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Water Sampling onto Filters for Nucleic Acids Sequencing

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ABSTRACT

This protocol will guide you through the process of filter water using a serial filtration approach to generate filters with microbial biomass. Later, nucleic acids will be extracted from these filters.

GUIDELINES

We stopped using RNALater to preserve our nucleic acids (even RNA), as we've learned that it does not improve the stability of nucleic acids. It also influences the community composition of the sequenced community, intriduces massive amount of salts that can interfere with downstream processsing, and if very expensive.

The gold standard approach is rapid collection of sample, immediate flash freezing, then rapid extraction upon thawing. If this can not be done, RNALater can offer some field scenarios an alternative. In the absence of a way to quickly freeze your samples, adding 1 ml of RNALater to the cryovial with your filter will preserve nucleic acids, even at room temperature, until they can be frozen.

MATERIALS

NAME	CATALOG #	VENDOR
Peristaltic field pump (yellow box, Perkin-Elmer)		
Tygon tubing of size that fits pump head		
4x 1 L Nalgene bottles for water collection (1 extra to collect filtrate)		
3 µm filters		Emd Millipore
0.22 µm filters		Emd Millipore
tweezers		
70% ethanol		Fisher Scientific
kimwipes		
Liquid nitrogen in dewar		
2 ml cryovials		Fisher Scientific
1 ml pipette		
1 ml pipette tips		
10% bleach, 0.5 L/filtered sample		
MilliQ water, 2-3 L/filtered sample		

Collect water

1

Pre-filter the water with a 20 µm mesh net and fill a clean carboy with several liters of this <20 µm-filtered water. If you are filtering one liter in triplicate (3 L total), collect at least 5 liters (~30% more than needed for filtration) at this stage to ensure enough water for system flushing.

Assembly of pump and filtration units

2

The goal of this step is to prepare unit such that direction of flow in tubing will be from water source, through pump head, through first filtration unit, through second filteration unit, and then into collection receptacle.

1. Attach tubing to both ends of a 47 mm filter unit.
2. Run tubing through pump head.
3. Dissable unit and leave the downstream part detached (the half on which the filter will be placed).
4. Pump approx. 200 ml of the <20 µm-filtered water through the system to clean the system.
5. Place a 3 µm filter into the filter unit and close it. Ensure that all O-rings are in place or the filter unit will leak when under pressure...losing your precious microbes.
6. Now the second filtration unit will be attached to the first. Disassemble the second filtration unit, such that only the top half is connected to the system. Again, pump approx. 200 ml of the <20 µm-filtered water through the system to clean it.
7. Replace the 3 µm filter (save this first filter for later itn he day when you need to clean the set-up again).
8. Place a 0.22 µm filter into the second filter unit and close it.
9. Now the system is set-up as described above: water source, pump head, first filtration unit, and second filteration unit. All the tubing has been flushed with station water of the approriate size fraction.

Note: If you were interested in collecting viruses, you would collect the <0.22 µm water that flowed out of the system (rather than discarding it).

Pumping the water (perform this in triplicate for each sample)

3

1. Pump water through the system at a moderate rate. You want to avoid high pressure in the system, so that things don't push through that would not naturally push the filters.
2. Collect the flow through in a waste collection bottle with graduated markings (or a hand-drawn line indicating 1 L volume). We measure the water collected in this vessel to know how much water has passed through the filters.
3. Pump 1 liter of water through. Stop the pump. Remove the 3 µm and 0.22 µm filters using sterile tweezers. Place the filters inside a cryovial. It is helpful to use two pairs of tweezers to roll or fold the filter. Shoving it into the cryovial is also acceptable if neccessary (e.g., running from a storm), but wrinking it will annoy you later when you do the DNA extractions.
4. Drop cryovial with filter into liquid nitrogen (LN2).
5. If the collection rate slows markedly or the pump appears to be building up high pressure in the system before 1 L has been fitlered, the filters (most likely the 3 µm) may be clogged. If this happens, stop the pump and replace the filters. You may use more than one filter if needed to get through 1 full liter of water. Filters can be conbined in the downstream processing. Be sure to note this process. It is useful to first start by replacing the 3 µm, as that is typically what limits the rate.

Cleaning the pump

4 Optimally, we clean the tubing and filtration units between all stations. If this is not possible logistically, flush with station water at each new station (described above).

1. Pump a small amount(ca. 0.50 L) of 10% bleach through the tubing and assembled filtration units (with not filters in place). Collect the outgoing bleach, as it can be reused to clean the pump in the future.
2. After the 10% bleach has been fully pumped through, pump another ca. 1-2 L of Deionized water (or MilliQ) through the pump to flush any bleach that remains. You know you are done flushing with water when the system no longer smells of bleach.