LIQUID LYSATE PHAGE PREPS.

There are two ways to start this prep. The first is fast but doesn't always lead to lysis. The second takes a day longer but nearly always works.

Either:

Day 1

1. Grow a 10 ml culture of host cells in LB + 10 mM $MgSO_4$ + 2% maltose overnight at 37°C.

Day 2

2. Add 100 µl phage eluate (fresh) to 100 µl overnight culture and incubate at 37°C for 20'.

3. Add 5 ml LB + 10 mM $MgSO_4$. Incubate 37°C for 1 –2 h.

4. Add to 500 ml LB + 10mM $MgSO_4$. Incubate overnight at 37°C.

Or:

Day 1

1. Grow a 10 ml culture of host cells in LB + 10 mM $MgSO_4$ + 2% maltose overnight at 37oC.

Day 2

2. Put cells at 4°C until the evening then
   a. Inoculate 10 ml LB + Mg with 0.5 ml host cells and 0.5 ml phage stock. Shake overnight at 37°C.
   b. Inoculate 500 ml LB + Mg + maltose in a 2 liter flask with 5 ml overnight culture. Shake at 37°C overnight.

Day 3.

3. a. If 2a is lysed, add 500 ml fresh LB + Mg and the 5 ml overnight phage culture (2a) to the 500 ml overnight culture (2b). Shake at 37°C until lysis occurs (normally 8 – 16 h).

   b. If 2a culture is not lysed, inoculate another 10 ml LB + Mg + maltose with 0.5 ml host cells from step 2b and add 50 µl lysate from the culture that did not lyse (2a). Shake again at 37°C overnight. Put flask (2b) at 4°C overnight (prewarm before use at 37°C). In the morning,

assuming lysis, pick up as at 3a.

Both preps.

1. Check culture is lysed (stringy bits in the bottom of flask). If not obvious, check by taking 2 x 1 ml culture in glass tubes. Add 1 or 2 drops chloroform to one tube and incubate both at 37°C for 5-10′ with intermittent shaking. Compare tubes. If one with chloroform clears, culture is about to lyse and it is OK to proceed.

2. Add 10 ml chloroform to flask and incubate at 37°C for 30′.

3. Pellet debris 10K 5′.

4. Chill supernate to room temperature and add DNAase and RNAase (solids) to 1 $\mu g ml^{-1}$. Incubate 30′ at room temperature.

5. Add NaCl to 1M (29.2 g/500 ml) and PEG 600 to 10% (50 g/ 500 ml). Dissolve by stirring at room temperature on a magnetic stirrer.

6. Cool in ice water and let stand for at least 1 h.

7. Spin at 11,000 g for 10′ at 4°C. Drain pellets well.

8. Resuspend in TE. Phenol /chloroform extract vigorously (have to remove DNAase as well as capsid coats).

9. Ethanol precipitate as normal.