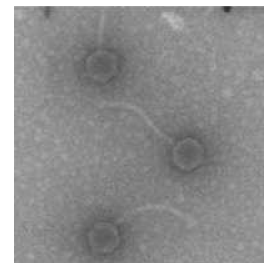


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Bacteriophage Propagation and Quantification via the Phage on Tap Method

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We use this protocol and it's working

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Abstract

The growing interest in bacteriophage (phage) applications across medical, industrial, and molecular fields necessitates rapid and efficient production of high-titer, homogeneous, and purified phage stocks. These stocks should be free from bacterial cells, debris, culture media, and endotoxins. Traditional phage concentration and purification methods are often time-consuming and may compromise phage yield or viability. The Phage On Tap (PoT) protocol offers a rapid, efficient approach for producing homogeneous, high-titer phage stocks. This protocol combines centrifugation, 0.22 μ m filtration, and chloroform treatment with ultrafiltration for concentration and washing, resulting in stable stocks stored at 4°C. This method has been tested on T4 (E. coli), T3 (E. coli), T5 (E. coli), and Spp1 (B. subtilis), is broadly applicable to other tailed phages, and supports laboratory-scale production of high-quality, reproducible phage stocks for research and therapeutic development.



Guidelines

- Phage propagation conditions are phage- and host-specific. The following protocol is optimized for SPP1 phage and *Bacillus subtilis* as host. Adjust conditions as needed for other phage-host systems;
- Proceed to Section 3 once a high-titer phage lysate is obtained;
- Propagate and purify only one phage at a time to avoid cross-contamination.
- The use of standardized, homogenous phage banks significantly reduces experimental variability in molecular phage characterization.
- The protocol is adapted from the Phage on Tap (PoT) method, which is validated for a wide range of bacteriophages and hosts.

Materials

Materials and Reagents

- Buffered Peptone Water (BPW)
- Plate Count Agar (PCA)
- Agar
- Calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)
- Magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$)
- Sodium chloride (NaCl);
- Magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)
- Tris hydrochloride (Tris-HCl, pH 7.4)

Equipment

- 37°C incubator with shaker
- 50 mL Falcon centrifuge tubes
- Microcentrifuge
- Magnetic stir plate
- Petri dishes with disposable lids
- 1.5 mL microcentrifuge tubes
- Small glass test tubes (13 × 100 mm)
- Serological pipettes
- Sterile syringe filters (0.02 μm)
- 0.22 μm filter units
- Amicon® Ultra-15 centrifugal filter units, Ultracel 100 kDa membrane
Note: The 100 kDa membrane pore size is suitable for retaining all known bacteriophages (Erickson, 2009).
- 500 mL screw-cap storage bottle
- 250 mL screw-cap storage bottle

Reagent Preparation

- Buffered Peptone Water (BPW): Dissolve 10 g BPW in 500 mL H_2O .
- BPW Top Agar: 4 g BPW, 1.5 g agar in 200 mL dH_2O .
- Plate Count Agar (PCA) Plates: 23.5 g PCA in 1 L dH_2O .
- SM Buffer: 5.8 g NaCl, 2.0 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 50 mL 1 M Tris-HCl (pH 7.4), in 1 L dH_2O . Autoclave, filter-sterilize (0.02 μm), and store at room temperature.
- Calcium chloride (CaCl_2): Prepare a 1 M stock solution. Add to BPW to a final concentration of 0.001 M for liquid lysate. Autoclave, filter-sterilize (0.02 μm), and store at room temperature.
- Magnesium chloride (MgCl_2): Prepare a 1 M stock solution. Add to BPW to a final concentration of 0.001 M for liquid lysate. Autoclave, filter-sterilize (0.02 μm), and store at room temperature.



1. Phage Plaque Assay for Titer Determination

- 1 Grow *B. subtilis* host overnight in BPW at 37°C (10 mL). 
- 2 Dilute phage stock or isolate in BPW to desired dilution (e.g., for 10⁸ PFU/mL stock, perform serial dilutions to 10⁻⁶ and 10⁻⁷ for countable plaques).
- 3 Melt BPW top agar in a microwave, then cool in a 56°C water bath until just warm to the touch. 
- 4 Add 100 µL (or 1 mL) of overnight host culture and 100 µL (or 1 mL) of phage dilution to a glass test tube; mix.
- 5 Add 2.5–3 mL of molten top agar to the tube.
- 6 Quickly pour the mixture onto a PCA plate and tilt to spread evenly. Allow agar to solidify (~5 min).
- 7 Invert and incubate the plate overnight at 37°C. 

- 8 Count plaques and calculate phage titer (PFU/mL) using:

$$\text{PFU/mL} = \text{Number of plaques} / \text{Volume plated (mL)} \times \text{Dilution factor}$$

Example: 20 plaques from 1 mL of 10⁻⁶ dilution yields 2.0 × 10⁷ PFU/mL.

2. Phage Propagation via Liquid Lysate

- 9 Grow *B. subtilis* host overnight in BPW at 37°C (10 mL). 
- 10 Prepare and autoclave 100 mL BPW supplemented with 0.001 M CaCl₂ and MgCl₂ in a 250 mL screw-cap bottle.
- 11 Inoculate supplemented BPW with 0.1 volume of overnight host culture.

Note: For fast-replicating phages (e.g., T3), increase host volume as needed.



12 Incubate with shaking at 37°C for 1 h.



13 Add 100 µL high-titer phage lysate ($>10^8$ PFU/mL).

14 Incubate at 37°C with shaking for ~5 h or until clearing is observed.



15 Harvest the phage lysate and store at 4°C until purification.

Optional: Determine titer by plaque assay to ensure high initial titer.

3. Phage Purification by Filtration

16 Transfer phage lysate to sterile 50 mL Falcon tubes and centrifuge at $4,000 \times g$ for 20 min.



17 Carefully collect the supernatant with a serological pipette and transfer to a labeled sterile tube.



18 Filter the supernatant through a sterile 0.22 µm filter to obtain a cell-free phage lysate.



19 Centrifuge again at $4,000 \times g$ for 5 min and transfer the supernatant to a 250 mL screw-cap storage bottle; store at 4°C until concentration.



Optional: Determine titer by plaque assay to ensure high initial titer.

4. Phage Concentration and Washing by Ultrafiltration

20 Add ~15 mL phage lysate to the upper reservoir of an Amicon filter device and centrifuge at $4,000 \times g$ for ~5 min.



Note: Centrifugation time varies by phage type and titer. Avoid drying the filter. Use shorter times and adjust as needed.

21 Discard filtrate and repeat with additional lysate until all is concentrated to <10 mL.



22 Add ~15 mL SM buffer to the upper reservoir and centrifuge at $4,000 \times g$ for ~5 min to wash.





Note: SM buffer is suitable for long-term storage of T phages, but other appropriate storage buffers may be used. Avoid drying the filter; adjust time as needed.

- 23 Repeat the wash step and concentrate to <10 mL.



- 24 Using a pipette, carefully collect the concentrated, washed phage lysate from the upper reservoir. Gently rinse the reservoir surface.



Note: Alternatively, vortex the device to assist phage recovery from the filter.

- 25 Collect <10 mL of concentrated, purified phage lysate. Determine and record titer (PFU/mL).

5. Phage Bank Storage

- 26 Dilute the concentrated phage lysate in SM buffer to prepare a high-titer working stock.

Note: Adjust dilution based on desired final concentration and volume. For higher-titer stocks, dilute less or omit dilution; for larger volumes, dilute more.

- 27 Determine titer of the diluted phage stock and record PFU/mL.

- 28 Aliquot working stocks into labeled cryovials and store at 4°C.

Note: This yields a homogenous, high-titer phage bank (up to 10^{10} – 10^{11} PFU/mL) suitable for laboratory assays.



Protocol references

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