# Southern Hybridizations

1. Place filters in a plastic container, add 150 μl/cm$^2$ of hybridization buffer, and incubate at 65°C with agitation for 1 to 2 hours.

2. Place radioactively labeled probe (at least 1.0x10$^8$ cpm/μg DNA) in boiling water for 5 minutes.

3. Pipette in the 10$^6$ cpm/ml into the solution (NOT directly onto the filter) and rock to evenly distribute.

4. Incubate at 65°C with constant agitation for 8 to 24 hours.

5. Rinse the filter with room temperature 2× SSC + 0.1% SDS. Discard this rinse into plastic radioactive waste container for decay prior to sewer release.

   Wash once with 0.1× SSC + 0.1% SDS for 30 minutes at 50°C. Discard this wash down the drain with copious amounts of water.

   Wash twice with 0.1× SSC + 0.1% SDS for 10 minutes each at 65°C. Discard this wash down the drain with copious amounts of water.

6. Rinse washed membrane with room temperature 2× SSC.

7. Wrap membrane in Saran Wrap™ (X-ray film cannot be allowed to become wet), and place in cassette. If desired, another sheet of Saran Wrap can be placed over all membranes to keep them in place. Turn out the lights, and place X-ray film on the membrane(s), place intensifying screens over the film, and close the cassette. Place in the ultracold freezer (<= -70°C).

8. Develop film after about 24 hours (repeat with longer exposure if necessary), and store membrane, still wrapped in Saran Wrap, at 4°C. Do not allow the membrane to dry as the probe will become permanently bound.

Hybridization Buffer

| Composition: | To make 100 ml: | 200 ml: | 300 ml: |
|---|---|---|---|
| Water | 56.5 ml | 113 ml | 169.5 ml |
| 6× SSC | 30 ml of 20x | 60 ml | 90 ml |
| 5× Denhardt's reagent (1 g/l each of Ficoll, Polyvinyl-pyrrolidone, Bovine Serum Albumin) | 10 ml of 50x | 20 ml | 30 ml |
| 0.5% SDS | 2.5 ml of 20% SDS | 5.0 ml | 7.5 ml |
| 100 μg/ml boiled sheared herring sperm DNA | 1.0 ml | 2.0 ml | 3.0 ml |

2× SSC + 0.1% SDS, 500 ml

50 ml 20× SSC
2.5 ml 20% SDS
water to 500 ml

0.1× SSC + 0.1% SDS, 1000 ml

5 ml 20× SSC
5 ml 20% SDS
water to 1000 ml

0.1× SSC, 500 ml

2.5 ml 20× SSC
water to 500 ml

## To strip membrane of probe after hybridization

1. Remove Saran Wrap, and place membranes in a container.

2. Add 0.1× SSC + 0.1% SDS at 100°C for 15 minutes. (If several membranes are done at once, use two changes for 10 minutes each.)

3. After stripping, wash by gentle agitation in 0.1x SSC + 0.5% SDS at 65°C for 2 to 24 hours.

4. Place membranes in covered plastic container, with layer of Saran Wrap on surface of solution to keep membranes in place, or air dry at room temperature.

0.1× SSC + 0.1% SDS, 1000 ml

5 ml 20× SSC
5 ml 20% SDS
water to 1000 ml

0.1× SSC + 0.5% SDS, 500 ml

2.5 ml 20× SSC
12.5 ml 20% SDS
water to 500 ml

## Autoclave Stripping Procedure

1. Remove Saran Wrap, and place membranes in a container.

2. Add 0.1× SSC + 0.1% SDS, autoclave for 5 minutes.

3. Remove from autoclave, and before solution cools, pour it off and add 0.1× SSC + 0.1% SDS.

4. Place membranes in covered plastic container, with layer of Saran Wrap on surface of solution to keep membranes in place, or air dry at room temperature.