

# eDNA PROTOCOL SAMPLE COLLECTION

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USGS Snake River Field Station



## 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 of 30 mL 1) 50% household bleach solution 2) distilled water in a holder (a foam drink holder [koozie] works well)
11. Polypropylene grab bottles and cooler (for off-site filtering) or Whirl-Pak® bags (for on-site filtering)
12. Water, bleach, scrub brush, and tubs (for decontaminating between sites)

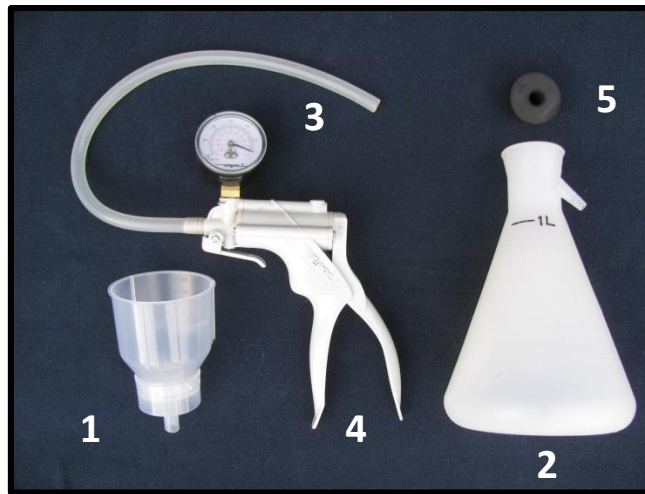


Figure 1. Filter funnel (1), vacuum flask (2), silicone tubing (3), vacuum pump (4), and rubber stopper (5).



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 solution distilled water (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 submerged in 50% bleach solution for at least 1 minute. Rinse thoroughly with clean water (fill, cap, shake, and rinse; repeat at least 3 times), let dry. At the sampling site, rinse again with sample water 3 times before collecting sample.
9. To test for field contamination, collect 1 field negative per site. 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.





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).
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. Make note of the volume of water filtered, whether samples were collected using whirlpaks 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).
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.
3. Fill grab bottle with water away from where rinsing occurred, as much as possible while standing in one place. Avoid stirring up sediment while collecting sample.
4. Cap firmly, label with site name and sample number, and place in a cooler.
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 funnel cup. Pull tab on side of funnel and gently twist to disconnect top and bottom units, exposing filter membrane (Figure 11).
3. After removing top of funnel, wear clean glove (nitrile or other single-use gloves) on hand that will touch filter paper.
4. Using decontaminated forceps and gloved fingers, fold filter paper in quarters by folding it in half and then in half again. Roll the folded filter into a cylinder that fits easily into the ethanol tube (Figure 12). Keep filter stable and from unrolling by using gloved finger. Place filter in 2 mL vial filled with 1 mL ethanol (Figure 13).
5. Cap vial and label with sample site, number, and date, using an ethanol-proof marker.
6. Remove filter funnel from rubber stopper and discard.
7. Repeat for each sample, making sure to empty vacuum flask before it overfills.
8. 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.