BACTERIAL COLONY LIFTS FOR CLONING SCREENING

Adapted from BV's Cookbook, TCH 1/27/02

1. Grow colonies as usual, ~ 100-1000 per 7.5 cm dish.

2. Punch extra hole in NEF-978X plaque screen discs;
   Mark plate # on concave side, within " ears" of discs.

3. Place convex side of filter on agar, and leave for 2-5 min.
   Use plastic Pasteur to such out agar through the four holes as fiducials.

4. Add 80 ml of 0.4M NaOH to 11 inch x 14 inch GB002 filter on cafeteria tray. Smooth out bumps in filter with glass test tube.

5. Place discs on GB002 with bacteria up, not contacting GB002. Leave for 10 min.

6. Wash 5 min, twice (total of 10 min) in 20 x SSC.

7. Do not dry filters, do not Stratalink filters, do not bake filters, do not pass go.

8. Place discs between two dry Whatman #1 filters in hyb. Bag. Add 20 ml Blotto-10 (or 4 ml per filter, whichever is more), and incubate at 60°C between glass plates for 3 or more hours.

9. As an optional step: if your probe is dirty or if you expect trouble, you can use 20 ml TE9 containing 1% SDS and 50 ug/ml Proteinase K instead of Blotto-10, for 3 hours at 60°C, then remove this and add Blotto-10 for 30 min.

10. Hybridize as usual in Blotto-10 at 60°C overnight. Use Placenta DNA in pre-annealing step if probe contains repeats. Use 40 ul/ml sssssDNA in blotto-10. Use 200,000-1,000,000 cpm/ml in Blotto-10 for oligo-labeled probes.

11. Wash as usual in Disc-Wisc, 65°C, 0.3x SSC, 0.1% SDS.

0.4M NaOH:

3.2 ml 50% (=19.5M) NaOH (Some people like to use 2.1 ml; it doesn't seem to matter).

+ 97 ml deionized water.