Propagation of marine eukaryotic viruses (Prasinoviruses)

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Abstract

Purpose: To reproducibly generate fresh preparations of virus for independent experiments.

Summary: Fresh virus sample for an experiment is generated by a primary infection of exponentially growing host cells from a master stock of virus. The infected host is allowed to lyse until the culture is cleared. The lysate is filtered to remove any large cellular debris, then the viral-size fraction is concentrated from the filtered lysate and washed with buffer using a centrifugal concentrator. The viral concentrate is stored at 4°C protected from light and should be used for an experiment within 1-2 days. On the day of (or the day before) the experiment, an MPN assay should be set up to assess infectivity of the fresh viral concentrate.

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Guidelines

Principle

Several external physical and chemical factors (e.g., temperature, salinity, acidity, light, and adsorption) influence the morphology, persistence, and function of marine viruses. Because viruses depend on their hosts for replication, biological parameters such as host physiology and growth rate, as well as viral and host abundances (i.e., associated encounter rate), likewise impact the properties of a particular viral population (e.g., infectivity, abundance). Standardized and characterized host growth prior to infection combined with consistent infection parameters (e.g., host density, viral volume added, time allowed for lysis, incubation conditions, etc.) should minimize variation among viral preparations and improve reproducibility of viral properties among experiments. Furthermore, using the same master stock of virus for each propagation instead of a serially-propagated virus stock should reduce the probability of introducing genetic mutations.

Host Preparation

Initiate a host culture for the primary infection to allow for at least 5 generations of characterized

exponential growth (10 generations is the gold standard). Avoid transferring cells on the day-of or the day-before the primary infection to minimize disturbance. Monitor growth and transfer/dilute host culture to pre-determined mid-exponential density daily (i.e., semi-continuous culture), including the day of the primary infection. If the host culture will be split to propagate multiple viruses simultaneously, make sure this is done at least 2 days prior to addition of virus. After final dilution of the host culture to pre-determined mid-exponential density, initiate primary infection as described below.

Example *Ostreococcus lucimarinus* (CCMP2972A) host preparation:

Growth conditions: 18°C, 14:10 hour light:dark cycle, light irradiance of ~100 µE m^{-2} s^{-1}

Growth media: L1 with natural seawater base

Exponential range: $7x10^5$ – $2x10^7$ cells mL^{-1}

For 2 viruses, intermediate volume (from Oct 2015): ~6 generations of characterized growth

Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Initial density (mL^{-1})	$3.5x10^7$	$9x10^6$		$1.7x10^7$	$7x10^6$	$9.4x10^6$	$9.4x10^6$
Growth Rate (d^{-1})	NA	0.68		0.69	0.53	0.62	0.68
Dilution/Transfer	Transfer	Dilute	Skip	Transfer	Transfer *(split)*	Dilution	Dilution
Final density (mL^{-1})	$4x10^6$	$4x10^6$		$5x10^6$	$5x10^6$	$5x10^6$	$5x10^6$ **(infect!)**
Volume (mL)	60	137*		200	140 *x2*	260* *x2*	490* *x2*

For 2 viruses, large volume (from Apr 2016): ~5 generations of characterized growth

Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Initial density (mL^{-1})	$2.2x10^7$			$1.8x10^7$	$8.7x10^6$	$8x10^6$	$8.7x10^6$
Growth Rate (d^{-1})	NA			0.760	0.516	0.481	0.487
Dilution/Transfer	Transfer	Skip	Skip	Transfer	Transfer *(split)*	Dilution	Dilution
Final density (mL^{-1})	$4x10^6$			$5x10^6$	$5x10^6$	$5x10^6$	$5x10^6$ **(infect!)**
Volume (mL)	120			400	330 *x2*	534* *x2*	840* *x2*

**Volume varies depending on growth*

Before start

Equipment and Materials.

Equipment:

Tube rack
200 and 1000 µL pipettes
Pipettor for serological pipettes
Refrigerated benchtop centrifuge capable of 1,000 x g
Swinging bucket rotor that can accommodate 50 ml conical bottom tubes
Vortexer

Materials:

Host culture
Virus master stock
Culture medium
200 and 1000 uL filter tips
Serological pipettes
0.45-µm-pore-size PES membrane Nalgene Rapid-Flow Sterile Disposable Filter Unit(s) (Thermo Scientific #166-0045)
100 kDa MWCO PES membrane VivaSpin20 ultrafiltration unit (Sartorius #VS2041)
15 or 50 mL conical tubes, sterile
Parafilm
0.02-µm-pore-size sterile Anotop 25 Syringe Filter Plus (GE Healthcare #6809-4102)
1X TE buffer pH 8.0 (Fisher BioReagents #BP1338-1), 0.02-µm-filtered (see below)

Preparation of TE buffer (1X, pH 8.0).

Dilute 0.5 mL 100X molecular grade Tris-EDTA (Fisher BioReagents #BP1338-1) in 49.5 mL MilliQ water. In hood, filter through 0.02-µm-pore-size sterile Anotop 25 Syringe Filter Plus (GE Healthcare #6809-4102) into sterile 15 mL tubes. Use within a few days of opening.

Protocol

Primary Infection

Step 1.

Primary Infection

1. Ethanol-clean culture hood as usual, then UV pipettes, tips, and tube rack for 10 min.
2. Work with one virus at a time, transferring the master stock from 4°C to the culture hood.
3. Initiate the primary infection by adding the master virus stock at 1% of the total culture volume (e.g., 5 mL virus to 500 mL exponentially-growing host culture).
4. Mix the infected flask and incubate at standard growth conditions until culture is mostly lysed (e.g., 5 days for *O. lucimarinus* viruses).
5. Thoroughly clean pipette and gloves with ethanol before repeating for additional viruses.
6. Ethanol-clean hood and UV pipettes, tips, and tube rack after use.

Cleaning and Concentration of Lysate

Step 2.

Cleaning and Concentration of Lysate

1. Once the primary infection culture has cleared, filter entire volume of lysate through a 0.45-µm-pore-size PES membrane sterile disposable filter unit to remove large cell debris.
2. Add 20 mL filtered lysate to the upper reservoir of a 100,000 Dalton MWCO PES membrane Vivaspin20 ultrafiltration unit.

NOTE: Appropriate MWCO will depend on virus capsid size. For maximum recovery select a MWCO at least 50% smaller than the molecular size of the particle of interest. 200 kDa is the equivalent of 10 nm.

3. Place unit with counter-balance in swinging bucket rotor in pre-cooled (4°C) benchtop centrifuge so that the printed side faces upwards/outwards.
4. Centrifuge at 1,000 x g (*do not exceed!*) for 5-10 minutes. Centrifuge time will depend on the amount of material in the sample. *Do not let the filter go dry.*
5. Record the volume retained in the upper reservoir.
6. Discard the filtrate by separating the upper reservoir from the bottom of the Vivaspin20 unit.
7. Repeat 2-6 (adding filtered lysate to the retentate in the upper reservoir up to 20 mL before re-centrifuging) until the entire volume of filtered lysate has been processed.

NOTE: Several Vivaspin20 units can be used in parallel for large lysate volumes, and pooled after concentration.

8. Transfer the concentrated virus sample (retentate) from the upper reservoir to a sterile 15 or 50 mL conical tube.
9. Remove the upper reservoir from the Vivaspin20 unit and cover the bottom with a double-layer of Parafilm.
10. Add 2 mL of 0.02-µm-filtered 1X TE buffer (pH 8.0) to the upper reservoir.
11. Vortex the upper reservoir (Parafilm side down) for 20 sec on 60% power to wash the Vivaspin20 filter.

12. Add the washed sample to the recovered viral concentrate.
13. Repeat 10-12 twice more for a total of 3 washes with TE buffer.
14. Store concentrated virus sample at 4°C protected from light.