GEN-FISH
Genomic Network for Fish Identification, Stress and Health
University of Windsor and Partners

GEN-FISH eDNA Sampling Protocol

Water sampling and on-site filtration using "DIY" peristaltic pump system

Overview: Parts to assemble this "DIY" water filtration system cost ~$600–800, plus the cordless drill (~$200) which powers it. Rate of filtration varies with turbidity, but three 0.5–1.0 L water samples plus a field blank can be filtered in ~20–45 minutes.

A. Materials (see Figure 1)

See ***Appendix B. Recommended Supplies List for eDNA Sampling*** for ordering details. Quantities per site based on three biological replicates (water samples) plus one negative control (NC/blank) per site. See the ***Packing List*** for an itemized list of all equipment that was sent to you.

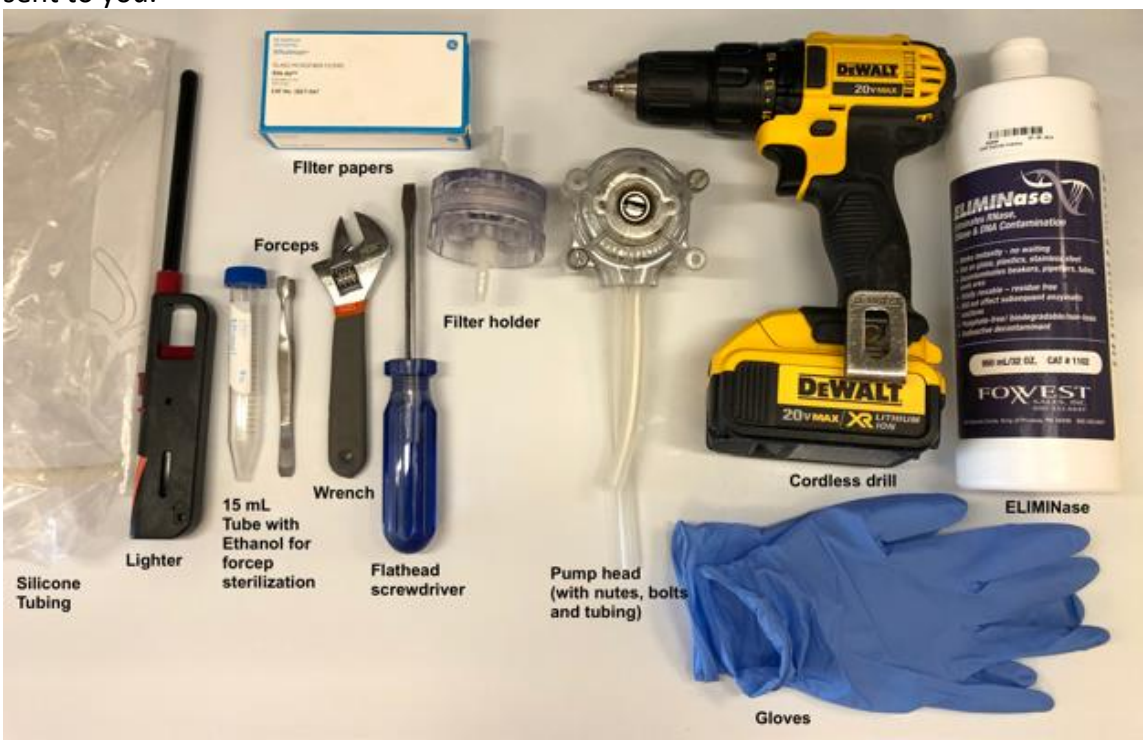

Figure 1*. Image of materials needed for assembling and operating DIY peristaltic pump.*

1. **Peristaltic pump head** with key, locknuts, and bolts (stored in an individual clean bag).
2. **Filter holder** (stored in an individual clean bag).
3. **Silicone tubing** cut into ~75 cm long pieces (stored in an individual clean bag).
4. **Adjustable wrench** for filtration system set-up.
5. **Handheld screwdriver,** flathead.
6. **Cordless drill** with flathead drill bit.
7. **Extra drill batteries.**
8. **Plastic sealable container** for transporting above items.
9. **Deionized water or sealed bottled water (500 mL)**, minimum four per site:
    i. Bottle #1: Rinsing tubing upon arrival at site—keep bottle for collecting water.
    ii. Bottle #2: Negative control filtering—keep bottle for collecting water.
    iii. Bottle #3: Rinsing tubing after sampling completed at site.
    iv. Bottle #4: Extra.
10. **Sterile metal forceps.** These are easily sterilized in the field with ethanol.
    i. **Sterile 15 mL tubes filled with 70–95% ethanol** for dipping forceps. Minimum one per sampling day and can be reused.
    ii. **Lighter,** such as a barbecue lighter.
11. **Sharpies and ethanol-proof markers.**
12. **ELIMINase or sodium hypochlorite solution;** concentrated bleach contains chlorine concentration of 5.25–8% or 52.5–80 g/L or 52,500–80,000 ppm; for example, one part 5% bleach to nine parts cold water will make a 10% bleach solution and the concentration of chlorine in the solution is 5,000 ppm, which is ideal for high level disinfection. Bleach solutions at 10, 20, or 50 % can be made daily and stored in an opaque container away from light. ~1 L of bleach solution per site or 30 mL ELIMINase. ELIMINase is non-toxic and does not leave behind harmful residues but all ELIMINase waste and bleach must be rinsed and the wastewater stored for safe disposal. If you opt to use bleach you will need to bring enough deionized water to be able to submerge the equipment as well as a large container to store waste bleach for safe disposal after sampling.
13. **Paper towels.**
14. **Clean containers or large bags** to keep everything in, as well as storing waste for disposal.
15. **Filter membranes,** four per site, plus backups.
16. If using **liquid preservative (ethanol or RNAlater)**:
    i. **Sterile screw-top tubes (skirted or non-skirted, Dnase and Rnase free)**, prefilled and prelabeled $^2/_3$ with molecular-grade ethanol (preferably 99%, no less than 95%) or RNAlater. Four per site, plus unlabeled backups.
    ii. **Storage boxes** or **falcon tube rack** to hold tubes upright.
17. If using **liquid-free preservative (self-indicating silica beads):**
    i. **Coin envelopes**, prelabeled. Four per site, plus extras.
    ii. **Plastic specimen bags**, sterile, prefilled with silica and use to store coin envelopes. Three bags per site (#1 for negative control, #2 for water samples and #3 for keeping together both bags #1 and #2 from a single site).

iii. **Sampler spoons**, sterile, to fill specimen bags with silica. One per site. One scoop holds ~15 g of silica.
iv. **Self-indicating silica**, prefilled or can fill specimen bags in field. ~150 g per site. ~30 g for negative control specimen bag #1, ~90–100 g for water replicate specimen bag #2. Extra to top up as needed as silica becomes saturated.

B. Cleaning Procedures (see *Equipment Sanitation Flowchart*)

Given the sensitivity of the eDNA techniques, avoiding contamination of field samples is critical:

- All sampling equipment must be kept separate from other field gear (e.g., nets, clothing, truck bed).
- The peristaltic pump tubing and filter holder are also possible sources of contamination since they are exposed to the surrounding environment at every sampling site. Do not handle these (or any other non-sterile items) while wearing sterile gloves.
- Always wear fresh gloves while handling sterile items and samples.
- When in doubt, change your gloves.
- Keep contaminated, used, or dirty field materials bagged and separate from clean equipment to minimize chances of contamination.
- **Always stay downstream of sampling locations.**

C. Preparation

Decontaminate bags and supplies

1. Prior to field sampling, replace or wash the bags, the pump head, filter holder, tubing, and tools. Equipment can be washed in a clean sink wearing gloves using the flowchart instructions provided in the ***Equipment Sanitation Flowchart*.**
2. Hang bags to dry in a freshly sanitized area, free of contaminant DNA. Preferably in a closed, indoor area away from sources of contaminants, such as fish DNA or areas where dissections or PCR are conducted.
3. If you have access to a PCR workstation with UV light irradiation equipment, it can then be UV treated and bagged in the workstation.
4. Wearing gloves, the pump head and filter holder can be placed in individual clean bags. Tubing pieces can be folded in half to form a “U-shape” and placed in another bag with the inflow and outflow ends of the U-shaped tube at the bottom of the bag. This way, when you need to take a tube out, you would be handling the tube from the middle of the bend in the U. Tools can be placed in another bag.
5. All items can be packed into a larger container or tote bag with heavier items at the bottom and lighter and more fragile components at the top. Having multiple bags or containers can help simplify packing. In one bag, you can place the tubing bag at the bottom, followed by the pump bag, filter holder bag and lastly with bag of tools for assembling filter. In a smaller bag, you can store the clean filters, forceps (in a plastic bag to prevent injury), tube of ethanol or bleach to sanitize forceps and lighter.
6. For each site you visit, you will want to have a piece of tubing, plus extras.

Prepare filter storage containers

Preparing filter storage ahead of time will save time in the field and prevent contamination.

- **Ethanol or RNAlater**
    1. Label the appropriate number of tubes with site name, date, ID, and any other important information.
    2. Prefill tubes $^{2}/_{3} - {}^{3}/_{4}$ full with DNA stabilizer.
    3. Store upright in boxes or racks, also labeled.
- **Self-indicating silica**
    1. Label the appropriate number of coin envelopes with site name, date, ID, and any other important information.
    2. Place three water sample coin envelopes into a plastic specimen bag, and the envelope for control into a secondary plastic bag
    3. Prefill specimen bag for three water samples with ~90–100 g (seven spoon-scoops) of silica
    4. Prefill specimen bag for one control sample with ~30 g (two spoon-scoops) of silica
    5. Store specimen bags in a secondary bag for each site.

D. Arrival at sampling site

"Housekeeping"

- Upon arrival at sampling site, determine who will be the Data Collector, DIY Sampling Assistant, and Water Sampler.
- The Data Collector will record the initial site metadata, such as starting time and any other parameters, except water chemistry.
- Meanwhile, the DIY Sampling Assistant and Water Sampler will start to assemble the filtration device.

Label ID for samples

Minimum requirements for sample labels for each 2 mL tube, 15 mL tube or sample container:

- the site name or abbreviation for site
- sub-sample information to distinguish between a blank/negative control and biological water samples
- the replicate number for biological water samples and date sampled

Any additional information can be given on the label as needed and more details can be provided on metadata sheets

Assembling DIY Filtration

1. Wearing sterile gloves, use the cordless drill with a flat head drill bit or the hand-held flat-head screwdriver to open the pump head. This may require the help of another person who can hold the nuts in place while the screw is undone, eliminating the need to place the filtration apparatus down. If the filtration system needs to be placed down, do so on clean paper towels.

2. Having a piece of tubing that is 75 cm long will allow you to comfortably assemble and filter water. Pick up a sterile piece of tubing from the middle of the tube. Avoid picking up tubing from the ends because the one end of the tubing is going to be placed in your water sample ("inflow") and you don't want to contaminate it, even if you're wearing gloves. Place a piece of sterile tubing in the pump. Leave enough room on one end of the tubing to allow it to reach the bottom of your water container; this will be the "inflow" end; the other end will be the "outflow."
3. To close the pump head, ensure that the tubing is tightly wrapped around the pump; you will need to hold it in place while you close the pump head.
4. Replace the nuts and screw on the pump head, tightening using the wrench and screwdriver. You should hear a "suction" sound when it's sealed.
5. To determine the top and bottom of the filter holder, see **Figure 5,** or find the top side of the filter holder by looking for the small white plastic screw. This small white screw can be loosened or tightened to adjust pressure build-up in the filter, if the filter starts to clog. Otherwise, the white screw should always remain *almost* screwed shut into the nozzle. If it's too loose, water will pour out from the top and not be filtered. Insert the outflow end of the tubing onto the nozzle on the top of the filter holder.

Flush the tubing

1. Take a sealed, 500 mL bottle of water or sterile, deionized, water (**Bottle #1**), and carefully place the inflow end of the tubing into the bottle of water until it reaches the bottom of the bottle. Ensure that you do not touch any portion of the tubing that will go into the water, even with gloved hands.
2. Place the appropriate flat head drill bit into the drill and insert drill head into pump head.
3. Set the gear selector switch on the drill to low gear or "1," and press on the speed trigger. Water will start to be drawn from the water bottle, into the tubing, and out through the outflow filter holder. If water does not begin to filter through and you're seeing bubbles forming in the water bottle, you need to reverse the switch to change the direction of the drilling by using the forward/reverse button. You can do this by pushing on the "REVERSE SWITCH" button on the drill (see **Figure 2**)
4. Increase the gear setting to the higher setting, "2," and continue to filter the water through the tube at a faster rate. Flushing the tubing with water at a fast speed with help to draw out any debris or contaminants.

Insert the filter into the filter holder

While one person holds the pump head and ensures the tubing inflow and outflow ends do not touch anything, the other will place a clean filter onto the filter holder. Do not touch any interior part of the filter holder unless it is with sterile forceps.

1. Unscrew the filter holder while holding the two parts vertically. Inside, there may be an orange-coloured O-ring that rests on the top part of the filter holder and holds the filter in place during filtration (it is helpful but is not a requirement; the pump will function without it). The bottom of the filter holder has a black surface that the filter rests on.

Holding the top and bottom of the filter holder vertically will prevent the O-ring from falling on the ground and getting contaminated (see **Figure 8**)

2. **DO NOT TOUCH ANY INTERIOR PART OF THE FILTER HOLDER UNLESS IT'S WITH STERILE FORCEPS**
3. **Sterilize Forceps**: If using metal forceps, dip them into your 15 mL tube of ethanol, holding the pincers of the forceps facing down so ethanol drips off the tips of the forceps and does not run down to the portion you're holding. Then flame them with your lighter to ensure sterility. It is crucial that the forceps dipped in ethanol are held in a manner that allows for the ethanol to drip down and not upright, which can expose your hands to high heat and could lead to burns. Alternatively, forceps can be sterilized by:
    - Dipping in a 50% bleach solution for 1–2 minutes, rinsing with deionized water, and wiped dry
    - Sprayed with ELIMINase and wiped dry
4. Use clean gloves to open the box of filters without touching the inside of the box. Using sterile forceps, pick up a filter and place it on the black filter area inside the holder.
5. Close the filter holder while holding the two parts vertically.
6. Using your sterile forceps, close the box of filters.

You're now ready to start filtering water samples.

Take a field blank or negative control

From this point, the Water Sampler (who will collect the water samples with gloves) must not come into contact with any filtration equipment until gloves are removed.

1. While one person holds the pump head and watches to ensure the tubing inflow and outflow end do not touch anything, the second person can use a sterile pair of forceps to place a clean filter onto the filter holder
2. Take a sealed, 500 mL bottle of water or sterile, deionized water (**Bottle #2**), and carefully place the inflow end of the tubing into the bottle of water, ensuring that you do not touch any portion of the tubing that will go into the bottle.
3. Change the gear setting on the drill back to low or "1" and keep this setting for the remainder of filtering water samples.
4. Press on the speed trigger and filter the water. Keep an eye on the tubing as it may become suctioned to the side of the bottle. If it becomes suctioned, move the water bottle down and away from the tubing to detach it.
5. Once the there is no water left in the bottle, continue to gently press the trigger to draw out any excess water from the filter.
6. One person can hold the pump head while the other carefully opens the filter holder.
7. Transfer the filter to the filter storage using sterile forceps. **Allow nothing to come in contact with the inside of the tube except for filter and sterile forceps**:
    a. Liquid preservative (ethanol or RNAlater): fold the filter in half on the side that contains the DNA, so the filtered material is enclosed, then fold in half two more times. Place the folded filter paper into preservative tube, being

careful to not touch the inner portion of the tube or lid without your forceps. Screw the cap tightly onto the vial and place into the storage box.

b. Liquid-free preservative (self-indicating silica beads): fold the filter and place into the coin envelope and into a plastic specimen bag with silica. The bag should ideally be pre-filled with ~30 g of silica, or can be added silica now. Seal the plastic specimen bag and set aside.

8. Using sterile forceps, place a new filter into the filter holder.

Collect water samples from the site

The Water Sampler will collect the appropriate number of water samples from the site, referring to your specific sampling design. To decrease the number of bottles needed, you can use the same bottle for each of the three bio-replicates. At this point you should have two empty water bottles that can be used to collect water (Bottles #1 and #2).
In most cases you will be collecting water from the water surface, without stepping into the water. If you must step into the water, ensure you are downstream from your sampling site, from an access point such as a bank

Filter water samples from the site

1. Keeping the drill gear setting on low or "1," press on the trigger and filter the water.
    - Keep an eye on the tubing, as it may become suctioned to the side of the bottle.
    - Filter the water as you filtered the blank or negative control; depending on the level of turbidity, you may need to slow down if you see water is being backed up in the top portion of the filter holder.
    - You can adjust the pressure in the filter by gently releasing the small white nozzle at the top of the filter.
    - Filter as much water as you can until the filter cannot filter any additional volumes.
2. Record the volume of water filtered using a graduated cylinder.
3. Remove the inflow end of the tubing from the water sample and continue to press on the trigger to without any water in the inflow to draw out excess water and moisture.
4. Transfer the filter using sterile forceps to:
    a. Liquid preservative (ethanol or RNAlater): fold the filter in half so the filtered material is enclosed, then fold in half twice more. Place the folder filter paper into preservative tube, being careful to not touch the inner portion of the tube or lid with your forceps.
    b. Liquid-free preservative (self-indicating silica beads): fold the filter and place into the coin envelope and into a plastic specimen bag with silica. This bag should ideally be pre-filled with ~90–100 g of silica (more may be needed). Seal the plastic specimen bag and set aside.
5. Using sterile forceps, place a new filter into the filter holder.
6. Repeat this procedure for each water sample from the site and preserve filters accordingly. For liquid-free preservative (self-indicating silica beads) storage, place the coin envelopes with filters into the same plastic specimen bag with silica with all other

water samples from the site The plastic specimen bag with blanks and the plastic specimen bag with biological water samples can both be placed into a third specimen bag to keep samples from a single site organized.

Once sampling at that site is complete

Have three bags ready:

- **Dirty Equipment Bag** (materials that will be cleaned back in the lab)
    - Filter holder
    - Pump head
    - Silicone tubing
- **Clean Bag** (materials that will remain in a clean area)
    - Binders and data sheets
- **Disposal Bag** (materials that will be stored away from clean equipment and will be disposed of upon return to lab or field station)
    - gloves, disposable forceps, contaminated filters, empty water bottles

1. After sampling at each and every site, disassemble the pump and place a sealed water bottle (**Bottle #3**) into the inflow end. Flush water through the empty filter holder by switching the gear speed to "2," until the water bottle (and tubing) is empty.
2. Remove the filter holder from the tubing by holding the outflow end of the tube in one hand and filter holder in the other.
3. Place the filter holder in a designated bag that remains in the Clean Bag.
4. Unscrew the bolts screw and nuts on the pump head using the screwdriver and wrench.
5. Gently pull apart the top and bottom portion of the pump head. This may be a bit difficult due to the suction created between the silicone tubing.
6. Remove the tube and place in the Dirty Equipment Bag, dedicated to dirty tubes that will not be reused until cleaned again.
7. Put the pump head together again, loosely, and place pump head in the Clean Bag.
8. Discard gloves into the Disposal Bag.
9. Filters should be stored in a cool, dry, and dark area until transported and stored accordingly upon return to lab, field-station, or house.
10. Collect any additional metadata and sampling data.
11. Repeat process at next site.

E. Notes and Precautions

- If you're going to be doing any other sampling in the water, please make sure that you take the biological water samples for eDNA first, followed by any other sampling in the water body. If you're going to be taking any water chemistry parameters, this should take place last so you're not carrying over contaminants from one site to another; cleaning sensitive water parameter equipment may not be possible in field.
- If any part from the filter holder (e.g., O-ring, etc.) becomes contaminated (e.g., fell to the ground, was touched with contaminated gloves, etc.), it will need to be cleaned in the field. **Do not submerge the peristaltic pump head in any liquid, including water—it will rust.** See the ***Equipment Sanitation Flowchart*** for appropriate sanitizing steps.
- Sanitizing any other part of unit except for peristaltic pump head:
    - With ELIMINase: dispense a small amount of ELIMINase onto clean paper towel and wipe down. Rinse with bottled or deionized water.
    - If ELIMINase is not available, using a 10–20% bleach solution: submerge the contaminated part for the allotted time (see below), and then rinse with bottled or deionized water. Perform a triple rinse with water to be sure there are not residues of bleach in the materials. Any residual bleach will degrade DNA in the sample. All waste bleach solutions must be transported back to lab or field solution for proper disposal.
        - 10% solution: 30 minutes
        - 20% solution: 15 minutes
        - 50% solution: 2 minutes
    - Note: the 50% bleach solution only requires a contact time of 1–2 minutes—but will require a large amount of bleach. All waste bleach solutions must be transported back to lab or field solution for proper disposal
- On a windy day, you may have difficulty placing the filter on the filter holder. Simply put any fallen filter papers in the Disposal Bag.
- Another challenge of a windy day is lighting the forceps for sterilizing. You can use a cardboard box to shield from the wind

Storage of Filters (Lab)

- Liquid preservative (ethanol or RNAlater): Filters that are preserved in ethanol can be stored at room temperature, but we suggest storing filters at -20 to -30°C.
- Liquid-free preservative (self-indicating silica beads): Filters that are preserved in silica can be stored at room temperature in a cool, dry location away from light, but we suggest storing at -20 to -30°C.

Appendix A. DIY peristaltic pump parts, assembly, and removing filter from holder for storage.

***Figure 2**. Components of cordless drill*

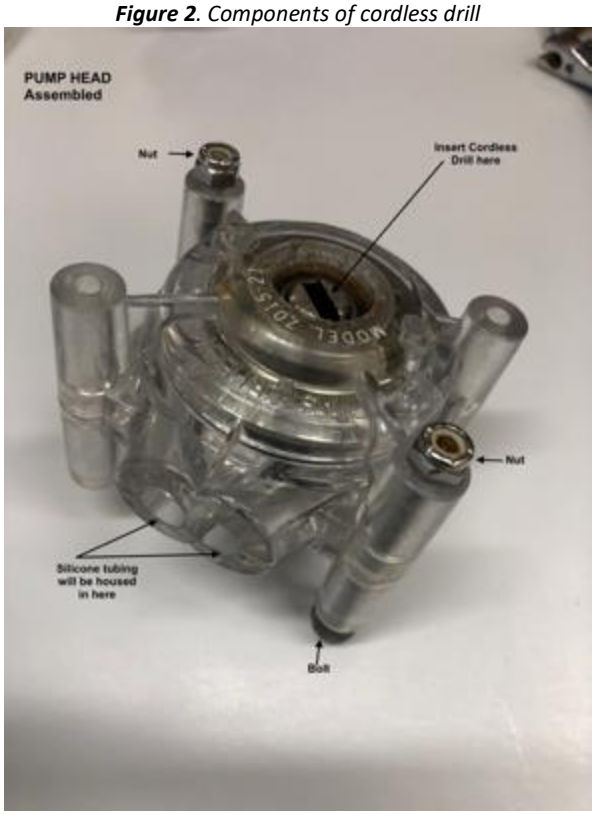

***Figure 3.** Pump head, assembled*

Figure 4. *Pump head, unassembled.*

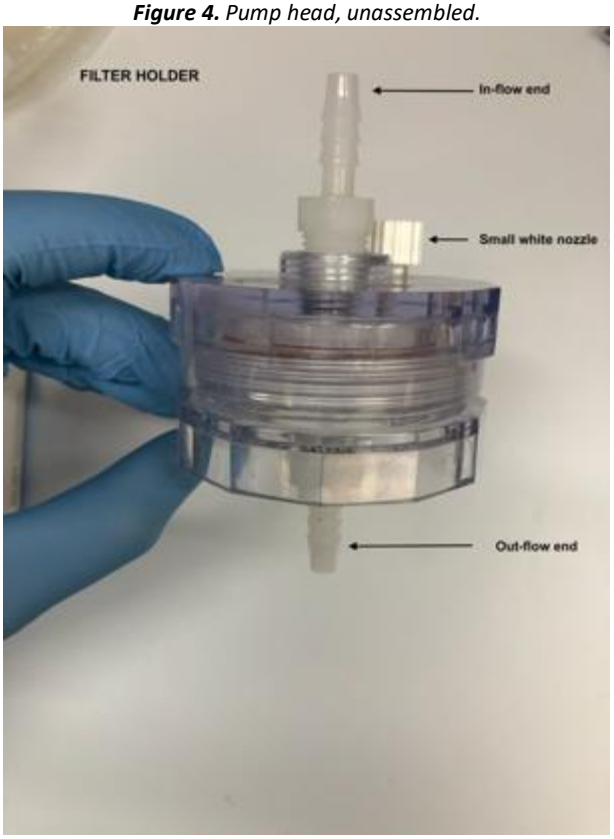

Figure 5. *Filter holder, assembled.*

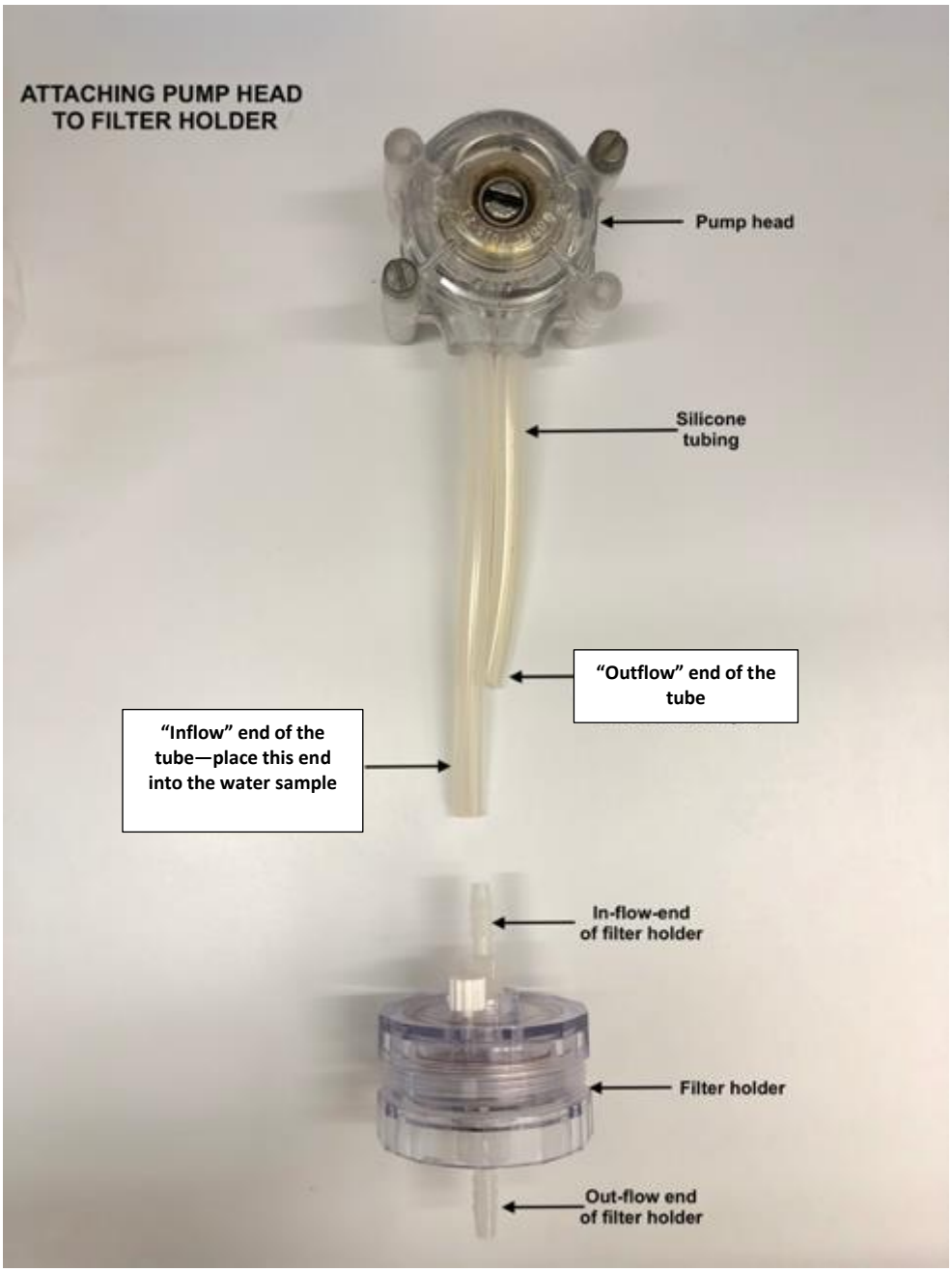

***Figure 6**. Pump head and filter holder, assembled. Parts are labelled to indicate site of attachment between peristaltic pump and in-line filter holder.*

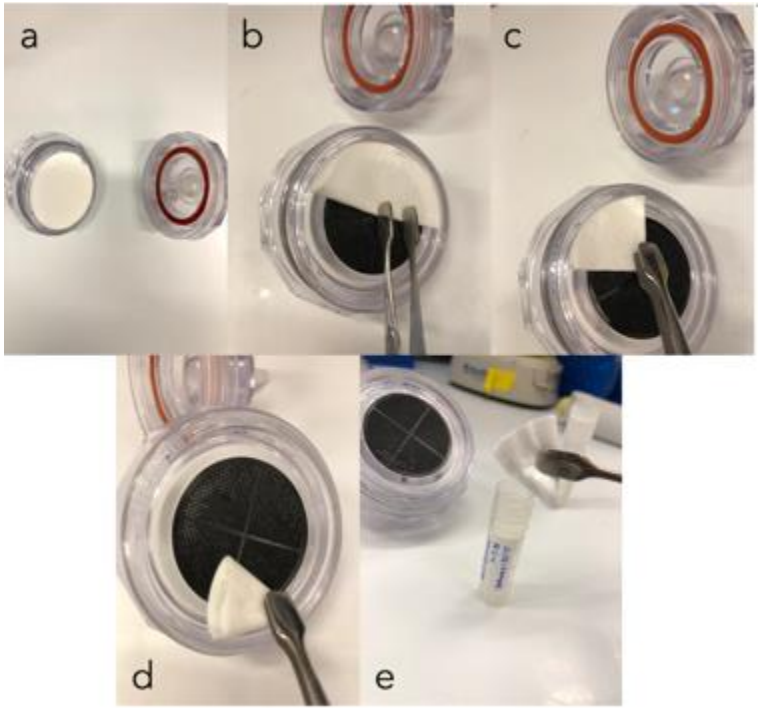

Figure 7. *Steps labelled from a–e showing process of filter folding to preserve in liquid preservative. a) open filter holder vertically; b) fold filter with sterile forceps, inwards, onto filtrant; c) and d) fold in half twice more; e) place folded filter into tube.*

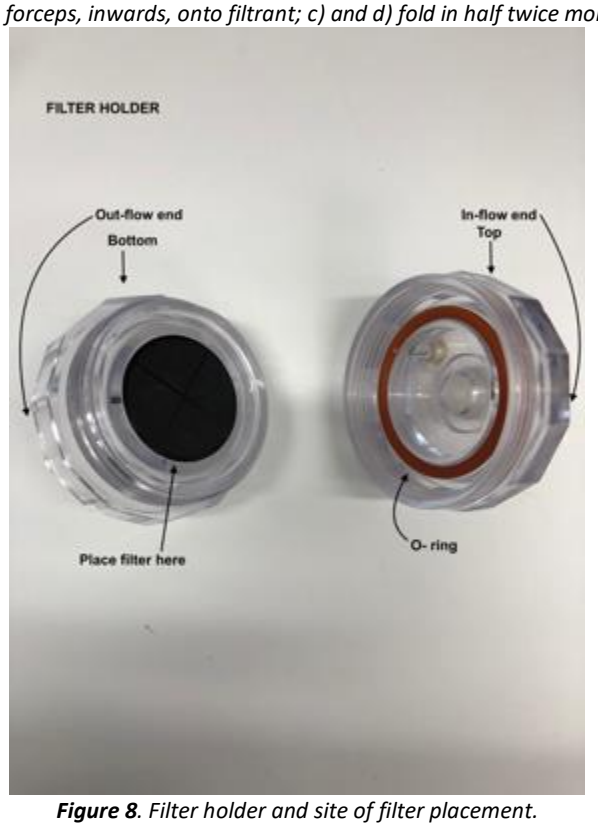

Figure 8*. Filter holder and site of filter placement.*

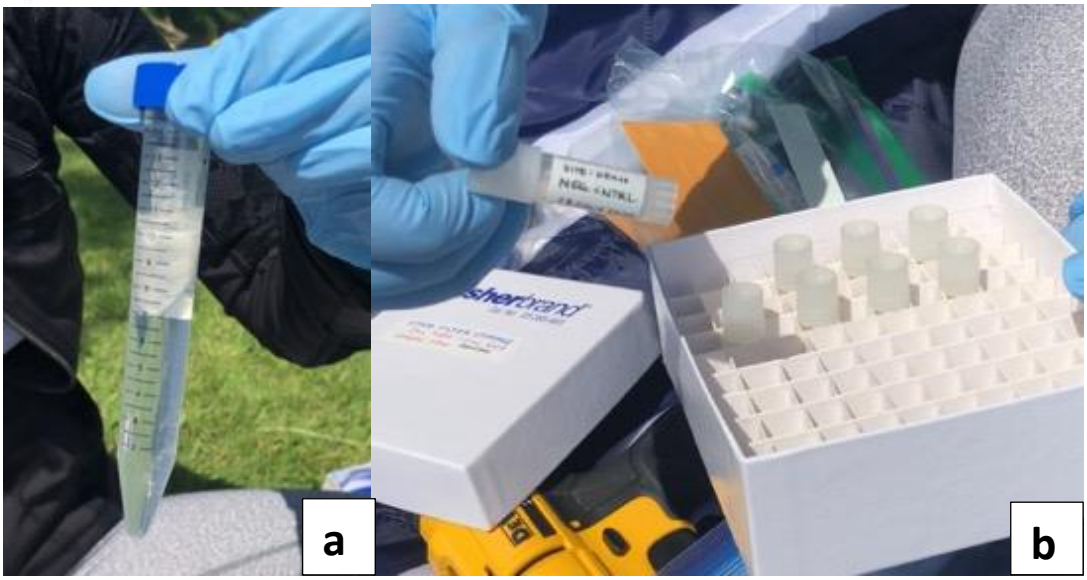

Figure 9. *Storing filter in liquid preservative in either a) 15 ml falcon tube or b) 2 mL screw-top tube, both pre-filled with liquid preservative.*

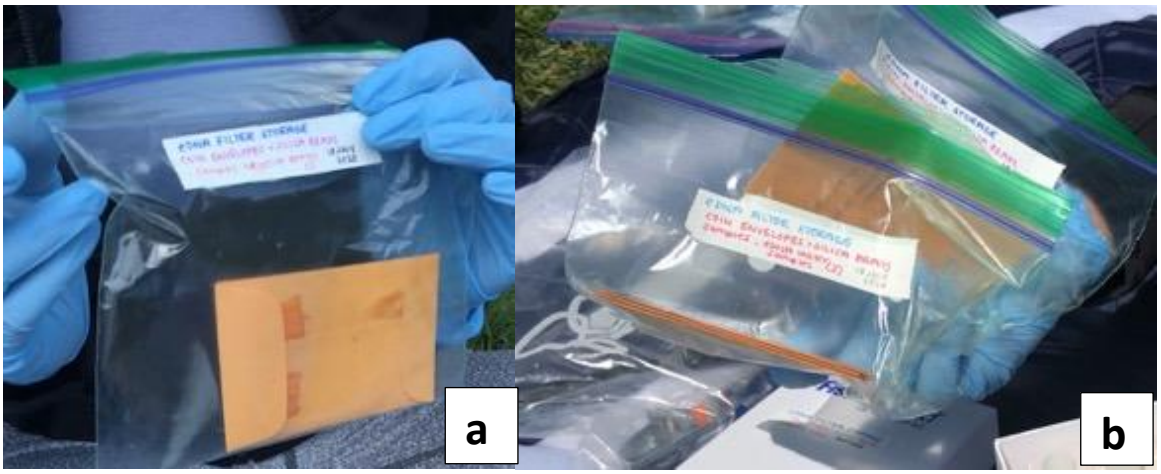

Figure 10. *Storing filter in self-indicating silica in coin envelopes. Blank or negative control filter can be placed in pre-labeled coin envelope inside a plastic specimen bag (a). Water control samples can be stored in three individually labeled coin envelopes in a plastic specimen bag (b). Bags should be filled with ~30 g and ~100 g of self-indicating silica, respectively, for bag in a) and b).*

Appendix B. Recommended Supplies List for eDNA Sampling (for emergency replacements); materials provided by central lab are in bold

Item	Description	Vendor and Item Number(s)
Peristaltic pump head	Masterflex L/S® Standard Pump Head for High-Performance Precision Tubing L/S® 15, Polycarbonate Housing, CRS Rotor	Cole Parmer: HV-07015-20 **$342**
Filter holder	Polycarbonate In-Line Filter Holder, 47 mm, Pall Laboratory	VWR: CA28144-257 **$275**
Silicone tubing, sterile	Masterflex L/S® High-Performance Precision Pump Tubing, Platinum-Cured Silicone, L/S 15; 25 ft, 4.8 mm ID	Cole Parmer: HV-96410-15 **$196**
Nuts (for fastening peristaltic pump head, need minimum of 6)	Paulin #8-32inch HEX Machine Nut	Suggested Vendor - Home Depot: Item model 848-217 Amazon: Hillman 140018 Hex Machine Screw Nuts, 8-32
Glass fibre filters	Cytiva Whatman™ 1.5 µm Binder-Free Glass Microfiber Filters, Grade 934-AH, 47 mm Circles	Fisher Scientific: 09-873DD
Forceps	Fisherbrand™ Filter/Membrane Stainless Steel Forceps	Fisher Scientific: 09-753-50 **$12**
Single-use nitrile gloves	Fisherbrand™ Powder-Free Nitrile Exam Gloves	Fisher Scientific: 191301597 (variety of sizes available)
Graduated container or cylinder	Fisherbrand™ Polypropylene Graduated Cylinders	Fisher Scientific: 03-007-44
1 L Nalgene water collection bottles	Fisherbrand™ Leakproof HDPE Wide-Mouth Bottles (1 L bottles)	Fisher Scientific: 02-896-2F
Chemically resistant markers	Fisherbrand™ Fine Tip Marking Pens	Fisher Scientific: 13-379-6

ELIMINase	Decon™ ELIMINase™ Decontaminant (50 mL spray bottle)	Fisher Scientific: 04-355-32
Wrench	Adjustable; use what you have available on hand that is comparable	Suggested Vendor: Home Depot Crescent 6 Inch Cushion Grip Chrome Adjustable Wrench Item: AC26CVS **$15**
Screwdriver	Flathead; use what you have available on hand that is comparable	Suggested Vendor: Home Depot STANLEY Push-N-Pick Screwdriver Item: 69-193P **$15**
Cordless drill	Use what you have available on hand that is comparable	Suggested: DEWALT 20V MAX Li-Ion Cordless Brushless Compact 1/2-inch Drill Driver w/ (2) Batteries 1.3Ah, Charger and Tool Bag
Extra cordless drill batteries	Use what you have available on hand that is comparable	Suggested: DEWALT DCB207 1.3 Ah 20V Li-Ion Compact Battery
FILTER PRESERVATION MATERIAL IN LIQUID PRESERVATIVE (ETHANOL OR RNALATER)—PROVIDED IF INDICATED		
Storage box, size appropriate to tube size.	Fisherbrand™ Cryo/Freezer Boxes (100- tube capacity)	Fisher Scientific: 03-395-465
Sterile tubes (15 mL or 2 mL)	United Scientific Supplies 15 mL Centrifuge Tubes National Scientific™ BioStor™ 2 mL Screw Cap Vials, Skirted	Fisher Scientific (15 mL): S99410 Fisher Scientific (2 mL): 11-844-18
RNAlater	Invitrogen™ RNA*later*™ RNA Stabilization Solution	Fisher Scientific (500 mL): **AM7021**
Ethanol	Ethyl Alcohol Denatured, MilliporeSigma™	Fisher Scientific (4 L): MEX02803

FILTER PRESERVATION MATERIALS IN LIQUID-FREE PRESERVATIVE (SELF-INDICATING SILICA)—PROVIDED IF INDICATED		
Coin envelopes, sterile	Coin Envelopes with Gummed Flaps, 2-1/4" x 3-1/2"	Suggested Vendor: Staples Item: 438346 Model: 530164
Self-indicating silica beads, sterile	Silica Gel, Honeywell Fluka (metal free) 2.5 kg	Fisher Scientific: 6002003
Plastic specimen bags, sterile	Minigrip™ Reclosable White Specimen Bags	Fisher Scientific: 22-310-032
Sterile sampler spoons	Bel-Art™ Sterileware™ Sterile Styrene Sampler Spoons (~15 mL capacity)	Fisher Scientific: 03-990-232

GEN-FISH
Genomic Network for Fish Identification, Stress and Health
University of Windsor and Partners

DIY Peristaltic Pump Packing List

Item *Italicized items are optional and may not be included*	**Quantity**	**Returned** *(check when packed)*	**Price** *(If GEN-FISH is required to replace)*
Pump head	1	☐	$342
Filter holder	1	☐	$275
Silicone tubing	12	☐	$195
Forceps	1	☐	$12
Chemically resistant markers *For GEN-FISH partners only / optional*	*2*	☐	$15
Screwdriver	1	☐	$15
Wrench	1	☐	$15
ELIMINase (spray bottle)	1	☐	$5
18% Gray Photographers card *For obtaining approximate turbidity estimates via the app "HydroColor" / optional*	*1*	☐	$10
Extra pieces Nuts and filter replacements for in-line polycarbonate filter holder		☐	$5

Additional items:

- Tubes pre-filled with self-indicating silica beads OR pre-filled with RNAlater or ethanol.
- Filter membranes
- *NOTE: Please indicate the entire amount(s) of the above preservation materials needed for all summer sampling on the GEN-FISH equipment booking form. Multiple requests will not be accepted, as this negatively impacts our shipping budget and available stock may be limited.*

You will need to provide the following to supplement sampling:

- Cordless drill (and flathead-bit that fits into pump)
- Batteries for cordless drill (Note: drill batteries will drain faster in colder temperatures)
- BBQ Lighter
- Sealed 500 mL water bottles or Nalgene sampling bottles
- Large bag or box to transport all equipment

GEN-FISH
Genomic Network for Fish Identification, Stress and Health
University of Windsor and Partners

Equipment Sanitation Flowchart

To be completed before and after field sampling, and before returning DIY unit. All cleaning steps must be performed while wearing gloves and in a space free of active fish dissections, PCR, or DNA amplification. *Check when each step is complete. Be prepared to submit this checklist upon return of equipment.*

- ☐ Rinse equipment with water
  - Tubing
  - Filter holder

  DO NOT SUBMERGE THE PERISTALTIC PUMP IN ANY LIQUID AS IT WILL RUST. Wipe down the pump with a Kimwipe or paper towel with ELIMINase to clean. The pump does not come into contact with water so should be free of DNA if all clean procedures are followed.
- ☐ Wash with soap and water
- ☐ Allow to air dry as paper towels can leave fibres behind
- ☐ Clean with ELIMINase
  - a few mL is put in each tube and swished back and forth
  - Kimwipes lightly sprayed with ELIMINase can be used to wipe equipment and outside of tools

  If ELIMINase is not available, bleach solution can be used.
- ☐ Clean with 70% Ethanol
  - Same procedure as with ELIMINase
- ☐ Rinse tubing with water
- ☐ Allow equipment to dry in a clean, DNA-free area. If laminar flow PCR workstation is available, dry in workstation.
- ☐ If UV irradiation is available, UV treat for 15 minutes (minimum).
- ☐ Handle sanitized equipment with clean gloves and after change of clothing
- ☐ Equipment can be stored in clean plastic bags until next use
- ☐ Check that all items from packing list are included in the shipment back to the central lab

Any steps of this sanitation protocol that are not met must be noted in this checklist, communicated via email, and included with shipment of unit to ensure the unit is properly decontaminated prior to further use.