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Spot assay

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We use this protocol and it's working

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Abstract

A variant of the plaque assay in which entire phage dilution ranges can be tested on a single plate.

Troubleshooting

1 Streak LB plate with E. coli (strain should susceptible to phage lambda. Strains that include a prophage lambda are likely to be resistant. Strains LE392 and DH10B have been used with this protocol) and incubate overnight at 37 °C

2 Pick a colony from this plate and use it to inoculate 10 mL of LB. Incubate this culture at 37 °C until OD reaches 2-3. Overnight culture is recommended.

3 Preheat LB agar plates at 37 °C . Store soft LB agar (LB agar with 0.7% agar) at 50 °C .

4 Make a phage dilution range in a 96 wells plate in LB medium. It is advisable to make a wide range of dilutions. 10^{-1} to 10^{-12} covers the range of possible titers.

5 Mix 200 µL of cell culture with 3 mL of liquid soft LB agar (50 °C).

6 Pour the mixture on the preheated LB agar plates and spread the mixture on the plate by moving it. Make sure to work quickly to avoid clumps of solidified agar. Incubate for 00:15:00 at room temperature.

7 Use a multichannel pipet with 8 channels to gently drop 3 µL drops of the phage dilution on the plates. 4 rows of 8 drops fit on a plate. Mark the plate beforehand to indicate the range of dilution. Be careful to not touch the plate with the pipet and to keep the plate steady. Avoid moving the plate to prevent the drops from spilling out.

8 Incubate the plates for 00:15:00 at room temperature . Check whether the drops have dried. If the drops are still clearly visible allow the plate to incubate for a longer time.

9 After the drops have dried, turn the plates over and incubate overnight at 37 °C .

10 Count the amount of plaques visible for the individual drops. Plaque Forming Units (PFU)/mL can be calculated with the following formula: PFU/mL = N × 1/DF × 1/V.
N is the number of plaques of lysis counted on the plate (expressed as PFU); DF is the dilution factor and V is the volume of phage dilution pipetted on the plate.