Protocol for collecting eDNA samples from streams

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Lone Bull Trout swimming the waters of Montana. Photo Credit: Aubree Benson

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Before Heading into the Field

The protocols contained in this manual have been designed and tested to minimize contamination. Please read the protocol carefully before heading into the field. Use the “Contents of eDNA Kit” on page 3 as a check-list to ensure that you have all required materials before going into the field.

Note: The kit contains all of the materials needed to collect eDNA except distilled water for the field control and a GPS unit to determine the sampling location. For each field control, you will need to bring 500 ml (0.5 liter) of distilled water (which can be purchased at a gas station or grocery store). Do not use regular tap water as a control because of the slight risk of contamination. Finally, please charge the pump batteries for 24 hours prior to sampling.

Choosing a Sampling Location at a Site

To avoid contaminating your field sample, it is important to make sure that you and all of the sampling equipment are downstream of the actual sampling location. Sampling should take place in the center of the current in small streams (i.e., the thalweg), or in well-mixed flow in larger streams. Be careful not to sample in an area near an eddy, backflow, splash pool, or whirlpool where DNA on your clothing may enter the water, flow upstream, and contaminate sample.
Contents of eDNA Sampling Kit

Figure 1. Complete environmental DNA kit

1. Duffle bag containing eDNA sampling equipment
2. Pump
3. Hose
4. Filter holder with filter
5. Filter holder adapter
6. Forceps (in sterile bag)
7. Sterile gloves
8. Outflow bucket
9. Sharpies
10. Sterile sample bags with desiccant (for storing filters after sample is taken)
11. Sample box with letter sized envelopes to separate and store samples from all sites
12. Black bag for used equipment (please return used equipment for sterilization and reuse)
13. White bag with unused site kits
14. Power cord
15. Pump battery
16. GPS unit (not provided)
17. 500ml distilled water for control sample (not provided)
18. Battery charger and adaptors for pump battery (not shown)

Note- The “site kit” is a one-gallon re-sealable bag containing all the materials needed to take a single field sample with companion control sample. It contains 2 sets of sterile gloves, 2 filter holders with filters (each in their own bag), 2 sterile sample bags containing silica desiccant, 2 pairs of sterile forceps. All unused, sterile site kits will be in the white bag. Sharpies, black “used equipment” bags, and extra hosing can be found in the duffle bag.
**Sterile Procedures and Avoiding Contamination**

The most important thing you can do to ensure the accuracy of your eDNA results is to avoid contamination of the control and field sample. The primary sources of contamination are anything that has come in contact with fish, or water that may have at some point been exposed to fish DNA. This includes (but is not limited to) your hands, clothes, waders, the field vehicle, used sampling equipment (such as forceps), and the environment around you (i.e., the field site).

The control sample taken at each site is used to ensure that DNA has not somehow entered into the sterile materials as a result of unsterile procedures. The field samples should only contain DNA found in the water from a particular site.

**A contaminated control or a false positive field sample could compromise an entire study.** If you suspect that any forceps, filter holders, samples, etc. have become contaminated, immediately discard the item and start over with a new, sterile item from the supply of extras. When removing an item from the bag of extras, do not reach into the bag. Instead, carefully work the needed item to the top of the bag with your hands on the outside of bag, and remove it once it is at the top. This handling procedure will help reduce the risk of contaminating the other materials in the bag of extras.

**Note:** The pump, hose, outflow bucket, and used equipment should all be considered sources of contamination since they are exposed to the surrounding environment at every sampling site. Avoid handling these potentially contaminated items with gloves, and only handle sterile items while wearing gloves. The contents of the white unused site kit bag are sterile. Sterile unused site kits and extra supplies stay in the white bag, and are only pulled out as needed. A site kit that leaves the white bag never returns. Used equipment goes into the black “used equipment” bag and is never touched again. Samples will be packaged individually and stored together in a plastic container until they are returned for analysis.
Field Collection Protocol

Step 1) Remove the pump and battery, and set in a stable area. Connect pump to battery using power cord.

Step 2) Put outflow end from pump into the outflow bucket to measure how much water has been pumped (Figure 2).

Figure 2. Outflow end of hose placed into outflow bucket

Step 3) Make sure the pump is set to full speed and in the FORWARD direction, then turn on the pump. Be sure that the end of the hose with the adaptor placed in an area where debris will not enter and clog the hose. Leave the pump running and do not touch it again until both the control and field sample have been completed. Be careful not to turn pump on in the reverse direction because this will contaminate the sample.

Step 4) Unscrew the top of the bottled water and leave the lid sitting lightly on top to keep any contaminants from entering the bottle. Place the bottle in an easily accessible area.

Step 5) Pull out a site kit from the white bag and immediately close the white bag. Put on a pair of gloves from the site kit. Once you have gloves on, be careful not to touch anything that may be contaminated with DNA (such as yourself, the pump, etc.)

Step 6) Remove one of the bags containing sterile forceps, and one of the sample bags containing silica desiccant. Unseal these bags without removing the contents and set them in an area where they are easily accessible, but not readily exposed to the
surrounding environment (i.e., where any dust, dirt, or water may enter the bag and contaminate these pieces of equipment).

**Step 7)** Remove one of the packaged filter holders from the site kit. Because the hose is a potential source of contamination, you will use the packaging from the filter holder as a glove.

Hold the filter cup in your left (non-dominant) hand. With the packaging bag on your right (dominant) hand, press the filter holder onto the filter holder adaptor on the hose (Figure 3).

Discard the plastic bag. From this point on, you will hold the filter in your left (non-dominant) hand while completing the sample. **To avoid contamination, be careful not to touch the adaptor or hose with either of your gloved hands.**

![Figure 3. Attaching filter holder adaptor and using packaging bag to handle hose](image)

**Step 8)** While running the pump, pour 500 ml (0.5 liter) of distilled water into the filter holder (Figure 4). Leave the filter in the holder with the pump running for ~30 seconds after all of the water has been filtered to help dry the filter paper.
**Figure 4.** Pouring 500 ml of distilled water through the field control

**Step 9)** Remove the filter holder, and separate the cup from the base (Figure 5). At this point you are done using this cup, and you can place it on the ground until you are ready to remove your gloves and throw all used materials into the black “used equipment bag”.

**Figure 5.** Removing cup from filter holder

**Step 10)** Use forceps to fold the filter paper in half, filtering side in (Figure 6). Once you have removed the filter paper from the holder, you can place the holder and hose
down, but remember that the pump is still running, so place it down in an area where dirt and debris will not be enter and clog the hose.

Figure 6. Folding the filter paper, filtering side in

**Step 11)** Drop the filter into the sample bag containing silica beads, force out any excess air from the bag, and seal completely. Label the bag as “Control” and include the date, stream identification (state, stream name, stream reach), GPS coordinates, and your initials. (Sharpies can be found in the duffle bag.)

**Step 12)** Label a letter-sized envelope in the **upper right hand corner** with the same information on the sample bag. Seal the plastic bag containing the sample and silica desiccant in the letter sized envelope and place in the plastic sample box.

**Step 13)** Discard your gloves. Place used forceps, filter holder, and filter cup together in one of the original packaging bags (to keep them organized), and place them in the black “used equipment bag” which can be found in the duffle bag. **Note**- we do not need you to return used gloves, or additional packaging materials.

You have just completed the control sample.

**Step 14)** If you have turned the pump off, turn it on again (as in Step 3) then put on a new pair of gloves. Prepare bags with forceps and silica beads. Attach a filter holder to the filter holder adaptor, again, using the packaging bag from the holder as a “glove” to avoid directly touching the hose and adaptor (as in Step 6).
Step 15) To collect the field sample, lower the filter holder into the stream pointing in the upstream direction (Figure 7). Scoop water from the surface of the water column. Be careful not to sample in any area with backflow that could result in contamination of the sample (see notes on “Choosing a Sampling Location at a Site” above). Continue to take scoops of water until 5 liters of water have been pumped. The outflow bucket is marked at 0.5 and 5.5 liters. The outflow bucket should already contain 0.5 liter from the control sample, so you will now pump until the water level meets the 5.5 liter mark.

Figure 7. Collecting the field sample in a large stream. Cup should be placed far enough into the current to ensure that only upstream water enters the cup.

Step 16) When 5 liters of water have been pumped, lift the cup up and away from the stream and leave the filter paper in the holder for ~30 seconds with pump running to allow the filter to dry. Then fold, remove, and store the filter paper as in Step 9. Label
as “SAMPLE 5L” (to indicate that a field sample of 5 liters was taken) and repeat all location and date information as in Step 10. Label a letter-sized envelope and store the sample as in Step 11.

**Step 17** If the filter becomes clogged (i.e., the flow of water is extremely slow) prior to completing the 5 liter sample you may need to use multiple filters from the extras provided to pump a total of 5 liters of water. In this case, label the plastic samples bags of each filter to indicate both the order of use and the approximate number of liters that were pumped through each filter. So for example, you may have one filter labeled “SAMPLE #1- 4L” and “SAMPLE #2- 1L”. Label the sample bags with the same date and identification number as in Step 10, **but store the filters together in the same letter-sized envelope.**

**Step 18** Discard gloves and turn off the pump. Put all used equipment (forceps, filter, holder, filter cup) into one of the packaging bags, and place them in the black “used equipment bag”. Tie off the used equipment bag to seal closed, and place it in the duffle bag. Finally, discard the water from the outflow bucket.

**Step 19** Environmental DNA samples are stable in silica desiccant beads for several weeks. However, the DNA may degrade after some time. Mail samples back to us for analysis within two weeks of collection using 2-3 day mail service with tracking. Please notify Kellie Carim via e-mail when samples have been shipped (kellie.carim@gmail.com).

To reduce risk of DNA degradation prior to shipping, filters may be stored in a freezer. It is not necessary to ship samples on ice.

**When returning the eDNA kit to the address below, don’t forget to include the black garbage bags of used equipment.** We will re-sterilize and reuse many of the components.

Please return eDNA kit and samples to:
Kellie Carim
eDNA Coordinator
U.S.D.A. Forest Service
Rocky Mountain Research Station
800 E. Beckwith Ave.
Missoula, MT 59801
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