**OBJECTIVE**
To amplify the phage as a high titer lysate.

**BACKGROUND**
A plate lysate is simply a liquid sample obtained by infecting a plate of bacteria with the phage of interest, letting the phage lyse the cells, then adding buffer directly to the plate surface to collect the phages. High titer phage lysates will yield sufficient quantities of DNA for sequencing. Plate lysates are the standard for long-term storage of a phage sample. Their long shelf-life at 4°C is years, if uncontaminated, and their reasonably high concentration make them useful. They are generally made from purified phage for use in experiments, but can be made as soon as a phage has been identified for archiving purposes.

**APPROXIMATE TIME NEEDED**
- Setting up the plates: **45 minutes**
- Allowing for phage to diffuse into lysate: ~**4 hours**
- Collecting lysate: **10 minutes**

**MATERIALS NEEDED**
**Equipment**
- Pipette Gun or equivalent with appropriate size pipettes
**Consumables/Reagents**
- Agar plates with a “Web Pattern” of phage
- Phage Buffer w/ 1mM CaCl₂
- Syringe with .22 μm syringe filter

**HELPFUL TIPS**
- Filtering the lysate helps prevent later contamination of the stock. Don’t skip this step!
- The highest-titer lysates will come from plates where individual plaques are nearly still visible, but are so densely packed as to cover the whole plate (a “web” pattern). This is an indication that several rounds of phage infection and lysis (and thus amplification) have taken place. If a plate is completely cleared it may mean that all bacteria were killed before multiple rounds of infection could occur, and the yield will be lower. If plaques are visible but sparse, enough rounds of infection probably occurred, but fewer phages are available for harvest. In either case, adjust the volume of phage initially added, and re-plate.
- Smeg’s doubling time is ~3 hours. That means that the bacteria will continue to grow for 30 – 36 hours on a typical lawn infection. Allow your lysate plates to incubate 30 – 36 hours (up to 48 hours) to obtain maximum phage yield.
• This protocol is written for a typical "small" plate (100 mm diameter), but lysates can also be made in the same way from "large" (150 mm) plates.

• Any plate of plaques will produce a lysate. To ensure that your phage live another day, you need to have a lysate to propagate the phage. Once you have a phage lysate, you can enter your phage into the database. (See PURIFICATION: Entering a Phage into PhagesDB.org.)

PROCEDURES

1. Once a titer is established, you can now manipulate the concentration so that you are putting enough phage on the plate to obtain a maximum phage yield. The goal of the empirical test based on the titer calculation is to determine the dilution of lysate necessary to form a web pattern of *M. smegmatis* growth (the appearance of a nearly cleared plate). This web requires about 6000 plaques per plate for an average-sized plaque. For very large or very small plaques, this number should be adjusted (<6000 for large plaques and >6000 for small plaques).
   a. 6000 plaques per plate / 1 x 10¹¹ plaques per ml = 6 x 10⁻⁸ mL lysate per plate
   b. 6 x 10⁻⁸ mL lysate per plate = 6 x 10⁻⁴ µL lysate per plate
   c. Based on the above calculations, add 6 µL of a 10⁻⁵ dilution of the lysate per plate.
      Depending on your pipetting technique, you may choose to add 60 µL of a 10⁻⁶ dilution instead.

2. We recommend that you ‘box’ your calculated amount of phage, because phages don’t do the math. Over-crowding of phage on a plate can modify how many phages are necessary to create the best yield. Empirically testing the number is necessary when amplifying the phage.

3. Once you obtain a plate (or plates) with the desired web pattern, add 5.0 mL Phage Buffer w/ 1mM CaCl₂ and swirl gently.

4. Let sit at room temperature for about 4 hours, or overnight at 4°C and then one hour at room temperature. Occasionally swirl the phage buffer gently to encourage mixing and diffusion, but do not splash.

5. When ready to collect, tilt the plate slightly by placing one edge on top of its lid to encourage the sample to pool on one side.

6. Using sterile technique, remove the buffer (now with phage) with an appropriate syringe.

7. Aseptically attach a 0.22 µm syringe filter to the tip of the syringe.

8. Filter the sample by pushing down the syringe plunger, collecting the filtrate in a 15 mL conical tube.

9. Label the tube with the phage name, your name/initials, sample type (small plate filtered lysate) and the date. You can now titer your phage lysate.