**eDNA Preservation Experiment II**

**Ethanol 4°C -- PCI**

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

Days 1-2 follows protocols of Piggott et al. 2016 and Deiner and Altermatt 2014. PCI protocol follows Renshaw et al. 2015 with an additional CI wash.

**Day 1**

1. Remove ethanol using a filtered pipette tip. Let air dry overnight.

**Day 2**

1. Add 500 µL of tissue lysis buffer to each tube [100 mM Tris-HCl pH 8.0, 5 mM EDTA, 0.2% SDS, 200 mM NaCl2]
2. Add 4 µL of 20 mg/mL Proteinase K (final concentration = 0.16 mg/ml)
3. Vortex samples gently for 10 seconds
4. Incubate overnight at 55℃ in shaker

**Day 3**

1. Remove PCI from refrigerator to acclimate to room temperature

1. Remove filter from tube
2. Add 500 µL of phenol:chloroform:isoamyl alcohol (25:24:1) to each tube
3. Vortex samples to thoroughly mix solution and filter for 10 seconds
4. Centrifuge tubes at 14,000 rpm for 5 minutes
5. Transfer 450 µL of aqueous layer to new 2-mL microcentrifuge tubes
6. Add 450 µL of chloroform:isoamyl alcohol (24:1) to each tube (1st wash)
7. Vortex samples for 5 seconds
8. Centrifuge tubes at 14,000 rpm for 5 minutes
9. Transfer 450 µL of aqueous layer to new 2-mL microcentrifuge tubes
10. Add 450 µL of chloroform:isoamyl alcohol (24:1) to each tube (2nd wash)
11. Vortex samples for 5 seconds
12. Centrifuge tubes at 14,000 rpm for 5 minutes
13. Transfer 400 µL of aqueous layer to new 2-mL microcentrifuge tubes
14. Add 1.1 mL of 100% (200 proof) **ice-cold** ethanol to each tube
15. Add 20 µL of 5 M NaCl to each tube
16. Precipitate samples at -20℃ overnight

**Day 4**

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