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Plaque assay T7 bacteriophage

Forked from a private protocol

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Duncan Whyte: Improved and adjusted protocol



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Plaque assay

- [Marijn Ceelen](#)
- iGEM Wageningen

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We use this protocol and it's working

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Abstract

This is a protocol for the quantification of T7 in a sample in Plaque Forming Units (PFU) per mL.

Image Attribution

Protocol image: Marijn Ceelen, iGEM Wageningen; from original protocol.

Phage safety work area image: Duncan Harry Whyte, iGEM TU Delft 2025

Materials

MATERIALS

 Liquid LB medium

 LB agar

Prepare a safe phage work area.

- 1 To avoid contaminating other lab work with phages, take the required measures. Always make sure to wear gloves when working with phages. Designate a certain lab bench area for phage work. Use a special phage incubator. Prepare and maintain *Virkon* to decontaminate the phage area after all work. Use filter tips for pipettes, and designate pipette(s) (required range:  11 μL -  111 μL) to remain phage-work pipettes. We recommend using fluorescent tape to mark pipettes and other phage-contaminated equipment.
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Here is an example of the phage area we used.





Create an *E. coli* culture

- 3 Streak LB plate with *E. coli*, e.g. BL21(DE3) and incubate overnight at  37 °C  
- 4 Pick a colony from this plate and use it to inoculate  3 mL of LB. Incubate this culture at  37 °C until the OD reaches 2-3. We recommend an overnight culture.

Prepare LBa and LBa plates

- 5 Prepare enough plates with LB and agar, one for every dilution sample and one for the control. For a first assay, we recommend 6 samples (10^0 to 10^{-4} and a control). For assays where the rough PFU is known, you might be able to leave out some of these dilutions.
- 6 Preheat the plates to  37 °C . Consider labelling the plates in advance.
- 7 We recommend preparing molten soft (0.5%) agar LB at  50 °C , but you can also make it before it is needed. You'll need  3 mL per dilution sample, but we recommend using a bottle with more than enough and reusing whatever is left. 

Make a range dilutions, by a factor 10 each, and a control

- 8 Make a range of dilutions . Mix  100 μ L of phage dilution with  200 μ L 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).
- 9 Prepare the required number of tubes. We recommend using  15 mL Falcon tubes, generically called *Screw-top centrifuge tubes*. Label these with (recommended) a combination of coloured stickers (per different T7 sample) and a marker (for different dilutions). Label the dilution samples with 0, -1, -2, etc.
- 10 Add  100 μ L of ddH₂O (MilliQ) to the control. Add  100 μ L of ddH₂O (MilliQ) to all dilution samples **except for the first dilution, labelled 0. Do not add any ddH₂O to this sample.**



- 11 Enter the phage area. Using a pipette tip with filter, pipette  111 μL of the desired T7 phage sample into the sample labelled 0.
- 12 With filter tips, pipette  11 μL from the sample labelled 0 to the next sample, -1. Mix the sample, or gently vortex it.
- 13 Now repeat this previous step for every dilution. That is, take  11 μL from the previous dilution sample and add it to the next one. Then mix or gently vortex.
- 14 Finally, discard  11 μL from the final sample (or leave it in and adjust the calculation at the end). Now every sample should contain  100 μL of T7, reduced in concentration by a factor of ten compared to the previous sample.

Add the *E. coli* and pour the plates

25m

- 15 Now add  200 μL of the *E. coli* culture to every dilution sample and to the control. Use filter tips. Gently mix. Wait  00:10:00 . Do not wait more than a phage replication time (rather conservatively,  00:20:00 at room temperature). 10m
- 16 Add  3 mL of liquid soft LB agar (LB with 0.5% agar) ( 50 $^{\circ}\text{C}$). Mix and quickly pour the resulting mixture on the LB plates (preheated at  37 $^{\circ}\text{C}$). Note: this means the plates will have two layers, the first without any *E. coli* or T7 and the second with. Incubate for at least  00:15:00 or more at  Room temperature , to let the second layer harden. Turn the plates upside-down. 15m
- 17 Incubate  Overnight in a specialised phage work area incubator at  37 $^{\circ}\text{C}$.  
- 18 Find the plate with the most easily identifiable number of plaques, e.g. 10-100. Calculate the Plaque Forming Units (PFU)/mL by the following formula: $\text{PFU/mL} = N \times 1/V_{\text{diluted}} = N \times 1/DF \times 1/V_0$.
 N is the number of plaques of lysis counted on the plate (expressed as PFU).
 V_{diluted} is the total volume of the original phage sample added to this plate (equal to $DF \times V_0$)
 DF is the dilution factor of this plate (i.e. 10^x , where x is the label 0, -1, -2, etc.).  



V_0 is the volume of phage dilution of the first plate, i.e.  100 μL .

When comparing PFU/mL, remember to take into account the strain of *E. coli* used.