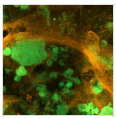

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Extraction and purification of viruses from stream biofilms V.3

PeerJ

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1 Works for me dx.doi.org/10.17504/protocols.io.32qgqdw

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ABSTRACT

This series of sequential protocols can be used to extract and purify viruses from stream biofilm samples for metagenomic analysis. We compared each critical step of the protocol to alternatives and evaluated their performance using epifluorescence counting of viral-like particles and DNA yields. The pipeline consists of concentration of sample using tangential flow filtration, extraction using tetrasodium pyrophosphate and sonication and purfication using DNase I treatment, filtration, and ultracentrifugation in a sucrose density gradient. Where appropriate, we provide links to similar protocols.

EXTERNAL LINK

https://doi.org/10.7717/peerj.8187

Before Starting

1 **Preparations**

- 200 x TE Buffer: 2M Tris-HCl (pH 8.5) + 0.2M EDTA.
- 1 x TE buffer: 10 mM Tris + 1 mM EDTA (pH 8.0).
- 10 x DNase buffer: 100 mM Tris (pH 7.5) + 5mM $CaCl_2$ + 25 mM $MgCl_2$.

Homogenization of biofilm sample

2 Centrifuge biofilm sample at 100 x g for 15 minutes at 4°C.
00:15:00

3 Recover the supernatant for downstream analyses.

Concentration using TFF

4 Use TFF to concentrate viruses following instruction posted here: https://www.protocols.io/view/Phage-Tangential-Flow-Filtration-c7yzpv/

Extraction using tetrasodium pyrophosphate and sonication

5 Add 5 mM (final concentration) of tetrasodium pyrophosphate to the concentrated biofilm sample.

6 Incubate in dark for 15 minutes.
00:15:00

7 Sonicate the sample on ice three times for 1 minute with 30 second intervals. Shake samples between sonication intervals.

8 Centrifuge at 3234 x g for 15 minutes at 4°C.
00:15:00

9 Filter sequentially the supernatant through 0.8 and 0.45 µm filter, respectively.

Purification using DNase I and sucrose gradient ultracentrifugation

10 ***DNase I digestion***
Add 0.1 volumes of DNase buffer 10 x.

11 Add 2.5 U/µL (final concentration) of DNase I enzyme.

12 Incubate for 3 hours at 37˚C.
03:00:00

13 Heat-inactivate DNase I enzyme at 65°C for 15 minutes.
00:15:00

14 Check for microbial contamination with a standard PCR using universal 16S rRNA gene primers and gel electrophoresis.
See for instance: https://www.protocols.io/view/16S-PCR-Universal-c5my45

15 ***Sucrose gradient ultracentrifugation***
Compare to protocol posted here: https://www.protocols.io/view/Purifying-Viruses-Using-Sucrose-Cushion-c3wypd

16 Pipette 3 mL of 66% sucrose and then 7 mL of 30% sucrose into the clean ultracentrifugation tube. Be careful not to mix the gradient.

17 Mark layers with a permanent marker to facilitate layer recognition.

18 Overlay the sample on top of the gradient and fill to the top. Be careful not to cause any mixing of the gradient.

19 Ultracentrifuge at 106800 x g for 2 hours at 4°C. Use a slow acceleration and no brake for the deceleration.
02:00:00

20 Using an 18 G needle, pierce carefully the tube between the 66% and 30% sucrose layers and extract approximately 2 mL.

Enumerating VLPs using epifluorescence microscopy

21 Compare to protocol: https://www.protocols.io/view/SYBR-Green-or-Gold-Staining-c7sznd

Because of high background noise in biofilm samples, it is important that VLP are counted after purification and DNase I treatment.

22 Stain sample with 2 µL 1000 x SYBR Gold and incubate for 30 minutes in the dark at room temperature.
00:30:00

23 Carefully filter the sample onto 0.02 µm pore size membrane filter (Anodisc).

24 Mount filter on glass slide using a drop of VectaShield.

25 Screen the filter under fluorescence microscope using blue light excitation (488 nm) and green emission (512 nm).

Nucleic acid extraction

26 Add 0.1 volumes of 200 x TE buffer, 0.01 volumes of 0.5M EDTA (pH 8) and 1 volume of formamide to sample. Mix well and incubate at room temperature for 30 minutes.
00:30:00

27 Add 2 volumes of 100% EtOH.

28 Incubate at 4°C for 30 minutes.
00:30:00

29 Centrifuge at 17000 x g for 20 minutes at 4°C to pellet the DNA.
00:20:00

30 Wash the pellet twice with 70% ice cold EtOH and allow to dry at room temperature.

31 Resuspend DNA with 567 µL of 1 x TE buffer.

32 **Optional stopping point:** store the sample at -20°C for up to one month.

33 Pre-warm SDS at 37°C and CTAB at 65°C.

34 Add 30 µL of 10% warm SDS and 3 µL of proteinase K (20 mg/mL).

35 Mix and incubate for 1 hour at 37°C.
01:00:00

36 Add 100 µL of 5M NaCl and mix.

37 Add 80 µL of warm CTAB solution.

38 Vortex and incubate at 65°C for 10 minutes.
00:10:00

39 Add equal volume of chloroform.

40 Vortex and centrifuge at 16000 x g for 10 minutes at room temperature.
00:10:00

41 Transfer top aqueous layer to new microcentrifuge tube.

42 Add equal volume of 25:24:1 phenol:chloroform:isoamyl alcohol.

43 Vortex, and centrifuge at 16000 x g for 10 minutes at room temperature.
00:10:00

44 Transfer top aqueous layer to new microcentrifuge tube.

45 Add equal volume of chloroform to supernatant.

46 Vortex, and centrifuge at 16000 x g for 10 minutes at room temperature.
00:10:00

47 Transfer top aqueous layer to new microcentrifuge tube.

48 Add 0.7 volumes of isopropanol to supernatant.

49 Gently mix and incubate at -20°C overnight to precipitate DNA.

50 Centrifuge at 16000 x g for 20 minutes at 4°C.
00:20:00

51 Wash the DNA pellet twice with 70% cold EtOH.

52 Resuspend DNA with 50 µL of DNase-free H_2O.

53 Quantify DNA (e.g. using Qubit according to manufacturer's instructions).

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