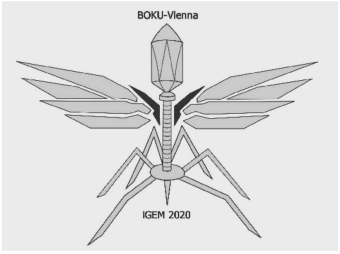

Infection Dose Assay

Aim of the Experiment:

This essay aims to determine the minimal dose of phages needed to clear a liquid culture of susceptible bacteria. This is useful to compare wild-type phages with engineered phages through differences in assembly efficiency. The required ratio of plaque forming units (PFU) per bacteria can be calculated. It represents the infectivity of the examined phage.

Preparation:

- Make an overnight culture of susceptible bacteria
- Determine phage concentration through plaque assay

Materials:

- 100 ml LB medium
- 21 glass test tubes (volume min. 6 ml)
- 1 ml overnight culture of susceptible bacteria
- Phage solution with determined concentration through plaque assay
- 200 ml shake flask
- Photometer
- 0.5 ml cuvettes for the photometer

Procedure:

This protocol and the described volumes are designed to create triplets of each sample. The phages will be added in 6 dilution steps.

1. Add 1 ml of the overnight culture to 100 ml LB medium in a shake flask and incubate in a shaker at 37°C until it reaches an OD_{600} of 0.4.
2. Dilution of the phages: prepare 6 dilution steps with concentrations ranging from 10^4-10^9 PFU/ml.
3. Add 3.9 ml of the bacterial culture to each of the glass test tubes.
4. Add 100 µl of sterile RO-water to 3 of the samples. These are the triplets for the negative control.
5. Prepare triplets for every dilution stage (18 samples in total) by adding 100 µl of the respective phage solutions to the remaining sample tubes.
6. Incubate at 37°C for 30 minutes and then measure the OD again.
7. An OD < 0.4 is defined as “cleared”.

Evaluation:

Assumption: An OD of 1 ≙ $5*10^8$ CFU/ml.
The Infection Dose is calculated according to this formula:

$$Infection\ Dose\ [Phage/Bacteria] = \frac{Phage\ Concentration^{1}\ [PFU/ml] * 0.1ml}{5 * 10^{8} * 4ml * 0.8}$$

[1]: Lowest Phage concentration which cleared the solution.