



## Rocky Mountain Research Station

### Protocol for collecting eDNA samples from streams Version 2.3- July 2015



Bull trout swimming exploring the complex habitat of a Montana stream. Photo Credit: Aubree Benson

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**Contact:**

Kellie Carim, Ph. D.  
eDNA Coordinator  
U.S.D.A. Forest Service  
Rocky Mountain Research Station  
phone: 406-542-3252  
e-mail: [kelliejcarim@fs.fed.us](mailto:kelliejcarim@fs.fed.us)

## **Table of Contents**

|   |     |
|---|-----|
| Before Heading into the Field.....                  | 2   |
| Collecting Control Samples.....                     | 2   |
| Choosing a Sampling Location at a Site .....        | 2   |
| Contents of eDNA Kit .....                          | 3   |
| Sterile Procedures and Avoiding Contamination ..... | 4   |
| Field Collection Protocol.....                      | 5-9 |
| Troubleshooting.....                                | 10  |
| Using Backup Battery.....                           | 10  |

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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** GPS unit to determine the sampling location.

## **Field Control Samples**

Throughout the 2014 field season, we had over two dozen biologist throughout the western US collect over 300 samples for eDNA analysis with paired controls. Control samples were collected by filtering 0.5 L of distilled water. No samples had any evidence of field contamination. This method of sampling verifies the cleanliness of the field equipment, as well as the handling methods of the field collector. However, it cannot measure any contamination that may occur in the process of collecting a sample from a stream or lake. For example, standing upstream of the sampling area in waders that have brook trout DNA on them while a sample is being collected may result in a false positive detection of brook trout in that sample. Similarly sampling for lake trout from a boat that was previously in a waterbody containing the fish may also contaminate a sample, leading to a false positive detection of lake trout in a field sample. As a result, we believe that a better approach for detecting false positives is to consider the local expert opinion of the area and verify results with repeated sampling where necessary.

Control samples are regularly collected in the laboratory to insure the field and lab equipment, as well as all laboratory analyses are free of contamination. However, there are some contexts under which you may wish to collect control samples. If you wish to collect control samples, please contact us and we will provide or help develop a protocol to meet your specific sampling needs.

## **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 with adaptor
4. Filter holder with filter
5. eDNA sampling protocol
6. Forceps (in sterile bag)
7. Sterile gloves
8. Outflow bucket
9. Sterile sample bags with desiccant (for storing filters after sample is taken)
10. Sample box with letter sized envelopes to separate and store samples from all sites
11. Black bag for used equipment (**please return used equipment for sterilization and reuse**)
12. White bag with unused site kits
13. Battery Charger
14. Pump battery (with backup battery)
15. Alligator clip adaptor for back up battery
16. GPS unit (**not shown; not provided**)

**Note-** The “site kit” is a one-gallon re-sealable bag containing all the materials needed to take a single field sample. Sterile forceps, gloves, filter cup and silica desiccate 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 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). **Always stay downstream of sampling locations.**

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 site kit.

**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 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. (See “Using Backup Battery” pg. 10 for how to use alligator clip adaptor).

**Step 2)** Thread the outflow end of the tubing from the pump through the hose clamp on the bucket to keep it in place for an accurate measure how much water has been pumped (Figure 2).



**Figure 2.** Outflow end of hose threaded through hose clamp and placed into outflow bucket

**Step 3)** Turn pump on and place adaptor end of hose in an area where debris will not enter and clog the hose. **Ensure hose has been loaded into pump following directions of the sticker placed on the pump and that the pump is turned on in the FORWARD direction.** Be careful not to turn pump on in the reverse direction because this will contaminate the sample and it must be discarded. If pump will not turn on or pull water, see “Troubleshooting” pg. 10.

**Step 4)** 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 5)** Remove the bag containing sterile forceps, and the sample bag containing the silica desiccant. Unseal these bags without removing the contents and set them in an area where they are easily accessible, but not where any dust, dirt, or water may enter the bag and contaminate these pieces of equipment.

**Step 6) Before attaching filter, place adaptor end of hose against back of hand to ensure suction is occurring.** If suction is not occurring, return to Step 3 and ensure that tubing is loaded in the proper direction. Remove the packaged filter holders from the site kit. Work the filter holder in the packaging so that it can be attached to the hose without removing it from the packaging. Press the filter holder on to the filter holder adaptor on the hose (Figure 3).



**Figure 3.** Attaching filter holder adaptor and using packaging bag to handle hose

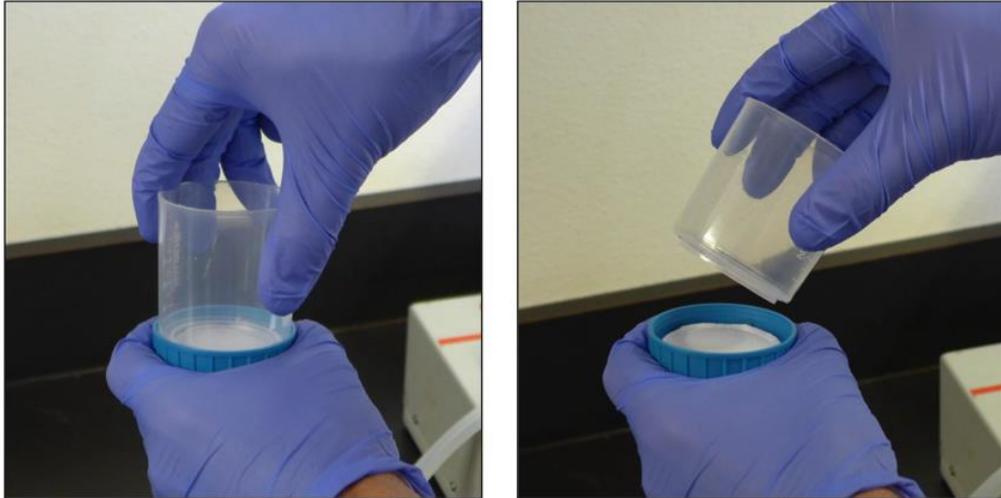
**Step 7)** To collect the field sample, remove the packaging from the filter and set aside. Lower the filter holder into the stream pointing in the upstream direction (Figure 4). Scoop water from the surface of the water column. **Be careful not to sample in any area with backflow that could result 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 into the outflow bucket.



**Figure 4.** 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 8)** 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. If the pump slows considerably due to filter becoming clogged, preventing a full 5 liters from being pumped, see “Troubleshooting” pg. 10.

**Step 9)** Remove the cup from filter holder (Figure 5; cup pops off, does not unscrew). 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)** Grab forceps out of bag, careful to not touch tips to anything except for filter itself. Use forceps to fold the filter paper in half, then in quarters filtering side in (Figure 6). Drop the filter into the sample bag containing silica beads, insuring that it is at the bottom of the bag and in contact with the beads. **Allow nothing to come in contact with the inside of the silica bag except for filter.** Push out any excess air from the bag and seal completely. Label the bag with the date, stream identification information (state, stream name, stream reach), GPS coordinates, and your initials.



**Figure 6.** Folding the filter paper, filtering side in

**Step 11)** Once you have removed the filter paper from the holder, turn off pump before placing it on the ground, to avoid clogging it with dirt and other debris, and place holder in black bag.

**Step 12)** Label a letter-sized envelope 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. Again, be careful to keep the envelopes flat and free from excess weight or handling that may cause the filter to crack or become damaged.

**Step 13)** Turn off the pump and 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. 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 14)** 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 ([kelliecarim@fs.fed.us](mailto:kelliecarim@fs.fed.us)). 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

## Troubleshooting

If the pump will not turn on or provide suction

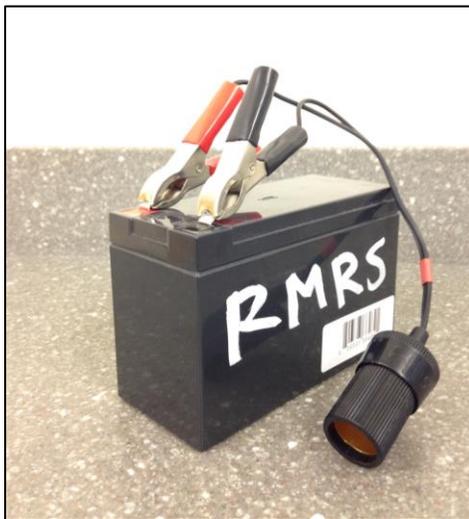
- Ensure battery is properly connected by turning connector gently at battery end.
- Turn speed control knob up to max.
- Try using backup battery if available, or charge original battery.
- Double check both hose direction (adaptor end is loaded in the way indicated on pump) and that the switch is in the FORWARD position, and lever on pump head is completely closed.

If the pump slows due to filter clogging

- If the 5 liter sample has not been completed, use additional filters 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. 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 12, **but store the filters together in the same letter-sized envelope.**

## Using Backup Battery

1. **Ensure pump is OFF to avoid getting shocked.**
2. Retrieve alligator clip adaptor and backup battery from kit.
3. Attach clips to matching color on battery (black with black, red with red), ensuring metal on metal contact between clips and battery (Figure 7).
4. Insert pump connector into adaptor



**Figure 7.** Alligator clips attached to backup battery