Purifying lambda DNA from liquid lytic growth/PEG precipitation of phage

See Lockett, Analytical Biochemistry **185**, 230-234 (1990).

1) Package phage as described in the Epicentre protocol.

2) Grow an o/n culture of LE392. Inoculate .4ml culture + .4ml (10mM CaCl2, 10mM MgCl2) with a single screened plaque.[1] Vortex briefly and incubate at 37°C for 15min.

3) Add this to 200ml of NZCYM broth (contains MgSO4) in a 2L flask (aeration is important). Grow at 37°C at 125rpm for 4hrs., at which point culture should be cloudy. Begin monitoring OD600: it will shoot up to above 1, and then drop rapidly. As soon as you reach ~.4, begin monitoring frequently until OD begins to climb again. This will happen ~.3, and in less than 8hrs.

Sample course:

time	OD_600
4:00 hrs	1.6
4:35 hrs	1.8
5:00 hrs	0.5
5:30 hrs	0.315
5:45 hrs	0.35

4) Add .5ml chloroform and shake at 80rpm for another 5min.

5) Transfer to a 200ml glass bottle and add dry NaCl to 1M; incubate on ice for 10min.

6) Transfer to 50ml tubes, avoiding chloroform. Centrifuge at 12,000xg for 10min. to remove cell debris.

7) Collect supernatant evenly in 50ml tubes and centrifuge again at 12,000xg for 5min. to remove remaining cell debris. Add solid PEG8000 (or PEG6000 or similar) to each (10% w/v); incubate on ice for 30min.

[1] Extracting the phage beforehand yields the same results as using a plug directly.

8) 12,000xg for 15min. to pellet phage. You will not get a pure-phage pellet, but rather a gunky smear containing cell debris... it's OK, because the phage is in there. Discard supernatant and spin again for 1min. to remove all the PEG-containing supernatant and concentrate the pellet.

9) Resuspend in as low a volume of suspension medium (SM) as possible and pool in a 15ml Falcon. Aim for about 2.5ml total volume. (Volumes of added buffers below for 2.5ml; scale up as necessay.)

10) Add RNase[2] and DNase[3] to 20µg/ml and 5µg/ml final concentrations; incubate at 37°C for 30min.

11) Add 2.5ml .3M Tris-HCl, EDTA to 100mM, and SDS to 1.25%; incubate 10 min. at 65°C

12) Add 2.5ml ice-cold 3M potassium acetate (forces SDS and associated proteins out of solution–will form thick, white gel); incubate on ice for 10 min.

13) 8,000xg for 10min.; switch centrifuge temp. to 25°C and save supernatant

14) Add 5ml isopropanol; 2min. at rt; 8,000xg for 10min. at rt.

15) Wash in a few mls 70% ethanol, transfer to an eppendorf and slowly resuspend in desired volume of Tris or TE.

N.B.: There will be RNA and genomic DNA contamination, but the lambda DNA will be in relatively high concentration and competent to package. A .6% agarose gel with a 1/10 dilution of product and 1/15 dilution of NEB lambda for comparison will give you some idea of how well the purification worked.

[2]Comes dry; make a 4mg/ml stock in water. R4875-500mg, Sigma.

[3]Use Sigma D5319-2mg. According to J, *via* Luke, other DNases will chew up packaged lambda DNA.