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Viral extraction from Sediment V.1

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Aditi K. Narayanan[1], Alon Philosof[1]

[1]California Institute of Technology

Aditi K. Narayanan: ORCID: 0000-0003-0627-1859
Alon Philosof: ORCID: 0000-0003-2684-8678

Orphan Lab

Victoria J Orphan
California Institute of Technology

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We use this protocol and it's working

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Abstract

Viruses are often undercounted in subsurface sediments due to their propensity to stick to surfaces. This protocol outlines a method for detaching viruses from sediment particles via a combination of chemical and physical agitation followed by density separation.

Aditi K. Narayanan and Alon Philosof

1 Based on *An Improved Method for Extracting Viruses From Sediment: Detection of Far More Viruses in the Subseafloor Than Previously Reported*

https://doi.org/10.3389/fmicb.2019.00878 (Pan et al 2019, Frontiers in Microbiology)

Materials

2
1. 20mM tetrasodium pyrophosphate solution (0.532g in water for 100mL final volume), CAS #7722-88-5, Millipore-Sigma Cat. #P8010
2. 10% NaCl solution (10g in water for 100mL final volume), CAS #7647-14-5
3. 30% Nycodenz solution (4.5g in water for 15mL final volume). Nycodenz is also called Histodenz or Iohexol), CAS #66108-95-0, Millipore-Sigma Cat. #D2158
4. 50% Nycodenz solution (7.5g in water for 15mL final volume)
5. 15mL Falcon tubes or similar (e.g. Fiusher Scientific Cat. #4-959-49B), capable of withstanding ~4000xg
6. Sonication wand (e.g. QSonica CL-188)
7. Centrifuge capable of holding 15mL Falcon Tubes (e.g. Beckman Coulter Allegra X-15R Centrifuge with SX4570 swinging bucket rotor)
8. Syringes, various volumes
9. Hypodermic needles, between preferably 3-4 inches in length if possible (otherwise 1.5 inches will do)
10. Glutaraldehyde solution (optional, for fixation)
11. Liquid nitrogen (optional, for storage after fixation)

Protocol

3 Take 0.1-0.33cm^3 of sample (or 1mL of slurry) and mix enough NaCl solution and tetrasodium pyrophosphate solution to get to a final concentration of 2.5% NaCl and 5mM tetrasodium pyrophosphate in a 6mL volume. Your stock solutions under the Materials section should be 4X concentrations. Get rid of any clumps by shaking. Works best in a 15mL Falcon tube.

For example, to each 1mL of slurry, add 1.5mL of 4X tetrasodium pyrophosphate, 1.5mL of 4X NaCl, and 2mL of virus-free water.

4 Sonicate on ice at 25 amps for 10 seconds. Rest for another 10 seconds. Repeat 2 more times for a total of 30 seconds of sonication. Our sonicator is a QSonica CL-188.

5 Create Nycodenz 30% and 50% gradient in a 15mL falcon tube using the underlay method:

1. Fill a syringe with 1mL of 30% solution. Attach a needle to the end.
2. Tap out any air bubbles in the syringe
3. Dispense the 30% solution into the very bottom of the Falcon tube
4. Repeat the above steps 1-2 with the 50% solution
5. Push the needle of the syringe containing the 50% solution all the way to the bottom of the Falcon tube
6. CAREFULLY dispense the 50% solution under the 30% solution, allowing the 30% layer to rise above it

6 Layer the sonicated sample over the gradient and centrifuge at 2900xg for 30min in a swinging bucket rotor.

7 Use a needle and syringe to remove the topwater, 30% fraction, and liquid portion of the 50% fraction. These fractions will contain your viruses, though you will need to stain with SYBR Gold to determine which of your fractions carries the largest number.

8 Filter the viral fraction through a 0.2µm membrane and store. If you choose to fix before storing, mix enough glutaraldehyde with the sample for a final concentration of 0.1% glutaraldehyde. Allow the samples to sit at 4˚C for 30 minutes, then flash freeze and store at -80˚C.

9 If you choose, you can repeat the pyrophosphate, sonication, and gradient steps on the sediment pellet to ensure maximum extraction efficiency.
1. Add 5-6mL of 1:1 solution (final concetration 5mM tetrasodium pyrophosphate and 2.5% NaCl)
2. Repeat steps 2-6

Protocol references

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