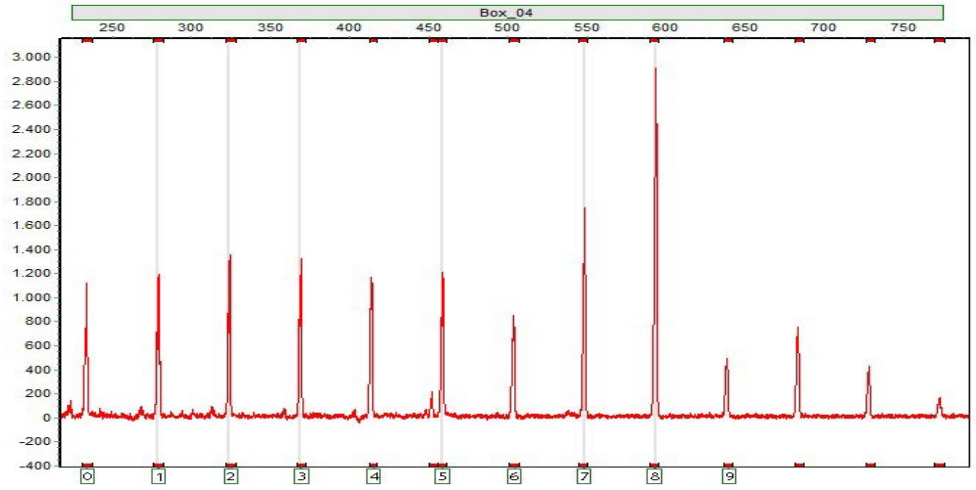


Figure 1. Peak profile created by the GeneMarker software for the BOX locus BOX_04 used in the *S. pneumoniae* MLVA.

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 BOX_04 locus. Nevertheless the regions flanking that normally flank the repeat units are present and consequently the PCR did yield a product. All other alleles are shown in the figure.
If you obtain the allelic ladders from us you can 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.

***Streptococcus pneumoniae* MLVA calibration set**
For the *S. pneumoniae* 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 BOX_06 allelic ladder had to be split up into 2 vials for each primer pair.
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 **2 µl** of one of the 9 vials and 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.

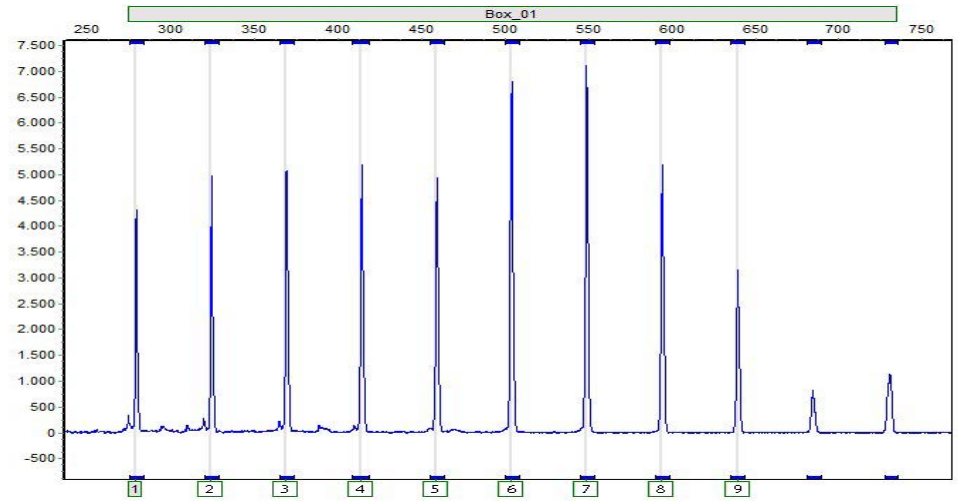
Remarks

− 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.

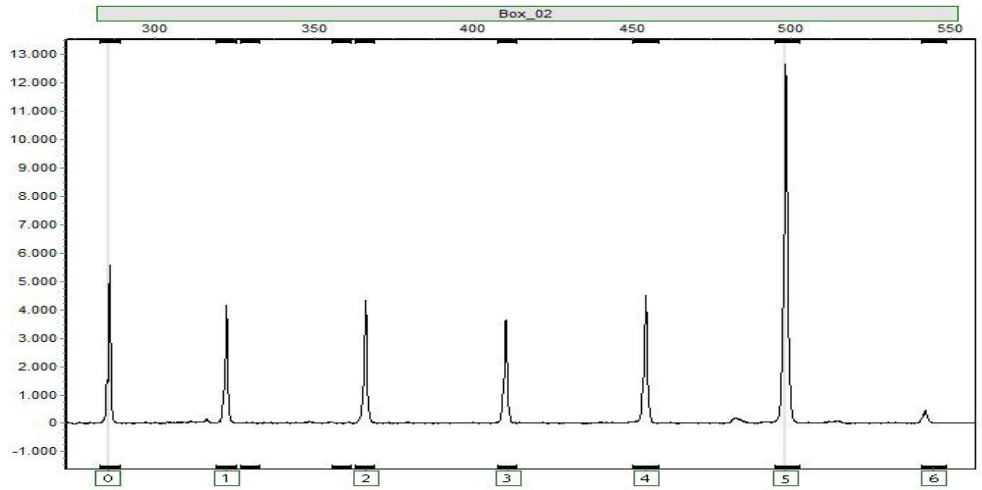
Composition of the *S. aureus* MLVA allelic ladders

Use in multiplex 1 PCR

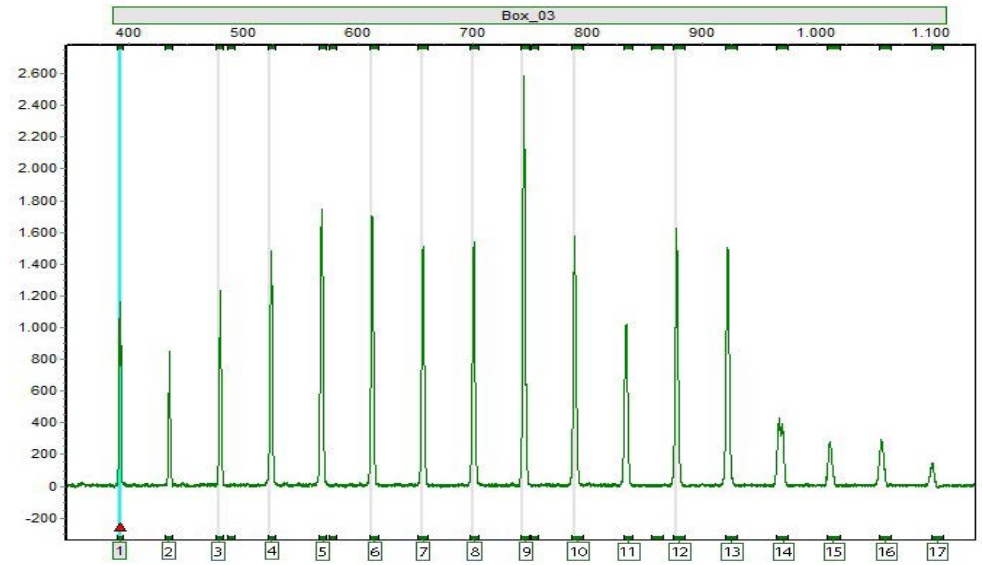
BOX_01



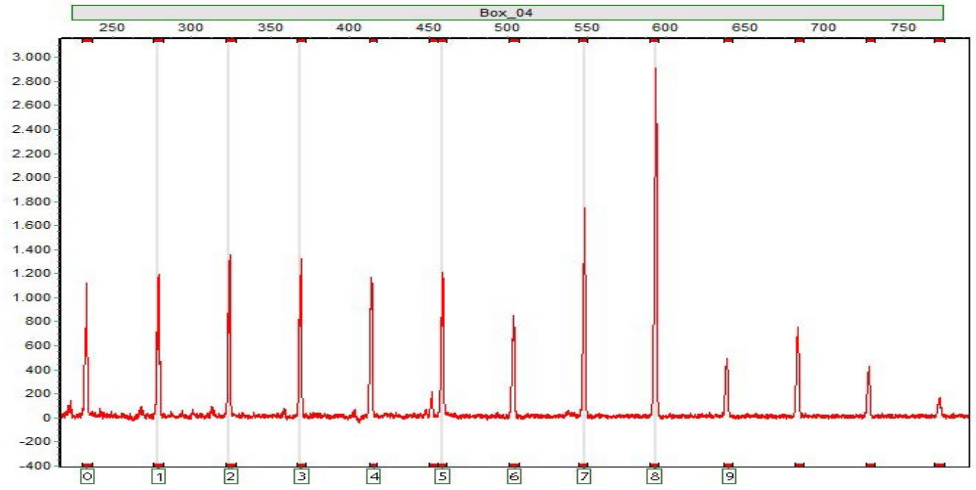
BOX_02



BOX_03

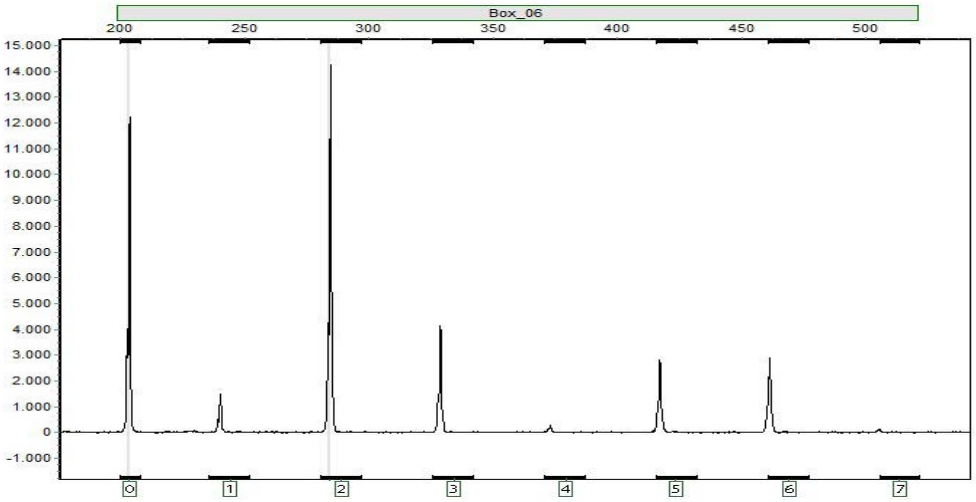


BOX_04

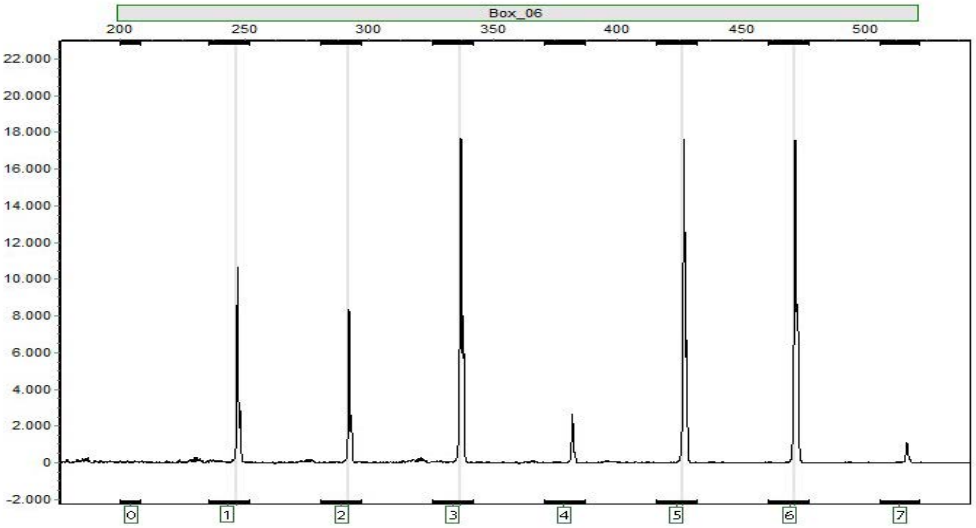


<u>Use in multiplex 2 PCR</u>

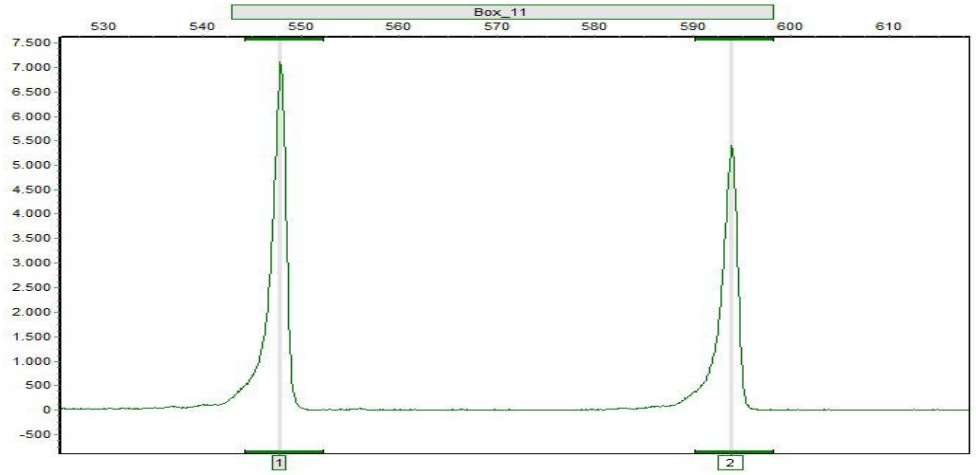
BOX_06 (1)



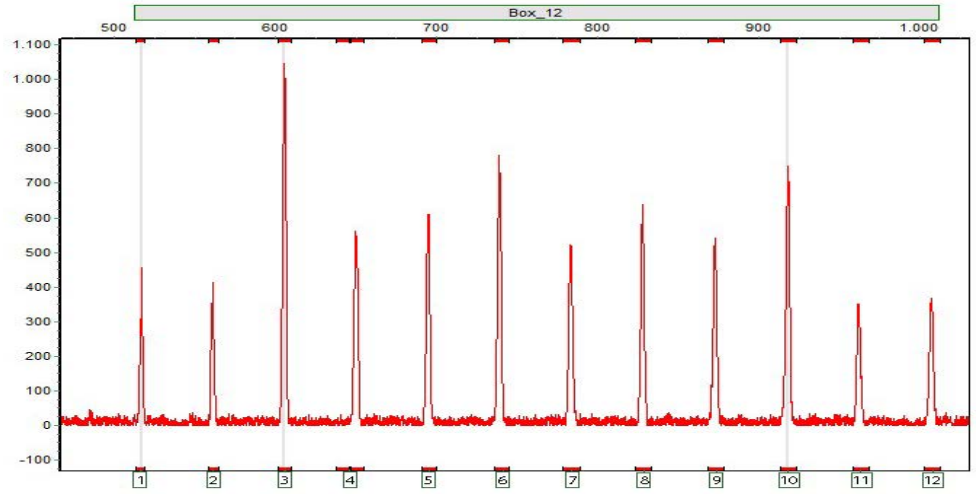
BOX_06 (2)



BOX_11



BOX_12



BOX_13