Extracting nucleic acids from viruses on a filter

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Abstract

This protocol is a minor modification of that reported by Culley and Steward (2007). As the starting point for this protocol, we assume that viruses have been collected on an aluminum oxide 0.02-µm syringe-tip filter (Anotop, Whatman), but other filters capable of capturing viruses may be substituted.

This is a protocol from:

Steward, G. F. and A. I. Culley. 2010. Chapter 16: Extraction and purification of nucleic acids from viruses. Manual of Aquatic Viral Ecology. Waco, TX:American Society of Limnology and Oceanography. doi:10.4319/mave.2010.978-0-9845591-0-7

Please see the published manuscript for additional information.

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Guidelines

Materials and equipment:
• Total nucleic acid extraction kit (MasterPure, Epicenter)
• Optional: polyacryl carrier (Molecular Research Center)
• Syringes (sterile, disposable, with Luer-Lok tips)
• Luer-Lok female–female adapter fittings
• Hybridization oven with rotisserie

Assessment: One caveat in extracting from aluminum oxide membrane filters is that they can irreversibly bind DNA under certain conditions (Dames et al. 2006). In particular, guanidinium-containing extraction buffers facilitate the binding of DNA to aluminum oxide (Gerdes et al. 2001) and are likely to result in low yields from the filters. For this reason, we do not recommend extracting from Anotop filters using the lysis buffers from any of the popular silica column–based kits. The Gentra PureGene Kit (Qiagen) is similar to the MasterPure kit and may work as well. These latter kits are based on a published protocol (Miller et al. 1988) that could be adapted for extraction from a filter. A version of that protocol designed for simultaneous DNA and RNA extraction (Yu and Mohn 1999) could also be used by employing heat (65°C, 15 min) instead of bead beating to facilitate lysis. It is worth noting that SDS and phosphate buffer appear to inhibit the binding of nucleic acid to aluminum oxide (Gerdes et al. 2001; Dames et al. 2006) and should aid in recovery. If one wishes to recover RNA using a self-made recipe, then we would recommend including an RNAse inhibitor (e.g., RNASecure, Ambion) in the extraction buffer.

We have recovered both viral RNA and DNA suitable for PCR amplification from aluminum oxide filters using essentially the procedure as described above (Culley and Steward 2007; Culley et al.

2008). A modification added here is the introduction and removal of the extraction buffer in a direction counter to that of filtration during sample collection (i.e., backflushing). According to the Anotop specifications, these filters are not designed to be backflushed, or to be operated at temperatures above 40°C. We have found that the filter can rupture if too much pressure is applied during backflushing, especially after incubating at 65°C. Injecting the extraction buffer slowly and removing the extract by gentle aspiration seem to avoid this problem. Although we have not tested the protocol described here on other filter types, the procedure should work as well for any direct flow filter capsule capable of retaining viruses.

Protocol

Step 1.
Add 1 mL T + C lysis buffer containing 100 µg/ml proteinase K to a low-volume (1–3 cc) syringe that has been fitted with a female–female Luer-Lok adapter (the injection syringe).

NOTES
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It is convenient to use a larger syringe (10–20 cc) as an extraction buffer reservoir. The injection syringe can be easily filled with the proper volume by connecting it tip to tip with the reservoir syringe via the adapter. The reservoir can be used to fill multiple injection syringes if more than one sample is to be extracted.

Step 2.
Ensure that there is minimal air in the injection syringe-adapter assembly, then connect it to the outlet of the filter.

Step 3.
Connect a second low-volume syringe to the filter inlet (the aspiration syringe).

Step 4.
Hold the filter-syringe assembly vertically with the injector syringe pushing upward from below.

Step 5.
Hold the filter securely to the injection syringe and gently, but firmly, push extraction buffer into the filter housing until liquid just begins to appear in the aspiration syringe.

Step 6.
Incubate the assembly (filter with two syringes attached) for 15 min at 65°C in a hybridization oven.

DURATION
00:15:00

NOTES
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It is helpful to connect the syringe-filter assembly to a rotisserie so that the entire filter surface is wetted in the event that bubbles are present in the housing. The syringes on either side of the filter can be secured to the clips of the rotisserie with elastic bands.

Step 7.
Allow the syringe-filter assembly to cool briefly.

Step 8.
Remove the extract by holding the syringe assembly vertically with the aspiration syringe underneath (and the filter upside down) and gently pulling on the plunger to pull the extract into the aspiration

syringe.

Step 9.
Detach the aspiration syringe.

Step 10.
Transfer the extract to a microcentrifuge tube.

Step 11.
Chill on ice for 2–3 minutes.

DURATION
00:03:00

Step 12.
Add one-half volume of MPC protein precipitation reagent (supplied in the kit) and vortex for 10 s.

DURATION
00:00:10

Step 13.
Pellet the debris by centrifugation at 10,000g for 10 min.

DURATION
00:10:00

Step 14.
Transfer the supernatant (containing the nucleic acids) to a sterile microcentrifuge tube; be very careful to avoid the pellet (containing the SDS-protein complex).

NOTES
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All or some of the sample may be archived at this point by freezing at –80°C.

Step 15.
Transfer up to 800 µl of the sample to a fresh tube; add 1 µl polyacryl carrier, and vortex briefly.

Step 16.
Add 1 µl polyacryl carrier, and vortex briefly.

NOTES
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Carrier is optional, but can improve yields when working with nanogram to subnanogram quantities of nucleic acid.

Step 17.
Add an equal volume of 100% isopropanol.

Step 18.
Mix by inverting the tube several times.

Step 19.
Centrifuge the sample at ≥10,000g for 15–45 min.

DURATION
00:45:00

NOTES
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Longer centrifugation times can improve the yields for small amounts of nucleic acids, especially in the absence of carrier.

Step 20.

Decant or aspirate the supernatant.

NOTES

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Use caution, the pellet can dislodge easily and be lost.

Step 21.

Wash the pellet twice, each time adding 70% ethanol, centrifuging for 1 min, and decanting (or aspirating) the ethanol.

DURATION

00:01:00

Step 22.

Air-dry the pellet, then dissolve in 10 µL of 0.02- filtered, sterile 0.5× TE buffer heated to 50°C.

Step 23.

If required, DNA or RNA can be selectively removed from the total nucleic acid precipitate by enzymatic digestion with DNase or RNase.