Burst size analysis (1:10 Transfection)

Aim of the experiment

The aim of this experiment is the analysis of the burst size of phages after the infection of their specific bacterial host. The burst size describes the number of phages which were amplified in one bacterium.

Materials

- Bacterial O/N culture
- NZCYM medium
- Phage solution with known Titer

Procedure

1. Inoculation (1:100) of O/N culture of T7 bacteria in MCZYM-Medium.
2. Incubate the culture at 37°C and 250rpm.
3. Growth till exponential growing phase (OD_{600} = 0.6 – 0.8).
4. Calculate the volume of phage stock solution that must be added.
5. Transfection for 12 minutes.
6. Gentle centrifugation with 3.300 rpm for 10 minutes at 4°C.
7. Supernatant contains non-adsorbed phages → continue with "Agar overlay plaque assay" for determining the titer "Non-ads. Phages".
8. Pellet from step 7 contains infected bacteria.
9. Resuspend pellet in same volume of NZCYM Medium as in step 1.
10. Incubation until medium becomes clear.
11. Centrifugation with 7,000 rpm, at 4°C for 10 minutes (pelleting cell debris). Pellet contains cell debris from lysed bacteria.
12. Supernatant with amplified phages.
13. Continue with "Agar overlay plaque assay" for determining the titer "Ads. Phages".