Phagehunting Program

Protocol: Checking Lysates for Lysogens

1. **Spot Test of Diluted Lysate**
   A. Make serial dilutions of your lysate from 10^{-1} to 10^{-10}. A 10^{-1} dilution is 9 parts phage buffer plus $CaCl_2$ and one part undiluted lysate. A 10^{-2} dilution is 9 parts phage buffer plus $CaCl_2$ and one part of the 10^{-1} dilution and so on.
   B. Put 0.5 ml *M. smegmatis* mc^2155 in a test tube. To this test tube add 4.5 ml 0.35% MBTA. Plate this mixture on 7H10/CB/CHX. Allow the liquid to cool and solidify, then spot 5 µl of each dilution so that none of the spots touch. Before moving on, allow this to dry so that the spots evaporate completely on the plate.
   C. Invert and incubate your plates at 37° C overnight.

2. **Purifying the Lysogen**
   A. With a sterile inoculating loop, pick a nearly cleared section from your spot test. Be sure not to touch the lawn of mc^2155 with the loop. Use the clear spot made with the most dilute sample possible.
   B. Streak this out on a new 7H10/CB/CHX plate. To streak, start at the top of the plate and move

back and forth horizontally until you are about one third of the plate. Heat your loop and cool it. Then move vertically on the lower left side of the plate until you reach the middle. Heat the loop again and then cool it Next make a 3rd pass. With each pass,, the number of cells will decrease, hopefully spreading far enough away from each other that isolated colonies form.

Refer to www.qiagen.com/literature/qiagennews/0598/985theqi.pdf for more information.

   C. Incubate the plate at 37° C for as long as needed (probably 3-4 days).
   D. If there is bacterial growth, use a sterile inoculating loop to pick a well-isolated sinlge colony.
   E. Repeat steps B, C, and D several times (3-5) to ensure that there is nothing but lysogen on your plate. (You are streaking for isolation of a pure colony.)

3. **Inoculating the Lysogen**
   A. With a sterile inoculating loop, pick a well isolated singlecolony from the most recent streaking. Swirl this into a test tube with 2 ml of 7H9/Tween/CB/CHX/ADC/$CaCl_2$. Vortex to ensure that there are no clumps of bacteria. Incubate at 37° C overnight or until there is noticeable growth.

B. Subculture this lysogen 1:50 to1:1000, (depending on how long you want it to grow before using it, several hours later that day or overnight) in 5 ml 7H9/CB/CHX/ADC/Ca. Shake at 37ºC.

4. Testing for immunity

A. Dilute your phage and an unrelated mycobacteriophage (for control) 10^{-1} to 10^{-9}.
Spot on both pahges lawns of both *M. smegmatis* mc^2155 and on the putative lysogen. Also spot some of the lysogen culture supernatant (see below) on booth lawns.

5.Testing for phage release

A. Spin 1 ml of the culture of lysogens at 14K RPM for 1 minute to pellet the cells.
B. Transfer the Supernatant to a fresh tube and repeat A.
C. Spot the dilutions of this supernatant on prepared lawns of *M. smegmatis* mc^2 155 and your lysogen.