GRADIENT PHAGE PREPS.

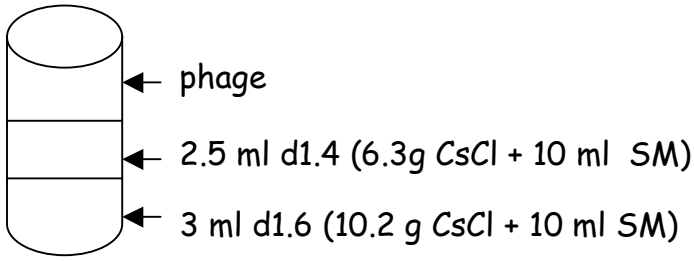
Do liquid lysate preps as normal - pick up at step 7.

1. Resuspend pellet in 3 ml SM.

2. Add equal volume chloroform. Vortex for 30 sec.

3. Spin 1600g 15' 4°C. Remove aqueous phase.

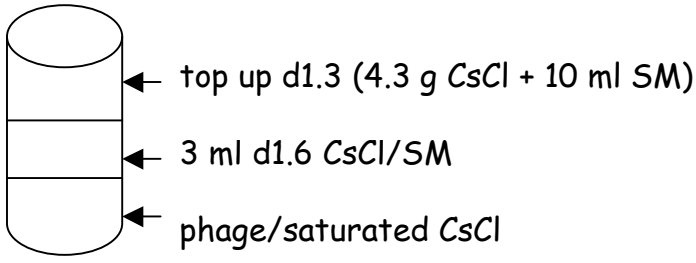
4. Load aqueous phase on a CsCl step gradient:



Mark interface between layers with a pen before spinning. Spin 2 h at 38,000 rpm in SW41 rotor. Protein will settle at interface between phage and d1.4, phage will settle at interface between d1.4 and d1.6.

5. Pull phage band with a needle and syringe (1 – 2 ml) and add an equal volume of CsCl saturated SM (25 g CsCl + 10 ml SM).

6. Set up a second gradient as follows:



Spin 1.5 h at 38,000 rpm in SW41 rotor. Pull phage band from interface between d1.3 and d1.6 layers.

7. Add:
   1/20 volume 0.5 M EDTA
   1/10 volume 2 M Tris base
   equal volume formamide

   Sit at room temperature for 30'.

8. Ethanol precipitate

9. Wash pellet with 70% ethanol, dry and resuspend in TE.