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③ Obtaining pure cyanophage stocks (plaque purification)



In 1 collection

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¹Manual of Aquatic Viral Ecology

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Protocol status: Working

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Troubleshooting

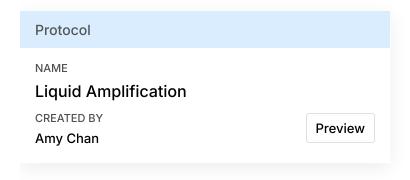


Make a dilution series of the lysate (assume 10⁴ to 10⁵ PFU per mL in the plaque lysate)

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.
- 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



- 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 Amy Chan	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. 10⁴ 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.