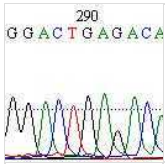

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16s rDNA sequencing reaction and precipitation protocol

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1 *Works for me* dx.doi.org/10.17504/protocols.io.brzpm75n

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MATERIALS TEXT

Materials

Polypropylene tubes of 1,5 mL
Absorbent paper
Tips of 10 µL, 200 µL, 1000 µL
Permanent marker for labeling
Nitrile gloves
96-well sequencing plates

Reagents

Primer 10f, 765f, 782r, 1100r and 1492r (20 µM each)
The BigDye® Terminator v3.1
EDTA (125 mM)
Sodium Acetate (NaOAc) 3M
Etanol 100%
Etanol 70%
Hi-Di™ Formamide - Thermo Fisher Scientific

Solutions

Save money buffer:
- 2 ml of tris HCl pH9.0, 1M
- 1 ml of MgCl 250 mM
- Fill with deionized sterile water up to a volume of 10 ml

Tris-HCl pH9.0, 1M:
-1,211 g of Tris and fill with deionized sterile water up to a volume of 8 ml
-Adjust pH by adding HCl 100%
-fill with deionized sterile water up to a volume of 10 ml

DNA template (10 - 20 ng/µL)
Sterile deionized water

Other

Micropipette of 10 µL, 200 µL, 1000 µL
Analytical balance
Freezer
Thermocycler
Refrigerated centrifuge for sequencing plates
Genetic Analyzer ABI 3500 series Applied Biosystems

DISCLAIMER:

Sequencing reaction

1

Consider a total volume of 10 µl

Add 6 µl of water for PCR

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2

3 Add 2 µl of save money buffer

4 Add 0.5 µl of primer

5 Add 0.5 µl of Byg Dye

6 Add 1 µl of DNA (10 - 20 ng/µl)

7 Place the reaction in the thermal cycler with the following cycling program:

7.1 Run an Initial denaturation step of 1 min at 96 °C.

7.2 Run 30 cycles of 15 sec at 96 °C for denaturation, 15 sec at 50 °C for annealing, 4 min at 60 °C for extension.

7.3 Store amplification products at 4°C until further use in precipitation reactions.

Precipitation

8 Add 1 µl of EDTA (125 mM) to each well

9 Add 1 µl of 3M Sodium Acetate (NaOAc) to each well

10 Add 25 µl of 100% cold Ethanol to each well, cap tightly and vortex slightly

11 Incubate at 4 °C for 15 minutes

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12 Centrifuge at 2250g for 30 minutes

13 Dispense the contents in the sink immediately and invert immediately on paper towels

14 If it is not dispensed immediately, centrifuge at 2250g for 2 min

15 Additional 35 µl of 70% chilled ethanol in each well

16 Centrifuge at 2250g for 15 minutes

17 Dispense the contents in the sink and centrifuge the inverted plate 15g for 1 minute

18 Incubate the uncapped plate in the thermal cycler at 95 °C for 5 minutes

19 Resuspend the samples in 10 µl of Hi-Di formamide, cover with the cap and vortex slightly

20 Incubate in the thermal cycler at 95 °C for 2 minutes

21 Put ice for 2 minutes

22 Place the card in the sequencer

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