

Processing eDNA water filters using Qiagen DNeasy and Zymo PCR Clean up  
Docker Lab UManitoba

[Qiagen DNeasy Extraction kit](#)

[Zymo One-Step PCR Inhibitor Removal kit](#)

*NOTE: Extraction process takes two days for isolation and all liquid waste must be disposed of in DNA buffer waste collected*

**DAY 1 – Approximately 2 hours**

1. Wipe down all equipment, including centrifuge, vortex, outside of pipettes, commonly contacted surfaces with 70% EtOH or ELIMINase
2. UV treat the hood that contains all the necessary equipment such as gloves, petri dishes, Kim wipes, Exacto knife pen. Make sure that the hood contains forceps, alcohol and ELIMINase for dipping or spraying between samples
3. Turn on the thermomixer and set temperature to 56°C, mixing or shaking speed to 350-400 rotations per minute (rpm)
4. Take out tubes with eDNA filters from freezer and keep away from UV hood
5. Once UV station treatment complete, take out 1.5 mL tubes (# of tubes = # of eDNA filters plus one extra to serve as the negative control)
6. Add **450 uL** of **Buffer ATL** to each tube (*check to see if Buffer has salt solids in bottom by opening carefully in UV station – if salt deposits are present, place in water bath for 5 minutes before pipetting*)
7. Add **50 uL** of **proteinase K** to each tube
8. Remove eDNA filter papers from tubes and place on petri dishes to dry for 30 minutes (or until ethanol has evaporated)
9. Label 1.5 mL tubes with sample ID and date
10. Start with the negative control sample. Cut the filter paper into 8 pieces and place into appropriate number of 1.5 mL labelled tube
11. Clean the razor, forceps and Exacto knife pen with ELIMINase after each sample
12. Vortex thoroughly – for 15 to 20 seconds
13. Place the 1.5 mL tubes in the thermomixer at 56°C, mixing 350-400 rpm
14. Leave overnight or anywhere between 18-24 hours

*At this point, if you want to process more sampling for different sites, you can clean up all the equipment, wipe down pipettes with a Kimwipe with ELIMINase, and UV the PCR station*

**DAY 2 – Approximately 3 hours**

**Set up station with DNA columns, Qiashredder columns, 1.5 mL tubes, collection tubes, forceps, ELIMINase and gloves**

1. Remove tubes from thermomixer and spin down at 13,000 rpm
2. Add **6 uL** of **RNase A** to each tube
3. Vortex briefly, 5-10 seconds
4. Spin tubes down, at 13,000 rpm for 10 seconds
5. Incubate in thermomixer for 5 minutes
6. Vortex thoroughly, 15 to 20 seconds
7. Spin tubes down - 13,000 rpm
8. Move each sample to a Qiashredder spin column by moving the filter with the sterilized forceps and pipette the rest of the liquid into the column
9. Spin for two minutes at 11,000 rpm
10. Remove columns and place liquid into a new 1.5 mL tube
11. Add **400 uL** of **Buffer AL** and **400 uL 100% ethanol** to each tube
12. Vortex tubes for 15-20 seconds
13. Spin tubes down at 13,000 rpm
14. Label the appropriate number of DNeasy Mini Spin Column or DNA collection columns (# of DNA columns = # of eDNA filters)
15. Pipette 600 to 700 uL of the mixture that is in the 1.5 mL tube along with the precipitate into the DNeasy Mini Spin Column
16. Centrifuge at 8,000 rpm for 1 minute
17. Discard the flow through into the waste container and place the tube back into collection tube
18. Pipette the remaining mixture into the spin column and repeat centrifugation and discard flow through
19. Keep the mini spin column and place into a *new* 2 mL collection tube, and discard the used collection tube
20. Heat Buffer AE on heating block at 70°C and set time for minimum 30 minutes without shaking

21. Add **500 uL** of **Buffer AW1**
22. Centrifuge for 8,000 rpm for 1 minute
23. Discard the flow through into waste and discard the collection tube
24. Transfer the mini column to a *new* 2 mL collection tube
25. Add **500 uL** of **Buffer AW2**
26. Centrifuge at 14,000 rpm for 3 minutes
27. Discard the flow through and collection tube
28. Keep the DNeasy Mini Spin Column
29. Pipette Buffer AE into multiple tubes and preheat in thermomixer for minimum 10-15 minutes at 70°C
30. Label the appropriate number of 1.5 mL tubes and 0.6 mL tubes (one-1.5 mL tube and two-0.6 mL tubes per sample)
31. Transfer the DNeasy Mini Spin Column into each 1.5 mL tube
32. Pipette **100 uL** of **HOT Buffer AE** into the spin column. *Make sure you're pipetting directly onto the filter membrane*
33. Let tubes sit at room temperature for 5 minutes
34. Centrifuge at 8,000 rpm for 1 minute
- 35. DO NOT DISCARD THE FLOW THROUGH - IT'S THE DNA**
36. Discard the mini spin column and close the tube
37. If you're going to be running an assay with DNA in the next few days, refrigerate DNA otherwise, store in -20°C or -30°C freezer

**OPTIONAL:** After DNA has been cleaned up using the Zymo PCR Inhibitor Removal kit.