Protocol: Subculturing phage with paper strips

Environmental enrichments or other phage mixtures must be purified to produce pure phage isolates suitable for characterization. Analogous to bacteria, each phage plaque is presumed to be clonal, having originated from a single virion. The picking and subculturing of plaques ensures that a phage population is descended from a single virion and is therefore clonal, or "pure". Streaking the phage on bacterial lawns is an efficient way of isolating single plaques from a phage suspension. As a general rule, phages should be subcultured (a plaque picked, streaked out and picked again) three times to ensure it is pure.

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 in sterile liquid culture medium
- Sterile paper strips
    - Many types of paper will work for this method, try using a heavy bond document paper or Whatman #1 filter paper. Cut the paper into strips of about 1 x 10 cm and autoclave to sterilize.
- 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)

Making "pickates":

1. Using standard aseptic technique, pipet 1 ml of sterile broth or SM buffer into sterile microcentrifuge tubes. Make one tube for each plaque you want to subculture. Close each tube to minimize airborne contamination.
2. First, on the back of a plate, circle and label the plaque you have chosen. Record in your notes the general morphology of the plaque (e.g., big, small, clear, turbid). Punch out the plaque using a sterile Pasteur pipette with a rubber bulb attached. Try to punch out the soft agar of the entire plaque; it does not matter if you also get some of the surrounding lawn, as long as you do not touch another plaque. Blow the plaque out into one of the tubes containing the 1 ml of diluent. Rinse the tip several times with the diluent by gently squeezing and releasing the bulb, but try not to blow too many bubbles (air can denature phage proteins). Close the tube and discard the pipette. Label the tube on the lid appropriately. Vortex the tube briefly, and store at 4°C.

Guidelines for choosing the plaques:

- First, examine your plates. If possible, pick plaques with different morphologies (plaque size, turbidity, "sharp" edged vs "fuzzy"). There is usually not much use in picking many identical-looking plaques from a single sample, as these are likely just clones of the same phage. Also, some phages produce plaques with

heterogeneous morphology, e.g. big and small plaques. Take note of the morphology of each plaque you pick and see if it looks the same ("plates true") in subsequent subcultures.

- If you have a choice, choose plaques which are the farthest away from other plaques. (This is to minimize cross-contamination of plaques; the virions are diffusing through the agar even as you are picking them!)

Streaking out plaques:

1. For each pickate you have, label a plate on the back, appropriately. Also, mark an X to indicate the initial spot for the pickate. Then use each labelled, marked plate to prepare a lawn (soft agar overlay, see the protocol *Plating out Phage*) of your indicator bacteria. This is done by adding 100 µl of a bacterial culture to a 4 ml aliquot of molten top agar in a small glass tube, vortexing and pouring over the agar surface of the plate.
2. Allow the lawn to solidify for ~5 min.
3. Spot 10 µl of the pickate over the spot marked by the X. Avoid touching the agar; just let the droplet touch it. It should transfer cleanly to the lawn. Allow this to soak into the lawn for ~5 min.
4. Choose a sterile paper strip from a glass tube. Holding it at the end that was sticking out of the tube, observe the two points at the other end. You can usually discern whether the corner tips are "uppies" or "downies". The idea is to dip one of the corner tips into the spot (marked X) where you deposited some of the pickate and then drag the (hopefully) phage-bearing tip across the surface of the soft agar, just like using a toothpick or sterile loop to drag bacteria from a colony across the surface of an agar plate. If you use a "downie" tip on the soft agar, it will gouge the agar and create micro-canyons where the phage will have a hard time making a nice plaque. So if you have an uppie, use that corner to dip into the X spot and then drag it carefully along the surface in several parallel paths. (You can always turn the paper upside down to make downies into uppies!)
    a. Now, take the other corner tip, which has not touched anything yet, and do a cross-streak across the first streaks, at approximately a right angle. Then take a new sterile paper strip and do a few more streaks at right angles to the second streaks. Hopefully, this will dilute the pickate enough to where single plaques will be visible on the second or third streaks.
5. Incubate these plates as appropriate. Use a Pasteur pipette to make new pickates from an isolated plaque on these streak plates, using the same technique described above.