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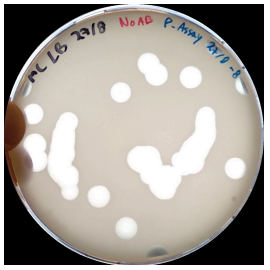
Plaque assay



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Marijn Ceelen[1]

[1]iGEM Wageningen

iGEM Wageningen 2019

Marijn Ceelen
Wageningen University

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Protocol status: Working
We use this protocol and it's working

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Abstract

This is a protocol for the quantification of phage lamba and T7 titers by counting plaques.

Materials

MATERIALS

Liquid LB medium

LB agar

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 Make a range of dilutions . Mix 100 µL of phage dilution with 200 µL of E. coli culture. Then incubate for 10 minutes at room temperature. After 00:10:00 add 3 mL of liquid soft LB agar (LB agar with 0.7% agar) (50 °C).

4 Pour the resulting mixture on LB plates (preheat plates at 37 °C). Spread the mixture on the plate by moving it. Make sure to work quickly to avoid clumps of solidified agar.

5 Incubate for 00:15:00 at room temperature. Afterwards turn the plates over and incubate overnight at 37 °C .

6 Use plates with 30-300 plaques to determine phage concentration. Calculate the Plaque Forming Units (PFU)/mL by 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 poured on the plate.