

# eDNA PROTOCOL SAMPLE COLLECTION

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## MATERIALS

1. Cellulose nitrate disposable filter funnels or other field-tested, disposable filter funnels
2. Vacuum flask (1L)
3. Silicone tubing
4. Vacuum hand pump (from auto parts store)
5. Rubber stopper with hole for funnel stem
6. Latex or nitrile gloves (non-powdered)
7. Forceps (filter forceps if possible)
8. High quality, o-ring screw cap 2mL tubes (e.g., Sarstedt brand) with 1mL 100% molecular-grade ethanol (not denatured)
9. Ethanol-proof laboratory pen (do not use a regular Sharpie marker)
10. 50 mL tubes with 30 mL of 50% bleach solution (15 mL household bleach and 15 mL distilled water) in a holder to stabilize tubes (a foam drink holder such as a koozie works well)
11. Polypropylene grab bottles and cooler with ice (for off-site filtering) or Whirl-Pak® bags (for on-site filtering)
12. Water, bleach, scrub brush, and tubs (for decontaminating between sites)

This protocol is adapted from  
Protocol Version 04/12/2012 (D.S. Pilliod, R.S. Arkle, and M.B. Laramie)  
USGS Snake River Field Station

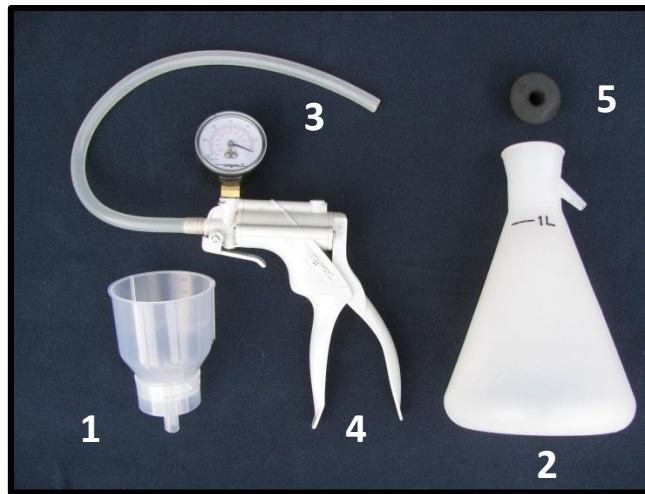


Figure 1. Filter funnel (1), vacuum flask (2), silicone tubing (3), vacuum pump (4), and rubber stopper (5).



Figure 2. Latex or nitrile gloves (6), forceps (7), 2 mL tubes with 1 mL ethanol (8), ethanol-proof lab marker (9), and 2 50 mL tubes with 50% household bleach/distilled water solution (10).

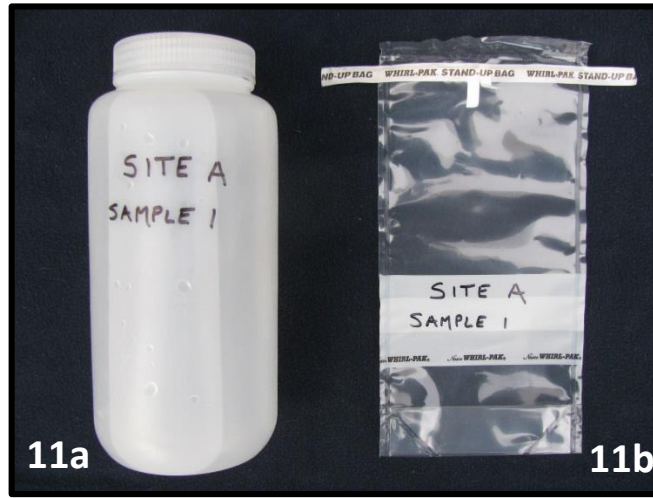


Figure 3. Polypropylene grab bottles (11a) and Whirl-Pak® bag (11b).



Figure 4. Water (12a), bleach (12b), scrub brushes (12c), and tubs (12d) for decontaminating boots and equipment between sites.

## CONTAMINATION PREVENTION

Avoid cross-contamination between samples! Contamination can result from a variety of factors at every step in the sample collection process. Be vigilant.

1. Wear gloves when removing filter and placing in ethanol storage tubes. Do not let gloves get contaminated before you handle the filter! Use non-powdered gloves only. Wear a glove when collecting water for sampling unless hands have been decontaminated while decontaminating boots and other gear between consecutive sites.
2. Be careful with gloves and other supplies. Do not leave them unprotected and do not toss them in a backpack. Keep everything clean and in plastic bags.
3. Open filter funnel package from bottom (stem end) and keep closed between sites.
4. When filtering samples, be careful not to touch the top or inside of the filter cup. No gloves are needed when handling the outside of the filter funnel, vacuum flask, and rubber stopper, as these are downstream from the filter (that is, they are below the filter and do not come into contact with sample water before it is filtered).
5. Decontaminate forceps in 50% bleach for at least 1 minute between each sample. Rinse well with distilled water (Figure 5).
6. Clean boots thoroughly between sites. Remove all dirt, pebbles, etc. from soles and sides of boots. Decontaminate in 10% bleach if they came in contact with water or mud during sampling. Rinse well in tap water (not water from the site) (Figure 6).
7. Bleach vacuum flask and stopper in 10% bleach between sites. Bleach pump and tubing if they got wet during sampling or filtering.
8. To re-use Nalgene grab bottles, bottles must be decontaminated prior to collecting new samples. Submerge bottles in 50% bleach/50% tap water solution for at least 1 minute. Rinse thoroughly with clean tap water (fill, cap, shake, and rinse; repeat at least 3 times). At the sampling site, rinse again with water from the water body 3 times before collecting sample.
9. To test for field contamination, collect 1 field negative per site. The field negative is distilled water that is filtered and preserved using the same equipment and procedures as the water samples. Fill collection receptacle (whirlpak or bottle, whatever is being used for the samples) with distilled water. Using methods for filtering samples as described in Step 3 below, filter the same volume of distilled water as the volume of samples. Remove and preserve filter as described in Step 4 below.



Figure 5. Decontaminate forceps in 50% bleach for at least 1 minute between each sample. Rinse well with distilled water.



Figure 6. Clean boots thoroughly between sites. Decontaminate with 10% bleach and rinse well with tap water.

## SAMPLE COLLECTION

### Step 1. Sample Site Selection

1. Determine criteria for selecting spot for water collection based on habitat use of the target species. Be consistent among sites.

In ponds, lakes, and wetlands, collect water at a site you predict your target species is most likely to occur. If wetland is >40 m diameter (assuming a round shape), collect additional samples from a second site 180 degrees across from first sample site. If wetland is >55 m diameter, consider using 3 sites.

In streams, many studies have collected water from the thalweg (area of strongest current, usually the deepest part of the channel) with success, but areas where the stream current is slower may contain more eDNA. This is currently being studied.

2. Take detailed notes about the sampling location within the pond, lake, wetland, or stream. Describe characteristics of the site relative to the water body as a whole. If the target species is observed before, during, or after collecting water samples, record the location of the species relative to the sampling location. Photos may also be helpful for later reference.

## Step 2: Filter Assembly (Figure 7)

1. Attach rubber stopper to top of the vacuum flask.
2. Attach disposable filter funnel directly to rubber stopper by inserting stem of funnel into hole in stopper, creating airtight seal.
3. Attach vacuum pump to tube on vacuum flask using silicone tubing.



Figure 7. Filter assembly.



### Step 3. Water Collection and Filtration

If filtering on-site:

1. Collect water in new Whirl-Pak® for filtering, one sample per pak (Figure 8a). Wear new gloves at each sampling site.
2. Pour sample slowly into filter funnel, filling funnel to 250 mL mark (Figure 9). Pause several times to swirl water in Whirl-Pak® or bottle before pouring remaining water into funnel.
3. Engage vacuum pump to begin filtration (Figure 10). During filtering, make sure vacuum pressure is sustained (monitor pump gauge if available, or watch water level to make sure water is flowing between the funnel and vacuum flask).
4. If >250 mL is being collected, disengage vacuum pump when adding more volume if you are using the funnel to measure volume. Otherwise, use mark on flask to determine when target volume has been reached. Do not use the pressure release on the vacuum pump or water from hose may contaminate the filter sample.
5. In some aquatic systems, the filter may clog before the target water volume has been filtered. The filtering rate may slow to individual drips separated by several seconds. Consider setting a cutoff time or drip rate for ending filtering. For example, you might end filtering when the drip rate slows to 3 drips every 10 seconds.
6. Make note of the volume of water filtered, whether samples were collected using Whirl-Paks® or grab bottles, and any unusual events, conditions, or problems.

If taking grab samples for later filtering off-site:

1. Collect water in sterile Nalgene bottle, one sample per bottle (Figure 8b). Wear new gloves at each sample site.
2. Rinse grab bottle 3 times with water from sample site. Cap and shake water during each rinse. Dispose of rinse water away from spot where you'll collect water sample.
3. Fill grab bottle with water away from where rinsing occurred, while standing in one place to the extent possible. Avoid stirring up sediment while collecting sample.
4. Cap firmly, label with site name and sample number, and place in a cooler with ice.
5. Filter as soon as possible (within 24 hours) using steps 2-4 described above for filtering on-site.



Figure 8. Collect water in (a) disposable Whirl-Pak® bag or (b) sterile Nalgene bottle.



Figure 9. Pour sample slowly into filter funnel, filling funnel to 250 mL mark.



Figure 10. Engage hand pump to begin filtration.

#### Step 4. Filter Membrane Removal

1. Decontaminate forceps by soaking in 50% bleach solution for at least 1 minute and then in deionized or distilled water, each stored in a 50 mL tube.
2. Remove silicone tubing from the vacuum flask to release vacuum pressure on the filter.
3. Remove funnel cup. Grasp funnel cup in one hand and the funnel base in the other. Gently twist and lift funnel cup to disconnect the funnel cup from the base, exposing filter membrane (Figure 11).
4. After removing funnel cup, wear clean glove (nitrile or other single-use gloves) on the hand that will touch filter membrane.
5. Using decontaminated forceps and gloved fingers, fold filter membrane in quarters by folding it in half and then in half again. In Nalgene cellulose nitrate and some other filter funnels, the filter membrane sits on top of a paper disc. Discard this thicker paper disc and preserve the thinner filter membrane.
6. Roll the folded filter membrane into a cylinder that fits easily into the ethanol tube (Figure 12). Keep filter stable and prevent it from unrolling by using gloved finger. Place filter in 2 mL vial filled with 1 mL ethanol (Figure 13).
7. Cap vial firmly and label with sample site, number, and date, using an ethanol-proof marker.
8. Remove filter funnel from rubber stopper and discard.
9. Repeat filtration and filter preservation for each sample and field negative, making sure to empty vacuum flask between samples to prevent it from overflowing.
10. Store sample vials at room temperature or colder, and away from light.

Note for shipping samples to a laboratory: Federal Express is currently the only major courier service that accepts ethanol in shipments.



Figure 11. Remove funnel cup.



Figure 12. Fold filter.



Figure 13. Place filter in 2 mL tube of ethanol.