Obtaining pure cyanophage stocks (liquid assay)

In 1 collection

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[1]Manual of Aquatic Viral Ecology

1 Works for me dx.doi.org/10.17504/protocols.io.dqm5u5

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EXTERNAL LINK

http://www.aslo.org/books/mave/MAVE_118.pdf

1 Determine the titer of the lysate using the 96-well assay as described earlier.

Lysate titer
by Amy Chan
PREVIEW RUN

1.1 Prepare end-point dilution series (10-fold serial dilutions, 5 to 6 levels).

1.2 Monitor plates for lysis every few days, recording the number and position of clear wells on the plate.

1.3 When clear or nearly clear wells no longer appear for 1 week, record the final “score” for each dilution level.

1.4 Use the MPN Assay Analyser program (Passmore et al. 2000) to determine the most-probable-number (Taylor 1962) of infective phages in the lysate.

2 Once the cyanophage titer is determined for the stock tube, proceed to purify a clonal virus.

Clonal virus purification
by Amy Chan
PREVIEW RUN

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

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

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

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

2.5 Monitor tubes for 2 to 3 weeks.

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

The probability that more than 1 infective unit occurred in a given culture is 0.0176.

2.7 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.

2.8 Propagate an aliquot from all the tubes to confirm the results.

2.9 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.

Add 0.1 mL instead of 0.2 mL.

2.10 Scale up each phage clone to make primary phage stocks.

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

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