Cyanophage plaque purification

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Abstract

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Protocol

Step 1.
Plate cells as described in Plating Prochlorococcus and Synechococcus strains in top agarose for plaque assays

Step 2.
When plaques begin showing up, pick them using the “coring” technique:

PROTOCOL

. Coring Technique

CONTACT: Bonnie Poulos

NOTES
Bonnie Poulos 17 Jun 2015
You’ll need to make sure that the appropriate cells are growing to inoculate the cored plaque into a liquid culture to obtain a cyanophage stock

Step 2.1.
Poke the plaque in the agarose with the tip of an autoclaved Pasteur pipette to core it out drawing agarose chunks into the pipette

NOTES
Bonnie Poulos 17 Jun 2015
I would pick a few (2-4?) plaques just in case one does not lyse

ANNOTATIONS
Chris Upton 10 Sep 2015
Can you use sterilized tooth-picks?
Would be cheaper.

Step 2.2.
Inoculate 2-5 ml dense cells (whatever is available) with the cyanophage plaque by pipetting up and down a few times

Step 2.3.
Let this sit in the incubator for 60 minutes to adsorb and then dilute with about 25 ml media

DURATION
01:00:00

Step 2.4.
Do this in parallel to a control tube of just cells and observe the cyanophage inoculated cultures for lysis (clearing) relative to the controls

Step 3.
To follow the liquid cultures, you just need to check (by eye) every couple of days to see if any of them have lysed

NOTES
Bonnie Poulos 17 Jun 2015
lysed = significantly reduced color relative to control

Step 4.
When you check them, give them a quick LOW-SPEED vortex (<4) to get the cells off the bottom of the tube (particularly the Syn) without spilling cells

Step 5.
When you go to “harvest” the cyanophage (after lysis), decant the tube into two 50 ml orange capped tubes

NOTES
Bonnie Poulos 17 Jun 2015
~15 ml each; spinning more than that can break the centrifuge tubes

Step 6.
Spin on the Beckman JA-17 rotor, 10K (13,800g), 17°C, 20 minutes

DURATION
00:20:00

NOTES
Bonnie Poulos 17 Jun 2015
NOTE: I’ve found that at these speeds and temperature, adding 25 or 30 ml to an orange capped tube results in breakage 50% of the time. Set the brake to max, compensation to -5.

Step 7.
Transfer the supernatant into two fresh pre-labeled acid-washed glass black-capped tubes (label with: cyanophage name, date, “spun”, 2xplaque purified) for storage. Work asceptically in the hood with flame.