

Environmental DNA (eDNA) Sampling Protocol

Field filtering samples in shallow (wadeable) water

General Field Supplies

- Small cooler with ice – if blue ice is used, wipe off with bleach solution before putting in cooler
- Small trash bag
- Distilled or lab water
- Spray bottle containing 10% bleach
- Small bucket with a 1L line marked inside*
- Hand or portable electric pump*
- Waders- if not sampling from a boat*
- Container to carry equipment*
- Extra gloves

*Clean with 10% bleach between sites.

Per site supplies – Collect all supplies for each site into a clean garbage bag, and tie closed

- 1 Ziplock baggie with paper towels
- 1 Ziplock baggie filled with disposable gloves (10 pairs/site)
- Pump tubing and connector
- Sample tube (1.5ml microcentrifuge tube with cap); labeled. 1 per sample, and 1 for blank.
- Disposable funnel with 1.5um filter (in a plastic bag-either the one it comes in or a Ziplock)
- Sharpie
- Sampling form
- Garbage bag

Before Sampling

Label the sample tubes (while wearing gloves) and place in separate ziplock bags for each site
Check field supplies, put fresh ice in cooler

Sampling

Designate one person as the Sampler. This person will collect the sample and will have direct contact with the water. A second person will be designated as ‘Handler’. This person will assist the Sampler, but will not have direct contact with water (this helps minimize transfer of contamination from the sample to the unused supplies).

1. Fill out field sampling form
2. The person designated as “Sampler” puts on waders that have been cleaned with water and 10% bleach prior to arrival
3. Put on gloves
4. Collect a field blank – Sampler opens one of the sealed filter funnels, exposes it to the site for few minutes, then folds it, and stores in a capped tube as described below
5. Collect sample - Handler sets up the pump, Sampler attaches the disposable funnel, then wades into the stream to collect a sample. Sample funnel should be held about 10cm

below the surface of the water, and moved gently around to maximize the region sampled. Handler operates the pump and holds the bucket.

6. When 1L has been filtered through, the Sampler removes the top of the funnel (can go into the trash bag), then removes and folds the filter (with his/her gloved hands and forceps) into half, then half again, then half again and one last time half again for a total of four folds.
7. The Handler puts on clean gloves, gets the sample tube from the kit, and holds it while the Sampler carefully places the filter into it.
8. The Handler closes the sample tube and places it in a ziplock bag in the cooler (on ice)
9. Disassemble and wipe down the pump, hose, and bucket with bleach solution and paper towels
10. The Sampler rinses the waders in the water, removing mud and other big debris and removes them. Waders should be sprayed with 10% bleach and wiped with paper towels.
11. Continue on to the next sampling site being careful to keep the waders, pump and bucket away from the rest of the supplies to avoid contamination from site to site

At the next site:

- The person designated as “Sampler” puts on waders that have been cleaned with water and 10% bleach prior to arrival
- From the shore, the Handler fills the bucket with water and then rinses the waders that the Sampler is wearing – make sure the water doesn’t run off into your sample site
- From here continue following the instructions as above, making sure to clean the waders, pump, bucket, and anything else that has contacted the water thoroughly between each sampling site

After sampling:

- Freeze filters as soon as possible
- Wash and decontaminate field equipment using tap water and bleach spray. Store sampling supplies in a clean area separate from possible contamination.
- If samples are being shipped for analysis, pack them in dry ice or blue ice to avoid meltwater during shipping. Samples should be sent overnight, and carefully tracked to ensure they arrive as soon as possible.

Notes:

Bleach destroys DNA, so be careful not to let it contact your sample or filter!

Sample Collection

Location: _____

Date: _____

Sampling Team: _____

Weather/comments: _____

Site ID: _____

Other ID: _____

Water Temp: _____

Sample Depth: _____

Samples collected:

Sample ID:	Volume:	Comments

Notes: _____
