Fall 2018

DNA EXTRACTION PROTOCOL OF ZOOPLANKTON (INCLUDING ARTEMIA)

DNA Kit utilized: Omega BioTek E.Z.N.A Tissue Kit

Benjamin D. Gallo & Brian F. Leydet

1. Collect zooplankton (methods may vary). Store collected zooplankton in 2.0 mL microcentrifuge tubes with approximately 1.5-1.75 mL Nucleic Acid Preservation Buffer (Camacho-Sanchez *et. al*  2013). Store at room temperature or in fridge.
2. Place a sterile 0.2 or 0.4 µm filter pad on a sterile water filtering pedestal (sterilize with 70% EtOH or bleach). Pour the stored zooplankton sample onto the filter and vacuum filter away the liquid. Try to dry the filter out as much as possible.
3. Turn off the vacuum, and carefully move the filter (with zooplankton) to a sterile weigh boat (sterilized with 70% EtOH) and scrape approximately 10 mg of zooplankton onto the weight boat.
4. Transfer the zooplankton contents to a sterile 2.0 mL screw-cap microcentrifuge tube using a sterile scalpel blade or sterile tweezers.
5. Add 200 µL TL Buffer and four sterile 3mm silica beads to the tube.
6. Bead beat the sample for 60 seconds on the beat beater in Dr. Whipps lab.
7. The TL buffer will become very bubbly/sudsy after bead beating. THIS IS OK. Return to the lab and centrifuge the tubes for one minute at *14,000+ x g* – this will help bring the bubbles down a bit, but some will still be present.
8. Add 25 µL OB protease enzyme to the tube. Vortex to mix thoroughly.
9. Incubate overnight in a standing water bath at 55˚C for 14-17 hours. This should make the bubbles subside.
10. After incubation, transfer ALL CONTENTS EXCEPT BEADS to a sterile 1.5 mL microcentrifuge tube. Save the beads in a separate closed container for future use.
11. Proceed forward with step # 5 on the E.Z.N.A Tissue DNA Kit Quick Guide.

MODIFICATIONS FOR ESOCID DRY FOOD

Dry food information:

#1 Crum BioVita Starter

Bio-Oregon, Westbrook, ME

Complete food for Salmonids

* Soak ample amount of dry food in sterile distilled water for one hour in 50 mL centrifuge tube at room temperature.
* After soak, place food on sterile filter and draw water off via vacuum filtration.
* Weigh out 10 mg of dry food from filter on a ethanol sterilized weigh boat.
* Add 100 mg Glass Beads X and 400 µL TL buffer.
* Vortex for 30 s – 60 s to mix thoroughly.
* Add 30 µL OB protease solution. Vortex to mix thoroughly.
* Continue with step #9 above.