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🌐 Deep-Sea eDNA Sampling from Seawater with Peristaltic Pumps and Sterivex Filters V.1

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We use this protocol and it's working

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Abstract

This protocol details methods that can be used to filter water directly from Niskin bottles to collect environmental DNA (eDNA) samples. This method uses peristaltic pumps, sterilized tubing, and Sterivex filters to filter directly from up to 12 niskin bottles, such as those on a standard water sampling rosette, simultaneously on the deck of a ship. This approach is advantageous as it uses a closed system to decrease chances of contamination and allows for quick filtration once the Niskins are on deck to avoid drastic temperature changes and subsequent DNA degradation (McCartin paper). This document contains three main sections: 1) Materials/ Parts List, 2) Equipment/work area sterilization, and 3) Sampling from Niskins in the field. Some sections, such as sterilization between sampling events and

Materials

Materials/Parts List

▪ **Peristaltic Pump parts**

- Masterflex L/S pump drive compatible that can accept up to four Masterflex Easy-Load II Pump head(s) (e.g. VWR - [MFLX07522-30](#))
- Any peristaltic pump drive that is compatible with Masterflex Easy-Load II pump heads and Masterflex tubing will work, but not all can pump water over four filters at once. We have also used [this pump drive](#) for sampling one sample at a time.
- Masterflex Easy-Load II pump head(s) (4) (VWR – [MFLX77200-60](#))
- Easy-Load II pump head mounting equipment for four pump heads (VWR - [MFLX77200-04](#))
- Pelican cases to ship and store the pumps when they are not in use. These cases can also be used as a surface to place the pump around the CTD rosette/ROV during the filtration protocol. ([iM2975 Storm Travel Case](#))
- Tubing and connectors
- Masterflex L/S High performance precision pump tubing, platinum-cured Silicone, size L/S 15 (inner diameter 3/16") (VWR - [MFLX96410-15](#))
- 3/8" ID Clear ND-100-65 Tygon PVC Plastic Tubing (McMaster Carr – [8349T25](#))
- 3/16" ID Clear ND-100-65 Tygon PVC Plastic Tubing (McMaster Carr – [8349T18](#)) or alternatively use short sections of the L/S 15 tubing.
- 3/8" inner diameter to 3/16" inner diameter plastic straight reducing coupler tube fitting (e.g. Cole-Parmer - [UX-50108-87](#))
- Luer Lock straight plugs, barbed tube adapters for 3/16" inner diameter tubing (e.g. polypropylene McMaster-Carr - [51525K125](#))
- Luer Lock straight sockets, barbed tube adapters for 3/16" inner diameter tubing (e.g. polypropylene McMaster-Carr - [51525K215](#))
- Tubing cutter (e.g. from [Amazon](#))

▪ **Filter and Filter Storage Supplies**

- MilliporeSigma Sterivex 0.22 um pore size polyethersulfone filter capsules, female luer lock inlet, male luer lock outlet (Fisher - [SVGPL10RC](#))
- 4 fl. oz Nasco Whirl-Pak Sample Bags (whirl-pak.com – [B00679](#))
- 2L Nasco Stand Up Whirl-Pak Sample Bags (whirl-pak.com - [B01451](#))
- 5 mL Luer Lock syringes (Fisher - [14-829-45](#))
- Zip-loc bags, small and large sizes.

▪ **Filtration Set-up Necessities**

- Nitrile gloves
- Weather-proof extension cord
- 1-5 gallon plastic buckets/milk jugs or similar containers for collecting effluent (need one per niskin).
- If using milk jugs or similar, it can be helpful to pack some plastic milk crates to hold them in during sampling.

▪ **Cleaning Supplies**

- Household Bleach (e.g. Chlorox 7.5% Sodium Hypochlorite)
- A source of ultrapure (e.g. MilliQ) or deionized water free of target eDNA.
- Be aware that water sourced from surface seawater and desalinated must be sufficiently filtered to remove any traces of eDNA from marine organisms.
- In a pinch, we have used bottled, spring, or distilled water and filtered it through 0.2-um-pore-size polyethersulfone filters to rinse tubing and conduct negative sampling controls.
- 2L graduated cylinder
- Two spray/wash bottles
- Three collapsible, plastic carboys (e.g. Reliance products - [5010-13](#))
- Two plastic containers with lids (e.g. Rubbermaid from [Amazon](#))
- Paper towels
- A portable table to use as a work surface on the deck of the ship
- We have also used the surface of Pelican cases that we ship our gear in.

Other recommended materials for sampling instrument flexibility

- 1/4" ID Clear ND-100-65 Tygon PVC Plastic Tubing (McMaster Carr – [8349T504](#))
- 3/8" inner diameter to 1/4" inner diameter straight reducing coupler tube fitting (e.g. nylon plastic McMaster-Carr - [5372K517](#))
- 1/4" inner diameter to 3/16" inner diameter straight reducing coupler tube fitting (e.g. nylon plastic McMaster-Carr - [5372K514](#))
- Plastic claw hair clips are another way to secure the tubing while pumping (example from [Amazon](#))
- Zip Ties to apply to the tubing to prevent the tubing from "walking" through the pump heads while pumping (example from [Amazon](#))
- Zip Tie cutters (example from [Amazon](#))

Troubleshooting



Preparing bleach solutions for equipment sterilization.

- 1 Put on a clean pair of nitrile gloves.
 - 1.1
- 2 In one of the spray bottles, create a 10% solution of household bleach in deionized or ultrapure water. Mix by shaking.
 - 2.1 Let sit for at least fifteen minutes before using.
- 3 In one of the clean, collapsible carboys, create a 10% solution of household bleach in deionized or ultrapure water.
 - 4 Shake to mix and wait for at least fifteen minutes.
 - 4.1 This is now your 10% bleach solution, which will be used to bleach-sterilize sampling equipment. Be sure to refresh this solution once every three days since bleach will lose its effectiveness after it is diluted.
- 5 In another clean, collapsible carboy, create a 10% solution of household bleach in deionized or ultrapure water.
 - 6 Shake to mix and wait for at least fifteen minutes. Discard the bleach.
 - 7 Rinse this second collapsible carboy three times by swirling/shaking a ~1-liter of deionized/ultrapure water and discarding it.
 - 8 Fill this second collapsible carboy with deionized/ultrapure water.
 - 8.1 This is now a DI/ultrapure water rinse that will be used to rinse the sampling tubing after each use after bleach sterilization. It will also be used to run the negative control during each sampling event.
- 9 Repeat steps 5-8 for the third, clean collapsible carboy.

- 9.1 This can also be used to rinse the sampling tubing after the bleach sterilization after each use. It can also be used to run the negative control during each sampling event.
- 10 Repeat steps 2 through 8, this time with the two Tupperware bins/lids, to create two more solutions of 10% bleach and deionized/ultrapure water in smaller volumes for sterilizing short tubing segments.
- 10.1 Be sure to refresh the 10% bleach solution once every three days since bleach will lose its effectiveness after it is diluted.
- 11 Repeat steps 2 through 8, this time with the two spray bottles, to create two more solutions of 10% bleach and deionized/ultrapure water for sterilizing surfaces.
- 11.1 Be sure to refresh the 10% bleach solution once every three days since bleach will lose its effectiveness after it is diluted.

Masterflex Pump Assembly (also refer to the Masterflex manual)

- 12 1. Screw the two rods into bottom holes on the front of the pump drive (make sure they are securely screwed tight), with the short, threaded end attached to the pump drive.



Masterflex pump drive with the two rods threaded onto it.

- 13 Slide the first pump head into place, threading the rods through the holes on either side of the tube rest- make sure that the key on the back of the pump head fits into the groove on the front of the pump drive (can rotate key to fit into place).



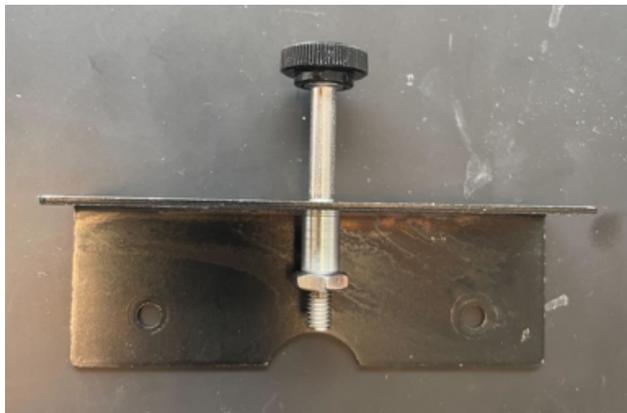
Sliding the Easy-Load II pump heads into place on the rods attached to the pump drive.

- 14 Slide next pump head into place the same way- make sure that the key on the back of the new pump head fits in line with the groove in the front of the previously placed one
- 15 Repeat with all four consecutive pump heads (will have long threaded end of rods exposed for kickstand assembly)



Four Easy-Load II pump heads in place and attached to the pump drive.

- 16 1. Assemble the kickstand by threading the pin through the center hole and tightening the nut until secure (adjustable for height of pump heads).



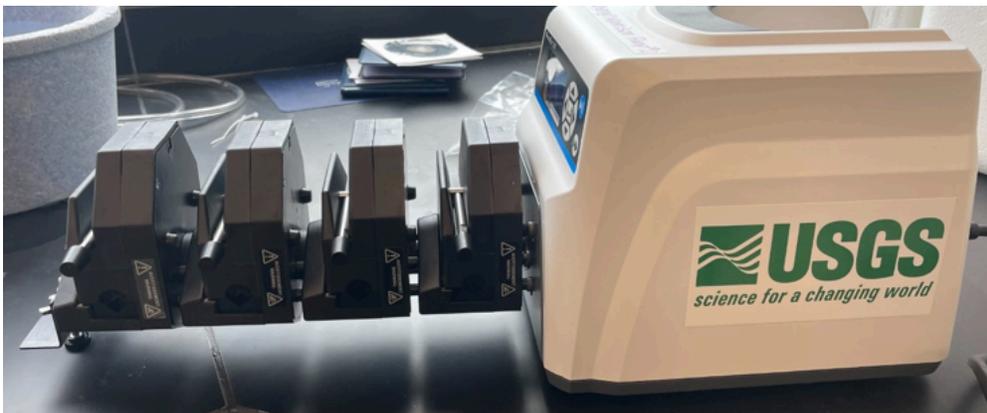
The kickstand, which will be used to secure the four pump heads in place.

17

- 18 Mount kickstand, threading rods through both holes (with pin facing outward and down), until it rests against the front of the fourth pump head
- 19 Thread washers onto the rods.
- 20 Tighten wing nut onto each rod until secure and make sure that the height of the kickstand keeps all four pump heads level



The kickstand with wingnuts tightened to secure the pump heads in place



The Matsterflex pump drive with the four Easy-Load II pump heads attached and secured in place.

Bleach sterilizing platinum-cured silicone sampling tubing

- 21 Put on a new pair of clean, nitrile gloves.



- 22 Sterilize your work surface by spraying it with a 10% bleach solution, distributing it with a paper towel wetted in the solution, and allowing it to air dry.
- 23 Rinse the residual bleach from the work surface by spraying it liberally with deionized/ultrapure water and wiping it with a paper towel.
- 24 Place your peristaltic pump with four pump heads onto the work surface.
- 25 Plug the power cord into the IEC connector on the back of the pump drive (use electrical tape to cover the connection between the pump plug and extension cord).
- 26 Plug into a wall outlet or extension cord.
- 27 Flip the power on (power switch on the back of the pump drive).
- 28 With the tubing cutter, cut four segments of the L/S 15 Platinum Cured silicone tubing long enough to reach from the pump heads to your Niskin bottle during field sampling (typically, we aim for 4' to have extra length if needed).
- 29 Securely clamp the four L/S 15 Platinum-cured silicone tubing segments in the pump heads.
- 30 Submerge the same end of each piece of tubing in the 10% bleach solution in the collapsible carboy.
- 31 Submerge the other end of each piece of tubing in the 10% bleach solution in the collapsible carboy. The bleach will be recirculating throughout this process, so you want all ends of the tubing to be in the carboy.
- 32 With the speed set to 100 RPM, activate the pump drive.
- 33 Recirculate the bleach solution in the four tubing segments for 15 minutes.
- 34 Deactivate the pump and carefully remove the upstream ends of the tubing segments from the 10% bleach solution in the collapsible carboy.



- 35 Activate the pump so air pushes the 10% bleach solution out of the tubing and back into the carboy.
- 36 Carefully remove the other end of the tubing from the carboy with the 10% bleach solution.
- 37 Spray off the outside ends of the tubing with deionized/ultra-pure water to remove as much of the bleach solution as possible from the outside of the tubing.
- 38 Place the same end of each piece of tubing in the deionized/ultra-pure water in another collapsible carboy.
- 39 Place the other end of each piece of tubing on a clean surface above a sink or container to collect water.
- 40 With the speed set to 100 RPM, activate the pump drive.
- 41 Deactivate the pump, and carefully unclamp your tubing from the pump heads.
- 42 Place the now sterilized tubing tubing in a clean, zip-loc bag.

Bleach sterilizing shorter tubing segments, tubing fittings, and Luer Lock adapters.

- 43
- 44 Put on a new pair of clean, nitrile gloves.
- 45 Sterilize your work surface by spraying it with 10% bleach solution, distributing it with a paper towel wetted in the 10% bleach solution, and allowing it to air dry.
- 46 Rinse the residual bleach from the work surface by spraying it liberally with deionized/ultrapure water and wiping it with a paper towel.
- 47 Cut ~1-foot long segments of the $\frac{3}{8}$ " inner diameter Tygon tubing with the tubing cutters.



- 48 Submerge these segments in the 10% bleach solution in the Tupperware.
- 49 Ensure that the ends are submerged so that the bleach solution passes through fully and stays in the tubing segments.
- 50 Let sit submerged in the bleach solution for at least 15 minutes.
- 51 Pour the 10% bleach solution from the tubing segments back into the Tupperware and transfer them to the deionized/ultra-pure water. Again, submerge the tubing segments so that the water flows through them.
- 52 Let the tubing sit submerged in the water to rinse indefinitely.
- 53 Repeat steps 4-9 for the 3/16" inner diameter Tygon tubing (or Platinum-cured Silicone L/S 15 tubing)
- 54 Once bleach-sterilizing and rinsing the tubing segments are finished, transfer them to a clean zip-loc bag.
- 55 Submerge the Luer Lock straight sockets, Leur Lock straight plugs, and straight reducing couplers in the 10% bleach solution in the Tupperware for at least 15 minutes.
- 56 Transfer and submerge the Luer Lock straight sockets, Leur Lock straight plugs, and straight reducing couplers in the deionized/ultra-pure water indefinitely.
- 57 Before using, remove the Luer Lock straight sockets, Leur Lock straight plugs, and straight reducing couplers from the water rinse, and transfer them to a clean paper towel to air dry.
- 58 Before using, transfer the rinsed Luer Lock straight sockets, Leur Lock straight plugs, and straight reducing couplers to a small, clean Ziploc bag.

Assembling tubing

59



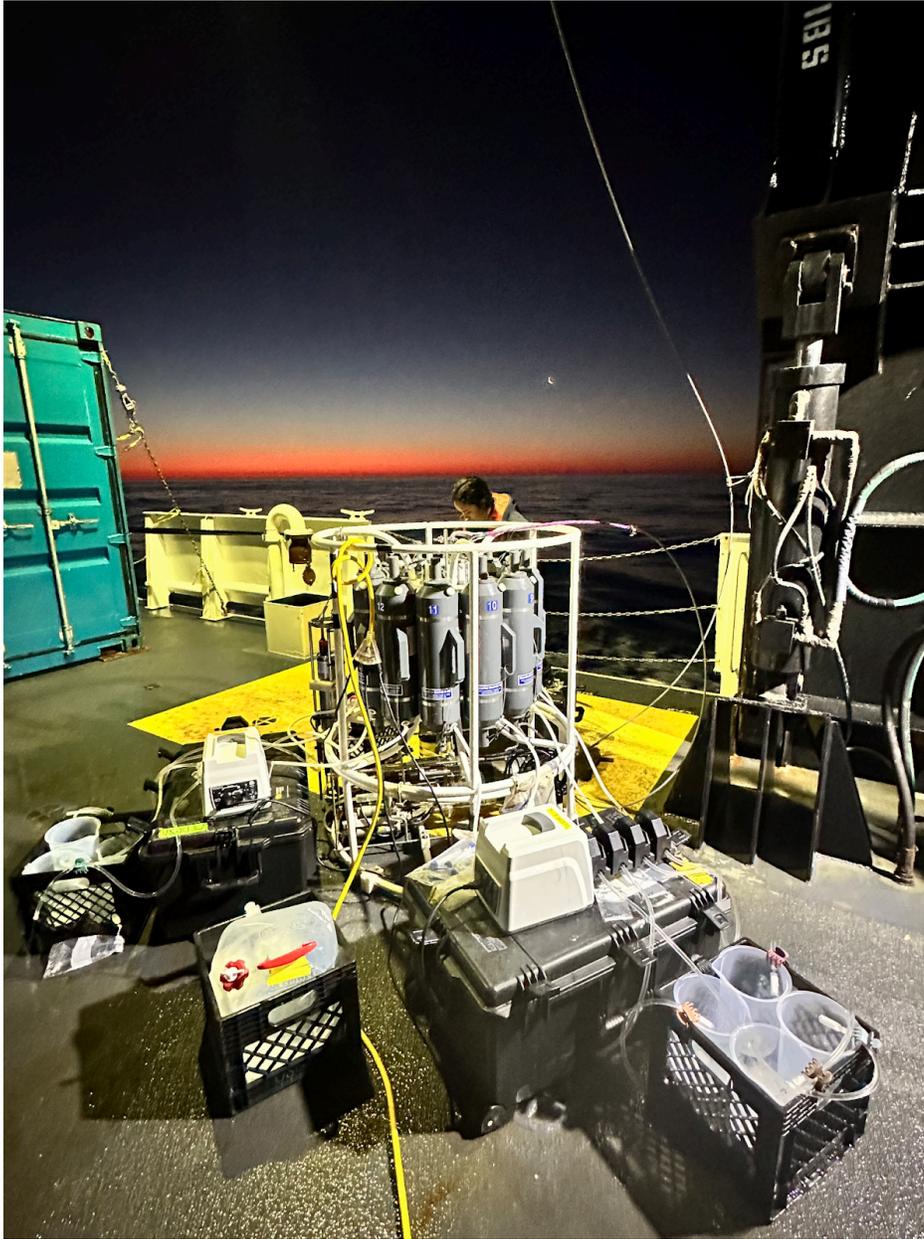
- 60 Take a ~1' segment of $\frac{3}{8}$ " inner diameter Tygon tubing and insert a $\frac{3}{8}$ " to 3/16" straight reducing coupler to one end of the tubing
- 61 To this barbed coupler, attach a ~4' segment of Masterflex L/S 15 Platinum-cured silicone tubing.
- 62 Insert a Luer Lock straight plug into the ~4' segment of Masterflex L/S 15 Platinum-cured silicone tubing. This is where the Sterivex filter will attach.
- 63

STEP CASE

Sampling water directly from Niskin bottles 48 steps



Three Masterflex pump drives, each outfitted with four pump heads, are used to simultaneously sample 12 Niskin bottles from a Niskin bottle rosette. A folding table holds all three pumps.



Three Masterflex pump drives, each outfitted with four pump heads, used for the simultaneous sampling of 12 Niskin bottles using Pelican cases as tables.



- 65 Put on a clean pair of nitrile gloves.
- 66 Sterilize your work surface by spraying it with 10% bleach solution, distributing it with a paper towel wetted in the 10% bleach solution, and allowing it to air dry.
- 67 Rinse the residual bleach from the work surface by spraying it liberally with deionized/ultrapure water and wiping it with a paper towel.
- 68 Place your peristaltic pump with four pump heads onto the work surface.
- 69 Plug the power cord into the IEC connector on the back of the pump drive (use electrical tape to cover the connection between the pump plug and extension cord).
- 70 Plug into the wall outlet or extension cord. Tip: use a waterproof extension cord with a three-female plug end.
- 71 Flip the power on (power switch on the back of the pump drive) and cover the back side with plastic to protect the power area from bleach solution and deionized/ultra-pure water.
- 72 Using a Sharpie, label a 2 oz Whirl-Pak with the cruise number, date, sample number, and Niskin bottle position (i.e., the bottle number on the CTD or submersible).
- 73 Label a clean, zip-lock bag with the cruise number and the samples to be placed inside. Tip: group samples from a single CTD cast or multiple submersible dives in the same bag.
- 74 Attach a ~1' segment of $\frac{3}{8}$ " inner diameter Tygon tubing to the petcock of each Niskin bottle.
- 75 Insert a $\frac{3}{8}$ " to 3/16" straight reducing coupler to the end of each tubing segment.
- 76 To this barbed coupler, attach a ~4' segment of Masterflex L/S 15 Platinum-cured silicone tubing.
- 77 Insert a Luer Lock straight plug into each ~4' segment of Masterflex L/S 15 Platinum-cured silicone tubing.



- 78 Open a new Sterivex filter capsule from its bag.
- 79 Attach the Sterivex filters to the Luer Lock plugs at each primed length of tubing.
- 80 Place the Masterflex L/S 15 Platinum-cured silicone tubing in a pump head, but do not clamp down on it!
- 81 Direct the outflow end of the filter into a 5-gallon bucket, milk jug, or similar container to collect the effluent.
- 82 Repeat for each of the up to 4 samples you are taking per pump.
- 83 To prime the tubing/filter capsule, loosen the top valve of the Niskin bottle and press the petcock to the open position.
- 84 Let the water flow from the Niskin bottles just until the Stervex filter is filled with water.
- 85 Next, clamp down the pump heads onto the L/S 15 tubing to stop the gravity flow. The filter capsule must be downstream of the pump head. Make sure that the pump head is closed correctly around the tubing such that the rollers are tightly sealed against the tubing.
- 86 Power on the pump drive.
- 87 With the speed to 100 RPM, activate the pump to push water over the Stervex filters.
- 88
- 89 When ~2L have been filtered, remove the tubing from the Niskin bottles to allow the water remaining in the tubing to go all the way to the end through the filter. When no more water is dripping from the Sterivex (it is empty), deactivate and turn off the pump.
- 90 Remove the Sterivex from the tubing.



- 91 Using a 5 mL Luer Lock syringe, push out any water remaining in the filter capsule from the inlet end.
- 92 Place each Sterivex in the appropriately labeled Whirl-pak.
- 93 Place all Whirl-Paks from as many samples as you've processed at once in the labeled Zip-Lock bag and freeze them together at -80°C.
- 94 Measure the effluent using the 2L graduated cylinder and record the volume filtered for each sample.
- 95 Disassemble the tubing segments.
- 96 Once all the filters have been stored in the freezer, return to the pump setup to clean the tubing before disassembling.
- 97 Bring one of the 1L containers of 10% bleach and the carboy containing deionized/ultrapure water next to one of the pump heads. Gather all the tubing ends attached to the Niskins and submerge them in the bleach solution.
- 98 Gather the output ends of the tubing (that had the Sterivex attached) and submerge them in the same bleach solution.
- 99 The bleach will be recirculating throughout this process, so you should put all the ends of the tubing in the bleach container.
- 100 With the speed set to 100 RPM, activate the pump drive.
- 101 Recirculate the bleach solution in the four tubing segments for 5 minutes.
- 102 Deactivate the pump and carefully remove the upstream ends of the tubing segments from the 10% bleach solution in the collapsible carboy.
- 103 Activate the pump so air pushes the 10% bleach solution out of the tubing and back into the 1L bleach container.



- 104 Carefully remove the other end of the tubing from the carboy with the 10% bleach solution.
- 105 Spray off the outside ends of the tubing with deionized/ultrapure water to remove as much of the bleach solution as possible from the outside of the tubing.
- 106 Place the same end of each piece of tubing in the deionized/ultrapure water carboy.
- 107 Clip the other end of each piece of tubing so that the end does not touch any surface. The water will flow onto the deck or into a bucket.
- 108 With the speed set to 100 RPM, activate the pump drive.
- 109 Run ~1 liter of the ultra-pure/deionized water through the tubing to rinse out any residual 10% bleach solution from the tubing, collect water in a bucket and discard.
- 110 Lift the end of the tubing out of the water and pump the remaining water out into a bucket to ensure no water remains in the tubing. Dispose of the water in the bucket.
- 111 Using medium or small Whirl-Paks, wrap the Whirl-Paks around each end of the tubing and tighten the twist ties securely to prevent contamination. Then, hang the tubing in the lab to allow further drainage before the next sampling event.