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16s rDNA sequencing reaction and precipitation protocol

Rene Flores Clavo¹, Cristian Daniel Asmat Ortega², Nataly Ruiz Quinones¹

¹Universidade Estadual de Campinas; ²Centro de Investigación en Innovación en Ciencias Activas Multidisciplinarias

Rene Flores Clavo: Centro de Investigación e Innovación en Ciencias Activas multidisciplinares-CIICAM;

Nataly Ruiz Quinones: Centro de Investigación e Innovación en Ciencias Activas multidisciplinares-CIICAM

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RENE FLORES

Tech. support phone: +55 (19) 991640041 email: renefloresclavo@gmail.com

[Click here to message tech. support](#)



Rene Flores Clavo

Universidade Estadual de Campinas, Centro de Investigación e...

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KEYWORDS

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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

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Sequencing reaction

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Consider a total volume of 10 µl

Add 6 µl of water for PCR

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- 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

- 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