

Video Article

Small Volume (1-3L) Filtration of Coastal Seawater Samples

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Abstract

The workflow begins with the collection of coastal marine waters for downstream microbial community, nutrient and trace gas analyses. For today's demonstration samples were collected from the deck of the HMS John Strickland operating in Saanich Inlet. This video documents small volume (~1 L) filtration of microbial biomass from the water column. The protocol is an extension of the large volume sampling protocol described earlier, with one major difference: here, there is no pre-filtration step, so all size classes of biomass are collected down to the 0.22 μm filter cut-off. Samples collected this way are ideal for nucleic acid analysis. The set-up, filtration, and clean-up steps each take about 20-30 minutes. If using two peristaltic pumps simultaneously, up to 8 samples may be filtered at the same time. To prevent biofilm formation between sampling trips, all filtration equipment must be rinsed with dilute HCl and deionized water and autoclaved immediately after use.

Protocol

1. Set up of filtration apparatus

1. Before filtering, first wipe away any condensation from the full bottles and weigh them
2. Place an autoclave bin on one side of the pump, and the waste flask on the other.
3. Then place a full sample bottle in the autoclave bin; however, do not remove the cap of the bottle yet.
4. Pass the tubing through the peristaltic pump so that the luer end of the tubing is in the flask.
5. Using tweezers, remove the cotton from a sterile 25 ml pipette, and attach the pipette to the other end of the tubing.

2. Filtration of seawater

1. To begin filtering seawater, open the bottle and insert the pipette. Turn on the pump and set the flow speed to 1. Watch to ensure that water is being pumped in the proper direction.
2. Allow ~100 ml of water to pass through the entire tubing.
3. Turn off the pump and attach a Sterivex filter to the male luer fitting at the free end of the tubing.
4. Continue filtering until no water is left in the bottles.

3. Storage of filters

1. To store the filters, first expel any remaining water with a 30-60 ml syringe.
2. Seal the bottom of the Sterivex filter with Parafilm. Using a pipette, add 1.8ml lysis buffer to the filter, keeping ~ 200 μl of space in the filter for later addition of reagents.
3. Then, seal the top of the Sterivex filter with a small piece of Parafilm, and label the filters with the date and sample identification.
4. Store the filters in pre-labelled 50 ml falcon tubes at -80°C

4. Clean up

1. Insert the 25 ml pipette into a flask containing 0.1% HCl, and switch on the pump.
2. After the HCl wash, rinse the tubing with autoclaved water.
Finally, measure and record the mass of the now empty 1L bottles in order to calculate the volume of water passed through the filter.

Representative Results: Each 1 L water sample yields one Sterivex filter, which contains biomass greater than 0.22 μm . This is stored in lysis buffer for subsequent DNA extraction and analysis. The final result consists of one Sterivex filter per 1-liter bottle stored in lysis buffer and ready for DNA extraction. The filter contains biomass greater than 0.22 μm . Filters can be stored at -80°C prior to eDNA extraction.

Discussion

It is imperative to wipe down benches and pumps with a damp towel after use to remove any lingering traces of salt water that would otherwise corrode equipment. Although the procedure is simple, the time requirement can be extensive depending on the concentration of biomass or debris in the sample being filtered.

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References