Concentration of viruses and preparation of FLVs for tracer assays

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Abstract

The steps describe how to prepare each virus concentrate. There are multiple options for many of the steps; in the case where there is more than one option they are noted in annotations.

Citation: André M. Comeau and Rachel T. Noble Concentration of viruses and preparation of FLVs for tracer assays. **protocols.io**
dx.doi.org/10.17504/protocols.io.dzv765
Published: 08 Feb 2016

Protocol

Step 1.
Collect up to 20 L using either four 5-L Niskin bottles (or other permutation) or by a triple acid- and sample-rinsed bucket into an acid-rinsed 20-L low-density polyethylene carboy.

Step 2.
The sample should be filtered at 5 kPa through a 142-mm–diameter, 0.22-µm–pore-size Durapore filter to remove bacteria and protists. The virus-sized fraction (material between 0.22 µm and 30 kDa) is concentrated to 150 mL using a spiral cartridge concentration system (Suttle et al. 1991). Further concentration should be conducted using Centriprep-30 centrifugal concentration units (Millipore) to a final volume of 5 mL.

Step 3.
The virus-sized fraction (material between 0.22 µm and 30 kDa) is concentrated to 150 mL using a spiral cartridge concentration system (Suttle et al. 1991).

Step 4.
Further concentration should be conducted using Centriprep-30 centrifugal concentration units (Millipore) to a final volume of 5 mL.

NOTES
Amy Chan 21 Oct 2015
Alternative: The sample can be directly concentrated using a tangential flow filtration spiral cartridge concentration system with either a 30- or 100-kDa cutoff (both have shown excellent recovery rates for marine viruses in past experimental procedures; e.g., Suttle et al. [1991], Breitbart et al. [2002]; GE Healthcare, Inc.) and then filtered using a 0.2-µm Sterivex-type filter (Millipore) to remove unwanted protists and prokaryotes. If desired, further concentration should be conducted using Centriprep-30 or similar centrifugal concentration units (Millipore) to a final volume of ~5 mL.

Step 5.
To each of the virus concentrates, SYBR Green I should be added at a final concentration of 2.5% vol/vol and incubated in the dark for at least 8 h at 4°C.

DURATION

08:00:00

NOTES

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Alternative: To each of the virus concentrates, SYBR Gold (Molecular Probes, Inc.) should be added at a final concentration of 2.5% vol/vol and incubated in the dark for at least 4 h at 4°C.

Step 6.

After the staining period, the unbound stain can be rinsed away by adding an equal volume of 0.02-µm filtered seawater (prepared by filtering fresh seawater from the same location through an acid-rinsed, autoclaved Nalgene filtration unit housing a 47-mm, 0.02-µm Anodisc filter) to the concentrate and centrifuging it in Centriprep-30 ultraconcentration units at 3,000g for 15 min.

DURATION

00:15:00

NOTES

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After labeling, the FLVs can be diluted into 1 L of 100-kDa filtrate from the sample site and reconcentrated using tangential flow filtration (TFF). This process is repeated three times to ensure removal of all stain.

Step 7.

This rinse is done three times. Each time, the labeled virus particles are resuspended in a total of 5 mL of 0.02-µm filtered seawater while reusing the same Centriprep-30 unit.

Step 8.

The final concentrates should be resuspended in a total of 5 mL of 0.02-µm filtered seawater.

Step 9.

To determine the concentration of viruses in the concentrate, 10 µL concentrate is diluted to a final volume of 2 mL with 0.02-µm filtered seawater, filtered through a 0.02-µm Anodisc, and counted by epifluorescence microscopy under blue excitation (Noble and Fuhrman 1998, Patel et al. 2007).

Warnings

All virus concentration steps should be performed either on ice or in a centrifuge held at <10°C, so as to minimize degradation of virus particles during the concentration steps.