Phage DNA Isolation

Materials

- Bacteriophage
- Bacterial host
- LB
- 5M NaCl
- PEG-6000 240 g/L (autoclaved)
- RNaseA (10mg/mL)
- DNaseI (NEB)
- Phenol:Chloroform:Isoamyl (25:24:1)
- Chloroform
- Sodium Acetate (3M, pH 5.2)
- 100% ethanol
- 70% ethanol
- Nuclease free water
- Agarose and TAE buffer

Protocol

1. Grow bacterial culture to OD 0.7 in 30 mL LB.
2. Infect with bacteriophage and incubate until the culture is lysed.
3. Add 1/10 volume of 5M NaCl to the lysate and mix.
4. Incubate at 4°C for 1h.
5. Centrifuge at 4800g for 10 min and keep supernatant.
6. Add 1/3 of volume PEG-6000.
7. Incubate at 4°C for 1h.
8. Centrifuge at 4800g for 30 min and keep pellet (containing phages).
9. Resuspend phages in 20 mL LB.
10. Add 10µL of RNaseA (10mg/mL) and 5µL of DNaseI (NEB).
11. Incubate for 1h at 37°C.
12. Add 1/3 of volume PGE-6000.
13. Incubate at 4°C for 1h.
14. Centrifuge at 4800g for 30 min and keep pellet (containing phages).
15. Resuspend pellet in 1mL nuclease free water and transfer to microcentrifuge tubes.
16. Add an equal volume of Phenol:Chloroform:Isomayl (25:24:1).
17. Mix well but avoid shearing of DNA.
18. Spin for 30 seconds at maximum speed in a microcentrifuge.
19. Pipette-off aqueous phase and transfer to a fresh microcentrifuge tube.

20. Add an equal volume of nuclease free water into the Phenol:Chloroform:Isomayl (25:24:1) tube and repeat (steps 17 and 18).
21. Pool the aqueous phases to the same microcentrifuge tube.
22. Add an equal volume of chloroform to the pooled aqueous phases.
23. Mix well but avoid shearing of DNA.
24. Spin for 30 seconds at maximum speed.
25. Pipette-off the aqueous phase and transfer to a fresh microcentrifuge tube.
26. Add 1/10 of volume sodium acetate (3M) and mix.
27. Add 2 volumes of 100% ethanol (ice cold) and mix.
28. Incubate at -80°C for 15 minutes.
29. Centrifuge at max speed for 15 minutes at 4°C.
30. Decant supernatant and dry pellet (do not over-dry).
31. Add 500µL of 70% ethanol and mix.
32. Centrifuge at room temperature for 10 min at max speed.
33. Pipette off the supernatant (pellet is slippery) and dry pellet (do not over-dry).
34. Resuspend pellet in desired volume of nuclease free water (rinse tube walls to collect all DNA but avoid shearing the DNA).
35. Analyze DNA in 0.4% agarose gel.
36. Check DNA for phenol contamination with a restriction digest and analyze bands in an agarose gel.

Yields about 10µg of phage DNA.