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Spot and Plaque Assay

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We use this protocol and it's working

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Abstract

This is a series of mini protocols for doing spot assays, plaque assays, and phage purification through plaque picking. This also has notes on how these different protocols can be used to measure phage titer.

Spot Assay

1 *Spot assays are usually performed to determine phage titer quickly and with minimal plates. It is not as accurate as doing a plaque assay to determine PFU. A spot assay can also tell you if there are phage in an environmental sample (either a direct or enriched sample).*

2 **Plate the bacteria** 30m
In a small test tube, transfer 100µL of bacteriophage to a test tube and using the pour plate method from the Amplifying Bacteriophage protocol (dx.doi.org/10.17504/protocols.io.36wgqpjdovk5/v1), plate the bacterial culture.

3 Wait for the top agar to solidify and label your plate with little dots. A 4×4 grid is usually the maximum amount you can fit onto a plate, but sometimes more can be applied.

4 **Spot the sample**
5-10µL of sample can be pipetted for each spot.

If you are determining a quick phage titer, perform a 10-fold dilution using SM buffer, and spot the dilutions onto the plate.

5 **Incubate** 1d
20˚C 24hrs

Plaque Assay

6 *Plaque assays are performed similarly, but instead of spotting, the phage will be incubated and spread across the entire pour plate.*

7 **Incubate Bacteria and Phage**
100µL of overnight culture
100µL of bacteriophage culture
You will need to adjust this accordingly based on the phage titer.
Time of incubation will also be dependent on the phage: ~00:10:00-00:30:00

8 **Plating the phage**
Take the 200µL phage and bacterial mix and mix with 3mL top agar (protocol here: dx.doi.org/10.17504/protocols.io.36wgqpjdovk5/v1) - pour immediately onto T-plate
Sit at room temperature to solidify

9 **Incubate**
20˚C 24hrs

Using a plaque assay to perform phage purification

10 **Plaque purification for isolation of one phage type**

Phage can diffuse to other parts of the plate, phage freshly isolated from environmental samples may not be a pure phage. Meaning that multiple different phage may be int the sample. Therefore, it is important to undergo a phage purification process by finding lone plaques with distance from other plaques to purify.

11 **Isolating the plaque pick**

It is important to pick a plaque pick that is a distance away from other plaques. Sometimes the phage titer can be too high from the environmental sample and the sample must be serial diluted before plating. This is the same for plaque picks. Alternatively, a smaller sample portion can be incubated with the bacteria.

"Cookie cutter technique"

Cut 1,000 µL pipette tips are useful to stab the agar with the plaque pick. If you use a razor blade to cut off the tips and then autoclave them, they will be sterile.

Place plaque pick in buffer

500µL SM buffer
1 plaque pick
Vortex (with caution because vortexing can destroy phage)
Alternatively, let the phage in the plaque pick diffuse into the buffer overnight at 4˚C

12 **Incubate Bacteria and Plaque Pick**

100µL of overnight culture
100µL of plaque pick
You will need to adjust this accordingly based on the phage titer of the plaque picks.
Time of incubation will also be dependent on the phage: ~00:10:00-00:30:00

13 **Plating the phage**

Take the 200µL phage and bacterial mix and mix with 3mL top agar (protocol here: dx.doi.org/10.17504/protocols.io.36wgqpjdovk5/v1) - pour immediately onto T-plate
Sit at room temperature to solidify

14 **Incubate**

20˚C 24hrs

15 This process should be performed 2-3 times with the new plate of your previous plaque pick in order to get a purified phage.