

## Protocol for collecting and filtering water for eDNA analyses

*This is a protocol for collecting and filtering water for eDNA analysis.*

### Collecting Water:

- **Before Starting:**
  - You will need sterile 1 L bottles, one for each sample, and a cooler large enough to fit the bottles. Water bottles can be reused but should be sterilized carefully between uses. Make sure to label the water bottles prior to collecting the water.
  - Bring one pair of gloves for each water sample that you will be collecting
- Make sure to use gloves and change gloves between samples
- Without disturbing the water, submerge the 1L collection bottle water until it is full, close the bottle, and place it in a cooler.
  - You may need greater volumes of water at sites with low target group density or if wishing to detect rare species.
- The water should be kept in the cooler or refrigerated until it is filtered, and filtration should occur **within 24 hours** of water collection.

### Filtering Water:

*We will use a peristaltic pump and single use analytical filter funnels to filter the water samples collected above. See the end of the protocol for catalog numbers.*

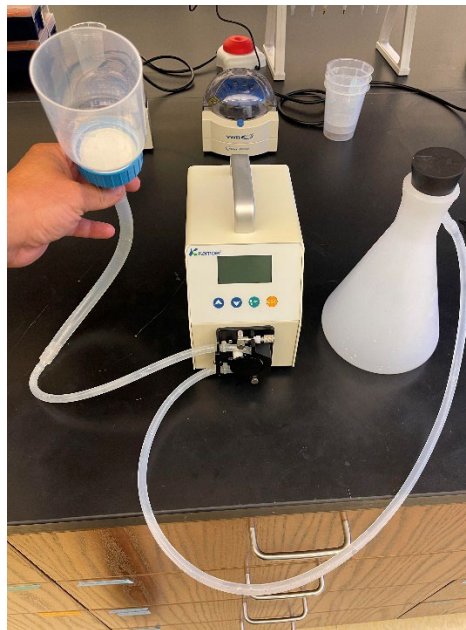


Figure 1: Peristaltic water pump and water filtration setup.

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- Set up the pump and filter as shown in the image above. Wear gloves.
  - When running the pump, it is useful to have the funnel high and the collection container low so that gravity assists with the filtration process.
  - Turn on the pump and make sure the water flow direction is correct. Water should flow from the filter funnel through the pump to the collection container. This can be tested with a used filter funnel and tap water.
- Add 250 ml of water into the top of the funnel and start the water pump running.
- Once the water level in the funnel is low, fill the funnel with water again. Repeat until all the collected sample water runs through the filter into the collection container.
- Take the top of the funnel off the base so that the filter membrane is exposed. Using membrane (flat-tip) tweezers, remove the filter membrane from the base of the funnel. Do this by folding the filter membrane several times with the tweezers so it fits in a 1.5-2 ml microcentrifuge tube with the preservative (usually 95% ETOH).
  - Alternatively, if the DNA extraction will be conducted immediately after the filtration, just put the filter membrane in a tube on ice without the preservative.
- Repeat for each water sample using a NEW sterile funnel/filter. Note that the cone attaching the funnel to the water tubing is NOT disposable. Do not discard the bottom cone!
- Clogging of the filter membrane is a very common problem. It is useful to let particles settle before beginning the DNA extraction or pre-filter with a larger pore membrane to remove some of the sediment.

#### Notes:

- For a useful video on collecting eDNA from water samples see: <https://youtu.be/VQ8zZDPR7OY>
- For a useful pdf guide see: <https://pubs.usgs.gov/tm/02/a13/tm2a13.pdf>

#### Materials and Reagents:

- Kamoer peristaltic pump: [https://www.amazon.com/gp/product/B01HBAMEGY/ref=ppx\\_yo\\_dt\\_b\\_search\\_asin\\_title?ie=UTF8&psc=1](https://www.amazon.com/gp/product/B01HBAMEGY/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&psc=1)
- Thermo Scientific™ Nalgene™ Single Use Analytical Filter Funnels Catalog # 09-740-30K
  - Membrane is 0.45 microns, funnel has 250ml volume capacity