SEP 21, 2023

DOI:
dx.doi.org/10.17504/protocols.io.ewov1qdopgr2/v1

Protocol Citation: Natascha Varona, Cynthia Silveira 2023. Seawater virome concentration with Vivaflow . **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1qdopgr2/v1

MANUSCRIPT CITATION: Productive viral infections in oligotrophic marine waters. Natascha Varona, Poppy Hesketh-Best, Alexandra Stiffler, Sofia Garcia, Yun Scholten, Andreas Haas, Mark Little, Felipe Hernandes Coutinho, Mark Vermeij, Antoni Luque, Cynthia Silveira. ResearchSquare https://doi.org/10.21203/rs.3.rs-3040647/v1.

Seawater virome concentration with Vivaflow

Natascha Varona[1], Cynthia Silveira[1]

[1]University of Miami Department of Biology

amz

amz

DISCLAIMER

You can reuse the Vivaflow cassette multiple times for different samples by cleaning between samples. However, you should always check the filtrate for VLPs using fluorescence microscopy to ensure the cartridge is still intact (no viruses going through the filter membrane).

ABSTRACT

Concentration of viral particles from seawater samples using a Vivaflow.

IMAGE ATTRIBUTION

Cynthia Silveira

GUIDELINES

This protocol is written for one sample only, however, with additional pumpheads and vivaflows you can run up to 4 viromes at the same time

Protocol status: Working
We use this protocol and it's working

Created: Sep 12, 2023

Last Modified: Sep 21, 2023

PROTOCOL integer ID: 87693

Keywords: seawater, bacteriophage, virome, concentration

MATERIALS

- Masterflex® L/S® Analog Variable-Speed Console Drive, 7 to 200 rpm; 115 VAC (MFL 07555-00) (Flow rate 0.42 to 2900 mL/min with L/S tubing---flow rate depends on drive rpm, pump head, and tubing size)
- Masterflex® L/S® Easy-Load® Head for Precision Tubing, 4-Roller, PARA Housing, SS Rotor (MFLX 07514-10)
- Sartorius Vivaflow™ 200 Crossflow Cassettes 100 KDa (VF20P4)
- MilliporeSigma™ Sterivex™ Sterile Pressure-Driven Devices 0.22 µm (SVGP01050) OR MilliporeSigma™ Sterivex™ ***OR*** Sterivex™ Sterile Pressure-Driven Devices 0.45 µm (SVHV010RS)
- Male Luer Lock 1/8" 3.2mm PP Hose Barb Adapter (B07W5S4728)
- 50 mL Corning™ Centrifuge Tubes with CentriStar™ Cap (REF# 430829)
- Bleach (NaOCl)
- Sodium Hydroxide (White Pellets), Fisher BioReagents™ (CAS# 1310-73-2)
- Chloroform
- DI Water
- Ethanol

Virome collection

1 Collect 500mL - 2L of seawater (depending on microbial density) in a sterile container.

2 Wipe down outside of all masterflex tubing with 70% ethanol.

2.1 Insert one end of the tubing into the sample and feed it through the Masterflex pump.

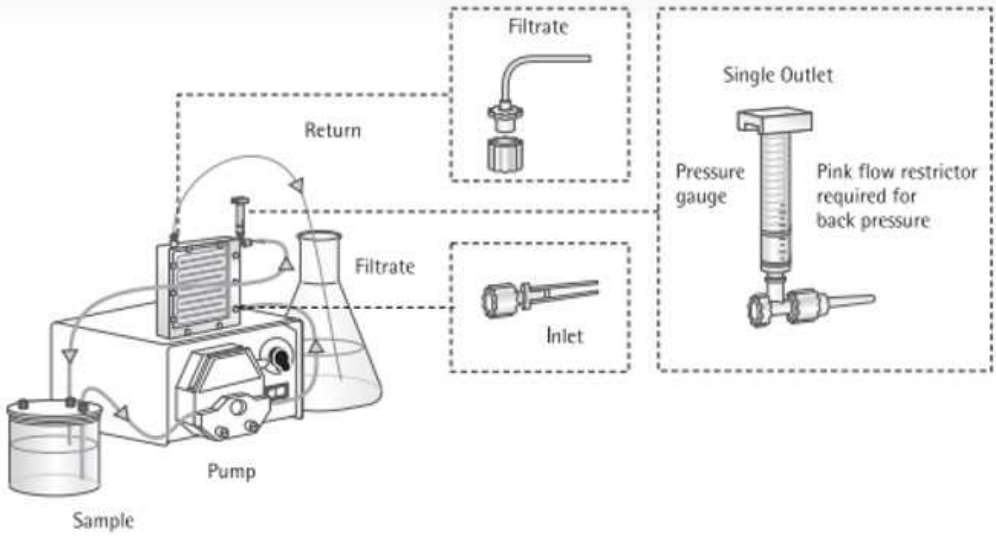
2.2 Attach 0.22 µm (or 0.45 µm depending on the viral size fraction you want to concentrate) Sterivex to the other end of the tube using a luer lock adapter.

3 Turn the Masterflex on a low setting (between 2-3).

4 Keep the flowthrough for virome concentration.

Virome concentration

5



Vivaflow cassete setup image from Sartorius manual (Sartorius Stedim Lab Publication No. SLU6097-e210810)

Set up Vivaflow per instruction according to the image above.

5.1 Before running the sample, rinse Vivaflow with 500 mL of DI water by inserting a sample tube into a container with DI water and placing the filtrate and return tubes in a waste container.

5.2 Pump water at a rate of 200-400 mL/min.

5.3 After 400 mL have passed into the waste, stop the pump, and check for leakage at connections.

5.4 Tighten if needed. Drain system. It is now ready for use.

6 Replace the DI water with the sample and collect the filtrate in a sterile container (this filtrate will be used for backflush later).

6.1 Move the return tubing to a waste container to rinse out residual DI-water before recirculating.

6.2 Pump liquid at a rate of 200-400 mL/min for a few seconds to ensure the DI water is removed and stop the pump.

6.3 Move the return tube to the sample container (note that the feed tube and return tube are now in the sample, this is necessary to recirculate and concentrate the sample).

7 Pump liquid at a rate of 200-400 mL/min. (If using a pressure indicator it should be approximately 2.5 bar).

8 When the sample container is nearly empty, remove the feed line, and collect the sample in a 50 mL conical tube. This usually contains about 30 mL of the sample.

9 For a more complete recovery, preform a backflush.

9.1 Remove and screw off filtrate line.

9.2 Take about 20 mL aliquot of the filtrate, and insert the feed tube into the filtrate.

9.3 Run the Vivaflow in reverse until the sample reaches about 50 mL .

10 Add chloroform to your virome sample for a final concentration of [M] 0.1 % (v/v) .
Example: 50 µL Chloroform for 50 mL of sample.

11 Samples can be stored at 4 °C for later extraction.

Vivaflow clean up

12 Reattach the filtrate line and flush the system with 200 mL of DI water with the filtrate going to the waste.

13 Place the feed, return and filtrate lines into a container with a cleaning solution of 250 mL of [M] 0.5 millimolar (mM) NaOCl and [M] 0.5 Molarity (m) NaOH. (250 mL DI-water + 133 µL NaOCl + 5 g NaOH)

14 Recirculate at 50-100 mL/min for 30 minutes.

15 Drain the system and recirculate 250 mL of DI-water through the system for 5-10 minutes.

16 Drain and rinse system with a further 500 mL of DI-water.

17 The system is now ready for the next sample, or for long term storage, fill the module with 10% ethanol and (in DI-water) store at 4 °C .