



PREPARATION



ISOLATION



PURIFICATION



EXTRACTION



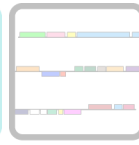
CHARACTERIZATION



SEQUENCING



ANNOTATION



PHAMERATION



FURTHER DISCOVERY

## Phage Sample Preparation for Electron Microscopy

### OBJECTIVE

To prepare phage sample for electron microscopy.

### BACKGROUND

The grids electron microscopy grids need to have little or NO charge, so that the EM stain (uranyl acetate) behaves correctly.

### APPROXIMATE TIME NEEDED

~20 minutes

### MATERIALS NEEDED

#### Equipment

- Micropipettor

#### Consumables/Reagents (See online media preparation guides)

- Appropriate micropipette tips
- Negative control plate
- 10-fold dilutions of phage

### HELPFUL TIPS

- The best phage pictures are be taken from a freshly CsCl banded sample. This alternative should only be considered if a CsCl band could not be obtained.
- Fresh samples are imperative to good pictures. Spots that are less than 48 hours old and are immediately put on grids and examined seem to contain numerous (100 – 200 per grid square) intact particles without as much of the cell debris as seen from a high-titer lysate. Spots that are not webbed will not yield nearly as many particles.

### PROCEDURES

1. Make a negative control plate (cells + top agar). Let dry.
2. Make ten-fold serial dilutions of the high titer phage stock in phage buffer + 1mM CaCl<sub>2</sub> (See **TOOLBOX: Making Serial Dilutions**).
3. Spot 5 – 10 µL of each dilution, including the neat stock with cells. Let the spots dry **fully**.
4. Incubate plates at 37°C overnight.
5. Identify the “webbed” spot, which contains the most number of plaques.

6. Carefully pipette 10  $\mu\text{L}$  of phage buffer onto the webbed spot. Wash the spot by VERY carefully pipetting up and down 7 – 10 times. **DO NOT** poke the agar or wash the surrounding lawn of cells.
7. Use 5  $\mu\text{L}$  of this on a glow-discharged grid. Stain with 1% uranyl acetate.
8. Observe the grid using the Electron Microscope. Refer to the Mycobacteriophage Database ([www.phagesdb.org](http://www.phagesdb.org)) for examples of EM photos.