16S rRNA Library Preparation Protocol

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Abstract

Citation: Kara Tinker,Elizabeth Ottesen 16S rRNA Library Preparation Protocol. **protocols.io**
dx.doi.org/10.17504/protocols.io.jz4cp8w
Published: 03 Oct 2017

Materials

Q5 Hot Start High-Fidelity DNA Polymerase - 500 units M0493L by New England Biolabs
EZNA Cycle Pure Kit D6492 by Omega Biotek

Protocol

Step 1.
Dilute sample DNA to 2-5ng/µl. If the sample is <2ng/µl, use sample as is.

Step 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 µlQ5 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.)

Step 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	

Step 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.

ANNOTATIONS

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Please note that the forward primer was accidentally omitted from the master mix ingredients. Be sure to include 1.5 µl 10µM 515_BCF.

Step 5.

Run on thermocycler as follows:

Temperature	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	

Step 6.

Verify PCR product by gel electrophoresis.

Step 7.

Purify reactions using Omega Bio-Tek's E.Z.N.A. Cycle-Pure Kit according to manufacturer's protocol.

Step 8.
Measure sample concentration and A260/A280 via nanodrop, record values. Store at -20°C.

Step 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.

Step 10.

Verify subpool products by gel electrophoresis.

Step 11.

Combine an equal amount of amplicons from each subpool and submit the pooled library for sequencing.