

## SCREENING $\lambda$ LIBRARIES

### 1° screen

1. Calculate how many 20 cm<sup>2</sup> plates you need to use based on 250,000 plaques per plate.
2. Pour plates and dry thoroughly.
3. Titer phage overnight and calculate pfu the following morning.
4. Aliquot 2 ml plating cells (use same cells as used for titering the night before) into 50 ml Falcon tubes (one tube per 20 cm<sup>2</sup> plate). Add equivalent of 250,000 pfu to each tube. Incubate 37°C 15' in a water bath.
5. Add 45 ml top agarose (LB + 0.7% agarose) to each tube and pour over dried plates. Leave lids off in laminar flow hood for 5' to dry top agarose thoroughly (this will prevent condensation forming later and smearing the plaques).
6. Invert plates and incubate at 37°C for 6 - 12 hours until phage are pinhead size.
7. Place plates in cold room overnight (or for at least an hour).
8. Drop a square of Hybond N on top of plate and leave for 2'. Orient filter with needle pricks through to agar. Mark position of the needle holes on the bottom of the plate with a marker pen.
9. Lift filter off and place in denaturing solution for 1' (1.5 M NaCl, 0.5 M NaOH). Transfer to neutralizing solution for 1' (3 M NaCl, 0.5 M Tris pH7.5) and then rinse in 2 x SSC for 2'.
10. UV X-link filter and hybridize as normal.
11. When film is exposed, use orientation holes in filter and pen marks on plate to work out which plaques have hybridized.

## 2° screen

1. Pick agarose plug from plate with the wide end of a flamed pasteur pipette. Put plug in 1 ml SM and shake at room temperature for 5'. For long term storage add 15  $\mu$ l chloroform.
2. Take 3 x 100  $\mu$ l plating cells and add 0.1, 0.5 and 1  $\mu$ l phage eluate.
3. Add 5 ml top agarose to each and plate out on LB. Incubate at 37°C overnight.
4. Lift and screen as above (7-11).

Continue screening until you have a single plaque pick.