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Environmental DNA Sampling Protocol—Filtering Water to Capture DNA from Aquatic Organisms

Chapter 13 of
Section A, Biological Science
Book 2, Collection of Environmental Data

Techniques and Methods 2–A13

U.S. Department of the Interior
U.S. Geological Survey

Cover: Okanogan River, British Columbia, Canada, 2012.

Insets from top to bottom:
Juvenile rainbow trout from Ratz Creek, Alaska, 2009. (c)2009 ADF&G, Division of Sport Fish. Used with permission.
Collecting eDNA sample from Boise River, Boise, Idaho, 2011.
USGS Technician Kevin Glueckert collects eDNA sample from Boise River, Boise, Idaho, 2014.
Preserving folded eDNA sample in ethanol vial-filled vial, 2012.

Photographs by Matthew B. Laramie, U.S. Geological Survey, unless otherwise noted.

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SALLY JEWELL, Secretary

U.S. Geological Survey
Suzette M. Kimball, Acting Director

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Contents

Abstract....1
Introduction....1
Contamination Prevention....2
Selecting the Best Protocol for Your Sample Sites....2
Protocol #1: Manual, Hand-Driven Vacuum Pump....4
Protocol #2: Rechargeable Cordless Driver/Drill with Peristaltic Pump Head....7
Protocol #3: 120-V AC Motor with Peristaltic Pump Head....10
Acknowledgments....13
References Cited....13
Appendix A. Suggested Supplies List for Environmental DNA Sampling....15

Conversion Factors

Inch/Pound to International System of Units

Multiply	By	To obtain
	Length	
inch (in.)	25.4	millimeter (mm)
	Volume	
ounce, fluid (fl. oz)	0.02957	liter (L)

International System of Units to Inch/Pound

Multiply	By	To obtain
	Length	
millimeter (mm)	0.03937	inch (in.)
micrometer (µm)	$3.93700787 \times 10^{-5}$	inch (in.)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
milliliter (mL)	0.03381402	ounce, fluid (fl. oz)

Environmental DNA Sampling Protocol—Filtering Water to Capture DNA from Aquatic Organisms

By Matthew B. Laramie[1], David S. Pilliod[1], Caren S. Goldberg[2], and Katherine M. Strickler[2]

Abstract

Environmental DNA (eDNA) analysis is an effective method of determining the presence of aquatic organisms such as fish, amphibians, and other taxa. This publication is meant to guide researchers and managers in the collection, concentration, and preservation of eDNA samples from lentic and lotic systems. A sampling workflow diagram and three sampling protocols are included as well as a list of suggested supplies. Protocols include filter and pump assembly using: (1) a hand-driven vacuum pump, ideal for sample collection in remote sampling locations where no electricity is available and when equipment weight is a primary concern; (2) a peristaltic pump powered by a rechargeable battery-operated driver/drill, suitable for remote sampling locations when weight consideration is less of a concern; (3) a 120-volt alternating current (AC) powered peristaltic pump suitable for any location where 120-volt AC power is accessible, or for roadside sampling locations. Images and detailed descriptions are provided for each step in the sampling and preservation process.

Introduction

Organisms produce an abundance of genetic material that can persist in aquatic environments as environmental DNA (eDNA) when expelled from the organism in the form of sloughed cells, feces, or other exogenous processes. The collection, concentration, and analysis of eDNA from water samples is an effective method of determining the presence of aquatic organisms such as fish (Jerde and others, 2011; Takahara and others, 2013; Laramie and others, 2014), amphibians (Ficetola and others, 2008; Goldberg and others, 2011; Pilliod and others, 2013a), and other taxa (Thomsen and others, 2012). For an overview of early (2008–11) methods and applications of eDNA, see Pilliod and others (2013b).

This publication is meant to serve as a guide for collection, concentration, and preservation of eDNA samples from lentic and lotic systems and to provide three sampling protocols and a list of necessary supplies similar to those used in past studies (Goldberg and others, 2011, 2013; Pilliod and others, 2013a; Laramie and others, 2015). These protocols have been used to reliably and consistently collect and concentrate eDNA from stream samples. However, adaptations to these protocols may be necessary, depending on target taxa or environmental conditions of the system being sampled. The protocols included in this document use cellulose nitrate filter membranes with a 0.45-µm pore diameter (see appendix A for supplies list) for water samples ideally ranging from 250 to 1,000 mL. Samples collected from streams or ponds with an abundance of impurities such as tannins and organic materials likely will be limited to 500 mL or less, using the described filter type. Filters with a greater pore diameter (from 0.45 to more than 3.0 µm) could reduce clogging of the filter, increasing the sample volume, when desired. Turner and others (2014) provides an isocline equation to calculate eDNA collection equivalents for various pore sizes and sample volumes using carp as a model organism. Essentially, greater pore sizes allow for filtration of greater volumes of water, but eDNA molecules could potentially pass through the filter without being collected. Examination of literature or empirical testing may be necessary to determine the most suitable materials for desired applications. Additionally, researchers also must consider the ecology of the target organism and the characteristics of the water body being sampled to determine the most suitable locations within a body of water to collect water samples (for example, stream margins or thalweg). Whenever possible, samples should be collected without entering a stream or pond to reduce the probability of contaminating the site, boots and clothing, and (or) sampling equipment.

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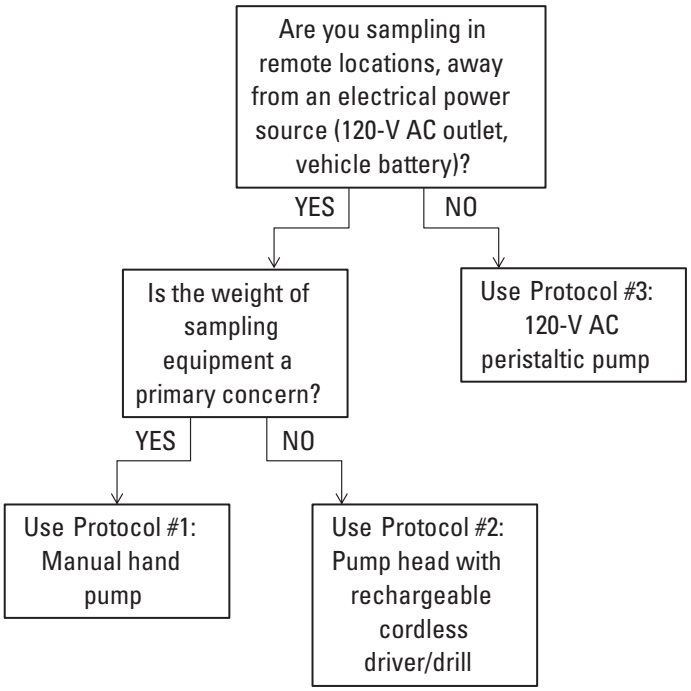
Contamination Prevention

Contamination can result from various factors at every step in the sample collection process. Be vigilant. Before initiating eDNA sample collection, the following field and laboratory practices should be reviewed to avoid contamination of samples and cross-contamination among samples:

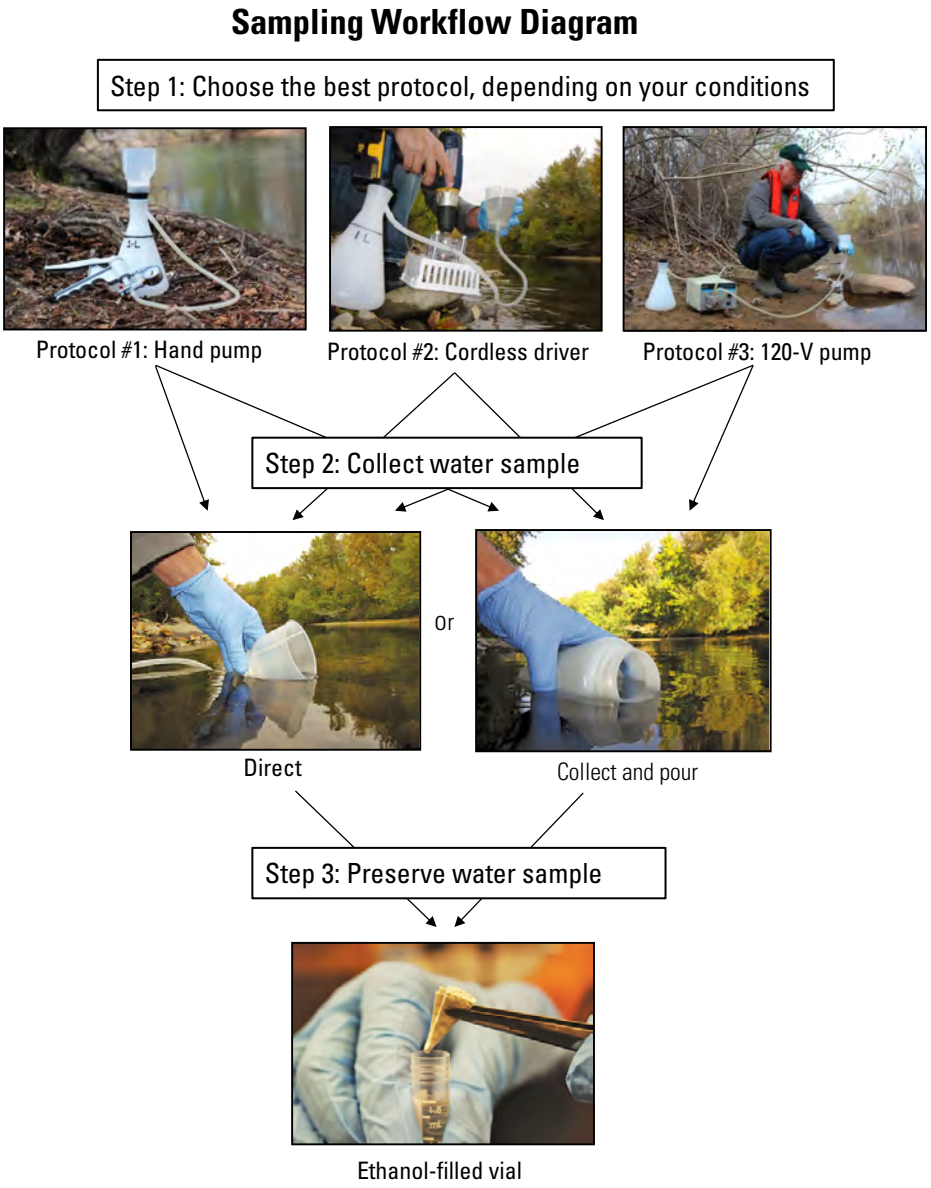
- Wear clean, non-powdered, single-use gloves when collecting samples and removing filters. Do not let gloves contact contaminated surfaces, such as any equipment that was not sterilized between sites, prior to handling the filter.
- Be careful with gloves and other supplies. Do not leave them unprotected and do not toss them in a backpack. Keep everything clean and in separate plastic bags, which should be discarded and replaced any time contamination is suspected.
- Open filter funnel package from bottom (stem end) and keep closed between sites. Be careful not to touch inside the filter funnel when removing it from its package.
- Decontaminate forceps between each sample by soaking in 50 percent commercial bleach for at least 1 minute. Rinse well with distilled water.
- To re-use grab bottles, decontaminate by submerging in 50 percent bleach solution and rinse thoroughly with distilled water (for example, fill, cap, shake, and rinse; repeat at least three times), let dry. At the sampling site, rinse again with sample water three times (cap and shake) to remove any remaining bleach before collecting sample.
- If it is necessary to enter the water at a sampling site, rubber boots should be used and must be decontaminated between sites. Remove all dirt, pebbles, and other environmental debris from soles and sides of boots. Decontaminate in 50 percent commercial bleach and rinse well with distilled water (or tap water, if necessary).
- If sampling a stream, collect sample upstream of your standing location to reduce potential contamination by boots, clothing, or equipment.

Selecting the Best Protocol for Your Sample Sites

Depending on the characteristics of your sample sites, samples can be collected and filtered in a variety of ways. A decision tree is provided to help you select the proper protocol before beginning.



Essentially, if working in remote locations without access to electricity or when the weight of your sampling equipment is a primary concern, we recommend Protocol #1, which uses a lightweight hand-driven vacuum pump. If you are willing to carry a rechargeable cordless driver/drill and a peristaltic pump head when working in remote locations, then we recommend Protocol #2. If your sample sites are near an electrical power source (vehicle battery) or if you prefer to collect the samples and then transport them to a centralized location near a power source, we recommend Protocol #3, which uses an 120-V AC motor unit with a peristaltic pump head.



The filtering procedure and filter paper preservation are essentially the same regardless of which sampling protocol is selected; only the configuration and assembly of the pump/filter differ. The sampling workflow diagram illustrates that, with all three protocols, water samples may be collected either by directly submerging the filter funnel (connected to a length of silicone tubing) into the stream or pond with the pump engaged or by using a sterile collection bottle (for example, a Nalgene™ bottle) or sterile sample bag (for example, Whirl-Pak®) to grab the sample from the stream or pond and pour it into the filter funnel with the pump engaged. Filter preservation is identical for all provided protocols and variations. Specifics for suggested pump options are listed in table 1. Suggested supplies list and vendor contact information are provided in appendix A.

Table 1. Suggested pump options for filtering environmental DNA samples.

[Environmental DNA (eDNA) sampling supplies manufacturer information is available in appendix A. **Abbreviations:** V, volt; AC, alternating current; DC, direct current; N/A, not applicable]

Protocol	Power	Pump/motor	Pump head	Tubing
Protocol #1: Manual, hand-driven vacuum pump	Manual, hand operated	Hand-driven vacuum pump	NA	Masterflex® platinum cured silicon tubing, L/S 15
Protocol #2: Rechargeable cordless driver with peristaltic pump head	Rechargeable battery powered	Cordless, handheld driver with standard flathead drillbit	Masterflex® L/S Standard Pump Head for L/S 15 tubing	Masterflex® platinum cured silicon tubing, L/S 15
Protocol #3: 120-V AC motor with peristaltic pump head	120-V AC (or 12-V DC, with power inverter)	Masterflex® L/S Economy Variable Speed Drive Motor	Masterflex® L/S Standard Pump Head for L/S 15 tubing	Masterflex® platinum cured silicon tubing, L/S 15

Protocol #1: Manual, Hand-Driven Vacuum Pump

This configuration requires no electricity and is most useful when working in remote sampling locations and when weight of sampling equipment is a primary concern. A hand-driven vacuum pump and filter assembly is shown in figure 1.1. Numbers in parenthesis in assembly instructions indicate parts shown in figure 1.1.

Figure 1.1. Necessary equipment: **1**, filter funnel; **2**, #8 Fisherbrand™ rubber stopper; **3**, Nalgene™ vacuum flask; **4**, Masterflex® L/S 15 silicone tubing; **5**, Nalgene™ hand-operated vacuum pump. **Note:** See appendix A for manufacturer's information.

Part 1. Pump and Filter Assembly

1. Seat rubber stopper (2) into top of vacuum flask (3), pressing to ensure a good seal.
2. Attach hand-driven vacuum pump (5) to tube connector on vacuum flask using section of silicone tubing (4). The vacuum flask allows for measurement of sample volume.
3. Secure filter funnel (1) to adapter stem.
4. *If collecting samples directly from stream or pond*: attach disposable filter funnel to rubber stopper using an appropriate length of silicone tubing by inserting one end of tubing into filter funnel adapter stem and the other end of tubing into hole in rubber stopper atop the vacuum flask, creating an airtight seal at both ends.

   or

   If first collecting samples in separate, sterile collection bottle or Whirl-Pak®: attach disposable filter funnel directly to rubber stopper by inserting adapter stem into hole in stopper, creating an airtight seal. Samples must then be poured into filter funnel for filtration.

Part 2. Filtration and Filter Preservation

Depending on your needs, water samples can be collected in two ways: (1) Directly; by submerging the filter funnel directly into the stream or pond with the pump engaged, or (2) Grab and pour; by collecting the water sample in a separate, sterile collection bottle (for example a Nalgene™ bottle or Whirl-Pak®) and then pouring it into the filter funnel, with the pump engaged.

Supplies needed (figs. 1.2 and 1.3):

- single-use gloves
- 50 percent bleach solution
- distilled rinse water
- 2.0 mL vials three-fourths filled with undiluted molecular grade ethanol (denatured ethanol will destroy the sample)
- forceps
- ethanol-proof marker
- optional: sterile Nalgene™ collection bottle or Whirl-Pak®.

Figure 1.2. Sampling supplies.

Figure 1.3. Optional supplies.

Filtration and Filter Preservation

1. Assemble filter funnel and pump as described in Part 1.
2. *If collecting sample directly from the stream or pond:* wearing clean gloves, collect 1 L (or desired volume) of stream or pond water by submerging the filter funnel (fig. 1.4) directly into the stream or pond surface with the pump engaged to filter the desired amount of water. Pre-marking the vacuum flask at the intended sample volume (for example, 1 L) is necessary to ensure consistent sample collection.

   or

   If first collecting sample in separate, sterile collection container: wearing clean gloves, collect desired volume (generally, 250–2,000 mL) of stream or pond water by partially submerging a sterile Nalgene™ bottle (fig. 1.5) or sterile Whirl-Pak® into the water surface. Slowly pour sample from Nalgene™ bottle or Whirl-Pak® into filter funnel (250 mL at a time) with pump engaged. Pause several times to swirl water in Nalgene™ or Whirl-Pak® before pouring remaining water into funnel. During filtering, make sure vacuum pressure is sustained to ensure that water is flowing unidirectionally from the funnel to the vacuum flask.

Figure 1.4. Filter funnel being submerged directly into stream with pump engaged to begin filtration.

Figure 1.5. Sterile Nalgene™ collection bottle being submerged in stream to collect desired volume of water. Sample must then be poured into filter funnel (250 milliliters at a time) with pump engaged to begin filtration.

3. Disinfect forceps by soaking in 50 percent bleach solution for at least 1 minute and rinsing thoroughly in distilled water stored in a sterile container (for example, 50 mL centrifuge tube).
4. Disconnect top and bottom units of the filter funnel to expose the filter membrane (fig. 1.6).

Figure 1.6. Disconnected top and bottom units of filter funnel and exposed filter membrane.

5. Using decontaminated forceps, fold filter paper in one-half four times (fig. 1.7). Keep filter stable and prevent it from unfolding by using gloved finger (change gloves first if glove has come into contact with anything except the stream or decontaminated equipment). Place folded filter in vial filled with 200-proof, molecular-grade ethanol for preservation and storage (fig. 1.8).

Figure 1.7. Method for folding filter paper in one-half four times using sterile forceps and clean gloves.

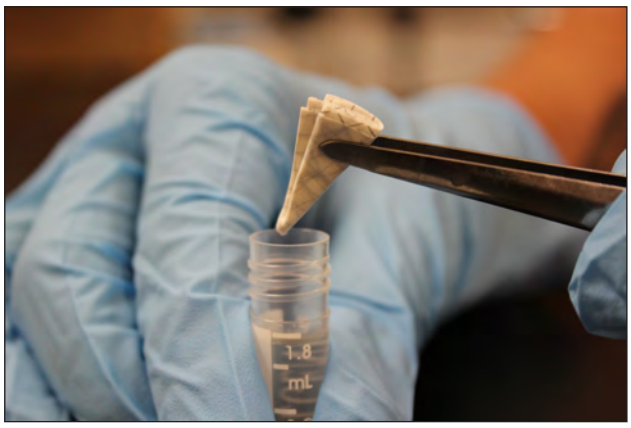


Figure 1.8. Folded filter being placed into 2.0 milliliter vial filled with 200 proof molecular grade ethanol for preservation.

6. Ensure that vial is labeled properly.
7. Remove filter funnel from rubber stopper.
8. Recycle plastic funnel when recycling is available. Do not reuse.
9. Discharge water can be poured back into stream, once sample collection is complete (if vacuum flask was decontaminated after previous use).
10. Store vials in a cool place away from light exposure.

Protocol #2: Rechargeable Cordless Driver/Drill with Peristaltic Pump Head

This configuration is most useful when working in remote locations when the ease of sampling takes precedence over the weight of the sampling gear. A peristaltic pump head driven by a rechargeable cordless driver/drill and filter assembly is shown in figure 2.1. Numbers in parenthesis in assembly instructions indicate parts shown in figure 2.1.

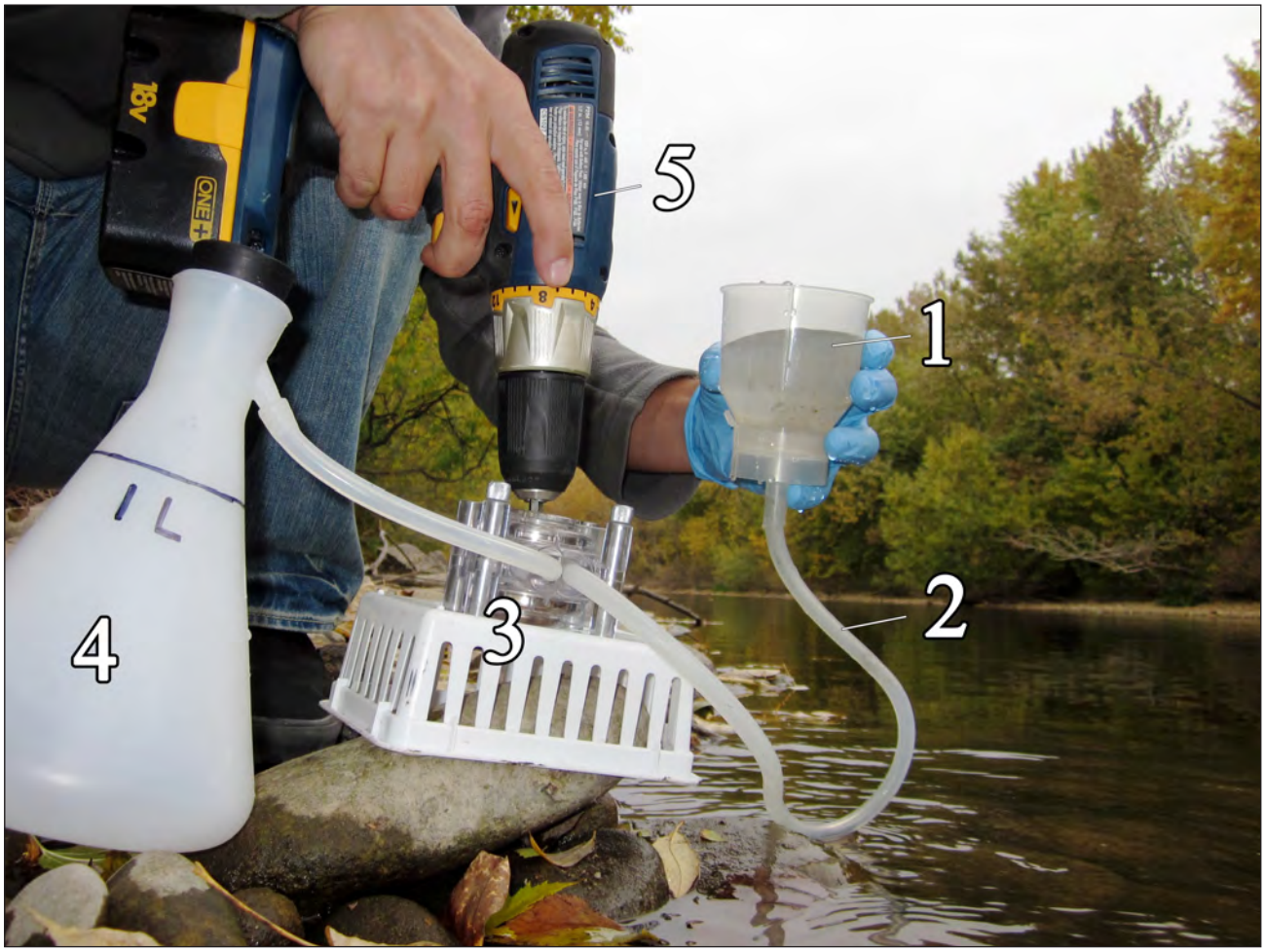


Figure 2.1. Necessary equipment: **1**, filter funnel; **2**, Masterflex® L/S 15 silicone tubing; **3**, Masterflex® standard pump head for L/S 15 tubing (shown mounted to piece of plastic); **4**, Nalgene™ vacuum flask; **5**, battery-operated driver/drill. **Note:** See appendix A for manufacturer's information.

Part 1. Pump and Filter Assembly

1. Run a section of tubing (2) through peristaltic pump head (3) and insert outflow end of tubing into a discharge bottle (4). A vacuum flask with desired volume marked on side (open, without vacuum) can be used as a means to measure discharge water so the amount of water pumped through the filter can be recorded.
2. Secure a filter funnel (1) to adapter stem.
3. Attach sterilized tubing (2) to filter funnel/adapter stem by inserting tubing into stem, creating airtight seal.
4. Engage the peristaltic pump head by driving it with the cordless driver/drill (5) using a standard flathead bit. Ensure that there is enough room beneath the pump head to allow for unrestricted rotation of the mechanism on the underside of the unit. As in figure 2.1, it may be useful to mount it to a piece of plastic or wood for this purpose.

Part 2. Filtration and Filter Preservation

Depending on your needs, water samples can be collected in two ways: (1) Directly; by submerging the filter funnel directly into the stream or pond with the pump engaged, or (2) Grab and pour; by collecting the water sample in a separate, sterile collection bottle (for example a Nalgene™ bottle or Whirl-Pak®) and then pouring it into the filter funnel, with the pump engaged.

Supplies needed (figs. 2.2 and 2.3):

- single-use gloves
- 50 percent bleach solution
- distilled rinse water
- 2.0 mL vials three-fourths filled with undiluted molecular grade ethanol (denatured ethanol will destroy the sample)
- forceps
- ethanol-proof marker
- optional: sterile Nalgene™ collection bottle or Whirl-Pak®.

Figure 2.2. Sampling supplies.

Figure 2.3. Optional supplies.

Filtration and Filter Preservation

1. Assemble filter funnel and pump as described in Part 1.
2. *If collecting sample directly from the stream or pond:* wearing clean gloves, collect 1-L (or desired volume) of stream or pond water by submerging the filter funnel (fig. 2.4) directly into the stream or pond surface with the pump engaged to filter the desired amount of water. Pre-marking the vacuum flask at the intended sample volume (for example, 1 L) is necessary to ensure consistent sample collection.

   or

   If first collecting sample in separate, sterile collection container: wearing clean gloves, collect desired volume (generally, 250–2,000 mL) of stream or pond water by partially submerging a sterile Nalgene™ bottle (fig. 2.5) or sterile Whirl-Pak® into the water surface. Slowly pour sample from Nalgene™ bottle or Whirl-Pak® into filter funnel (250 mL at a time) with pump engaged. Pause several times to swirl water in Nalgene™ or Whirl-Pak® before pouring remaining water into funnel. During filtering, make sure vacuum pressure is sustained to ensure that water is flowing unidirectionally from the funnel to the vacuum flask.

Figure 2.4. Filter funnel being submerged directly into stream with pump engaged to begin filtration.

Figure 2.5. Sterile Nalgene™ collection bottle being submerged in stream to collect desired volume of water. Sample must then be poured into filter funnel (250 milliliters at a time) with pump engaged to begin filtration.

3. Disinfect forceps by soaking in 50 percent bleach solution for at least 1 minute and rinsing thoroughly in distilled water stored in a sterile container (for example, 50 mL centrifuge tube).
4. Disconnect top and bottom units of the filter funnel to expose the filter membrane (fig. 2.6).

Figure 2.6. Disconnected top and bottom units of filter funnel and exposed filter membrane.

5. Using decontaminated forceps, fold filter paper in one-half four times (fig. 2.7). Keep filter stable and prevent it from unfolding by using gloved finger (change gloves first if glove has come into contact with anything except the stream or decontaminated equipment). Place folded filter in vial filled with 200-proof, molecular-grade ethanol for preservation and storage (fig. 2.8).

Figure 2.7. Method for folding filter paper in one-half four times using sterile forceps and clean gloves.

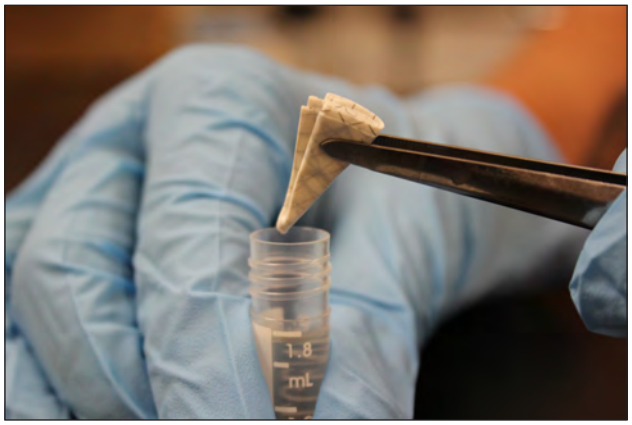


Figure 2.8. Folded filter being placed into 2.0 milliliter vial filled with 200 proof molecular grade ethanol for preservation.

6. Ensure that vial is labeled properly.
7. Remove filter funnel from rubber stopper.
8. Recycle plastic funnel when recycling is available. Do not reuse.
9. Discharge water can be poured back into stream, once sample collection is complete (if vacuum flask was decontaminated after previous use).
10. Store vials in a cool place away from light exposure.

Protocol #3: 120-V AC Motor with Peristaltic Pump Head

This configuration is most useful when sampling near an electrical power source (e.g. vehicle battery) or if you prefer to collect the samples and transport them to a centralized location near a power source prior to filtration. A 120-V AC motor unit with attached peristaltic pump head and filter assembly is shown in figure 3.1. Numbers in parenthesis in assembly instructions indicate parts shown in figure 3.1.

Figure 3.1. Necessary equipment: **1**, filter funnel; **2**, Masterflex® L/S 15 silicone tubing; **3**, 120-V Masterflex® L/S economy variable speed drive motor; **4**, Masterflex® standard pump head for L/S 15 tubing; **5**, Nalgene™ vacuum flask. **Note:** See appendix A for manufacturer's information.

Part 1. Pump and Filter Assembly

1. Connect peristaltic pump head (4) to 120-V AC motor (3) using supplied bolts.
2. Run tubing (2) through peristaltic pump head (4) and insert outflow end of tubing into discharge bottle (5). A vacuum flask with desired volume marked on side (open, without vacuum) can be used as a means to measure discharge water so the amount of water pumped through the filter can be recorded. Discharge water container does not need to be sterile (but if it is not decontaminated between sites, discharge water should not be emptied in aquatic environments).
3. Secure filter funnel (1) to adapter stem.
4. Attach tubing (2) to filter funnel/adapter stem by inserting tubing into stem, creating airtight seal.
5. Connect motor unit to 120-V AC power source such as a wall outlet. Motor unit can also be powered by a 12-V DC battery (such as a vehicle battery) with the use of a power inverter (12-V DC to 120-V AC).

Part 2. Filtration and Filter Preservation

Depending on your needs, water samples can be collected in two ways: (1) Directly; by submerging the filter funnel directly into the stream or pond with the pump engaged, or (2) Grab and pour; by collecting the water sample in a separate, sterile collection bottle (for example a Nalgene™ bottle or Whirl-Pak®) and then pouring it into the filter funnel, with the pump engaged.

Supplies needed (figs. 3.2 and 3.3):

- single-use gloves
- 50 percent bleach solution
- distilled rinse water
- 2.0 mL vials three-fourths filled with undiluted molecular grade ethanol (denatured ethanol will destroy the sample)
- forceps
- ethanol-proof marker
- optional: sterile Nalgene™ collection bottle or Whirl-Pak®.

Figure 3.2. Sampling supplies.

Figure 3.3. Optional supplies.

Filtration and Filter Preservation

1. Assemble filter funnel and pump as described in Part 1.
2. *If collecting sample directly from the stream or pond*: wearing clean gloves, collect 1-L (or desired volume) of stream or pond water by submerging the filter funnel (fig. 3.4) directly into the stream or pond surface with the pump engaged to filter the desired amount of water. Pre-marking the vacuum flask at the intended sample volume (for example, 1 L) is necessary to ensure consistent sample collection.

   or

   If first collecting sample in separate, sterile collection container: wearing clean gloves, collect desired volume (generally, 250–2,000 mL) of stream or pond water by partially submerging a sterile Nalgene™ bottle (fig. 3.5) or sterile Whirl-Pak® into the water surface. Slowly pour sample from Nalgene™ bottle or Whirl-Pak® into filter funnel (250 mL at a time) with pump engaged. Pause several times to swirl water in Nalgene™ or Whirl-Pak® before pouring remaining water into funnel. During filtering, make sure vacuum pressure is sustained to ensure that water is flowing unidirectionally from the funnel to the vacuum flask.

Figure 3.4. Filter funnel being submerged directly into stream with pump engaged to begin filtration.

Figure 3.5. Sterile Nalgene™ collection bottle being submerged in stream to collect desired volume of water. Sample must then be poured into filter funnel (250 milliliters at a time) with pump engaged to begin filtration.

3. Disinfect forceps by soaking in 50 percent bleach solution for at least 1 minute and rinsing thoroughly in distilled water stored in a sterile container (for example, 50 mL centrifuge tube).
4. Disconnect top and bottom units of the filter funnel to expose the filter membrane (fig. 3.6).

Figure 3.6. Disconnected top and bottom units of filter funnel and exposed filter membrane.

5. Using decontaminated forceps, fold filter paper in one-half four times (fig. 3.7). Keep filter stable and prevent it from unfolding by using gloved finger (change gloves first if glove has come into contact with anything except the stream or decontaminated equipment). Place folded filter in vial filled with 200-proof, molecular-grade ethanol for preservation and storage (fig. 3.8).

Figure 3.7. Method for folding filter paper in one-half four times using sterile forceps and clean gloves.

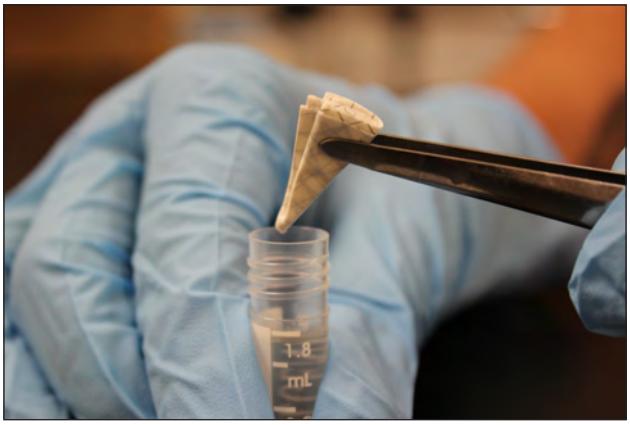


Figure 3.8. Folded filter being placed into 2.0 milliliter vial filled with 200 proof molecular grade ethanol for preservation.

6. Ensure that vial is labeled properly.
7. Remove filter funnel from rubber stopper.
8. Recycle plastic funnel when recycling is available. Do not reuse.
9. Discharge water can be poured back into stream, once sample collection is complete (if vacuum flask was decontaminated after previous use).
10. Store vials in a cool place away from light exposure.

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Appendix A. Suggested Supplies List for Environmental DNA Sampling

[Supply list represents author suggestions. Alternative manufacturer's products may be substituted. **Product No.:** Valid if ordering through http://www.FisherSci.com, http://www.coleparmer.com, as noted. **Abbreviations:** AC, alternating current; in., inch; L, liter; µm, micrometer; mL, milliliter; mm, millimeter; oz, ounce; V, volt; N/A, not applicable]

Product No.	Description
Fisher Scientific Cat.#: 02-893D, Vendor Cat. #: 2105-0032	1-L Nalgene™ bottle.
Fisher Scientific Cat. #: 01-81-8C, Vendor Cat. #: B01196WA	Whirl-Pak® polyethylene sample bag, write-on style, 24 oz.
Fisher Scientific Cat. #: 10 182 50B, Vendor Cat. #: DS4101-1000	1,000 mL[1] Nalgene™ polypropylene vacuum flask with tabulation.
Fisher Scientific Cat. #:14 135M	#8 Fisherbrand™ rubber stopper (must be drilled with 5/8 in. drill bit to accommodate stem of filter funnel).
Fisher Scientific Cat. #: 13 310 110, Vendor Cat. #: 96410 15	Masterflex® platinum-cured silicon tubing (size L/S 15).
Fisher Scientific Cat. #:09 740 30K, Vendor Cat. #: 145-2045	Nalgene™ analytical filter funnel (disposable) with 47 mm diameter cellulose nitrate (0.45 µm pore diameter) filter membrane with filter funnel adapter.
Fisher Scientific Cat. #: 13-874-612A, Vendor Cat. #: 6132-0010	Nalgene™ hand-operated vacuum pump.
Fisher Scientific Cat. #: 09-753-50	Fisherbrand™ filter forceps.
Fisher Scientific Cat. #: 14-432-22, Vendor Cat. #: 352070	Falcon™ 50 mL centrifuge tube.
Vendor Cat. #: 72.609.001 and	Sarstedt™ micro tube, 2 mL (tubes only).
Vendor Cat. #: 65.716.002	Sarstedt™ o-ring screw caps (for 2 mL micro tube)
N/A	Cordless handheld, rechargeable driver/drill with standard flathead drill bit.
ColeParmer Cat. #: WU-07015-21	Masterflex® standard pump head for L/S 15 tubing.
ColeParmer Cat. #: WU-07554-90	Masterflex® L/S economy variable-speed drive motor (120-V AC).

[1]A 1,700 mL flask and #10 stopper could be substituted if additional volume is preferred.

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