Plating Lambda Libraries

1. Preparation of host bacteria
   a. Inoculate LB (+ 10mM $MgSO_4$ and 0.2% maltose added after autoclaving) with a single colony. Grow at 37C, shaking, for 4-6 hours (OD_{600} max=1.0) or 30C overnight.

   b. Pellet bacteria at full speed (setting 4) in table top centrifuge, 10 min.

   c. Gently resuspend in 10 mM MgSO4 at about $0D_{600}$=0.5.

2. Plating phage.
   (scale up these volumes as needed for your experiment)
   a. assuming that a single plaque will contain about 10^5 phage (or a plate lysate 10^9/ml), calculate the required dilutions for your experiment.

   b. To a sterile 16 ml plastic tube, add the lambda phage (in 100 µl SM) to 200 µl bacteria (in 10 mM MgSO4). Be sure not to transfer any chloroform to the mixture! Incubate at 37C for 15-20 min to allow the phage to attach to the cells.

   c. Add 3 ml of top agar (NZY broth gives bigger and clearer plaques), melted and cooled to 48C (±2C). QUICKLY mix by inverting 4X and pour IMMEDIATELY on prewarmed bottom agar plates (NZY).

   d. Wait a few minutes for the plates to solidify. Incubate the plates 7-12 hrs at 37C, inverted.

SM (1 liter)
5.8 g NaCl
2 g $MgSO_4$-$7H_20$
6.05 g Tris base
water
pH to 7.5 with NaOH
Add 0.1 g gelatin
autoclave

NZY broth (1 liter)
5 g NaCl
2 g $MgSO_4$-7H20
10 g NZ amine (casein hydrosylate) water
pH to 7.5 with NaOH
for top agar: add agarose to 0.7%.
for <u>bottom agar</u>: add agar to 1.5% (standard applications) OR agarose (for DNA preps).