Preparing a lawn of bacteria

Plaques form when bacteria are immobilized in a lawn of top agar while phages can slowly diffuse, leading to the formation of a plaque from a single phage-infected cell. Top agar is low concentration agar medium (typically 0.5-0.7% agar).

Once your plate has been labeled and marked you are ready to pour your lawn. For each lawn, you will require 4 mL of top agar that has been melted by boiling and then kept at 55°C. Note that 55°C can kill many host bacteria so you must work quickly - get your working zone set up so you can work efficiently. And once you have poured the top agar lawn it will start to set in a few seconds. You should be able to complete the following operation in a little under 10 seconds.

Attach a fresh 200 µL tip onto your P200 pipettor and aspirate 100 µL of bacterial culture. Take a top agar aliquot and eject the culture into the agar. Vortex the tube for a few seconds to evenly distribute the cells while replacing the pipettor on its holder. Open the lid of the Petri dish and quickly, but smoothly, pour the mixture over the solid agar surface. Your main objective is not to introduce bubbles into the lawn either from the vortexing or pouring steps. Replace the lid of the Petri dish, and put the empty tube back in the hotblock, then gently swirl the plate for 2-3 seconds so that the molten agar forms an even layer. If there are any bubbles in the top agar they can be removed with a sterile pipet tip, but working quickly before the agar is set. Alternatively, once the top agar is set, the location of any bubbles can be marked on the underside of the Petri dish.

Set the plate to one side and allow the top agar to set. About 5 minutes, but be careful when you check - gently lift one side of the plate very slightly and if you see any movement of the top agar then place the plate back flat on the bench and wait a few more minutes. Be careful because if the partially set agar moves too much the lawn will break apart and the plate will have to be discarded.

Notes:

The cell inoculum volume can be adjusted as required.

This same technique is used when phages and cells are premixed, such as in whole plate titering. Be sure to adjust the inoculum volume so to allow for the required volumes of both cells and phage.