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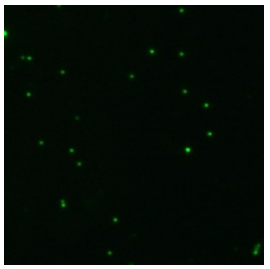
Propagating T5-phages for Fluorescent Staining



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The Aquatic Microbial E...

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Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

Materials

STEP MATERIALS

Magnesium sulfate heptahydrate

Magnesium sulfate heptahydrate

Protocol materials

Magnesium sulfate heptahydrate

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Magnesium sulfate heptahydrate

Troubleshooting

Propagating T5-phages

1 Grow a culture of E.coli ATCC11303 in LB media overnight

2 The next morning, prewarm LB-agar plates to 37ºC

3 Pellet 1 mL of the cells in an Eppendorf tube at 10,000 xg, 2 min
00:02:00

4 Discard the medium

5 Resuspend the pellet into 1 mL 10 mM $MgSO_4$
1 mL
Magnesium sulfate heptahydrate

6 Melt 0.6% top agar in the microwave

7 Prepare sterile 5 mL tubes and pipet 3 mL of melted top agar into them
3 mL

8 Keep tubes in 45ºC heat block or water bath so that the agar stays in a liquid state, but not too hot to kill the cells.

9 Add 10 µL T5 from the stock (except the control) to 100 µL of resuspended cells

10 Incubate 5 min at room temperature
00:05:00

11 Add the infected cells to the molton top agar

12 Vortex briefly

13 Pour onto prewarmed LB agar plate and tilt the plate to spread

14 Let top agar solidify ~30-60 min
01:00:00

15 Evert plates and return to 37ºC incubator

16 Let grow ~6-8 hrs
08:00:00

17 When confluent plaques are seen on the plate, add 5 mL of sterile phage buffer (10 mM $MgSO_4$; 1 mM $CaCl_2$; 10 mM Tris-HCl, pH 7.5; 1% gelatin)

18 Let the plates sit overnight at 4ºC with the buffer on top to gather the phages

19 The next day, transfer the liquid from the plates to a sterile tube and add 100 µL chloroform and 5 mL phage buffer

20 Shake for 1 min to let chloroform settle out
00:01:00

21 Transfer 4 mL of the supernatant to a fresh tube
4 mL

22 Filter the solution with 0.22 µm pore size syringe filter to get rid of all the cellular debris

23 Store phage at 4ºC. They will stay infective for >1 yr.

Protocol

NAME

Fluorescent Staining of T5-phages

CREATED BY

Steven W Wilhelm

Preview

23.1 Pellet phage in an ultracentrifuge at 28,000 rpm, 90 min, 10ºC in 35 mL polycarbonate tubes, filled to the rim, balanced with phage buffer so that the difference is <0.01 g.

01:30:00

Note

Use the SW-28 rotor, or equivalent, cooled down in the fridge prior to using.

23.2 Discard the supernatant

23.3 Resuspend the pellet with phage buffer

800 µL

23.4 If the pellet still seems to have some cellular debris in it, filter the resuspension using a 1 mL syringe and a small 0.2 µm pore size syringe filter.

23.5 Re-pellet the viruses in a Beckmann TL-100 ultracentrifuge at 35,000 rpm, 2 hr, 6ºC using the TL-55 rotor cooled down befoer use using 1.4 mL polycarbonate tubes, filled and balanced as the bigger tubes in step 1.

23.6 Resuspend the pellet with phage buffer and transfer into 1.5 mL screw cap tubes

100 µL

23.7 Store at 4ºC

23.8 Add YO-PRO stain to each tube containing 100 µL resuspension

0.5 µL

Note

Make sure to work in the dark, because the stain is photosensitive.

23.9 Cover the tubes with aluminum foil and let sit at 4ºC for 48 hrs

23.10 After incubation, increase the volume to 1.4 mL

23.11 Centrifuge in the Beckmann TL-100 as in step 5

23.12 Discard the supernatant

23.13 Suspend the viruses in Milli-Q water

100 µL