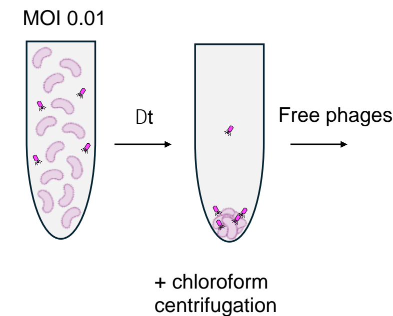
# ADSORPTION TEST

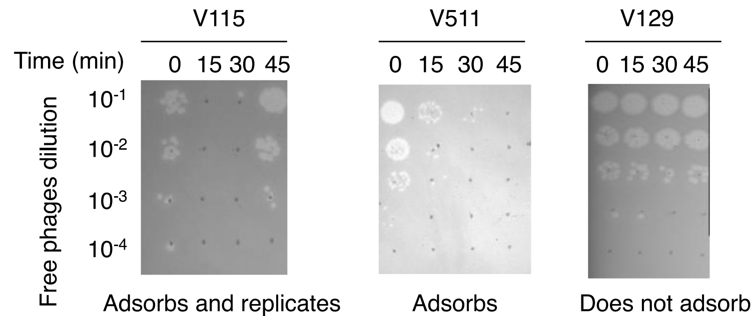
## Introduction

The initial stage of infection, termed the external stage, involves the adsorption of the phage to a receptor (polysaccharide or extra-membrane protein). This adsorption traps the phages on the surface of cells, leading to a decrease in the quantity of free phage in the medium. This phenomenon is measured in the assay described (refer to the figure below). Ideally, the assay is conducted at a low Multiplicity of Infection (MOI) to observe the complete disappearance of free phages. Following the necessary adsorption time (Left photo, 15 min), an increase in free phages can be observed (45 min), attributed to the release of progeny. This assay also allows for the detection of resistance mediated by intracellular mechanisms. In such cases, a decrease in free phages is observed (center photo, 30 min), but never an increase (45 min). Chloroform is employed to lyse the cells (thereby halting the assay); after centrifugation, the phages trapped on the membrane are found in the pellet.

**Method**




**Example of results**




## Materials

- Host bacteria
- Phage
- Marine Broth (MB) filtered with 0.2mM to eliminate the aggregates
- Marine agar (MA)
- Erlenmeyers (control phage alone ; bacteria plus phages at MOI 0.01)
- Tubes Eppendorf 1.5mL labelled
- 1 medium petri dish with MA to titrate the host bacteria
- 1 big petri dish with MA to titrate phages
- 20 mL of Top agar in MB (0.2% for the schizo phages ; 0.4% for all other phages)
- Chloroform

## Method

**Day-1**

- Launch the bacterial culture in 5mL of Marine Broth (MB), ON, 20°C in soft agitation (100 rpm).

**Day 0: Absorption test**

- Dilute the initial bacterial culture to 1/100 in 50 mL of MB in an Erlenmeyer.
- When the suspension has arrived to a DO of 0,3, take 1mL of the culture, and do a limit dilution ($10^{-1}$ to $10^{-8}$) and put a drop of 5 ul of each dilution in a MA gel=> this will allow the confirmation of CFU/mL.
- Put 10 mL of the bacterial suspension at a DO 0,3 in a small Erlenmeyer.
- Idem for the control of the phage alone. Put 10 mL of the MB media in a small Erlenmeyer.
- To the bacterial suspension, add the phage to have a MOI 0.01, mix and let it sit statically at 20°C.
- Put the same volume of phages in the control, mix and take 1mL to be transferred to an Eppendorf tube containing 100uL of chloroform. **This will be the T0.**
- At the following times: **T5, T10, T15, T30, T60, T120, T180, T240** take 1mL of the mix of bacteria+phages and place them in an Eppendorf tube containing 100uL of chloroform, mix with vortex, and save at 4°C until the titration of the phage.

When the cinematic is done:

- Centrifuge for 5min at 13 000 rpm.
- Obtain 100 ul of the supernatant (well above the choloform) for a limit dilution in a 96- well plate (pure at $10^{-7}$).
- Apply **5ul** onto a bacterial lawn in top agar.

## Important tips!

- Each phage and host behave differently; therefore, it is crucial to determine their growth rates, the OD 0.3 to CFU/ml ratio, phage titer considering decay, etc. Thus, before commencing:

i) Evaluate the OD 0.3 to CFU/mL ratio in three separate experiments.

ii) On the day before the adsorption experiment, titrate the phage.

- It is likely that the growth phase determines receptor expression. If experiments fail at OD 0.3 (after at least three attempts), consider testing other OD values.

- Results may also be achievable with higher MOIs (0.1 to 1) or much lower (<0.001).

- In the initial experiment, conduct long-term assays. For the second and third experiments, focus on the window of decline in free phages (maximum adsorption time) and subsequent increase (start of lytic replication). Adsorption time varies significantly, ranging from approximately 1 minute to 15-30 minutes for our phages.

- Chloroform may alter certain phages; thus, confirm on the phage stock that there is no effect on infective titer.

- For some phages, a decrease in the quantity of free phages is observed but never a disappearance.

- This protocol provides a rough estimate of the time required for phage production. However, since chloroform is used to lyse cells, this time corresponds to the eclipse (time between phage genome injection and particle production in the cell) rather than the latency (time between phage genome injection and particle release from the cell). To estimate latency, do not treat with chloroform; instead, centrifuge the cells and filter the supernatant through a 0.2 μm filter.

**Reference**

Hyman, P. & Abedon, S. T. Practical methods for determining phage growth parameters. Methods Mol Biol 501, 175-202, doi:10.1007/978-1-60327-164-6_18 (2009).