# COLONY HYBRIDIZATION PROTOCOL

*Adapted from* Sambrook, Fritsch, and Maniatis *Molecular Cloning (1989) by Vinh Pham.*
*Last modified: June 7, 1999*

MATERIALS:

**Soln A (10% SDS)**
**Soln B (0.5N NaOH, 1.5M NaCl)**
**Soln C (0.5M Tris-Cl, 1.5M NaCl pH 7.4)**
**Soln D (2X SSC)**
**Nylon-backed Membrane** (1), cut to size of 100mm petri dish
**Whatman 3MM** or other **Blotting Paper** (5)
**15 x 150mm petri dish** (4)

PROCEDURE:

1. Grow up cells to approximate concentration of 50-100 colonies per plate. If desired, replica plate before proceeding (highly recommended).

2. Cut a piece of nylon membrane to the size of the petri dish (plate) to be probed.

3. Soak 4 of the pieces of blotting paper into one of the 4 solutions (A, B, C, or D). Avoid overwetting the paper. It is useful to keep each soaked paper in a 15 x 150mm petri dish.

4. Press the piece of nylon membrane onto the plate to be probed. Mark 3 spots on both the membrane and plate for orientation.

5. Put the piece of nylon membrane, cells side up, over the 4 blotting papers soaked in their respective solutions in the order, and for the incubation times, indicated below:

| ORDER | SOLUTION | INCUBATION TIME |
|---|---|---|
| First | Soln A | 3 minutes |
| Second | Soln B | 5 minutes |
| Third | Soln C | 5 minutes |
| Fourth | Soln D | 5 minutes |

6. Place the membrane, DNA side up, over a dry piece of blotting paper and air dry for 30'.

7. Wrap membrane with saranwrap and UV-crosslink (for 3') DNA onto membrane.

8. Probe and wash blot under same conditions as with standard Southern protocol.

9. Expose to phosphorimager plate, scan on Storm machine, and identify candidate colonies.

10*. Pick-and-patch candidate colonies.

11*. Repeat steps 1-6 for the pick-and-patch plate.

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* If needed.