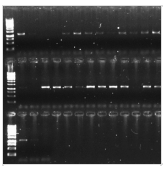

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16s rDNA gene amplification protocol

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PCR amplification, universal bacterial primers, 16s rDNA

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MATERIALS TEXT

Materials

Polypropylene tubes of 1,5 mL
Polypropylene tubes of 0,5 mL
Absorbent paper
Tips of 10 µL, 200 µL, 1000 µL
Permanent marker for labeling
Gloves

Reagents

Taq buffer (10X)
Taq DNA polymerase (5 U/µL)
$MgCl_2$ (50 mM)
dNTP (2.5 mM each dNTP)
Primers (20 µM)
Syber safe (10000X)
Loading buffer dye (6X)
DNA Ladder 1Kb (50 ng)
TBE 1X (89mM Tris-borate, 89mM boric acid, 2mM EDTA)

Solutions

DNA template (50-100 ng/µL)
Sterile deionized water

Other

Micropipette of 10 µL, 200 µL, 1000 µL
Analytical balance
Freezer
Thermocycler

DISCLAIMER:

Master mix preparation

1 In a polypropylene tube, mix the following reagents for the final volume of 25 µL per reaction

1.1 Add 2.5 µL of 10X Taq buffer (final concentration 1X)

1.2 Add 0.75 µL of MgCl2 (50 mM) (final concentration 2.5 mM)

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1.3 Add 0.2 µL dNTP (2.5 mM each dNTP) (final concentration 0,02 mM)

1.4 Add 0.5 µL of each primer (20 µM) (final concentration 0,4 µM)
Primer 10f 5'-GAG TTT GAT CCT GGC TCA G-3'
Primer 1401r 5'-CGG TGT GTA CAA GGC CCG GGA ACG-3' or 1492r 5'- ACC TTG TTA CGA CTT-3' or
Primer 1492r 5'- ACC TTG TTA CGA CTT-3'

1.5 Add 5.0 µL of the DNA template (50-100 ng/µL) (final concentration at 1-2,5 ng per reaction)

1.6 Add 0,2 µL of Taq DNA polymerase (5 U/µL) (Final concentration 0,04 U)

1.7 Add sterile deionized water until filling at 25 µL per reaction.

PCR reaction

2 For PCR amplification, use the following cycling program

2.1 Run an Initial denaturation step of 5 min at 95°C

2.2 Run 30 cycles of 1 min at 94°C for denaturation, 1 min at 55°C for annealing, 3 min at 72°C for extension

2.3 Run a 3 min at 72°C for the final extension

2.4 Store amplification products at 4°C until visualization.

Visualization

3 To analyze and visualize the amplified products, put 2 µL of the PCR product with 2 µL of loading buffer dye in agarose gel electrophoresis with 1X TBE stained with SYBER safe at 1:10000.

4 To compare, add 1 µL of 1kb DNA ladder (final concentration 0,5 ng/µL) and observe a band of 1500 pb approximately.

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