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DNA EXTRACTION FROM POLYCARBONATE (PC) FILTERS (47mm)

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

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1. Collect water samples (minimum tested amount = 25 mL) and filter on 47 mm polycarbonate filter paper within 24 hours. Keep water samples cool until filtration. Note that amount of water for filtration is dependent on the project scope and source of water. Tested samples included: pond water, aquarium water, distilled water.

2. Store filter samples in 1.5-2.0 mL of nucleic acid preservation (NAP) buffer (Camacho-Sanchez *et. al* 2013) within sterile 2.0 mL microcentrifuge tubes (it is necessary to fold up and stuff the filter to put it inside the small tubes). The NAP buffer allows for storage of samples at room temperature or refrigeration. Samples can be stored for up to 10 months at ambient temperature.

3. When ready to commence with the DNA extraction, remove the folded filter paper and place in an ethanol sterilized container.

4. Cut the filter into small pieces (>5 pieces) and equally divide the filter pieces between two new sterile 2.0 mL microcentrifuge tubes.

(Optional): If additional filters were stored in the NAP buffer in the same tube (e.g. in order to filter the water, two filters were needed due to clogging). Repeat step #3-4.

5. Take the NAP buffer in the original tube and filter the material through a new 47 mm polycarbonate filter paper.

6. Cut the filter into small pieces (>5 pieces) and equally divide the filter pieces into the two 2.0 mL microcentrifuge tubes noted in step #4.

7. Place four sterile 3 mm glass beads into each of the tubes.

8. Add 100 mg of Glass Beads X (available in the Omega BioTek E.Z.N.A Water DNA Kit) to each tube.

9. Dispense 400 µL of TL buffer into each tube.

10. Homogenize the tubes via bead-beating for 15 minutes. The TL buffer will turn very foamy during this process. That is normal.

11. Centrifuge the samples for 10-15 seconds (speed variable) to reduce the foam.

12. Add 30 µL OB Protease solution to each tube. Vortex to mix thoroughly, then quickly centrifuge down to remove liquid from the top of the tube.

13. Incubate the samples at 55˚C for 14-17 hours.

14. Transfer as much of the supernatant as possible from the incubated tube to a new 1.5 mL microcentrifuge tube. Supernatent transfer amounts range between approximately 200-300 µL.

15. Continue at step #5 on the E.Z.N.A DNA Tissue Kit. Use 420 µL for both the BL buffer and 100% Ethanol steps.

**References:**

Camacho-Sanchez, M., Burraco, P., Gomez-Mestre, I., & Leonard, J. A. (2013). Preservation of RNA and DNA from mammal samples under field conditions. *Molecular Ecology Resources*, **13**(4), 663–673. https://doi.org/10.1111/1755-0998.12108.