

Burst size analysis (Heinemann, 2007)

Aim of the experiment

This protocol is used to determine the number of phages produced per infected bacterium.

Materials

- NZCYM Medium
- 2 x 250 ml shaking flasks
- Bacteriophage solution with known titer
- 2 x 50 µl Chloroform
- 2 x 1.5 ml microcentrifuge tubes
- 3 x PCR tubes

Procedure

Preparation of samples

1. Mix 9.5 ml NZCYM Medium with 500 µl O/N culture of T7 bacteria in 250 ml shaking flask (culture 1).
2. Growth at 37°C and 300 rpm until OD reaches 0.25.
3. Add 10^6 bacteriophages to the culture.
4. Start the timing.
5. After 5 minutes incubation at 37°C, without shaking transfer 100 µl of culture 1 to fresh 10 ml of pre-warmed (27°C) NZCYM Medium (culture 2).
6. After 5.5 minutes from start of infection, transfer 1 ml from culture 2 into a 1.5 ml centrifuge tube.
7. Quickly transfer 20 µl sample into a PCR tube → Titer 1 (non adsorbed).
8. Add 50 µl Chloroform and vortex.
9. Transfer the 20 µl sample into PCR tube → Titer 2
10. After 17.5 minutes incubation: cell lysis → only free phages
11. Transfer 1 ml from culture 2 into a 1.5 ml tube
12. Add 50 µL Chloroform and vortex.
13. Transfer 20 µL sample into PCR tube → Titer 3.

Calculation

1. Calculate the number of initially infected cells (N_i).

$$N_i = \text{Titer 1} - \text{Titer 2}$$

2. Calculate burst size

$$\text{burst size} = \text{Titer 3} / N_i$$