Tissue derived DNA extractions - using Qiagen DNeasy Blood and Tissue kit 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. 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)
5. Add **180 uL** of **Buffer ATL** to each tube *(check to see if Buffer has salt solids in bottom by opening carefully in UV station – it salt deposits are present, place in water bath for 5 minutes before pipetting)*
6. Add **20 uL** of **proteinase K** to each tube
7. Label 1.5 mL tubes with sample ID and date
8. Place pre-cut tissue for DNA extraction into labelled tube

   NOTE: Remember to ALWAYS: Clean the razor, forceps and Exacto knife pen with ELIMINase after each sample See Qiagen protocol to see amount and size of tissue
9. Vortex thoroughly – for 15 to 20 seconds
10. Place the 1.5 mL tubes in the thermomixer at 56°C, mixing 350-400 rpm
11. Leave overnight or anywhere between 18-24 hours

DAY 2 – Approximately 3 hours
Set up station with DNA 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 **3 uL** of **RNase A** (PureLink™ RNase A (20 mg/mL) 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

OPTIONAL Qiashredder steps

8. Pipette each sample to a Qiashredder spin column
9. Spin for two minutes at 11,000 rpm
10. Discard top purple column. A pellet may have formed, dislodge the pellet with pipette tip or by pipetting up and down.
11. Pipette liquid and pellet mix into a new 1.5 mL tube

12. Add **200 uL** of **Buffer AL** and **200 uL 100% ethanol** to each tube
13. Vortex tubes for 15-20 seconds
14. Spin tubes down at 13,000 rpm
15. Label the appropriate number of DNeasy Mini Spin Column or DNA collection columns (# of DNA columns = # of eDNA filters)
16. 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
17. Centrifuge at 8,000 rpm for 1 minute
18. Discard the flow through into the waste container and place the tube back into collection tube
19. Pipette the remaining mixture into the spin column and repeat centrifugation and discard flow through
20. Keep the mini spin column and place into a *new* 2 mL collection tube, and discard the used collection tube

21. Heat Buffer AE on heating block at 70°C and set time for minimum 30 minutes without shaking
22. Add **500 uL** of **Buffer AW1**
23. Centrifuge for 8,000 rpm for 1 minute
24. Discard the flow through into waste and discard the collection tube
25. Transfer the mini column to a *new* 2 mL collection tube
26. Add **500 uL** of **Buffer AW2**
27. Centrifuge at 14,000 rpm for 3 minutes
28. Discard the flow through and collection tube
29. Keep the DNeasy Mini Spin Column
30. Pipette Buffer AE into multiple tubes and preheat in thermomixer for minimum 10-15 minutes at 70°C
31. Label the appropriate number of 1.5 mL tubes
32. Transfer the DNeasy Mini Spin Column into each 1.5 mL tube
33. Pipette **60 uL** of **HOT Buffer AE** into the spin column. *Make sure you're pipetting directly onto the filter membrane. It's better to have a higher concentration of DNA rather than too dilute, so start with 60 uL Buffer AE.*
34. Let tubes sit at room temperature for 5 minutes
35. Centrifuge at 8,000 rpm for 1 minute
36. **DO NOT DISCARD THE FLOW THROUGH - IT'S THE DNA**
37. Discard the mini spin column and close the tube
38. 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.