Obtaining pure cyanophage stocks (plaque purification)

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Abstract

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Protocol

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

NOTES
Amy Chan 02 Sep 2015
Use this to perform a second round of plaque assays to purify the phage.

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

Step 3.
Repeat the plaque purification procedure again.

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

Step 5.
Method A: liquid amplification

PROTOCOL

. Liquid Amplification

CONTACT: Amy Chan

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

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

Step 5.3.
Filter sterilize the stock.

Step 5.4.
Store at 4°C until further analysis.

Step 6.
Method B: plate amplification

PROTOCOL

. Plate Amplification

CONTACT: Amy Chan

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

NOTES

Amy Chan 03 Sep 2015
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.

Step 6.2.
Add 5 mL sterile seawater to the plate.

Step 6.3.
Scrape off the top agar layer into the seawater.

Step 6.4.
Leave at 4°C overnight.

DURATION
18:00:00

Step 6.5.
Remove agar and cell debris by centrifugation.

Step 6.6.
Filter sterilize the stock.

Step 6.7.
Store at 4°C until further analysis.

Step 7.
Titer the final stock via plaque assay.

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