Experienced User Protocol

Total time: 1-2 hrs
Prep time: 30 min – 90 min

PCR Purification (aka Bead Cleanup)

CALeDNA Metabarcoding Wet Lab Protocol *V.4.0*
Questions or Comment? Email anworth@ucsc.edu

Meyer Lab edit –03/03/2023

Important notes before starting:

a. ***Precision is key for this protocol. Pipette slowly and carefully making sure you are pulling up the full amount of liquid each time, that there are no drops of liquid on the outside of your tips, and that all of the liquid has been expelled from your pipette tip.***

***** If you accidentally pull up beads (the solution in your pipette tips would have brown in it) pipette the liquid back into the well and allow the magnets to clear the solution. Always check your tips for any hints of brown by holding them against a white background. If you discard liquid with beads inside you are throwing away the DNA that is bound to those beads. ****

b. **Check that the bead to PCR product ratio is not going to remove any of your amplicons. For the MagBio PCR cleanup beads, a 1.2:1 (beads:sample) removes primer dimer and fragments less than 100bp. A 1:1 ratio works fine too and removes fragments <200bp.**

Suggestion: Aliquot your own tube of beads from the lab stock. Make sure they are warmed up and mixed thoroughly (not vortexed!) before

Prepare MagBio beads, ethanol and tubes:

1. Put on gloves. **Wipe counter with 10% bleach and 70% ethanol.**
2. **Allow MagBio beads to come to room temperature** for approximately 30 minutes. Cold beads can affect how well DNA can bind to them.
3. Label enough 96-well plates or strip tubes for all of your samples.
4. Once the beads are at **room temperature, invert and shake beads thoroughly (~60s)** until there are no beads (brown spots) stuck to the bottom of the tube. Thorough mixing ensures our MagBio beads remain at the correct concentration.
5. **Prepare an 80% ethanol solution daily.** Pour 40mL of molecular grade ethanol (200 proof) into a 50mL falcon tube. Add 10mL of Ultra Pure H_2O. Invert to mix.

Purify your PCR product using MagBio beads:

6. Remove samples from the freezer or fridge and allow to fully thaw. **Gently vortex and spin down,** about 3 seconds each.

7. **Determine what quantity of beads you will need for each sample.** With MagBio beads for metabarcoding PCR cleanup we use a 1:1 to 1.2:1 ratios, and for indexing PCR cleanup, we use a 1:1 ratio:

   (1.2 ul of beads) x (# ul of sample) + 10% = # of ul of beads/sample

8. **Multiply this ratio with the amount of PCR Product. Multiply by the total number of samples you have and add 10% for error:**

   (n of samples) x (# of µL of beads/sample) + 10% = total volume of beads

9. Prepare a sterile reservoir for the beads so that you can use a multichannel throughout this protocol. **Pipette the needed volume of beads into the reservoir.**
10. **Carefully pipette your calculated volume of beads** (# of µL of beads/sample) into the PCR product. Mix by pipetting **at least 20x up and down with pipette.**
11. Allow the PCR product with added beads to **incubate at room temperature for at least 5 minutes.** This incubation step is required for the DNA to bind to the beads, if you rush this step you will discard some of your DNA with the supernatant in step 13.
12. Move your open sample tubes or plates on to a **magnetic plate for 2 minutes, or until the solution has cleared.**
13. **Remove the supernatant and discard.** Be careful not to discard any supernatant with beads inside. **If the solution is brown do not discard.** Pipette back into the well or tube and wait for the solution to become clear again.
14. Pour your **freshly made 80% ethanol** into a new sterile reservoir.
15. **Add 180µL of ethanol** to each sample and let them **incubate for 30 seconds. You must add enough ethanol to completely cover the beads.**
16. **Remove ethanol with a pipette and discard.** Since this wash will be followed by a second ethanol wash it is okay to leave some residual ethanol in your tubes/plate.
17. **Add 180µL of ethanol** to each sample and **let incubate for 30 seconds.**
18. **Remove ethanol with a pipette and discard.** This is the last wash, be extra careful to not leave any residual ethanol behind.
19. Once you have removed as much of the ethanol as possible, **remove your samples from the magnetic plate and allow to dry (with lids open) for 2-5 minutes until residual ethanol is gone.**
20. **Monitor your beads closely. It is <u>critical</u> that you avoid allowing the beads to turn dull while waiting for the ethanol to evaporate.** If your beads start to look dull, immediately close the lids of the tubes or go on to the next step. If your beads are dried to the point of being dull and cracking, you can shear the DNA when you try to elute with water.
21. Pour enough **dH_2O** into a new sterile reservoir for your elution step.
22. **Elute your DNA in sterile water. Add 20 to 30µL of Ultra Pure dH_2O and mix at least 20 times by pipetting up and down with pipette**. You can add more or less water, depending on your samples. Generally, not below 20µL or above 40µL of water. The less water you elute into, the higher your concentration will be.
23. **Incubate at room temperature for at least 5 minutes.**
24. Place on **magnetic plate for 2 minutes, or until solution has cleared.**
25. **Transfer supernatant with a pipette into new, clean, labeled strip tubes or plates.**
26. Close tubes/plate and **spin down**.

Store cleaned PCR and clean workspace:

27. Store cleaned PCR product.

Where to store your PCR? If you will use your PCR product within 1 week, it is safe to leave in the fridge (4^{o}C).If it will be more than 1 week, store your PCR in a -20 ^{o}C freezer to avoid DNA degradation.

28. Clean up and wipe bench with **10% bleach and 70% ethanol.**