Jun 15, 2018

Purification of Cafeteria roenbergensis virus particles

DOI
dx.doi.org/10.17504/protocols.io.qz2dx8e

Matthias Fischer[1]

[1]Max Planck Institute for Medical Research, Heidelberg, Germany

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Matthias Fischer

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Protocol Citation: Matthias Fischer 2018. Purification of Cafeteria roenbergensis virus particles. **protocols.io https://dx.doi.org/10.17504/protocols.io.qz2dx8e**

Protocol status: Working
We use this protocol and it's working

Created: June 15, 2018

Last Modified: June 15, 2018

Protocol Integer ID: 13082

Keywords: giant virus, CroV, virus purification, Optiprep, purification of giant dna virus, particles of the giant marine virus crov, cafeteria roenbergensis virus, heterotrophic flagellate cafeteria roenbergensi, giant marine virus crov, giant dna virus, purification, smaller bacteria, bacteria, tangential flow filtration, centrifugation, combination of centrifugation, gradient ultracentrifugation, particle size, only separation method

Abstract

The concentration and purification of giant DNA viruses from bacteria-containing cultures can present problems because the particle size of giant viruses overlaps with that of smaller bacteria, hence filtration as the only separation method is not possible.
In this protocol, a combination of centrifugation, tangential flow filtration, and density-gradient ultracentrifugation is used to concentrate and purify particles of the giant marine virus CroV that infects the heterotrophic flagellate *Cafeteria roenbergensis*.

Materials

MATERIALS

- Optiprep (Iodixanol) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D1556-250ML
- F/2 medium **Catalog #**MKK50L
- Sorvall Lynx 4000 Centrifuge **Thermo Fisher Scientific Catalog #**75006581
- BD Bacto™ Yeast Extract **Becton Dickinson (BD) Catalog #**212750
- Cafeteria roenbergensis culture, from culture collections such as Roscoff or CCAP
- SW40 Ti (with Beckmanultracentrifuge) rotor with ultra-clear tubes (14×95 mm) **Beckman Coulter Catalog #**344060
- Slide-A-Lyzer 3 mL Dialysis Cassettes, 20 kDa MWCO **Thermo Fisher Scientific Catalog #**66003
- Vivaflow 200 tangential flow filtration unit (0.2 µm, PES), including size 16 tubing for Masterflex peristaltic pump **Sartorius Catalog #**VF20P7

Troubleshooting

1 Grow cultures of *Cafeteria roenbergensis* to an initial density of 7E+05 to 1E+06 cells/mL in f/2 artificial seawater medium containing 0.03% (w/v) yeast extract to stimulate bacterial growth (*Cafeteria* feeds on the bacteria).
If needed, dilute denser cultures to the desired cell concentration with f/2-yeast medium.
Aliquot the cultures, e.g. 50 ml aliquots per 250 ml polycarbonate Erlenmeyer flask, or 500 ml per 3 L polycarbonate Fernbach flask.
Infect host cultures with a Cafeteria roenbergensis virus (CroV) suspension at a multiplicity of infection (moi) of ~0.01.
Incubate at 20-23 °C (constant shaking not required).
Leave one culture uninfected as a negative control.

2 Monitor host cell density daily by counting on a hemocytometer until lysis occurs (e.g. at 4-6 days post infection).

3 When cell densities of infected cultures drop below 1E+05 cells/ml, centrifuge the lysates to remove cell debris.
Use 1 L centrifuge bottles, e.g. in a Sorvall F9-6×1000 rotor (used with Sorvall Lynx centrifuges), at a speed of 8000 rcf for 20 minutes and a temperature of 4 °C.
Save the virus-containing supernatant and discard the pellets.

4 Concentrate the CroV-containing supernatant by tangential flow filtration, e.g. in a Vivaflow 0.2 µm PES unit.
Concentration of up to 15 L is possible with a single Vivaflow unit (can take several days).
Cool the unit and reservoir during concentration, check the tubing regularly for wear and make sure the connections are tight.
Do not exceed a back pressure of 2 bars.
The filtrate is discarded, the CroV-bacteria mixture is concentrated to a final volume of 30-50 ml.
Color may vary depending on the bacteria present in the host cultures.
Save the concentrate in a 50 mL Falcon tube.

5 To remove most of the bacteria and further concentrate CroV particles, use ultracentrifugation on a two-step Optiprep cushion.
Fill Beckman SW40 ultraclear tubes with ~6 ml of CroV-bacteria concentrate, then slowly underlay with 2.5 mL of a 23% Optiprep solution in 50 mM Tris-Cl, pH 8.0, 2 mM $MgCl_2$, 400 mM NaCl, and then with 1 mL of a 40% Optiprep solution in 50 mM Tris-Cl, pH 8.0, 2 mM $MgCl_2$, 400 mM NaCl. The 23% cushion will stop the migration of bacteria, the 40% cushion will stop CroV particles and pellet them gently.
Fill the tubes carefully to the top with CroV-bacteria concentrate and balance them against each other.
Centrifuge in a Beckman SW40 swinging bucket rotor (always load all buckets, even if empty!) at 100,000 rcf for 2 hours at 15 °C.

6 Collect the opaque CroV-containing band at the 23%-40% interface by puncturing the tube with a 20G needle and syringe (1-2 mL per tube).
Dialyze the virus particles in 3 mL dialysis cassettes (Slide-A-Lyzer, 20 kDa cutoff) against 1 L of 50 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$, 400 mM NaCl at 4°C overnight.
Volume in the dialysis cassette will increase 2-3fold.

7 Prepare 10%-50% continuous Optiprep, 50 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$, 400 mM NaCl gradients in SW40 ultraclear tubes, e.g. using the Gradient Master (settings:81.5° angle, 35 rpm, 1 min 15 sec). Mark tubes at upper half edge and fill with 6.5 mL 10% Optiprep in 50 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$, 400 mM NaCl.
Load up to 2 mL of dialyzed CroV suspension per tube by removing gradient solution from the top and replacing it with virus suspension.
Centrifuge for 4 h in a SW40 rotor at 100,000 rcf, 4°C.
Extract CroV-containing bands using a 21G needle and syringe.

8 Dilute the CroV-Optiprep suspension 3-fold with 50 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$, 400 mM NaCl and spin in a tabletop centrifuge for 20 min, 4°C, 10,000 rcf. Lower g-forces and longer centrifugation times are required if the particles should all be intact, e.g. for structural studies.
Save supernatant, dissolve opaque pellets in 50 µl of 50 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$, 400 mM NaCl, combine the dissolved pellets and spin them again for 15 min at 8,000 g, 4°C.
This should result in a clearly visible white pellet. A yellow color indicates the presence of free lipids, e.g. released from broken virus capsids.
Carefully resuspend the virus pellet..
The saved supernatants can be centrifuged again for 1 h at maximum speed (20,000 rcf), 4°C to collect the remaining virus particles, e.g. for DNA extraction.