Release of nucleic acids with heat, chelator, and detergent

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Abstract

If one has a purified stock of viruses obtained, for example, by banding in a buoyant density gradient, or even a relatively pure viral concentrate obtained by size fractionation, it is possible to release the DNA in a high molecular weight form suitable for some applications (e.g., pulsed-field gel electrophoresis [PFGE] for sizing or probing, or nucleic acid quantification by fluorescence) relatively simply. This method involves exchanging the buffer in which the viruses are suspended with one containing EDTA and SDS, followed by heating. The method is similar to that described previously (Steward 2001), but with the optional addition of detergent to facilitate disintegration of the viral capsid.

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:
• Centrifugal ultrafiltration device (30,000 molecular weight cutoff, e.g., Millipore Ultracel YM-30, cat no. 42410)
• TE buffer (10 mM Tris, 1 mM EDTA, pH 8) or TEGED buffer (10 mM Tris, 1 mM EDTA, 1 mM EGTA)
• Optional: 6× SDS-EDTA loading buffer (1% SDS, 60 mM EDTA, 0.03% bromophenol blue, 0.03% xylene cyanol, 60% glycerol)

Assessment: This method is similar in strategy to the simple protocol for assaying the DNA content of bacteriophage λ stocks described by Sambrook and Russell (2001, p. 2.45–2.46). SDS is added to facilitate the release of DNA from the capsids and to minimize DNA–protein interactions during electrophoresis. The protocol described here, but without SDS, has been used in a number of studies to investigate genome size distributions in viral communities (e.g., Steward and Azam 2000; Steward et al. 2000; Riemann and Middelboe 2002; Jiang et al. 2003; Jiang et al. 2004; Filippini and Middelboe 2007). One can carry out essentially the same procedure by pelleting viruses in an ultracentrifuge rather than using centrifugal ultrafiltration. If one assumes a minimum sedimentation coefficient for

viruses (e.g., 80S), the time needed to pellet the virus can be determined from the k-factor of the rotor being used (Lawrence and Steward 2010, this volume). The centrifugation time required in a swinging bucket rotor, which will produce the most compact pellet, can vary from 30 min (6 × 4 mL sample in a Beckman SW 61 rotor) to 3 h (6 × 38.5 mL sample in a Beckman SW 28 rotor). In this case, the supernatant is drained completely and carefully from the pellet. Residual liquid on the walls can be removed using the tip of a twisted lint-free absorbent wipe (e.g., KimWipe, Kimberly Clark) or sterile cotton swab. TE is added to the pellet, and the tube is sealed with plastic wrap to minimize evaporation and heated to 60°C with occasional gentle agitation for 10 to 15 min.
If the samples are handled carefully (to minimize shearing, pipette slowly, use wide-bore pipette tips, and avoid vortex mixing), the DNA should be of high molecular weight suitable for sizing by PFGE (Steward et al. 2000; Steward 2001). DNA prepared by the centrifugal ultrafiltration method has resulted in no noticeable shearing of bands up to several hundred thousand base pairs. The alternative ultracentrifugation method has sometimes resulted in slight smearing of bands, indicating some shearing. Even in the former case, some small amount of shearing of the higher molecular weight nucleic acids might be expected from handling them in solution.

The sensitivity of viral DNA to shearing will depend on its size, composition, and conformation. Most viral genomes are small enough that they can be extracted in solution without appreciable shearing if handled gently. Bacteriophage genomes up to 100 kb produced crisp bands with no evidence of shearing when extracted using a protocol similar to that described here (Steward et al. 2000). A large algal virus genome (320 kb) was found to be fragmented when subjected to standard phenol-chloroform extraction procedures (Lanka et al. 1993). When treated gently, however, Chlorella viruses ranging in size up to 380 kb tolerated limited pipetting in liquid and produced crisp single bands by PFGE (Rohozinski et al. 1989). If shearing must be minimized to the greatest possible extent, one should consider embedding the viruses before extraction as described in protocol C. Even embedded viral DNA, however, may be susceptible to some degree of fragmentation (Lanka et al. 1993), perhaps due to premature disintegration of viruses during the embedding process (see Extracting DNA from viruses embedded in agarose, “Assessment”).

It may be possible to obtain intact viral RNA using the same general approach as described here by simply including an RNase inhibitor in the TE buffer, but we have not explicitly tested this. If more purified nucleic acids are required, the simple release step described here can be followed by purification by organic extraction (Cottrell and Suttle 1991; Wilson et al. 1993; Sambrook and Russell 2001) or purification with any of a number of commercially available nucleic acid purification kits. If one wishes to purify both RNA and DNA free from proteins, the appropriate extraction buffer from a suitable kit (QIAamp MinElute or UltraSens virus kits, Qiagen; MasterPure total nucleic acid extraction kit, Epicenter) can be substituted for the TE after concentrating the viruses by centrifugal ultrafiltration or ultracentrifugation.

Protocol

Step 1.
Concentrate the viruses by centrifuging in the centrifugal ultrafiltration device at 1000g until only a small volume (ca. 10 µL) remains.

Step 2.
Add 100 µL TE (or TEGED).

Step 3.
Concentrate the sample again to ca. 10 µL.

Step 4.
Repeat steps 2 and 3 once more.

Step 5.
Recover the final concentrate.

Step 6.
Rinse the membrane in the device by adding a small volume of TE or TEGED (5–10 µL).

Step 7.
Recover the rinse and pool with the concentrate.

Step 8.
Optional: If conducting electrophoresis on the sample, add SDS-EDTA loading dye to a final concentration of 1×.

Step 9.
Heat the recovered sample (with or without loading buffer) to 60°C for 10 min to release the nucleic acid.

DURATION
00:10:00