3.PCR clean-up for 16S

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ABSTRACT

This protocol is to purify the 16S V3 and V4 amplicon away from free primers and primer dimer species using AMPure XP beads.

This protocol is adapted from https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html from page 8-9 with some minor volume edits which are bolded.

MATERIALS TEXT

Item	Quantity	Storage
10 mM Tris pH 8.5 or PCR grade water	30µl per sample	-15° to -25°C
AMPure XP beads	20 µl per sample	2° to 8°C
Freshly Prepared molecular grade80% Ethanol (EtOH)	400 µl per sample	
96-well 0.2 ml PCR plate	1	
PCR plate film	1	
magnetic plate	1	
Reagent reservoirs	3	
Pipetts	p20, p200, p100	

BEFORE STARTING

- Bring the AMPure XP beads to room temperature.
- Prepare fresh 80% ethanol

Cleanup

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1. Centrifuge the Amplicon PCR plate at 1,000 × g at 20 °C for 1 minute to collect condensation, carefully remove seal.The sample can remain in the 96-well PCR plate.
2. Vortex the AMPure XP beads for 00:00:30 sec to make sure that the beads are evenly dispersed. Pour an appropriate volume of beads to a reagent reservoir depending on the number of samples processing.
3. Using a multichannel pipette, add 20 µl of AMPure XP beads to each well of the Amplicon PCR plate and gently pipette entire volume up and down 10 times. Change tips between columns.
4. Incubate at room temperature without shaking for 00:05:00 min.
5. Place the plate on a magnetic stand for 00:02:00 minutes or until the supernatant has cleared.
6. With the Amplicon PCR plate on the magnetic stand, use a multichannel pipette to remove and discard the supernatant. Change tips between samples.
7. With the Amplicon PCR plate on the magnetic stand, wash the beads with freshly prepared 80% ethanol (poured onto a reagent reservoir) as follows: a. Using a multichannel pipette, add **195 µl** of freshly prepared 80% ethanol to each sample well. b. Incubate the plate on the magnetic stand for 00:00:30 seconds. c. Carefully remove and discard the supernatant.
8. With the Amplicon PCR plate on the magnetic stand, perform a second ethanol wash as follows: a Using a multichannel pipette, add **195 µl** of freshly prepared 80% ethanol to each sample well. b Incubate the plate on the magnetic stand for 00:00:30 seconds. c Carefully remove and discard the supernatant. d Use a P20 multichannel pipette with fine pipette tips to remove excess ethanol.

9. With the Amplicon PCR plate still on the magnetic stand, allow the beads to air-dry for 00:10:00 minutes.
10. Remove the Amplicon PCR plate from the magnetic stand.
11. Using a multichannel pipette, add **30 µl** of 10 mM Tris pH 8.5 or molecular grade water (poured onto a reagent reservoir) to each well of the Amplicon PCR plate and gently pipette mix up and down 10 times, changing tips after each column. Make sure that beads are fully resuspended.
12. Incubate at room temperature for 00:02:00 minutes.
13. Place the plate on the magnetic stand for 00:02:00 minutes or until the supernatant has cleared.
14. Using a multichannel pipette, carefully transfer **25 µl** of the supernatant from the Amplicon PCR plate to a new 96-well PCR plate. Change tips between samples to avoid cross-contamination.
15. Seal and store it at -15° to -25°C for up to a week.