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🌐 Propagating T5-phages for Fluorescent Staining

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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 **Contributed by users**

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Propagating T5-phages

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

- 2 The next morning, prewarm LB-agar plates to 37°C
- 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 MgSO₄
1 mL
Magnesium sulfate heptahydrate **Contributed by users**
- 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 45°C 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 37°C 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 MgSO₄; 1 mM CaCl₂; 10 mM Tris-HCl, pH 7.5; 1% gelatin)
- 18 Let the plates sit overnight at 4°C 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 μ L 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 μ m pore size syringe filter to get rid of all the cellular debris
- 23 Store phage at 4°C. They will stay infective for >1 yr.

Protocol

NAME

Fluorescent Staining of T5-phages

CREATED BY

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PREVIEW

- 23.1 Pellet phage in an ultracentrifuge at 28,000 rpm, 90 min, 10°C 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, 6°C using the TL-55 rotor cooled down before 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 4°C

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 4°C 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