Amplicon Library Preparation

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1 Works for me
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ABSTRACT

18S and 16S amplicon library preparation protocol.

DNA or RNA is usually extracted with our automated protocols from sterivex but other types of samples are occasionally used. If using RNA, first generate cDNA with Invitrogen's SuperScript III First-Strand Synthesis System. Blanks from the nucleic acid extraction should always be used as negative controls. Normally, DNA is diluted 10-fold after extraction and 1 µL of the diluted DNA or the cDNA is used for template in the PCR reaction. If DNA concentrations are very low, 1 µL of the undiluted DNA may need to be used.

The library is generated with a 1-step PCR, i.e. amplicons are amplified and barcoded simultaneously. Amplicons can be generated for 16S (515F-Y/926R primers from Parada et al. 2015), 18Sv4 (V4F/V4RB primers from Balanzo et al. 2015), and 18Sv9 (1389F/1510R primers from Amaral-Zettler et al. 2009). Barcoded primers for all of these amplicons are attached to this protocol. Typically, the primers are pre-mixed into 96-well plates such that one set of 8 unique F primers (plate rows) and one set of 12 unique R primers (plate columns) are mixed to create 96 unique combinations. Reactions can then be run in a 96 well plate where template plate positions correspond to unique barcode plate positions. The i5 barcode is inline to generate higher sequence quality but also requires a customized demultiplexing workflow. When sequencing, only demultiplex based on the i7 index first. Scripts to then demultiplex based on the i5 index can be found on our Github here.

We also highly recommend including mock communities in every 96-well plate.

ATTACHMENTS

16SV4V5 primers.xlsx 18SV4 primers.xlsx 18SV9 primers.xlsx

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16S, 18S, amplicon, metabarcoding, environmental microbiology

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GUIDELINES

Please see the information in the abstract about demultiplexing. You will not be able to properly demultiplex a library using these primers with other software.

MATERIALS

NAME	CATALOG #	VENDOR
Agencourt AMPure XP	A63880	Beckman Coulter
High Sensitivity D1000 Reagents	5067-5585	Agilent Technologies
Quant-iT™ PicoGreen™ dsDNA Assay Kit	P11496	Thermo Fisher
Qubit dsDNA HS Assay Kit	Q32851	Invitrogen
TruFi DNA Polymerase Kit	AZ-1710	

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1 Setup a **25 µl** PCR reaction as follows:

Reagent	1x reaction (uL)
5x buffer	5.0
F + R primer combined (10 uM)	1.0
DNA polymerase	0.25
Template (10 pg - 500 ng)	1.0 - 2.0
Molecular grade H2O	to 25 uL

2 Run the PCR reaction:

Step	Temperature (degrees C)	Time

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Initial Denaturation	95	1 minute
30 Cycles	95	15 seconds
	56	15 seconds
	72	30 seconds
Hold	4	

3 Run **2.5 µl** of the PCR product on a 2% gel to confirm amplification in all samples except negative controls.

4 Clean up the PCR products with Agencourt AMPure XP beads with their standard PCR purification protocol and eluting in **35 µl** of elution buffer.

5 Quantify **1 µl** of each sample in duplicate with the PicoGreen ensuring that you have a good standard curve and samples have duplicates CV values.

6 Pool **10 ng** purified PCR product. For small libraries, you may want to pool **20 ng** .

7 Clean and concentrate the final library with XP beads. Elute in **45 µl** .

8 Analyze the final library on the TapeStation and quantify with the Qubit.

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