Extracting DNA from viruses embedded in agarose

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Abstract

If one wishes to have a stock of high molecular weight viral DNA that can be stored for long periods of time with minimal shearing or degradation, the viruses can be embedded in agarose before extraction. Extraction of embedded cells is the standard procedure for sizing the genomes of bacteria and yeast by PFGE (Sambrook and Russell 2001), and a similar protocol can be used for embedded viruses (Rohozinski et al. 1989; Lanka et al. 1993; Wommack et al. 1999; Sandaa et al. 2010, this volume).

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:
• Agarose (molecular biology grade, low gelling temperature; InCert® Agarose, Lonza)
• SE buffer (75 mM NaCl, 25 mM EDTA, pH 8)
• TE buffer (10 mM Tris, 1 mM EDTA, pH 8)
• Lysis buffer (TE pH 8; 1% SDS)
• Proteinase K
• Optional: casting wells
• Optional: phenylmethanesulfonylfluoride (PMSF)

Assessment: One of the main purposes of extracting nucleic acids from embedded viruses or cells is to avoid shearing of high molecular weight DNA. The use of a low-melting/gellingpoint agarose in SE buffer is recommended to minimize premature disruption of viral capsids by thermal and osmotic shock. DNA released before the casting of the gel plugs has the potential to be sheared during mixing and pipetting.

Use of a low-gelling-temperature agarose also allows one to recover nucleic acids from the agarose plug using an agarase enzyme (β-agarase, Lonza or New England BioLabs). DNA can be recovered from other types of agarose using silica-based gel extraction methods, by electroelution, or by organic extraction (Sambrook and Russell 2001), so it is possible to use molecular biology–grade agaroses with higher gelling temperatures. In this case, however, the agarose must be maintained at a higher

temperature before mixing with the sample. Gelling temperatures for other pulsed-field grade agaroses are around 36–42°C, so maintaining at 50–60°C before mixing with sample should be adequate. One should bear in mind that some viruses may disintegrate at this temperature. For some applications (e.g., shotgun cloning), some fragmentation of the DNA is not an issue. If sheared DNA is not an issue for one's application, then one might consider a less cumbersome extraction protocol that results in DNA in solution.

The release of viral DNA in plugs is commonly used for sizing of large viral genomes either intact (McCluskey et al. 1992) or after digestion with a restriction endonuclease (Rohozinski et al. 1989; Lanka et al. 1993). Variations of the above method have been used for analyses of genome size distributions in viral assemblages using PFGE (e.g., Wommack et al. 1999; Larsen et al. 2001; Øvreås et al. 2003; Sandaa and Larsen 2006; Parada et al. 2008; Sandaa et al. 2010, this volume). Viral community DNA has also been recovered from agarose plugs for subsequent sequence analysis by shotgun cloning (Bench et al. 2007).

The disadvantages of the method for community genome size analyses are that the preparation time is longer and the resolution of bands will typically be lower when performing PFGE from viral DNA in plugs (depending on the thickness of the plug) compared to that achievable with DNA in solution prepared by protocol B (Steward 2001). The considerable advantage of the method is that the DNA appears to be more stable at 4° when embedded in agarose (many months) than when dissolved in buffer (up to a few days), so embedding is recommended for storing extracted viral DNA that will not be used right away. One of the authors (G. F. Steward) has observed that a high molecular weight PFGE standard embedded in agarose that was accidentally frozen on dry ice resulted in a banding pattern that was indistinguishable from that of parallel standard that had never been frozen. Freezing the plugs at –80°C may therefore be useful for long-term archiving of samples. Freezing is not recommended for samples that will be accessed more than once or twice, since repeated freezethaw cycles are likely to degrade the DNA.

Protocol

Step 1.
Add agarose to SE buffer for a final concentration of 1.5% (wt/vol).

Step 2.
Melt the agarose in a microwave oven, then cool and maintain at 37°C in a water bath.

Step 3.
Warm the viral concentrate to 37°C in the water bath, then immediately mix with an equal volume of molten agarose and quickly transfer the mixture to casting molds.

NOTES

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Casting in rectangular plug molds is preferred if the embedded DNA is to be analyzed by electrophoresis, since this results in plugs of uniform height and thickness that fit the wells without extensive trimming. Electrophoresis using plugs that are not uniform will result in bands with uneven intensity. Special rectangular plug molds for PFGE are available from BioRad. Alternatively, one can draw the molten mixture into a 1-cc syringe that has had the tip cut off, or simply pipette the mixture as drops onto a sheet of plastic wrap or Parafilm M®.

Step 4.
Once the agarose has gelled, transfer the plug (or noodle from the syringe, or buttons from the

parafilm), into a tube containing 5 volumes of lysis buffer amended with proteinase K (1 mg mL^{-1} final concentration).

Step 5.
Incubate at room temperature overnight.

DURATION
18:00:00

Step 6.
Decant the lysis buffer, being careful not to lose the plugs.

Step 7.
Optional: Rinse the plugs twice, each time adding 25 volumes of fresh TE containing 1 mM PMSF, incubating for 1 h with gentle agitation, then decanting the rinse fluid.

DURATION
01:00:00

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This step will inactivate the proteinase K, which is recommended if the DNA in the plug is to be further manipulated with enzymes (e.g., digestion with restriction endonucleases).

Step 8.
Rinse the plugs, add 50 volumes of fresh TE with no PMSF, incubate for 30 min. with gentle agitation, then decant the rinse fluid.

DURATION
00:30:00

Step 9.
Once more, rinse the plugs, add 50 volumes of fresh TE with no PMSF, incubate for 30 min. with gentle agitation, then decant the rinse fluid.

DURATION
00:30:00

Step 10.
Store the plugs at 4°C submerged in TE.