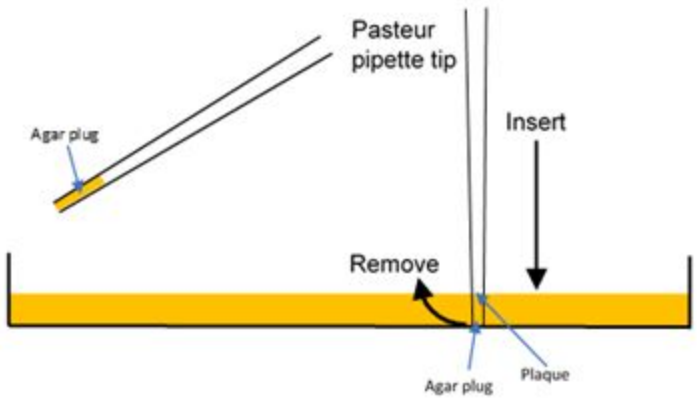
Making a pickate

A pickate is when a plaque is ‘picked’ from a Petri dish and resuspended in buffer. As such it is usually the first sample of a newly isolated phage and is used to generate larger volume stocks for any subsequent work.

To make a pickate, first identify which plaque(s) to pick. Remember that the visible edge of a plaque is not where the phages end, they can diffuse farther into the lawn than the plaque boundary. So choose a plaque that is as well separated from other plaques as possible. Mark the plaque by drawing a circle around it on the underside of the plate. Label two tubes for each plaque to be picked and add 1 mL of λ-dil buffer to one of each pair of tubes.

Take a sterile Pasteur pipette and attach a bulb to the top. Now hold the pipette like a pencil, with your fingers not too far from the tip. Take the lid off the Petri dish and hold the plate up to the light so that you are looking directly through the lawn. Take the Pasteur pipette and push it into the plaque, perpendicular to the lawn, so that the tip encompasses the center of the plaque as much as possible. Take care with small plaques that the pipette tip surrounds the plaque, it can be easy to miss them entirely.

To remove the plaque with the pipette tip the seal between the base of the agar plug and the Petri dish must be broken. Do not remove the pipette from the lawn in the perpendicular direction, instead move the tip very slightly along the base of the dish, for no more than a couple of millimeters, before removing the tip.



The Pasteur pipette tip should be inserted into the agar perpendicular to the lawn, but then removed with a slight sideways movement to detach the agar from the dish. If there are other nearby plaques, it can be useful to note the direction you want to remove the tip when you mark the plaque(s) on the underside of the dish.

Check the tip visually to see if the plug is present. Place the tip just into the very top of the λ-dil buffer of a labeled microcentrifuge tube and gently squeeze the bulb to eject the plug. Pump a small volume of buffer in and out of the tip to flush out any residual phages. Discard the Pasteur pipette and keep the bulb. Close the microcentrifuge cap and vortex the tube for 1 minute to resuspend the phage particles.

To filter sterilize your pickate, take a 5 mL Luer-lock syringe and a sterile 0.2 μM syringe filter. Completely remove the plunger from the syringe and attach the filter. Then, using a P1000

pipettor, take the pickate from the tube and eject it into the syringe body. Place the filter tip into your fresh, labeled microcentrifuge tube and insert the plunger. Gently push down on the plunger to force the pickate through the filter. If the filter clogs, don't push too hard, change filters or discard any excess unfiltered pickate as needed.

Titer your pickate following the instructions here. Your pickate is now ready for use.