

Phage T7 DNA Purification

Aim of the experiment

This protocol is specially for purify DNA from the Phage T7 for further analysis.

Materials

- Bacterial culture
- NZCYM media
- Phage solution
- Phage Buffer
- Chloroform
- 0.45 µm filters
- 15/50 ml Falcons Tubes

Procedure

1. Overnight bacterial culture dilute 1:100 in 200 ml NZCYM media.
2. Incubate at 37°C until OD₆₀₀=0.8
3. Cool down the Centrifuge 5430R to 4°C.
4. Invert Phage solution (Stock in the fridge) and add 20 µl to the bacterial culture.
5. Incubate for 32 minutes.
6. Alternative: Prepare two flasks and infect one 10 minutes earlier than the other. Wait until cells in first flask got lysed, now harvest cells from second one.
7. Transfer total volume of bacterial-phage-solution to 50 ml Falcons / 15 ml falcons better for phase separation in step 10.
8. Centrifugation at 7000 rcf 4°C for 10 minutes.
9. Discard supernatant and resuspend pellet in 5 ml Phage Buffer + 250 µl Chloroform.
10. Centrifugation at 4000 rcf at 4°C for 10 minutes (you get 3 phases: upper: phages solution, middle: bacterial cell lyses, down: chloroform).
11. Empty tubes.
12. Filter the supernatant (upper phase) with 0.45µm filters.
13. Check number of phage by dynamic light scattering machine.

14. Add 1µl/ml RNase solution or 1µg/ml of lyophilized RNase A and incubate for 30 minutes at RT, shaking.
15. PEG precipitation 1200 µl Phage solution + 300 µl "20% PEG/2.5M NaCl in Phagenpuffer" (in the fridge) incubation on ice for 30 minutes.
16. Centrifugation at 16 000 rcf for 10 minutes at 4 °C; discard supernatant.
17. Centrifugation at 16 000 rcf at 4°C for 5 minutes; discard supernatant with pipette.
18. Resuspend pellet in 100 µl nfH₂O.
19. Continue with Phenol-chloroform Precipitation Protocol.