IN VIVO EXCISION λZAP

1. Pick plaque into 500 μl SM. Add 20 μl chloroform and vortex. Leave at room temperature for 1 – 2 h.

2. In a 50 ml Falcon tube put:

   200 μl OD_{600} = 1 XL1Blue cells
   100 μl phage stock (> 1 x 10^5 pfu)
   1 μl exassist helper phage (> 1 x 10^6 $pfuml^{-1}$)

   Incubate at 37°C 15'.

3. Add 3 ml **2 x YT** and shake for 2.5 h at 37°C. (Cloudy growth is not always seen at this stage.

4. Heat tube at 70°C for 20' then spin for 15' at 4000g.

5. Decant supernate into a sterile tube.

6. To plate out phagemid:

1 eppendorf:	200 μl OD_{600} = 1 SOLR cells 1 μl phage stock (from step 5)
1 eppendorf:	200 μl OD_{600} = 1 SOLR cells 50 μl phage stock (from step 5)

7. Plate 100 μl of each on LB + Amp plates and incubate at 37°C overnight.

8. Pick colonies from whichever plate produces them.

2 x YT – 16g tryptone, 10 g yeast extract, 10 g NaCl per litre.