VERSION 1

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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)

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Protocol status: Working
We use this protocol and it's working

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21767

MATERIALS

50 ng of total DNA, 1U of Taq DNA polymerase Cellco Biotec, 0.2 μ M 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 μ L.

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

Table 1 - Primer Sequences for primers used in this Protocol

cl3309s ubF	TGTTGTAGGAGGAACAAT CC
cl3309s ubR	TTCTAATGTCAGCAACAT GC
cl3309c liF	GCTATTCCTAGATGCACT
cl3309c liR	TTTAAAGTATGACATGAAT G
cl3316F	ACGCAAAACCCCTTTACTA GT
cl3316 R	GCAACAACCTAGTAGGAGT GA
LipoP- F-CS	GYTTTGCGAAAACGTTAG AYATGTA
LipoP- R-C	TGCCTTCATCATTAATTGG ACCAATC
LipoP- F-CS	GYTTTGCGAAAACGTTAG AYATGTA
LipoP- R-CS	GGTAAAYTCAATGTYCTTA TRTCC

primers cl3309sub F/R

- 1 Initial denaturation: 95.0 °C for 5 min;

2 35 cycles of denaturation at 95.0 °C for 45 s,

3 Annealing: 55 °C for 45s

4 Extension: 72 °C for 45 s

5 final extension at 72.0 °C for 10 min

primers cl3316F/R

6 initial denaturation: 95.0 °C for 5 min;

7 35 cycles of denaturation at 95.0 °C for 45 s,

8 Annealing: 55 °C for 45 s

9 Extension: 72 °C for 45 s

10 final extension at 72.0 °C for 10 min.

primers cl3700 - LipoP FCS/RC

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.

cl33009cli F/R

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 cl3700 - 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

25 final extension at 72.0 °C for 10 min.

Analyzing the amplified fragments

26 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.