Feb 02, 2016

Roseobacter Screening Of Surface Waters For Viruses

DOI
dx.doi.org/10.17504/protocols.io.dbt2nm

Matthew Sullivan[1]

[1]Matthew Sullivan Lab, University of Arizona, Ohio State University

VERVE Net | Sullivan Lab

Verve Team
University of Arizona

DOI: https://dx.doi.org/10.17504/protocols.io.dbt2nm

Protocol Citation: Matthew Sullivan 2016. Roseobacter Screening Of Surface Waters For Viruses. **protocols.io https://dx.doi.org/10.17504/protocols.io.dbt2nm**

Protocol status: Working

Created: July 08, 2015

Last Modified: November 09, 2017

Protocol Integer ID: 1107

Keywords: roseobacter screening of surface water, roseobacter screening, surface water, virus

Guidelines

Notes:

Goal initially is to screen several seawater samples on several roseobacter hosts to determine breadth of rosephage present at each location and then to focus on a single site.

Nomenclature for viruses should include a designation for the site of seawater collection followed by a number and the host it was isolated on.

Put information into an Excel worksheet. Create a separate Excel file for each source of virus. Use a different tab in each worksheet for different hosts.

Turbid plaques indicate possible lysogenic phage that may be of interest to Feng Chen's laboratory in Maryland. Keep the plaque plug at 4°C. Take 50-100 µl from a few and grow up small 1 ml cultures to cryopreserve (glycerol) them. Here trying to get the putative lysogenic cells (prophage containing confers resistance to colonies of cells within the plaque = turbid).

Troubleshooting

Growth

1 Pull Roseobacter cultures from freeze and grow in 5 ml broth cultures

Note

Matt has 14 isolates from Wendy Ye in Mary Ann Moran's lab in Georgia. Two of them are from open ocean (CCS1 and CCS2) and they are grown at 20-22°C in 1/10 YTSS. All others are from coastal waters and are grown at 30°C in 1/2 YTSS. For virus screening, grow them to log phase (OD_{600} = 0.6 - 0.8). Some will grow quickly (overnight) while others will take 1-4 days to reach a log phase.

Screening

2 Perform virus screening in sterile 96 well flat-bottom tissue culture treated microtiter plates

Note

Viruses will be screened from seawater collected from various sources.

3 Store seawater filtrates at 4°C

Note

Ice crystal formation at -20°C **bad**.

Absorption

4 Absorb the viruses to the bacteria

5 Place 15 µl of log phase cells and 15 µl of seawater filtrate (containing virus) into wells of a 96 well plate

Note

Uninfected controls should surround infected wells on each plate in a checkerboard pattern.

6 Allow the viruses to absorb to the bacteria for 1 hour

01:00:00

7 Add 200 µl growth media (1/2 or 1/10 YTSS) to the wells

8 Seal the plate with parafilm and incubate with shaking

9 Look for infected wells that show lysis with respect to uninoculated controls

Enrichment

10 Enrich for viruses by transferring from initial 96 well plate to another 96-well plate containing fresh liquid media ("frogging" into new wells)

> Note
>
> Does not require fresh log-phase host cells, the cells come with the transfer. Ideally frog **before** cells into stationary phase.

> Note
>
> Enrichment for viruses may take several rounds of culturing because we're assuming initial concentrations of phages in the seawater are low.

11 Once enriched sufficiently, filter out cells with 0.2um filtration into 1.5ml microfuge tubes

12 Spin for 5 mins. at max to pellet cells

00:05:00

13 Transfer supernatant to fresh tube for storage

14 Store at 4°C

Plaque Purification

15 Plaque-purify wells containing virus by growing cells plus virus on solid media

16 Prepare solid agar media and 0.5% overlay agar

17 Absorb virus to cells using as a starting point, 25 µl virus from 96 well plate enrichments and 200 µl log-phase host bacteria for 1hr

01:00:00

Note

Try growing lawns first, maybe needs to be up to 1 ml cells.

18 Mix the virus-absorbed cells in the 5 ml overlay agar

19 Pour over the solid media

20 Incubate and look for plaque formation

Note

Will have several possible results: no plaques (not likely if using virus-enriched cultures from 95 well plates), too many plaques to purify them (in which case, a dilution of virus is made and plaque assay repeated), or well-resolved plaques. If well-resolved plaques, then cored from agar using Pasteur pipet and dispensed into 100µl YTSS broth in 1.5ml tubes.

21 Pick representatives of all plaque types present and record appearance of the plaques

Note

Clear, well-lysed plaques are of the most interest to us. Turbid plaques may indicate lysogenic phage and some should be picked, but these will be sent to another laboratory for further study (see guidelines).

22 Store the broth that contains the agar plugs at 4°C and allow the viruses to diffuse out of the agar into the broth

DNA Purification Determination

23 Once the viruses are plaque-purified they can be scaled-up in 5 ml broth cultures for DNA purification

24 Take 25 µl of the plaque-purified virus (agar plug in ~100 µl broth) and absorb to 200 µl log-phase bacteria for 1 hr
01:00:00

25 Add to 5 ml broth and grow with shaking

26 Centrifuge out cells and filter culture through 0.2 µm filter

27 SYBR stain a portion of the filtrate to determine how much virus is present

Note

If a lot of virus is present, then may be enough to purify DNA.

DNA Purification

28 Purify DNA using the Promega Wizard Lambda DNA kit

Note

Goal is to obtain about 1 µg DNA for future work. If 5 ml does not yield that amount, will need to grow up more (e.g. 225 ml volume) and repeat DNA purification.