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WORKS FOR ME 1

Phage amplification and concentration

COMMENTS 0

In 1 collection

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ABSTRACT

This protocol details methods to amplify bacteriophages T4 and SPO1, and concentrate using either PEG precipitation or filtration.

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PROTOCOL CITATION

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COLLECTIONS

Protocol collection: Phage DNA isolation and chemical analysis

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phage, gDNA, nucleoside, genome modification, HPLC, Mass spectrometry

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PARENT PROTOCOLS

Part of collection

Protocol collection: Phage DNA isolation and chemical analysis

Phage amplification

1 For each host, prepare a 30 mL subculture at 1:100 dilution (300 µL in 30 mL). Grow for 2 h at 37 °C in a shaking incubator. Subculture media (LB or ENB) should be supplemented with 1 mM $CaCl_2$ and 1 mM $MgCl_2$.

2 Grow a 3 mL overnight culture of phage host at 37 °C with shaking. *Escherichia coli* strain B (ATCC Strain 11303) should be propagated in Lysogeny Broth (ATCC Medium 1065), and *Bacillus subtilis* strain 168M (ATCC Strain 27370) is propagated in Enriched Nutrient broth (ATCC Medium 265).

Note

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Note

3 Add ~10^7 PFU of phage T4 (ATCC Strain 11303-b4) to the *E. coli* subculture, and ~10^7 PFU of phage SPO1 (ATCC Strain 27370-b1) to the *B. subtilis* subculture. Return the infected subcultures to the incubator, at 37 °C with shaking. Grow until the culture is cleared (around 5 h). If the culture doesn't completely clear within 5 h, you can let the infection proceed overnight.

Phage isolation and concentration

4 Move the phage lysate to a 50 mL conical tube, and add ~1 mL of chloroform. Seal the tube tightly, and shake on a platform shaker for 10–30 min. Transfer to a new conical tube. Then, spin the lysate down in a centrifuge at maximum speed for 30 minutes.

5 Carefully pipette off the supernatant from the spun-down culture into a new 50 mL conical tube, avoiding any debris at the chloroform interface. Also avoid the chloroform. Move to a new 50 mL conical tube, and spin again at max speed for another 30 min or until supernatant is clear. Move to a new 50 mL conical tube.

6 To concentrate the phage lysate, we have used both PEG precipitation and a filter-concentrator based protocol. The PEG protocol requires an overnight incubation, but the filter-concentrator generally requires more hands-on time. Use whichever phage concentration protocol works best for your circumstances.

Step 6 includes a Step case.

PEG prep

Filter prep

step case

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PEG prep

PEG prep requires an overnight incubation step at 4 °C.

7 To PEG-precipitate phage, first prepare 5× PEG precipitation solution containing 2.5 M NaCl and 20% w/v PEG8000.

Note

8 Add PEG precipitation solution to the phage lysate to obtain a 1× concentration of 0.5 M NaCl and 4% w/v PEG8000. Mix by inverting the tube several times and refrigerate overnight at 4 °C.

9 After the overnight incubation with PEG precipitation solution at 4 °C, pellet the PEG precipitated phage particles by centrifuging at 19,000 × g for 60 min at 4 °C. A small white pellet should form at the bottom of the 50 mL conical tube.

10 Remove the supernatant, being careful not to disturb the PEG-precipitated phage pellet.

11 Resuspend the PEG-precipitated phage pellet in 300 µL SM buffer. Store at 4 °C.

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