# SINGLE-STRAND DNA PREP. LARGE SCALE

1. Grow a 5 ml overnight culture of the plasmid in an F' E. coli host e.g. LE392F'. For uracil-containing DNA, use a dut-ung- strain such as CJ236 F`.
2. Next day add 0.5-1.0 mls M13 phage suspension and transfer culture to 500 mls LB + antibiotic. Incubate 8-16 hours at 37C with vigorous shaking. For uracil-containing DNA supplement the medium with 0.25 ug/ml of uridine.
3. Spin down the bugs at 4000 rpm 15 mins. Transfer supernatant to fresh centrifuge bottle(s) and add 125 mls 20% PEG8000/2.5 M NaCl or add solids to give 5% PEG8000/0.5 M NaCl, mix and put on ice 2-16 hours. Make dsDNA from the bacteria pellet if desired (see BUGS-4).
4. Spin down the phage precipitate 6000g for 10 mins(7000 rpm in Hermle; 4000 rpm in JA-10 rotor in Beckman centrifuge). Decant most of the supernatant but leave a few mls to resuspend the pellet. Resuspend phage and transfer to 12 ml tube. Spin again and this time remove as much supernatant as possible.
5. Resuspend the phage in 3 mls TE, spin 4000 rpm 5 mins to remove any residual bacteria, transfer supernatant to fresh tube.
6. Extract supernatant twice with an equal volume of chloroform, twice with an equal volume of TE saturated phenol. Add 0.1 vol 7 M ammonium acetate and 2.5 vol ethanol, leave on ice 15 mins then spin down ssDNA 10,000 rpm 10 mins.

   NOTE: **PHENOL CAUSES SEVERE BURNS. WEAR GLOVES, LAB COAT AND EYE PROTECTION. READ MSDS SHEET BEFORE HANDLING.**
7. Wash pellet with 70% ethanol and leave to air dry.

   NOTE: **ETHANOL IS HIGHLY FLAMMABLE. USE CAUTION NEAR AN OPEN FLAME.**
8. Dissolve the DNA in 200-500 ul TE, read the O.D.260 of a 1/100 dilution to determine the concentration. (1.0 O.D.260 = 40 ug/ml single strand nucleic acid.)
9. Check relative yield of plasmid:M13 DNA by agarose gel electrophoresis. Store at -20øC.

NOTE 1: For any F` bacteria, it is a good idea to grow them in the presence of chloramphenicol as this selects for the retention of the F`, which is required for M13 infection. The M13 phage enters the host bacterium through sex pili which are encoded for by the F` factor. However, chloramphenicol should not be added when growing a ssDNA prep since it prevents the synthesis of ssDNA

NOTE 2:You may also check to make sure that your DNA is single-stranded by doing a digest with S1 nuclease, an enzyme which will only digest single-stranded DNA. See DNA section for protocols.