COLONY HYBRIDIZATION

1. Transform or streak out colonies to LB + antibiotic plates (don't bother with blue/white selection). Try for a density of about 1000 to 2000 colonies per small plate or 10,000 to 20,000 colonies per 20 cm^2 plate.

2. Grow cells during the day at 37ºC or overnight at 30ºC and put plates in the fridge for one hour when the colonies are still small i.e 1 mm in diameter.

3. Place a piece of Hybond N on the plate for 1' and stab through the membrane to orient the filter. Ink is not necessary. Lift the membrane slowly trying to take as much of the colony as possible. Put the plates back into 37ºC incubator for 3-5 hr.

4. Place the membrane colony side up onto Whatman paper soaked in denaturation solution (1.5 M NaCl, 0.5 M NaOH). Don't let the solution go ov the top of the membrane or everything starts running away.

5. After 7' transfer to Whatman soaked in neutralization buffer (3 M NaCl, 0.5 M Tris pH7.5).

6. After 5' transfer to Whatman soaked in 2 x SSC (300 mM NaCl, 30 mM Na citrate pH7.6).

7. After 3' remove filters to paper towels and let air dry.

8. UV cross link the membranes or bake for 1 hour at 80°C.

9. Rinse filter for 15 minutes in 2 x SSC at room temperature.

10. Hybridize overnight as for Southerns, expose film for 3 hr and develop.