Protocol 5.3: Plaque Assay for Direct Isolation

1. Add 500 microliters of the direct isolation sample into 250 microliters of the bacterial host sample *Mycobacterium smegmatis* using a micropipettor
2. Tap tube and let sit for 7 minutes
3. Remove a bottle of agar from the 55° C bath
4. Using a 5 ml pipette, remove 3 ml of top agar and insert into tube including the host bacterium and phage sample
5. Immediately aspirate mixture and transfer onto plate
6. Gently jiggle plate to spread agar

For negative control:

1. Add 10 microliters of phage buffer into bacterium host sample tube
2. Tap tube and let sit for 7 minutes
3. Remove a bottle of agar from the 55° C bath
4. Using a 5 ml pipette, remove 3 ml of top agar and insert into tube including the host bacterium and phage sample
5. Immediately aspirate mixture and transfer onto plate
6. Gently jiggle plate to spread agar

Let plates sit undisturbed for 15 minutes to solidify. Invert plates and incubate at 37° C starting at 2:25 on 9-7-17.

Day 2: no plaques were seen.