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Working

Polymerase Chain Reaction

Version 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)

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

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 $MgCl_2$ was excluded from thePCR reactionsthat contained the primers LipoP-F-CS/LipoP-R-C.

Table 1 - Primer Sequences for primers used in this Protocol

cl3309subF	TGTTGTAGGAGGAACAATCC
cl3309subR	TTCTAATGTCAGCAACATGC
cl3309cliF	GCTATTCCTAGATGCACT
cl3309cliR	TTTTAAGTATGACATGAATG
cl3316F	ACGCAAAACCCTTTACTAGT
cl3316R	GCAACAACTAGTAGGAGTGA
LipoP-F-CS	GYTTTGCGAAAACGTTAGAYATGTA
LipoP-R-C	TGCCTTCATCATTAATTGGACCAATC
LipoP-F-CS	GYTTTGCGAAAACGTTAGAYATGTA
LipoP-R-CS	GGTAAAYTCAATGTYCTTATRTCC

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 Anealing: 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.