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Enriching and isolating phages on agar plates

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We use this protocol and it's working

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Abstract

This protocol explains how we isolate phage from microbial communities on solid agar plates. For a protocol on liquid enrichment and isolation, check out our **companion protocol**.

Troubleshooting

Solid-plate phage isolation

1 Set up cultures of your target host strains. We use this protocol for cheese bacterial isolates, which grow best in LB at 25 °C with shaking at 200 rpm, and reach high density after two days.

2 Prepare your phage extracts to infect your host strains. There are a couple different ways to go about setting up your experiment:

- If you have one extract per strain that you want to test, we recommend using between 10–100 µL of phage and pre-infecting the bacterial culture before plating it to spread plaques across the full plate. (See **pre-infection** step-case below)

- If you have many extracts per strain and you do not care about which plaques come from which source, you can pool your phage extracts and use 10–100 µL of pooled phage per host. You can then use this pooled extract to pre-infect the bacterial culture before plating it to spread plaques across the full plate. (See **pre-infection** step-case below)

- If you have many extracts per strain and you want to be able to track which plaques come from which source, we recommend preparing a 96-well plate with each concentrated extract in one well, and using 3 µL of phage per host and spotting in a grid pattern across the plate. (See **spotting** step-case below)

STEP CASE

Pre-infection 5 steps

Pre-infect cells before plating them.

3 Add 10–100 µL of phage (pooled or individual extracts) to 200 µL of host cells.

4 Add the infected sample to 3 mL of molten (but not overly hot) top agar. Pour top agar onto a room-temperature LB plate, swirling to evenly distribute the agar across the plate before it cools.

Note

Top agar recipe
100 mL LB
0.25 g agarose (0.25%)
100 µL 1 M $MgSO_4$ (1 mM)

Autoclave to sterilize. Reheat to melt in the microwave, then make sure you let it cool to a point where it is warm to the touch but not scalding, or you will kill your bacterial hosts.

5 Incubate plates at room temperature. Plaques will show up in 24–72 hours.

6 Pick plaques using a pipette tip, and transfer into 300 µL of SM buffer with 50 µL of $CHCl_3$.

7 Purify these phages by diluting the picked plaque down to a concentration that will give single plaques, then infecting 200 µL of host culture with 10 µL of diluted phage, and finally plating out using the double agar overlay method. Repeat 2×.

Protocol references

Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. (2009). Enumeration of Bacteriophages by Double Agar Overlay Plaque Assay. https://doi.org/10.1007/978-1-60327-164-6_7