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Phage amplification and concentration

In 1 collection

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Adair Borges[1], Januka Athukoralage[1]

[1]Arcadia Science

Arcadia Science

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We use this protocol and it's working

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Abstract

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

Troubleshooting

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

ATCC Medium 265: Enriched Nutrient Broth

Heart Infusion Broth (BD 238400) – 12.5 g
Nutrient Broth (BD 234000) – 5.4 g
Yeast Extract – 2.5 g
DI Water – 1000 ml

Autoclave at 121 °C.

Note

ATCC Medium 1065: LB Agar/Broth, Miller

LB, Miller Composition
Tryptone – 10.0 g
Yeast extract – 5.0 g
Sodium chloride – 10.0 g
*Agar – 15.0 g

Final pH 7.0 ± 0.2. Autoclave at 121 °C.

*Omit agar for broth medium.

Note

Note: In subsequent experiments we propagated *Bacillus subtilis* strain 168M in LB broth, and amplified phage SPO1 under identical conditions as *Escherichia coli* strain B and phage T4 with no noticeable difference in bacterial or phage growth.

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 CASE

Phage precipitation with PEG 5 steps

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

5× PEG solution (500 mL)

5 M NaCl stock solution – 250 mL
PEG8000 – 100 g
DI water – to 500 mL

Stir until dissolved. Sterilize with with 0.22 µm filter. Store at room temperature.

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.

Citations

Step 8

Bonilla N, Rojas MI, Netto Flores Cruz G, Hung SH, Rohwer F, Barr JJ. Phage on tap-a quick and efficient protocol for the preparation of bacteriophage laboratory stocks.
https://doi.org/10.7717/peerj.2261