

Amplicon Pooling and Cleanup protocols (Modified from EMP protocol*)

Pooling Amplicon Libraries

1. Using the pooled PCR samples combine an equal amount of amplicon from each sample into a single, sterile, DNase free tube. Typically between 100ng -240ng of each sample can be pooled.
2. Label pooled library and proceed to Cleanup step

Library Cleanup (adapted from manufacturer's protocol)**

Note: Depending on the number of pooled samples and the concentration of the pools it may be necessary to split pools into multiple cleanups to avoid overloading the filter.

1. Shake to mix the SpinBind before use. Add 5 volumes of SpinBind to your PCR reaction.
2. Mix well by pipetting.
3. Transfer PCR/SpinBind mixture to a Spin Filter unit.
4. Centrifuge 30 seconds at a minimum 10,000 x g in a tabletop microcentrifuge.
5. Remove the Spin Filter basket and discard the liquid flow-through from the tube by decanting.
6. Replace the Spin Filter basket in the same tube.
7. Add 300 µl SpinClean® Buffer to the Spin Filter.
8. Centrifuge 30 seconds at a minimum 10,000 x g.
9. Remove Spin Filter basket and discard liquid flow through by decanting then replace basket back into the same tube.
10. Centrifuge 60 seconds at 10,000 x g.
11. Transfer Spin Filter to a clean 2 ml Collection Tube (provided).
12. Add 50 µl of Elution Buffer solution provided directly onto the center of the white Spin Filter membrane.
13. Centrifuge 30-60 seconds at a minimum 10,000 x g.
14. Discard Spin Filter basket from the inside of the Spin Filter unit. Purified DNA is now in the 2 ml Collection Tube. The DNA will be free of all reaction components such as primers or linkers, enzyme, salt, and dNTP's.
15. Measure the concentration of the pooled library using the Qubit hsDNA protocol.
16. Record the concentration and give to the TAs along with the pooled libraries.

Note: The final concentration of the library must be 10nM before it is handed off to the CGRB for sequencing.

*Earth microbiome project(EMP; <http://www.earthmicrobiome.org>)

**MOBIO (MoBio UltraClean PCR Clean-Up; <http://www.mobio.com>)