An Aguirre Lab Protocol
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Water Filtration for Environmental DNA (eDNA) Assays

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Foreword

Environmental DNA (eDNA) assessments have become an increasingly popular tool in ecology and molecular biology for detecting and monitoring species presence and abundance in aquatic ecosystems. To maximize eDNA capture and ensure accurate results, it is crucial to filter and pre-filter water samples before DNA extraction. This is because eDNA can rapidly degrade or be adsorbed onto particles in the water column, reducing the concentration and quality of DNA available for detection. Non-target DNA such as microbial and algal DNA can compete with target DNA for amplification during PCR, leading to false-negative or false-positive results.

Therefore, utilizing dual step filtration can help to remove particles and non-target DNA, reducing background noise during sequencing and increase sensitivity and specificity of eDNA detection.

Several studies have demonstrated the importance of filtering and pre-filtering water samples for eDNA assessments. Hunter et al. (2017) found that filtering water samples through a 0.45 µm membrane filter prior to DNA extraction significantly increased the detection rate of eDNA for aquatic species. Similarly, Kelly et al. (2014) showed that pre-filtering water samples through a 20 µm mesh filter before using a 0.22 µm filter for DNA extraction improved the accuracy of eDNA detection for fish species. These studies highlight the need for careful sample preparation and filtration protocols in eDNA assessments to ensure the reliability and accuracy of the results.

This protocol addresses these limitations and involves the use of gravity drip apparatus with multiple filtration layers (see **Image I**) and a peristaltic pump attached to a collection cup internalizing a 0.22 µm Sterivex-GP PES filter (see **Image III**).

Part A:

Materials Needed:

- Gravity-drip apparatus with multi-sized filters (Newstar 110 mm coffee filter of Grade 103, 0.22-0.44 μm Sterivex filters, Newstar 100 mm coffee filter) see **Image I**)
- Sealed Water Samples;
- 5% Bleach solution
- Sterile bottle

Sample Disinfection

- ☐ Apply diluted 75% bleach solution to the bench and clamp apparatus.
- ☐ Allow solution to sit for a 10-minute duration, then wipe clean.

Preliminary Filtration

- ☐ Set up the gravity-drip apparatus with the filters according to size (see **Image II**).
- ☐ Allow the water samples to pass through the apparatus.
- ☐ Capture the filtrate in a sterile bottle placed beneath the apparatus.

Image I. Gravity-drip apparatus with multi-sized filters (Newstar 110 mm coffee filter of Grade 103, 0.22-0.44 μm Sterivex filters, Newstar 100 mm coffee filter.

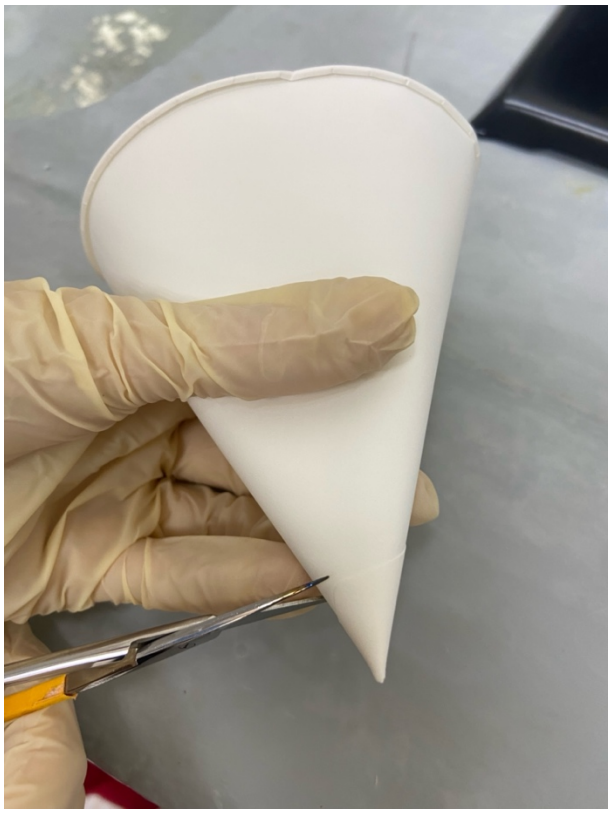


Image II: Cut was made ~1 cm up from the bottom of the paper cup to allow for water flow that would not expand past the collection bottle's mouth.

Part B:

Materials Needed:

- Peristaltic pump(s) see **Image III**
- 0.22 µm Sterivex-GP PES filter
- 95% Ethanol

Image III. Peristaltic pump connected to filtration cup.

Primary Filtration: Peristaltic Action

☐ Using the peristaltic pump, draw the pre-filtered water samples through the 0.22 µm Sterivex-GP PES filter.

Recommended Settings:

Volume	260.0 mL
Times	300
Flow	360 mL/m
Interval	1s

Note:

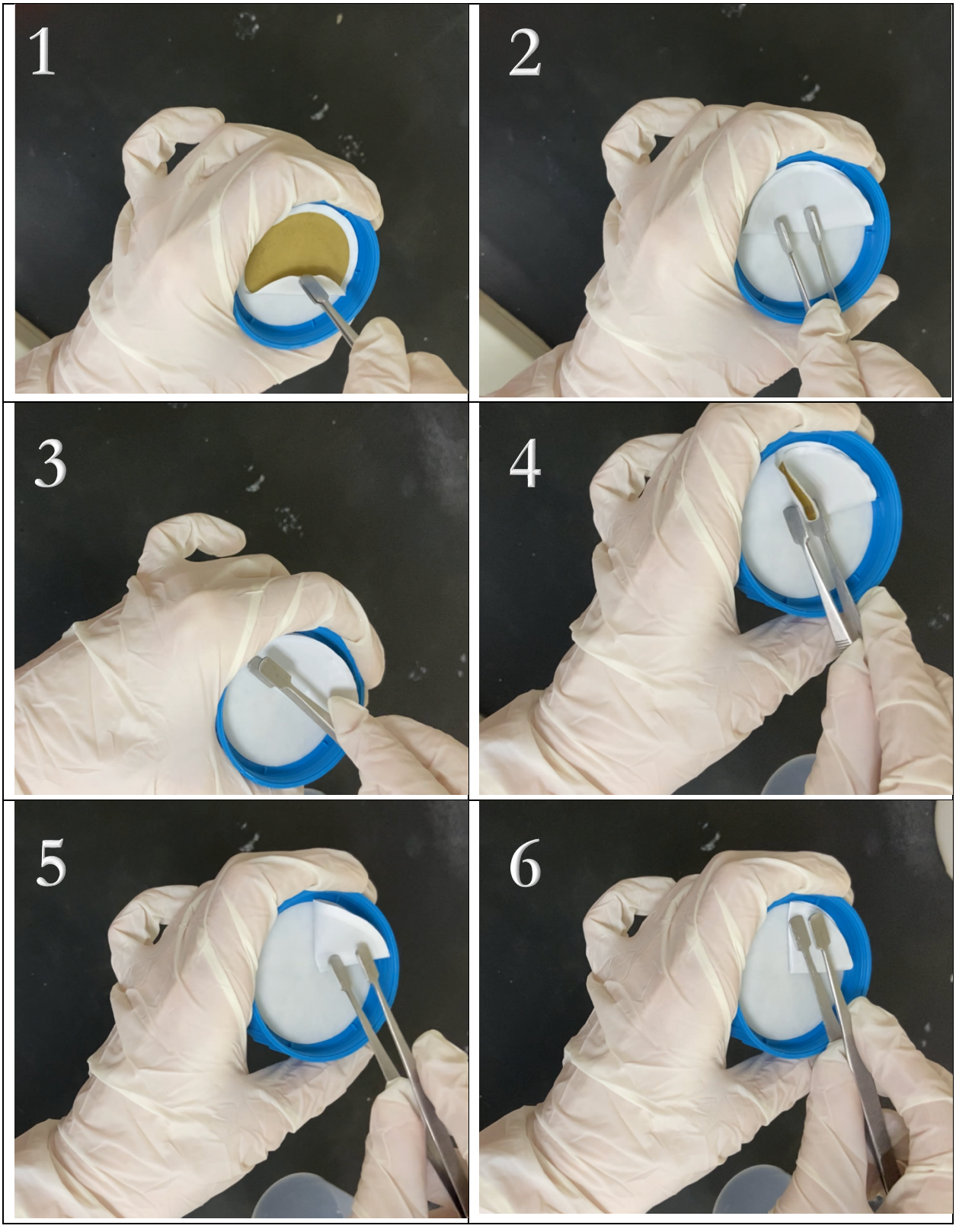
The characteristics of the substrate at the sampling location can exert influence over the pump's ability to maintain a steady throughput of water volume. Filters may be subject to clogging if the substrate at a sampling locality contains a high density of fine sediment or organic matter. Due to substrate-specific differences, a flexible approach to sample collection and processing might be necessary.

Filter Folding, Preservation

☐ Carefully fold the filter post-filtration (see **Image IV** for guidance).

☐ Store the filter in a 95% ethanol solution for preservation, 1.5 mL tubes with screw-caps preferable if samples are traveling internationally.

Image IV. Step by step images of the filter-folding process.



References Listed

Hunter ME, Dorazio RM, Butterfield JS, Meigs-Friend G, Nico LG, Ferrante JA. Detection limits of quantitative and digital PCR assays and their influence in presence-absence surveys of environmental DNA. 2017. Mol Ecol Res. 2:221-229. doi: 10.1111/1755-0998.12619

Kelly RP, Port JA, Yamahara KM, Crowder LB. 2014. Using Environmental DNA to Census Marine Fishes in a Large Mesocosm. PLoS ONE 9: e86175. https://doi.org/10.1371/journal.pone.0086175