



# Phagehunting Program

## Outline of Procedure for the Isolation and Purification of a Phage Stock

### I. Sample Collection and Preparation

1. Collect environmental samples in 15-50 ml conical tubes
2. If solid, add phage buffer with 1mM CaCl<sub>2</sub> and vortex
3. Remove 1 ml aliquot and spin to pellet debris
4. Filter sterilize supernatant with 0.22 μ filters

### II. First Round of Infection: Plaque Screening

1. Infect 0.5 ml *Mycobacterium smegmatis* mc<sup>2</sup>155 with 50 μl of filtered sample
2. Add 4.5 ml MBTA top agar/CaCl<sub>2</sub>
3. Plate on 7H10/CB/CHX/ADC/CaCl<sub>2</sub>
4. Incubate at 37° C overnight
5. **Next day: Check for plaques**

### III. Spot Test for Verification of Putative Plaques

1. Pick putative plaques into 100 μl phage buffer/Ca Cl<sub>2</sub>
2. Mix 0.5 ml *M. smegmatis* mc<sup>2</sup>155 with 4.5 ml MBTA/CaCl<sub>2</sub>
3. Plate on 7H10/CB/CHX/ADC/CaCl<sub>2</sub>
4. Allow top agar to dry completely
5. Spot 5 μl of each sample into appropriate labeled grid box
6. After spots dry, incubate at 37° C overnight
7. Check spot plate for plaques

### IV. Additional Rounds of Infection for Plaque Purification

1. Use a pipette tip to pick a single plaque into 100 μl phage buffer plus calcium
2. Serially dilute sample to 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>
3. Infect 0.5 ml *M. smegmatis* mc<sup>2</sup>155 with 10 μl of each sample dilution
4. Add 4.5 ml MBTA/CaCl<sub>2</sub>
5. Plate on 7H10/CB/CHX/ADC/CaCl<sub>2</sub>
6. Incubate at 37° C overnight
7. Twenty-four hours later, many plaques should be visible
8. Repeat steps IV-1 through IV-7 several times to isolate and purify a single phage

### V. Final Plaque Purification

1. Use a pipette tip to pick a well-isolated plaque from one of the dilution plates into 100 μl phage buffer plus calcium
2. Serially dilute phage sample to 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>

3. Infect 0.5 ml *M. smegmatis* mc<sup>2</sup>155 with 10 µl of neat (undiluted) and dilutions
4. Add 4.5 ml MBTA
5. Plate on 7H10/CB/CHX/ADC/CaCl<sub>2</sub>
6. Incubate at 37° C overnight

## VI. Plate Lysate Production

1. To a nearly cleared plate, add 4.5 ml phage buffer/CaCl<sub>2</sub>, swirl gently
2. Let sit at room temperature for 2-3 hours
3. Siphon liquid lysate and filter through a 0.22 µM filter

## VII. Titer Determination

### “Quick and Dirty” Spot Tests

1. Mix 0.5 ml *M. smegmatis* mc<sup>2</sup>155 and 4.5 ml MBTA/CaCl<sub>2</sub>
2. Plate on 7H10/CB/CHX/ADC/CaCl<sub>2</sub>
3. Serially dilute lysate 10<sup>-1</sup> through 10<sup>-10</sup> in phage buffer/CaCl<sub>2</sub>.
4. Spot 5 µl of each dilution on the plate after it has cooled and dried
5. After the spots evaporate, incubate at 37° C overnight
6. Calculate titer and compare to small plate results.

### Small plate titer

1. Serially dilute lysate 10<sup>-1</sup> through 10<sup>-10</sup> in phage buffer/CaCl<sub>2</sub>.
2. Infect 0.5 ml *M. smegmatis* mc<sup>2</sup>155 with 10 µl of each dilution.
3. Add 4.5 ml MBTA
4. Plate on 7H10/CB/CHX/ADC/CaCl<sub>2</sub>
5. Incubate at 37° C overnight
6. Review your plates at 24 hours and choose a plate with between 20 to 200 plaques. Since the counting error is proportional to the square root of the number counted, the greater number that you count the smaller, the error. The uncertainty in a count of 16 is ± 4 plaques with an error of ± 25%. The uncertainty of 169 plaques is ± 13 or ± 7.7%. At the other end, it becomes cumbersome to count more than 300 plaques on a plate without losing count, so again you begin to introduce a significant counting error into the mix.
7. Calculate titer and compare to “quick and dirty” results.

## IX. Empirical Test of Lysate Concentration

1. Use the titer to calculate amount of phage lysate necessary to infect one large plate
2. Choose additional volume/dilution combinations of lysate to test which dilutions will form a web effect of plques that almost cover the bacterial lawn.
3. Serially dilute lysate to create necessary dilutions
4. Infect 1 ml *M. smegmatis* mc<sup>2</sup>155 with each volume/dilution combination
5. Add 9 ml MBTA/CaCl<sub>2</sub>
6. Plate on large-size 7H10/CB/CHX/ADC/CaCl<sub>2</sub>
7. Incubate at 37° C overnight
8. Determine lysate volume/dilution that yields the best web effect of *smeg* growth

## X. Big Plate Infection

1. Calculate volume/dilution of lysate needed to infect 30 plates based on your empirical test
2. Prewarm 30 large 7H10/CB/CHX/ADC/CaCl<sub>2</sub>
3. Infect 30 ml *M. smegmatis* mc<sup>2</sup>155 with appropriate amount of freshly diluted lysate
4. Make 300 ml 0.35% MBTA/CaCl<sub>2</sub>

5. Add 300 ml top agar to infected *M. smegmatis* mc<sup>2</sup>155 and swirl
6. Quickly plate on large-size 7H10/CB/CHX/ADC/CaCl<sub>2</sub>
7. Allow plates to harden, then incubate at 37° C overnight