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Plaque Assay For Screening Viral Concentrates for Bacteriophage

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ABSTRACT

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

Culture Preparation

- 1 Grow a 4 mL liquid culture of the bacterial isolate of interest in an appropriate growth medium overnight

4 mL

- 2 Dilute the culture 10-20% and let it grow for ~4 hrs prior to plaque assay

04:00:00

- 3 Melt 0.6% top-agar in the microwave and let cool ~10-15 min

00:15:00

- 4 Pipet 1.5 mL liquid culture into 1.5 mL Eppendorf tubes (or enough liquid culture for 500 μ L for each plate sample that you want to make).

- 5 Centrifuge 2 min at 14,000 rpm

00:02:00

Note

Some bacterial isolates do not require centrifugation. This is something that needs to be empirically determined for each putative bacterial host.

- 6 Discard the supernatant

- 7 Resuspend the remaining cells in 1.5 mL BG-11 media and mix well with pipet

1.5 mL

- 8 Pipet 4 mL of molton top agar into sterile culture tubes

4 mL

- 9 Keep top agar in a 45°C heat block to keep in a liquid state
- 10 In 500 µL of resuspended cells, add 100 µL of 0.22 µm filtered viral concentrate
- 11 Incubate for 15 min at an appropriate growth temperature for the bacterium in use
00:15:00

Plaque Assay

- 12 Pipet infected cells into molten top agar
- 13 Vortex briefly avoiding bubbles
- 14 Pour top agar onto the plate and spread
- 15 Let agar solidify at room temperature ~30-60 min
01:00:00
- 16 Flip plates over and place in an incubator at an appropriate growth temperature and light conditions for the bacterium in use

17 Check plates daily until a confluent lawn appears on plates. Plaques will be obvious clearings.