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16S rRNA Library Preparation Protocol

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Protocol status: Working

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Attachments



16S_Library_Preparat...

104KB




16S_Library_Preparat...

93KB

Materials

MATERIALS

 Q5 Hot Start High-Fidelity DNA Polymerase - 500 units **New England Biolabs Catalog #M0493L**

 EZNA Cycle Pure Kit **Omega Biotek Catalog #D6492**



- 1 Dilute sample DNA to ~2-5ng/μl. If the sample is <2ng/μl, use sample as is.
- 2 Sample PCR should be done in duplicate, one serving as the base sample and another as a technical replicate. Prepare reaction mix for #of reactions +1 to prevent shortage of mix. Reagents for one 10μl reaction are as follows:

H₂O – 4.7 μl

5X Q5 Hot Start Buffer - 2.0 μl

10μM Fprimer (505F)- 0.5 μl

10μM Rprimer (806R)- 0.5 μl

10mM dNTPs- 0.2 μl Q5 Hot Start DNA Polymerase- 0.1 μl

Distribute 8 μl of reaction mix to reaction tubes and then add 2 μl of diluted DNA (total 10μl). (Note: Add reagents as listed. 5X buffer and primers may be thawed at room temperature but dNTPs should be thawed on top of ice and polymerase should be kept on ice at all times.)

- 3 Run on thermocycler as follows:

Temperature	Time	Cycles
98°C	30 s	1
98°C 52°C 72°C	10 s 30 s 30 s	15
72°C	2 min	1
10°C	hold	

- 4 Prepare 2nd reaction mix while the first reaction is running. Prepare reaction mix for # of reactions +1. Reagents for one 30ml reaction are as follows:

H₂O- 11.1 μl

5X Q5 Hot Start Buffer- 6.0 μl

10μM 806_BCR primer- 1.5 μl

10mM dNTPs- 0.6 μl

Q5 Hot Start DNA Polymerase – 0.3 μl

Remove reactions from thermocycler once the first reaction is completed. Keep reactions on cold block. Distribute 21µl of 2nd reaction mix to each new reaction tube. Add 9µl of reaction 1 product to the appropriate 2nd reaction tube. Pipette up and down to mix.

- 5 Run on thermocycler as follows:

Temp eratur e	Time	Cycle
98°C	30s	1
98°C 52°C 72°C	10 s 30 s 30 s	4
98°C 72°C	10 s 1 min	6
72°C	2 min	1
10°C	hold	

- 6 Verify PCR product by gel electrophoresis.
- 7 Purify reactions using Omega Bio-Tek's E.Z.N.A. Cycle-Pure Kit according to manufacturer's protocol.
- 8 Measure sample concentration and A260/A280 via nanodrop, record values. Store at -20°C.
- 9 Pool together an equal amount of amplicons (generally 50ng, but adjust if necessary) from each sample into subpools composed of 15-30 unique samples.
- 10 Verify subpool products by gel electrophoresis.
- 11 Combine an equal amount of amplicons from each subpool and submit the pooled library for sequencing.