GENERAL PROCEDURES FOR PHAGE PREPARATION AND PURIFICATION

Materials:

SM:	NaCl		5.8 g
	$MgSO_4$ $7H_2O$	2.0 g	
	1M Tris HCl pH7.4	50 ml	
	2% gelatin		5 ml
	q.s. to 1 liter, sterilize by autoclaving		

LEM:	Bacto-tryptone		10 g
	Yeast extract	5 g	
	NaCl		5 g
	1M $MgSO_4$		10 ml
	q.s. to one liter, sterilize by autoclaving		

Top agarose: 0.7% agarose in LEM
Bottom agar: 1.5% agar in LEM, pour into 100 mm petri dishes after autoclaving.

Bacterial Cultures

Overnight culture can be stored for approx. 1 week and is used to inoculate either a 3 hour culture or to prepare another fresh overnight.

3 hr culture: A 1:10 dilution of an overnight culture in LB grown for 3 hours at 37°C. This can be used for approx. 24 hours. A fresh overnight culture can also be used to plate out bacteria.

General Procedure for Plating and Titering Lambda stocks.

The bacteria on which the strain is to be grown should be cultured overnight by sterile transfer from a stab or slant to a medium size culture tube containing 5 ml LB (+0.1 M $MgSO_4$). Any necessary additions should also be included (to a final concentration of 1 ug/ml for thiamine or 5 ug/ml for thymine or thymidine). This culture should be grown about 14 hr. at 37°C. The overnight will plate phage but the plaque morphology will be better with a 3 hour culture. The overnight can be used 1 week, but the 3 hour culture is best when used within a day after subculture.

When bacteria are ready, dilute the phage in SM to a final concentration of about 2 x 10^3/ml. If the concentration is unknown, plate several dilutions, assuming 10^{10}/ml for a new stock and 10 x less for every 2 years of age of an older stock. Dilutions are usually done by adding 0.05 ml* to 5 ml of SM for a 10^2 dilution or by adding 0.5* ml to 4.5 ml SM for a 10 x dilution. If the dilutions are to stand longer than 1/2 hour, keep them on ice.

Add 0.1 ml of the phage dilution to 0.25 ml of the 3 hour culture and preabsorb at 37°C for 5-10 min (the incubation time, once chosen, should remain constant from day to day). Add

2.5 ml top agarose (top agarose which has been melted and is at 45°C-47°C) to each tube and pour onto bottom agar plates. If thiamine is a requirement it should be added to the top agar. The plates should be grown at 37°C overnight. If the bacterium is temperature sensitive, the pre-absorption and overnight incubation should be done at an appropriate temperature.

*Always use a new pipette for every step of the dilution.

General Procedure for Purification of Strains

When purifying a single strain from a heterogeneous stock pick a single, well isolated plaque. The plaque is removed from the plate using a sterile open-ended capillary tube or pasteur pipette to pierce the agar (both top and bottom layers). Withdraw the 'plug' by holding the index finger over the open end. Expel into 1.0 ml SM plus a drop of chloroform; shake briefly to suspend the phage. An average of 10^5 - 10^6 phage/plaque should be expected, so plate a 10^3 and 10^4 dilution of this suspension of the next cycle of purification. At least 2 cycles should be carried out.

Procedures

Small lysate

1. Core a single plaque and add to 1 ml SM. This can be used within 30 minutes. Store at 4°C.
2. Add 0.5 ml phage to 0.15 ml 3 HR culture of appropriate bacteria. Incubate 37°C, 20 min.
3. Add 3 ml top agarose and plate out on a fresh agar plate.
4. Within approx. 4 hours bacteria growth should be apparent. In 1-2 more hours, substantial clearing will be apparent. When almost all, but not all, bacteria have been lysed, add 3 ml SM to the plate and store at 4°C. After 1 hour to overnight, take off the SM with a sterile pasteur pipet. If all of the SM has been absorbed by the plate (particularly common with older plates), add 3 ml SM more and wait as above. This stock of phage should have a titer of approx. 5 x 10^9.

Large Lysate

1. Grow up fresh overnight.
2. Preabsorb bacteria and phage in the following ratio at 37°C for 15-20 min.
   5 ml bacteria
   500 ml LEM
   2 x 10^7 phage
   It may be necessary to vary the ratio of phage to bacteria for different clones. More phage may be required for slower growing clones.
3. Pre-warm media to 37°C. (500 ml in a 2 liter flask).

4. Add inoculant to media and shake vigorously at 37°C until lysis is complete (4-5 hours but I usually allow it go overnight).
5. Add chloroform (4 ml/500 ml media) to lyse remaining cells and add NaCl to make lysate 1M. Shake 15 min. at 37°C.
6. Chill.
7. Spin 8 K for 15 min.
8. Add Carbowax PEG 6000 to 7% to the supernatant.
9. Shake to dissolve PEG, and put in cold room for at least 1 hour, but overnight is also fine.
10. Spin 8 K for 15 min. and discard supernatant.
11. Invert bottle and wipe out all residual liquid.
12. Resuspend pellet (which is quite large and contains mostly unwanted bacterial debris) in 7 ml SM. You do not have to break up the large clumps of debris.
13. Transfer to a disposable centrifuge tube. Include the clumps.
14. Add crude DNase to 20 µg/ml and incubate for 20 min at room temperature.
15. Spin 8K for 15 min and, if supernatant is still cloudy, transfer to another centrifuge tube and spin again. Discard the centrifuge tube(s).
16. Pour supernatant into a disposable, graduated screw-top tube. (The Corning 15 ml orange top tubes are perfect.) Adjust volume of each sample to a convenient volume.
17. Add 0.78 gm CsCl per ml of solution. Density should be 1.5000 gm/cc, but I never check anymore.
18. Spin 35K for 20 hours.
19. Take off band. You need at least 3 x 10^{11} phage to see a band. 2 x 10^{10} phage yields 1 µg DNA.
20. Dialyze against SM.
21. Save 50-100 µl of the phage as a high titer stock for future endeavors.
22. To the remainder, make the solution 10 mM excess EDTA, 0.1% SDS. Add proteinase K to 100 µg/ml and incubate at 65°C for 45 min.
23. Cool and Phenol (50:50 phenol:chloroform) extract. Do not vortex. Solution should become viscous. Ether extract.
24. Ethanol precipitate either by spooling out the DNA (very effective at removing RNA) or by spinning down. Wash in 70% ethanol and dry the tube with kimwipes to remove excess ethanol. If you have spooled out the DNA, rub the DNA against the side of the tube to remove as much liquid as possible. Under no circumstances allow the DNA to become completely desiccated. Take the DNA up in DNA buffer and allow it to go into solution (up to 1 day at 4°C). If you feel there is excess ethanol or ether in your sample, you can put the tube in the vacuum dessicator to drive off the organic liquids. Do this in a tube with very high walls as the solution will bump under the vacuum.

Prepared by Ron Blackman--July, 1982

References :

1) Meselson Cookbook

2) Molecular Cloning: A laboratory Manual (1982) T. Maniatis, E. F. Fritsch, T., Sambrook p. 76-85, 63-67.