



Phagehunting Program

Mycobacteriophage Genomic DNA Preparation Protocol

The phage particle is basically a tough protein shell around a single wonderful DNA chromosome. We want to purify the DNA away from the proteins. We start with 100,000,000,000 or so phages (and chromosomes) and isolate the DNA. Phenol is a chemical that denatures proteins. It also has the properties of being immiscible (doesn't mix) with water or aqueous liquids and is more dense than water. We use these properties to sequentially break up the phages and separate the DNA from the denatured proteins.

NOTE: Be sure to remove the phenol from below the phenol/buffer interface in its container.

SAFETY NOTICE: Since phenol denatures proteins you must wear gloves and be sure not to spill any on yourself. It is poisonous if absorbed. Phenol will produce contact burns and acts as an anesthetic.

General note about mixing: To avoid mechanical damage to the DNA, use tube gentle inversion, rather than a vortex. Don't use small-bore pipettes when transferring DNA mixtures.

1. Add 600 μ l dialyzed CsCl phage to eppendorf Tube #1
2. Add an equal volume buffer equilibrated phenol and mix by gentle inversion. The liquid should turn white and goopy upon mixing.
3. Spin at 14K RPM at Room Temperature for 5 minutes.
4. Transfer aqueous (top) phase to fresh Tube #2, avoiding the white interface. There will be two layers with some white interface (denatured phage protein) between them. Use a 1000 μ l pipettor to pipette up slowly.
5. Add 500 μ l phenol to Tube #2 and gently mix.
6. Add 600 μ l TE to Tube #1 and gently mix.
7. Spin Tube #1 and Tube #2 at 14 K RPM at Room Temp for 5 minutes.
8. Transfer the aqueous phase from Tube #2 to the fresh Tube #3, avoiding the white interface.
9. Transfer the aqueous phase from Tube #1 to Tube #2, avoiding the white interface, and gently mix Tube #2.
10. Add 500 μ l phenol to Tube #3 and gently mix Tube #3
11. Spin Tube #2 and Tube #3 at 14 K RPM at Room Temp for 5 minutes.
12. Transfer the aqueous phase from Tube #3 to fresh Tube #4, avoiding the white interface.
13. Transfer the aqueous phase from Tube #2 to Tube #3, avoiding the white interface, gently mix Tube #3.
14. Add 500 μ l phenol to Tube #4 and gently mix Tube #4.
15. Spin Tube #3 and Tube #4 at 14 K RPM at Room Temp for 5 minutes.
16. Repeat steps 12-15 (adding one to each Tube #X) until there is no white goopy interface.

17. Transfer the final aqueous phases to a fresh tube(s). If the total volume of this aqueous phase is >700µl, two tubes will be needed. At step 31, they may be combined in one tube.
18. In a fume hood, add an equal volume of (phenol:chloroform:isoamyl alcohol) (25:24:1) and mix gently.
19. Spin at 14K RPM Room Temperature for 5 minutes.
20. Transfer the aqueous phase to a fresh tube.
21. In a fume hood, add an equal volume of chloroform and gently mix.
22. Spin at 14K RPM at Room Temperature for 5 minutes.
23. Transfer the aqueous phase to a fresh tube.
24. Add a 1/10 volume of 3 M sodium acetate (NaOAc) and 2.5 Volume of 95% ethanol (EtOH).
25. You should see a fine ball of string (DNA!) appear upon mixing.
26. Mix very well.
27. Freeze on dry ice. After freezing, you may continue with the protocol or leave the tube in the freezer to finish later.
28. Spin at 14K RPM at 4°C for 30 minutes
29. A white pellet should have precipitated. Remove (decant or pipette) the EtOH and wash the pellet with 1 ml of 70% ethanol. Be careful that the pellet stays in the tube!
30. Air dry the pellet but do not over dry. A pellet that is too dry will be difficult to dissolve.
31. Dissolve in 200 µl TE.
32. You can gently pipette up and down to mix, and incubate at 37°C or 42°C to help it dissolve. Be careful not to accidentally aspirate and remove the pellet in this process. The final solution should be viscous but homogeneous. You can leave the DNA in TE at room temperature or 37°C overnight to dissolve completely with no mixing. (You can also do the final dissolve step at 65°C for 10 minutes, then put on ice.