Polymerase Chain Reaction V.1

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ABSTRACT

This was the PCR protocol used for each set of primers in the study Comparative genomics of *Staphylococcus aureus* associated with subclinical and clinical bovine mastitis (Rocha et al., 2019)
MATERIALS

50 ng of total DNA, 1U of Taq DNA polymerase Cellco Biotec, 0.2 µM of each primer, 0.2 mM deoxynucleotide triphosphate mixture, 1X reaction buffer containing 2.0 mM MgCl₂, extra 1.0 mM MgCl₂, and Milli-Q water to increase the reaction volume to a final volume of 25 µL.

The extra 1 mM MgCl₂ was excluded from the PCR reaction that contained the primers LipoP-F-CS/LipoP-R-C.

Table 1 - Primer Sequences for primers used in this Protocol

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>cl3309s ubF</td>
<td>TGTTGTAGGAGGAACATCC</td>
</tr>
<tr>
<td>cl3309s ubR</td>
<td>TTCTAATGTCAGCAACATGC</td>
</tr>
<tr>
<td>cl3309c liF</td>
<td>GCTATTCCTAGATGC</td>
</tr>
<tr>
<td>cl3309c liR</td>
<td>TTTTAATGATGACATGAG</td>
</tr>
<tr>
<td>cl3316F</td>
<td>ACGAAAAACCTTTACTAGT</td>
</tr>
<tr>
<td>cl3316R</td>
<td>GCAACACTAGTGGAGTA</td>
</tr>
<tr>
<td>LipoP-F-CS</td>
<td>GYTTTGCAGAAACGTTAGAYATGTA</td>
</tr>
<tr>
<td>LipoP-R-CS</td>
<td>GGTAATCAATGTYCTTA</td>
</tr>
<tr>
<td>LipoP-F-CS</td>
<td>GYTTTGCAGAAACGTTAGAYATGTA</td>
</tr>
<tr>
<td>LipoP-R-CS</td>
<td>GGTAATCAATGTYCTTA</td>
</tr>
</tbody>
</table>

1 Initial denaturation: 95.0 °C for 5 min;
35 cycles of denaturation at 95.0 °C for 45 s,

Annealing: 55 °C for 45 s

Extension: 72 °C for 45 s

final extension at 72.0 °C for 10 min

primers cl3316F/R

initial denaturation: 95.0 °C for 5 min;

35 cycles of denaturation at 95.0 °C for 45 s,

Annealing: 55 °C for 45 s

Extension: 72 °C for 45 s
10 final extension at 72.0 °C for 10 min.

11 initial denaturation: 95.0 °C for 5 min;

12 35 cycles of denaturation at 95.0 °C for 45 s,

13 Annealing: 54 °C for 45 s

14 Extension: 72 °C for 45 s

15 final extension at 72.0 °C for 10 min.

16 initial denaturation: 95.0 °C for 5 min;
17 35 cycles of denaturation at 95.0 °C for 45 s,

18 Annealing: 45 °C for 45 s

19 Extension: 72 °C for 30 s

20 final extension at 72.0 °C for 10 min.

primers cI3700 - LipoP FCS/RCS

21 initial denaturation: 95.0 °C for 5 min;

22 35 cycles of denaturation at 95.0 °C for 45 s,

23 Annealing: 50 °C for 45 s

24 Extension: 72 °C for 1min
final extension at 72.0 °C for 10 min.

Analyzing the amplified fragments

Analyze the amplicons by electrophoresis in 1X Tris-acetate-EDTA on a 1.0% agarose gel and visualize imagen under UV light after staining with 2 mg.ml-1 ethidium bromide.