SOP#102: Filtration of Water Samples for eDNA Extraction

Purpose: To prepare and preserve filters from environmental water samples for later extraction of eDNA.

This method has been published in Allison, M.J., Round, J.M., Bergman, L.C., Mirabzadeh, A., Allen, H., Weir, A., and Helbing, C.C. 2021. The effect of silica desiccation under different storage conditions on filter-immobilized environmental DNA. BMC Research Notes, 14: 106. doi: 10.1186/s13104-021-05530-x.

Equipment Required:
Nalgene Analytical Test Filter Funnels with 0.45 µm Cellulose Nitrate Filters [CAT No. 145-2045]
Vacuum pump (e.g., Welch 2546 vacuum system (rate 45 L/min; Vacuum: 60 torr; Pressure: 100 psi) or similar)
1 Rubber hose long enough to connect hose barb of 1st Büchner flask with mouth of second Büchner flask (~2 ft)
1 Rubber hose long enough to connect hose barb of 2nd Büchner flask with vacuum pump (~5 ft)
2 Non-serrated medium tipped forceps
2 – Autoclaved 400 mL glass beakers
2 – 1 to 2 L Büchner flasks with one-holed rubber stoppers (one of the stoppers has a part of a plastic graduated pipette through it to serve as an attachment point for the rubber hose between the flasks)
Large collection beaker (For water disposal)
Fresh package of paper towels/Kimwipes
Whatman™ filter papers (qualitative, circles, 150 mm diameter) [CAT No. 1001 055]
Black, alcohol-resistant marker
Manila coin envelopes, Kraft (#1; 5.5 x 9 cm): 1 per filter sample
Small heavy-duty freezer resealable plastic baggies: (1 baggie for 3 coin envelopes per site) or you can use smaller baggies and pack one coin envelope per baggie
Large heavy-duty resealable plastic baggies
Orange colour-indicating Silica Beads (1 tbsp/coin envelope up to three coin envelopes with 3 tbsp silica beads in a resealable plastic baggie). These beads can be purchased in 1 quart (2 lb) containers on Amazon from Dry & Dry 1 quart orange premium dessicant indicating silica gel beads (industry standard 2-4 mm) reusable (Product #CRH-16036).  • Beads will **turn from Orange (Active) to Dark Green (Water Saturated)** when 50-60% absorbed with moisture. • Free of Cobalt Chloride (there are blue ones that turn pink when saturated – don't get those as they are toxic). • Beads can be reactivated by placing in the oven for 0.5-2 hours at 200-250°F or microwave for about 10 min at DEFROST. Don't use over 250°F in the oven. Depending on beads' condition, they may take shorter than the recommended time. • Check the beads' color periodically.
Reagents Required:
~500 mL Autoclaved dH_2O
Javex 12 Bleach by Clorox (10.3% sodium hypochlorite by weight)
10% (v/v) bleach solution for cleaning equipment (made fresh daily)

Estimated time: 2-4 hours depending upon number of samples

Important Notes or Considerations:

1. Wear safety glasses, nitrile gloves and a lab coat.

2. Benchtop must be wiped with 10% bleach solution prior to set-up and after finishing for the day. All bleach solutions should be made fresh each day. Wipe work area with 70% ethanol between each sample.

3. Pre-label the coin envelopes with their respective sample IDs and dates using the black, alcohol-resistant marker. Double-check correct sample labelling.

4. Both forceps should be submerged in a 50% bleach solution, then completely rinsed with water and dried with a clean paper towel between each sample handling.

Set-up:

1. Set up vacuum filter apparatus by connecting the hose adaptor of the first Büchner flask to the shorter length of rubber hose and connect the other end of this hose into the rubber stopper of the second Büchner flask that will serve as a moisture trap. Use 70% ethanol as a joint lubricant to ensure a tight seal.

2. Connect the longer length of rubber hose to the hose adaptor of the second Büchner flask and connect the other end of this hose to the input adaptor of the vacuum pump. Ensure all hoses are firmly attached and rubber stoppers form tight seals to Büchner flask mouths.

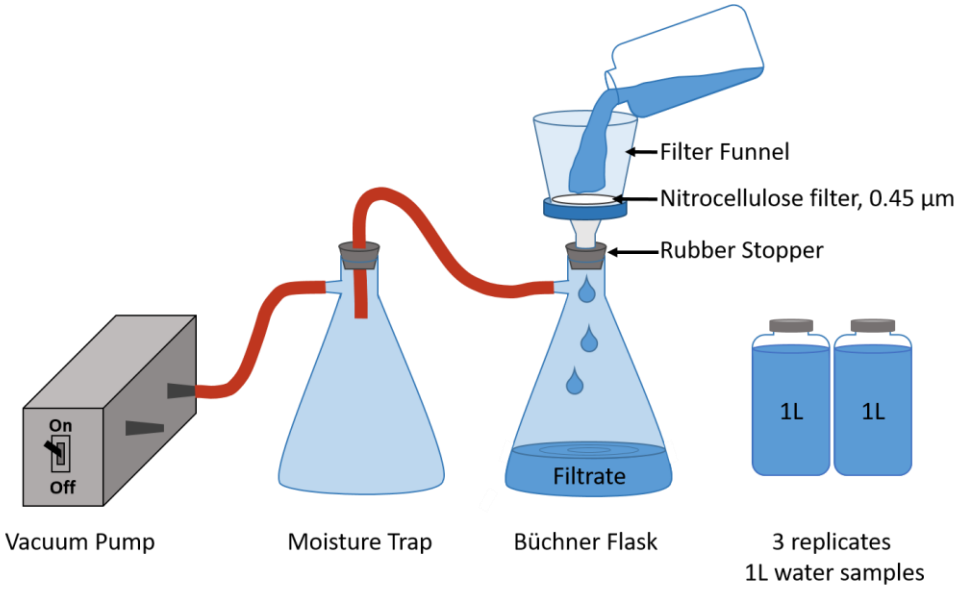

Figure 1 The water sample filtration set up.

3. In a clean area of the benchtop, lay out two overlapping clean paper towels, and lay a third paper towel on top. On this paper towel, lay one Whatman™ filter paper. This will be used for processing the eDNA sample filters after filtration.

4. Fill one autoclaved 400 mL beaker with 150 mL of bleach and 150 mL autoclaved dH_2O to create 300 mL of a 50% (v/v) bleach solution. Fill another autoclaved 400 mL beaker with ~350 mL autoclaved dH_2O.

5. Gently place the two forceps into the 50% (v/v) bleach solution for 30 sec, allow excess bleach to drip off forceps then transfer them to the dH_2O wash beaker and swish briefly to thoroughly remove all the bleach. Wipe them well with paper towel to dry. This will be repeated prior to handling each filter sample.

Methods:

1. Connect a sterile filter funnel to a filter adapter (included with funnels) and insert adapter firmly into rubber stopper. Filter adaptors are re-used for multiple filter funnels.

2. Lift top of filter funnel and pour ~250 mL of sample into funnel. Turn vacuum pump on and check that there are no air leaks in the filtration system. Water should be filtering into the first Büchner flask. The vacuum pump pressure knob is easily turned down, make sure that the vacuum pump is operating at high suction by ensuring the pressure knob is turned all the way up.

3. Top up the filter funnel as the sample water filters through until the entire sample has been filtered. If the filter begins to clog, stop filtering at 30 min, carefully remove the partially full filter funnel, pour off remaining fluid, and re-attach the filter funnel to the Büchner flask. For samples with especially high particulate matter, you may insert a clean, non-bleached coffee filter into the filter funnel for use as a pre-filter. Continue with step 4.

4. Once the water sample has been completely filtered, leave vacuum pump running for one minute to remove as much water from the filter as possible.

5. Detach filter funnel from the flask. Carefully snap open the filter funnel and use clean (properly prepared) forceps to remove the filter from the supportive backing.

6. On the clean Whatman™ filter paper, use both forceps to gently fold the sample filter in half with the filtride on the inside, and insert it into its corresponding pre-labelled coin envelope.

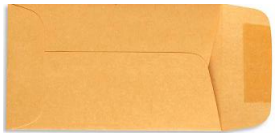

Figure 2. Example of a #1 coin envelope.

7. Insert the coin envelope into a sealable plastic baggie and add ~1 tbsp silica beads to the baggie. Zip up completely while removing air in the bag. Three coin envelopes may be stored in one small heavy duty resealable freezer baggie provided there is a tbsp of silica beads for **each** sample (so you could have your three replicates per site in one baggie containing 3 tbsp silica beads). You can store small sample baggies from multiple sites in a larger resealable bag to reduce the chance of air exchange and to enhance sample organization.

Figure 3. A filter membrane is folded in half with the filtride side in and placed inside a paper coin envelope. This, in turn, is placed in a sealable plastic bag containing silica gel beads, the air removed and firmly sealed before storage. It is easy to monitor the moisture content of the sample. The orange beads turn dark green when there is too much moisture and need to be replaced or the beads regenerated as per the manufacturer's instructions.

8. Put used filter funnel in plastic recycling. Dispose of the used Whatman™ filter paper and top paper towel and replace with clean filter paper and paper towel. Pour filtrate from 1st Büchner flask into the appropriate disposal beaker, or into the sink if contents are not harmful.

9. Repeat Steps 1-8 for each water sample.

10. Store sample baggies in a larger resealable bag to reduce the chance of air exchange. You may insert multiple sample baggies into a single larger bag for this purpose.

11. Store the filter samples in the dark in a refrigerator or cooler at ~ 4°C for short term storage (up to ~1 month). For longer term storage, store at -20°C in a NOT frost-free freezer. Monitor the colour of the beads to ensure that the filters remain properly dessicated. Replace the beads if more than 50% of the beads have changed colour.

Last Updated: May 2021 **Update Author:** eDNA team