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Obtaining pure cyanophage stocks (plaque purification)

In 1 collection

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[1]Manual of Aquatic Viral Ecology

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Troubleshooting

1 Make a dilution series of the lysate (assume 10^4 to 10^5 PFU per mL in the plaque lysate)

Note

Use this to perform a second round of plaque assays to purify the phage.

2 Repeat the plaque purification procedure 2 more times to ensure that the cyanophage isolated is clonal.

3 Repeat the plaque purification procedure again.

4 Finally, prepare a primary cyanophage stock using lysate from the final purification via method A "**Liquid Amplification**" **OR** method B "**Plate Amplification**"

5 Method A: liquid amplification

Protocol

NAME

Liquid Amplification

CREATED BY

Amy Chan

Preview

5.1 Add some of the lysate to target host in liquid culture.

5.2 After the culture has lysed, remove cell debris via centrifugation.

5.3 Filter sterilize the stock.

5.4 Store at 4°C until further analysis.

6 Method B: plate amplification

Protocol

NAME

Plate Amplification

CREATED BY

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Preview

6.1 Prepare plaque assays with a dilution series of lysate from the final purification.

Note

Plates with confluent lysis of the host lawn (typically ca. 10^4 PFUs) can then be used to obtain cyanophage stocks by elution of phages from the plates.

6.2 Add 5 mL sterile seawater to the plate.

6.3 Scrape off the top agar layer into the seawater.

6.4 Leave at 4°C overnight.

18:00:00

6.5 Remove agar and cell debris by centrifugation.

6.6 Filter sterilize the stock.

6.7 Store at 4°C until further analysis.

7 Titer the final stock via plaque assay.

8 Cyanophage stocks stored at 4°C in the dark are stable for at least a year.