



WATER SAMPLE COLLECTION AND FILTRATION

We do not recommend freezing water samples, as cell lysis occurs and results in less than optimal data. However, filtering the samples and freezing the filters for shipment preserves integrity of microbial DNA if done properly. General instructions are given below for sample collection and filtration. Please contact project@phylotech.com with any questions you may have.

COLLECTING AND FILTERING WATER SAMPLES

Materials

Sterile 1-liter wide-mouth screw capped plastic collection bottles.

Any chemical-free plastic water bottle that meets standard EPA bottle cleanliness guidelines will suffice. The bottles must be clean. See USGS and EPA manuals for details.^{1,2}

Sample Collection

Sample collection procedures will depend on the location, onshore or offshore, and the depth of the desired sample.

Onshore beach water sampling protocol

1. Attach a sterile 1 L collection bottle to the end of a long pole using bungee cord e.g. a painter's pole works well.
2. Walk into water to ankle-deep depth, taking care not to kick up sand or sediment.
3. Remove bottle cap and extend pole into water to grab the sample.
4. Immediately cap the bottle.
5. Cool samples, place on ice or refrigerate and maintain at 2-8°C. Do not freeze.

For offshore sampling the method is depth dependent. For surface water, simply dip the sampler off the side of the boat. For deep water sampling please use samplers that are specially designed to capture and seal water samples at depth.

To obtain optimal target yield and good data quality please carefully follow these collection and shipping instructions.

FILTERING THE WATER SAMPLE

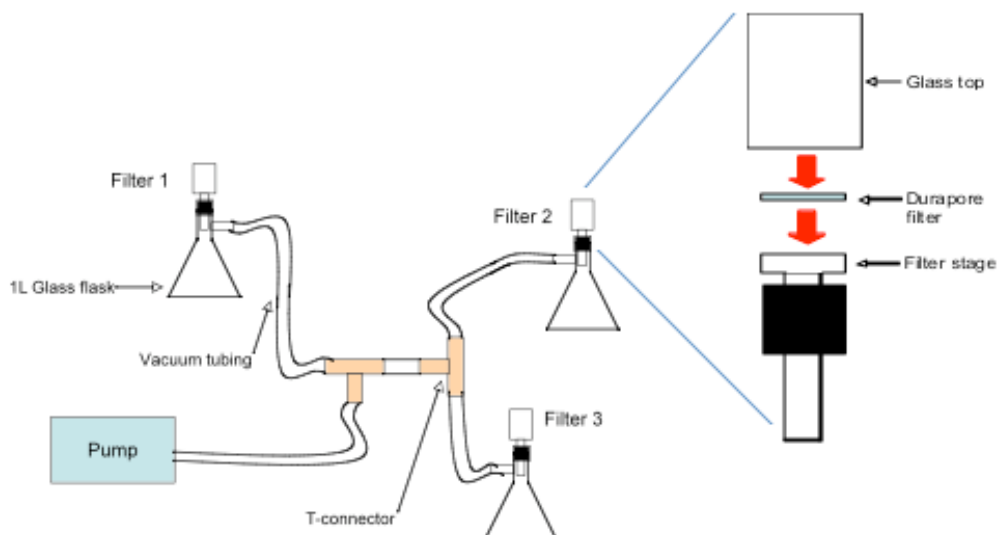
Materials

- Millipore Durapore PVDF Membrane Filters: 0.22 µPore Size; Dia.: 47mm (P/N GVWP04700)
- 1L flasks for filtration
- Vacuum Pump
- Vacuum Tubing
- T adapters
- Filter setups (glass filter stage, glass top, metal clamp)
- Whirlpak bags (Nasco/VWR, P/N B01009WA)
- Bar coded labels for each bag
- Paper towels
- 70% alcohol solution
- Forceps

FILTRATION

It is optimal to collect multiple aliquots of each sample in parallel using the same vacuum pump.

1. Pre label WhirlPak bags with barcodes.
2. Set up filtering apparatus according to the figure below. Check each connection point to ensure components are snug and pressure loss is minimized.



3. Clean each filter setup (glass top and filter stage) using 70% ethanol or isopropanol. Wipe dry with paper towels.
4. Gently place Durapore filter on each filter stage using alcohol-cleaned forceps. Place glass top onto filtering stage and use metal clamps to hold components together.
5. Start vacuum pump; allow it to run ~5 sec before adding samples.
6. Homogenize seawater sample by shaking gently.
7. Pour ~350mL of seawater from a single sample onto each filter.
8. Continue to operate pump until a vacuum pressure of 25 inches of mercury (inHg) is obtained. The pump can be shut off at this point to minimize strain on the motor. Pump should be turned back on if pressure drops below 20 inHg.
9. Once sample has completely been filtered through all three of the Durapore filters gently remove the glass tops, and carefully peel Durapore filters from filter stages using forceps.
10. Carefully place all three filters into individual WhirlPak bags labeled with sample name, data, and operator.
11. Freeze filters at -20C (-80C if possible).
12. Separate a 500mL portion of seawater for further lab tests to be performed at LBL. Store liquid samples at 4C.
13. Discard remaining seawater in the sink.
14. If additional samples will be filtered, rinse filter stage and glass top with de-ionized water and wipe down with 70% alcohol solution. Filter next sample as described above.
15. Once filtration of all samples is complete disassemble sampling apparatus. Rinse filter-setups and glassware with de-ionized water and 70% alcohol. Store tubing, vacuum pump, and connectors. Autoclave all glass ware if possible.

REFERENCES

1. U.S. Geological Survey Book 9 Handbooks for Water-Resources Investigations, National Field Manual for the Collection of Water-Quality Data
<http://water.usgs.gov/owq/FieldManual/Chapter7/Cover-Contents-Introduction.pdf>
2. www.epa.gov/waterscience/methods/
3. [www.doh.state.fl.us/lab/PDF Files/GUIDELINES WATER BACTERIOLOGICAL.pdf](http://www.doh.state.fl.us/lab/PDF%20Files/GUIDELINES%20WATER%20BACTERIOLOGICAL.pdf)