Protocol: Making plate lysates

Plate lysates are the most efficient means of making small volumes (< 50 ml) of high-titer phage stock, sufficient for long-term storage or DNA isolation. If you have a large number of phage isolates, typically a single plate of lysate is enough to make a 5-6 ml "founder" stock of your isolates. For DNA extraction, 4-5 plates are sufficient. A "well-behaved" phage will achieve titers of about 10^{10} PFU/ml from plate lysates.

Materials required:

- Host culture
- SM buffer (100 mM NaCl, 25 mM Tris-HCl pH 7.5, 8 mM $MgSO_4$, 0.01% (w/v) gelatin) or sterile liquid culture medium
- Petri plates containing solid culture medium appropriate for the host bacterium
- Aliquots of molten soft agar for overlays (4 – 5 ml each, enough to cover one plate), held at ~50 ºC in a heat block or water bath
- Tube racks, pipettors, sterile tips
- Aseptic working area (near a flame or in an aseptic cabinet)

Setting up plate lysates

1. Label 1-5 plates for each phage isolate you will be propagating. The number of plates depends on how much lysate you need, but generally 1 plate will yield about 6-7 ml of lysate. Label the plate(s) with the phage isolate name.
2. Aliquot 4 ml of top agar into glass tubes and keep in the heat block. Make enough aliquots depending on how many plates per phage isolate you will be propagating.
3. In a second set of glass tubes, add 100 µl of host culture and 100 µl of appropriately diluted phage. Mix briefly.
    a. Ideally, a plate lysate should be inoculated with enough phage to cause nearly-confluent lysis: the plate should be covered in plaques, with just a lacey remnant of the lawn visible. This usually translates into 10^4 – 10^5 PFU/plate for a standard 10-cm Petri plate, depending on the size of the plaques. Therefore, it is helpful to know at least the approximate titer of the phage stock or pickate you are using to inoculate the plate lysates. If you do not know, make your best guess on the phage concentration, remembering it is better to be too high than too low.
4. Pour a top agar aliquot into a tube containing the host culture/phage mixture, vortex briefly and pour over the appropriately labeled plate. Allow the lawns to set for 5 min, then invert and incubate as appropriate for your host organism.

Harvesting plate lysates

1. Place the plate face-up and, using a pipette, flood the plate with 6-8 ml of broth or SM buffer. Use the pipette to GENTLY disrupt the lawn surface and then aspirate the resulting slurry back into the same pipette. Be tidy! Don't slosh the

lysate out of the plate! You only want to disrupt the soft top agar layer…do not gouge into the firmer bottom agar layer.

2. Place the lysate into a clean 15 ml Falcon tube and label it with the isolate name.
    a. To avoid cross-contaminating your phage isolates, flood plates and transfer the slurry to tubes one isolate at a time. Periodically wipe the work surface with sanitizer or EtOH.
3. Centrifuge the lysates at 8000 x g, 10 min.
4. Filter sterilize the phage-containing supernatant.
    a. Remove a 10 ml syringe from its wrapper and remove the plunger from the syringe; place the syringe barrel on the bench and stand the plunger upright.
    b. Place a 0.22 µm syringe filter onto the syringe barrel, and twist until it is locked on securely.
    c. Pour the supernatant lysate into the syringe barrel, avoid disturbing the pelleted agar and cell debris.
    d. Holding the tip of the syringe over a new tube, place the syringe plunger back into the barrel, and press to force the phage lysate through the filter.
    e. Label the new tube containing the filter-sterilized phage lysate with the isolate name and date. Store at 4 ºC.

Materials

PYE broth	6 x 100 ml bottles
PYE plates	4 sleeves
PYE top agar	6 x 50 ml bottles, melted
CB15 o/n culture	4 x 4 ml
Sterile t-top tubes	150
10 ml syringes	80
0.22 µm syringe filters	80
10 ml pipettes	80
15 ml Falcon tubes	3 packs

Swing-bucket centrifuge