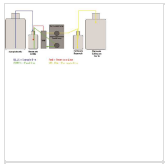

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Sample collection and DNA purification from virus-enriched tangential flow filtration retentates

Paul Den Uyl[1], Ed Delong[1]

[1]University of Hawaii at Manoa

Paul Den Uyl: Currently affiliation: Cooperative Institute for Great Lakes Research (CIGLR) - University of Michigan

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DeLong Research Group SCOPE

Paul Den Uyl

ABSTRACT

To collect and concentrate viral particles, seawater samples were collected and prefiltered to remove bacterioplankton cells. The resulting virus-enriched filtrate was concentrated via tangential flow filtration (TFF) before DNA extraction. For each sample, 90 - 110 L of seawater was collected using a Niskin bottle rosette attached to a conductivity-temperature-depth (CTD) package. The seawater was pre-filtered by peristatic pumping through either a 0.22 um filter (Sterivex GV) for the 25 m (higher biomass) sample, or a 0.1 uM Supor cartridge filter (Acropak 500, Pall, USA) for the 117 m and 250 m (lower biomass) samples. The resulting virus-enriched filtrate was concentrated by tangential flow filtration (TFF) over a 30 kDa filter (Biomax 30 kDa membrane, catalogue #: P3B030D01, Millipore). Subsequently, the retentate was reduced to a volume of ~200 mL, the tangential flow filter was backflushed with 100 mL of permeate to release virus particles trapped in the filter, and the concentrated viruses were recovered in a final retentate volume of ~300 mL. The virus-containing retentates were stored at 4°C until final processing and DNA extraction.

ATTACHMENTS

dmnvdbpn.docx

PROTOCOL CITATION

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DNA purification, Tangential flow filtration (TFF)

LICENSE

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GUIDELINES

Concentrating:

- When the external sample has been emptied and only the 2 L retentate volume remains, it may be preferable to reduce the filtration speed. I typically reduce the feed pump to 280 RPM and adjust the permeate pump accordingly to maintain 3 psi backpressure.
- Make sure to record the pump settings, as you will need to return to these after each backflushing procedure.

Filtration Completion:

- As the concentrate in the retentate bottle draws down, it can help to stabilize the bottle in a slanted position to allow filtration.

SAFETY WARNINGS

Concentrating:

Warning!: From now on, when stopping and starting the filtration, the feed AND permeate pumps need to be stopped/started simultaneously.

Beware!: When the external sample bottle is empty (and thus the retentate bottle is no longer pulling sample from it), the permeate back pressure will increase. Make sure to adjust accordingly.

Filtration Completion:

Warning: Always make sure the feed line is immersed in sample. DO NOT allow air to inter the feed line, which will lead increased feed pressure, and will expose the filter to air, which may be harmful to viruses still attached to the filter.

BEFORE STARTING

Concentrating:

- Make sure permeate tubing is not clamped with permeate pump (permeate line needs to flow freely).
- Make sure all tubing is attached in a way to prevent bending and crimping (which could lead to high pressure buildup and tubing/filter breakage).

Pre-Cleaning 30m

1 Recirculate 0.1N Sodium Hydroxide at **330 rpm** feed speed for **00:30:00** . 30m

2 Flush with **30 L** MilliQ.

3 Drain the system (see sample recovery).

Concentrating

4 Fill retentate reservoir.

5 Attach retentate reservoir.

5.1 Connect feed line.

5.2 Connect retentate line.

5.3 Place sample tubing into external sample bottle.

> Make sure this tubing extends to the bottom of the bottle. Attaching a serological pipet to the end of the tubing can help with this.

6 Attach permeate collection bottle.

6.1 Connect permeate line.

6.2 Place permate over-flow line into external collection/waste bottle.

7 Start the feed pump at around **50 rpm** .

> Make sure air is only coming out of the small retentate line inside the retentate bottle. This will ensure the tubing is connected properly, and that the feed line extends to the bottom of the bottle.

8 Gradually increase the feed pump to approximately **330 rpm** . Gradually increasing the speed will help prevent high pressures while the tubing and filters are clearing themselves of air. Once at **330 rpm** , additional air can be cleared by gently and quickly pinching the permeate and retentate lines (make sure not to exceed pressures of 5 psi on either the permeate/retentate gauges

9 Lower feed pump to **100 rpm** .

10 Clamp permeate line into permeate pump.

11 Slowly increase feed pump until permeate backpressure guage reads 2-3 psi.

Now, as you continue to slowly increase the feed pump, also simultaneously increase the permeate pump (starting at **10 ml/min**), so that the permeate backpressure remains at 2-3 psi while increasing the feed pump. The goal is to increase the feed pump back up to 330 psi, while maintaining 2-3 psi of permeate backpressure. This back pressure will help prevent clogging while filtering. Make final adjustments so that feed pump is at approximately **330 rpm** , while permeate backpressure is at 3 psi (approximately **180 ml/min** - **200 ml/min** on the permeate pump). While filtering, the permeate backpressure may change. Adjust the permeate pump accordingly to maintain 3 psi.

Filtration Completion

12 Continue filtering until you feel you can no longer safely filter without accidentally introducing air bubbles.

Beware: Sometimes the rententate line in the retentate bottle can force air bubbles into the path of the feed line. **Be prepared to stop the filtration ahead of time. BOTH pumps will need to be turned off simultaneously.**

13 Once filtration has stopped, begin back flushing.

Back flushing

14

OPTIONAL: Disconnect the permeate overflow tubing from the permeate reservoir (this is the tubing that carries permeate overflow from the permeate reservoir to either a collection bottle or a waste container). Depending on the setup, disconnecting this tubing can prevent introducing contaminants into the permeate reservoir during the back flushing procedure. Make sure to also partially unscrew the permeate reservoir cap to allow air displacement, preventing permeate from flowing onto the benchtop.

Simultaneously turn off both feed and permeate pumps.

15 Reduce feed pump to **100 rpm** to allow mild circulation of the sample.

16 Start feed pump.

17 Reduce the permeate pump to the lowest setting (**10 ml/min**). Switch the direction of the permeate pump, so that permeate is flowing back into the TFF filter.

18 Start the pump.

19 Increase the speed of the permeate pump, but **do not exceed 5 psi backpressure**.

20 Continue backflushing for a few hundred milliliters, which will be seen as an increase in the retentate volume.

21 Turn off both pumps.

22 Return the pumps to their previous settings for filtration (**also make sure to switch the permeate pump direction back to normal**).

23 Turn both pumps on simultaneously, and filter sample to further concentrate.

24 Stop the filtration (both pumps simultaneously) before completely filtered, and repeat the backflushing protocol.

25 Perform the backflusing protocol for a total of 3 times, and after the third time, recover the sample.

On the 3rd backflush, you can backflush with less fluid (e.g. **50 mL** - **100 mL**) to achieve a smaller recovery volume.

Sample Recovery (recovery volume typically between 250-400 mL)

26 Turn both pumps OFF.

27 Disconnect the permeate tubing at the joint between the downstream end of the pump head and the permeate reservoir.

28 **Drain the System:**

Release and remove the feed tubing from the feed pump head, to allow fluid to freely flow.

29 Switch the direction of the permeate pump so that fluid is flowing back into the TFF filter. Set the speed to **50 ml/min** .

30 Turn ON permeate pump.

31 Monitor the permeate backpressure gauge. When the needle approaches 1 psi backpressure, turn the pump OFF.

32 Release the permeate tube from the permeate pump head, so that fluid/air can flow freely.

33 Reconnect the feed tubing to the feed pump head. If the pump can only filter in one direction, make sure to orient the tubing so that it is pumping in the reverse direction (out of the TFF filter)—otherwise, simply switch the direction of the pump.

34 Set the feed pump to **50 rpm** .

35 Turn on the feed pump, and tilt the TFF filter as necessary to assist pumping out all the remaining fluid from the filter.

36 As soon as all the sample has been pumped out, turn the feed pump OFF.

37 Detach the retentate bottle and store the concentrate.

38 Proceed with the post-cleaning.

Post-Cleaning 30m

39 Flush filter with MilliQ (up to **330 rpm** feed pump speed).

40 Recirculate 0.1N Sodium Hydroxide at **330 rpm** feed speed for **00:30:00** . 30m

41 Flush filter with MilliQ (up to **330 rpm** feed pump speed).

42 Drain system (see sample recovery).

43 Store filters in 0.05N Sodium Hydroxide solution.