Clonal virus purification

Mathias Middelboe, Amy M. Chan, and Sif K. Bertelsen

Abstract

For use in "Obtaining pure cyanophage stocks (liquid assay)"

Citation: Mathias Middelboe, Amy M. Chan, and Sif K. Bertelsen Clonal virus purification. **protocols.io** dx.doi.org/10.17504/protocols.io.dqp5vm
Published: 08 Feb 2016

Protocol

Step 1.
Use 13-×-100–mm culture tubes (or 24-well plates).

Step 2.
Prepare exponentially growing target cells (e.g., >100 mL).

Step 3.
Dilute some of the titered lysate to 1 infective virus/mL.

Step 4.
Add 0.2 mL (0.2 infectious units) to each of 20 tubes of susceptible host cells.

Step 5.
Monitor tubes for 2 to 3 weeks.

Step 6.
Cultures in which lysis occurs are assumed to be the result of a single-virus infection.

NOTES
Amy Chan 02 Sep 2015
The probability that more than 1 infective unit occurred in a given culture is 0.0176.

Step 7.
If lysis occurs in 4 tubes or less of 20, it is assumed that lysis in each tube was caused by one infectious unit, therefore each tube would contain a separate phage clone.

Step 8.
Propagate an aliquot from all the tubes to confirm the results.

Step 9.
If lysis occurs in more than 4 tubes, repeat the clone out procedure by reducing the volume of diluted lysate added to the 20 tubes.

NOTES
Amy Chan 05 Oct 2015
Add 0.1 mL instead of 0.2 mL.

Step 10.
Scale up each phage clone to make primary phage stocks.

NOTES

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e.g., add 5µL of the lysate to 40 mL of cells.

Step 11.

Centrifuge, filter, and titer the stock, store at 4°C in the dark.