eDNA Extraction from Filters using the Qiagen DNeasy Kit
Goldberg Lab WSU
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All liquids used must be disposed of in a hazardous waste bottle. Use only filter tips.

Day 1 (for filters stored in ethanol)

1. Set out your UV-sterilized tubes (1.5mL) for filter drying/DNA digestion in a tray and close them. The number of tubes should be the number of samples **plus one negative control.**

2. Fill one beaker with 50% bleach/50% DI H_2O about ¾ of the way up; fill another with just DI H_2O to the same level.

3. Bleach the bench you'll be working at and set out a pile of paper towels.

4. Take out one paper towel to wipe off liquid from sterilized tweezers and another to work on. Take out 2 pairs of tweezers from the bleach, swish them in the water, and wipe them off on the first paper towel.

5. Open the first pop-top tube and the first sample. Take out the filter carefully with the tweezers and open it onto your working paper towel.

6. Use the tweezers to score down the exact middle of the filter (use the grid). Pull apart along the scoring. Put one half of the filter back in the original tube. Tear the other half into about 4 pieces and put them in the open pop-top tube.
    a. **Make sure all pieces can be air dried** – that none is forming a seal at any point in the tube that won't let air in or out. Shift things around with tweezers as necessary.

7. Close the cap, label the tube, and take the tweezers that are still sitting in the bleach and put them in the beaker with water, then put the tweezers you've been using back in the bleach. Change the paper towel you worked on for a new one.

8. Complete for all samples in the batch – change gloves at any point you touch a filter or get ethanol from a sample on your gloves **(or think you may have).**

9. Put a label on the tray to indicate that these are eDNA samples drying – please do not disturb, and the date.

10. Leave overnight.

Day 2

1. Turn heatblock to 55˚C.

2. Add 180 µL ATL to each tube.

3. Add 20 µL ProK to each tube individually. Use the tip to push all the filter material down into the liquid at the bottom. It should be fully immersed but seem to take up the whole amount of liquid. Squish it around a little. **Vortex each one as you finish with it.**
    a. If there really doesn't seem to be enough liquid to cover the filter material, set it aside and top it off with ATL at the end – make a note.

4. Incubate at 55˚C in a heatblock and vortex a few more times that day. Incubate overnight.

Day 3

1. Put storage tubes under the UV light to sterilize while you work.

2. Remake the bleach and water beakers like in day one. Get out 2 pairs of tweezers and put them in the bleach. Get a paper towel for wiping off sterilized tweezers.

3. Remove samples from the heatblock and turn up to 70˚C. **Put AE Buffer on heatblock**.

4. Set out 1.5 mL pop-top tubes and label. Set Qiashredder spin columns into those pop-top tubes and label those as well.

5. Vortex samples 15 seconds. Move each sample to a Qiashredder spin column by moving the filter with the sterilized tweezers and pipetting the rest of the liquid. Try to get all of it.

6. Spin for 2 minutes at 11,000 RPM.

7. Remove columns and add 200 µL Buffer AL to each sample. **Vortex immediately.**

8. Incubate at 70˚C for 10 minutes.

9. Add 200 µL 100% EtOH to each tube, vortexing immediately each time.

10. Add the mixture to a DNeasy spin column (~600 µL). Centrifuge at 8000 RPM for 1 minute.

11. Place the column in a new collection tube, pour the filtrate into the collection bottle, and discard the old collection tube.

12. Add 500 µL AW1 to each sample. Centrifuge at 8000 RPM for 1 minute.

13. Place the filter in a new collection tube, pour the filtrate into the collection bottle, and discard the old collection tube.

14. Add 500 µL of AW2 and spin at 11,000 RPM for 3 minutes.

15. Remove the spin column carefully so no ethanol splashes on the column. Place the spin column in new tube with no top.

16. Elute the DNA with 100 µL Buffer AE (preheated to 70˚C). Incubate at room temperature for 5 minutes then spin at 8000 RPM for 1 minute.

17. Pipette this final elution into a permanent storage tube and label with sample name, date extracted, and your initials.

18. Store in the refrigerator or freezer until ready for PCR.