Protocol: Plating out phage

Materials required:

- Host culture
- Phage stock, serially diluted in SM buffer (100 mM NaCl, 25 mM Tris-HCl pH 7.5, 8 mM $MgSO_4$, 0.01% (w/v) gelatin) or in sterile liquid culture medium
- Petri plates containing solid culture medium
- 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)

I. Spot titration of phage

1. Pour a bacterial lawn
    a. Label the bottom surface of a culture plate with the phage and lawn strain names, and mark the positions where phage dilution spots will be deposited.
    b. Place the plate face up near a flame or in an aseptic cabinet.
    c. Retrieve a top agar aliquot from the water bath.
        i. The agar will start to solidify when it cools to below about 40 ºC, so you must work quickly for this procedure.
    d. Transfer 100 µl of the host culture into the molten agar.
    e. Vortex the molten agar on medium speed, briefly (about 1 second)
    f. Open the plate and pour the contents of the tube onto the agar surface. Swirl the plate gently to distribute the molten agar across the plate.
    g. Replace the plate lid, and allow the plate to sit undisturbed for a few minutes to let the agar solidify.
2. Apply phage spots to lawn from serial 10-fold dilutions
    a. Place the plate containing the bacterial lawn you prepared in step 2 face up. Open the lid and set it aside.
    b. Aspirate 10 µl of the lowest phage dilution and deposit it over the corresponding label marked on the bottom of the plate.
    c. Repeat this procedure for each of the phage dilutions, depositing a 10 µl drop of diluted phage over its corresponding dilution marked on the bottom of the plate.
    d. When done, leave the lid off of the plate and allow the plate to dry near a flame or in an aseptic cabinet until the drops are absorbed, about 10-15 min.
    e. Incubate the plates, inverted, at the optimal growth temperature of the host until plaques appear in the lawn. The lower phage dilutions will yield confluent spots of lysis, which should give way to individual plaques in the spots at higher dilutions.
        i. Individual plaques may be counted in these spots but be aware that the counting error will be high.

II. Full-plate titration of phage

1. Determine the approximate titer of your phage stock, by spot titration or previous experience. Determine which serial dilution of your phage stock will yield ~50 to 500 plaques in one 100 µl aliquot. Use this dilution, the dilution above it and the dilution below it for plating.
2. Prepare 3 plates by labeling them with the bacterial strain name, the phage name and the dilution to be plated. Place them face-up near the working area.
3. Place 3 empty sterile top agar tubes into a rack, label them with the three dilutions you will be plating.
    a. Aliquot 100 µl of the host culture into each tube.
    b. Aliquot 100 µl of the phage dilutions you will be plating into their corresponding tubes containing host culture. Vortex the tubes briefly to mix.
4. Retrieve one molten agar aliquot from the water bath.
5. Pour the aliquot from its tube into one of the tubes containing the host cell-phage mixture, vortex briefly, and pour the contents onto the appropriately labeled plate. Swirl gently, replace the lid and allow to sit undisturbed until the lawn sets.
    a. Repeat this step with the other dilutions.
6. Incubate the plates, inverted, at the optimal growth temperature of the host until plaques appear in the lawn.