PLATE METHOD FOR λ DNA PREP

1. Plate 4 plates to confluence. Incubate 37°C, overnight.

2. Add 5 ml of SM per plate. Swirl plates gently for 2 hrs at room temperature.

3. Pool the liquid from the plates in a 50 ml tube. Add 500 μl of chloroform (crude cleaning step), vortex, and leave for 30 minutes (vortexing every now and then).

4. Transfer contents to a fresh tube, leaving chloroform behind. Add 6 μl of 10 $mgml^{-1}$ RNAase and 4 μl of 10 $mgml^{-1}$ DNAase. Leave at room temperature for 30 minutes.

5. Precipitate the phage by adding 1.2 g of NaCl. Dissolve salt and leave at 4°C for at least 1 hr.

6. Transfer sample to 30 ml tube and spin in SS34 rotor at 7000 rpm, 4°C, for 20 minutes.

7. Transfer supernatant to a fresh 30 ml tube and add 2 g PEG6000. Dissolve the PEG (takes 10-30 minutes to fully dissolve), and leave sample at 4°C overnight.

8. Spin at 7000 rpm for 20 minutes, as before. Remove as much supernate as possible and discard. Resuspend pellet in 500 μl of SM and transfer to eppendorf.

9. Extract the DNA, using first an equal volume of chloroform, then phenol, then phenol-chloroform, and lastly chloroform again.

10. Precipitate DNA with 2 volumes of 100% EtOH, spin and
wash with 70% EtOH. Dry pellet and resuspend in appropriate volume (about 100 μl) of 1x TE buffer.