**eDNA Preservation Experiment I**

**Phenol-Chloroform-Isoamyl (PCI) Extraction Protocol**

Filters preserved in Longmire’s Buffer solution are stored at room temperature and are extracted using the phenol-chloroform-isoamyl (PCI) extraction protocol described by Renshaw et al. 2015. *Molecular Ecology Resources*.

**Day 1**

1. Turn heating block to 65℃
2. Move 850 µL of Longmire’s solution from the original sample to a new 2 mL tube (samples were stored in 1.7 µL LM instead of 900 µL)
3. Incubate microcentrifuge tubes containing filter and Longmire’s Buffer at 65℃ for 10 minutes
4. Add 850 µL of phenol:chloroform:isoamyl alcohol (25:24:1) to each tube
5. Vortex samples to thoroughly mix solution and filter for 10 seconds
6. Centrifuge tubes at 14,000 rpm for 5 minutes
7. Transfer 750 µL of aqueous layer to new 2-mL microcentrifuge tubes
8. Add 750 µL of chloroform:isoamyl alcohol (24:1) to each tube (1st wash)
9. Vortex samples for 5 seconds
10. Centrifuge tubes at 14,000 rpm for 5 minutes
11. Transfer 600 µL of aqueous layer to new 2-mL microcentrifuge tubes
12. Add 600 µL of chloroform:isoamyl alcohol (24:1) to each tube (2nd wash)
13. Vortex samples for 5 seconds
14. Centrifuge tubes at 14,000 rpm for 5 minutes
15. Transfer 500 µL of aqueous layer to new 2-mL microcentrifuge tubes
16. Add 1.3 mL of 100% (200 proof) **ice-cold** ethanol to each tube
17. Add 20 µL of 5 M NaCl to each tube
18. Precipitate samples at -20℃ overnight

**Day 2**

1. Centrifuge tubes at 14,000 rpm for 10 minutes
2. Decant liquid using a filtered pipette tip, **making sure not to disrupt the pellet**
3. Dry pellets in a vacuufuge at 45°C for 15 min
4. Air dry until no visible liquid remains
5. Rehydrate pellets in 100 µL of 1xTE Buffer
6. Combine separated samples into one tube so final elution is in 200 µL of 1xTE Buffer
7. Dilute sample 10x-fold in new screw-top tube: 10 µL eDNA sample (2x) + 90 µL H20 = final dilution of 20x