PREPARATION OF PHAGE LAMBDA DNA

1. Prepare cells by growth overnight in LB + maltose. Concentrate two fold in 10 mM $MgSO_4$.

   2λ phage
   200λ Hfl-
   18 ml NZCYM

2. Incubate cells (0.2 ml) and phage (2×10^6 or 2 µl of lysate) at 37°C for 15 minutes, then add them to 50 ml of NZC Broth. Incubate culture at 37°C till lysis (approx. 8 to 12 hours; O.N. is O.K.).

3. Add 0.2 ml of chloroform, shake and let separate. Decant super into 40 ml SS-34 centrifuge tube and clear at 12K RPM for 20 min. Decant super to large SW-28 centrifuge tube, leaving tube about 1/2 inch from full.

   9500 RPM 10min

4. Underlay lysate with a pasteur pipette full of 40% glycerol in TM buffer (50 mM Tris-HCl, 10 mM $MgCl_2$). Balance tubes with fresh NZC Broth. Spin tubes in SW-28 rotor at 25K RPM for 2.5 hours. SW41 1hr 40K RPM

5. Pour off super and invert tubes for about 10 min. Wipe excess liquid from sides of tube and add 0.5 ml of TM buffer. Resuspend with gentle shaking on rotary shaker. Transfer to an eppendorf tube (if there is considerable debris then spin briefly and transfer super to fresh tube.

   .200 ml TM

6. Add 0.5 µl DNAse (10 mg/ml) and 2.5 µl of RNAse (10 mg/ml) and incubate at R.T. (37°C) for 20 minutes, then add 50 µl of 0.5 M EDTA and heat at 65°C for 5 min.

   for .5ml TM: 5λ DNase 1 mg/ml, 3λ RNase 10 mg/ml, 37° 20'

   for 200: 3λ DNase, 2λ RNase

7. Add 10 µl of 0.1 mg/ml sol. of Proteinase K: hold R.T. for 5 min. Then add 25 µl of 10% SDS (mix by inverting) and continue incubation at ~~R.T.~~ 50° for another 15 min (solution clears). 5λ of 20%

   4λ of 2 mg/ml Prot K

8. Add 100 µl of 3M Potasium Acetate (precipitate forms). Invert to mix.

9. Heat at 65°C for 20 minutes (precipitate dissolves). Invert to mix.

10. Chill in wet ice slurry for 15 min (guess what?.. Precipitate forms).

11. Spin (@ 4°C) in microfuge for 15 minutes and pipette super to new tube.

12. Add 1 vol. (0.6 ml) of isopropanol, mix slowly to form floculent DNA precipitate. "Fish" precipitate with a drawn micropipette and wash in 1 ml of 70% ETOH. "Touch Off" excess ETOH on side of tube and transfer DNA to 100 to 200 µl of TE (10 mM Tris-HCl, 0.1 mM EDTA).

    pellet DNA in microfuge, resuspend in 100λ TE, add 50λ NH4OAc

This DNA has been used not only for restriction analysis, but has also been used for various in vitro reactions involved in phage construction (DNA polymerase fill-in and linker ligation, end labeling, etc.) Rarely, one sees some nuclease contamination in these DNA preparations, when noted this can be corrected by phenol extraction, precipitation, and "re-fishing".

plate lysate
50 µl of 1 ml plaque
100 µl Hf1
plate on small plate 5-6 hrs 37°
overlay c̄ SM O/N 5 ml
store at 4° c̄ $CHCl_3$
use 10 λ of 10^{-2} dilution for miniprep