DNA/RNA BLOTTING & HYBRIDIZATION

I. **Introduction:**

Nucleic Acids can be transferred from an agarose gel onto nitrocellulose by blotting fixed onto the nitrocellulose by baking in vacuo and used to probe for homologous DNA sequences with ^{32}P nick translated DNAs. Such a method is described.

II. **Materials:**

20 x SSC 3.0M NaCl
0.3 M Sodium citrate

Denaturation solution	1.5 M NaCl
(for Southerns)	0.5 M NaOH
Neutralization solution	1.5 M NaCl
(for Southerns)	0.5 M Tris pH 7.4

Prehybridization solution
6 x SSC
5 x Denhardt
0 x Denhardts:
5 g Ficoll
5 g PVP
5 g BSA fractionV
dH_2O to 500 ml

0.5% SDS
100 µg/ml denatured salmon sperm DNA
(50% formamide if hybridizing at 42°C for RNA blots)

Hybridization solution
6 x SSC
10 mM EDTA
^{32}P labeled probe-boil with carrier DNA 5 minutes.
(quick-cool)
add remaining ingredients:
5x Denhardts
0.5% SDS
100 µg/ml carrier DNA

Procedure:

1. Excise gel segment to be blotted. Measure and immerse in denaturation solution at room

temperature. Soak for 30 minutes with occasional shaking. Make the denaturation solution immediately before use.

2. After the denaturation step, rinse gel segment briefly in water and transfer to neutralization buffer. Soak in this solution for 30 minutes with intermittent swirling.

3. For RNA gel, transfer gel directly to nitrocellulose as below without soaking first.

4. Prepare the blotting setup while the gels is being processed.
   a. Cut a segment of nitrocellulose (S+S BA-85) the same size or slightly larger then the gel segment. Immerse nitrocellulose in 2 x SSC and monitor for even hydration. Areas that do not wet well will blot poorly.

   b. Cut pieces of Whatman 3 mm of 6-7 inches square that will be used as the bed support for the gel.

   c. Just before the neutralization step is completed wet the 3mm pieces (6-7" sq) in 20 x SSC. Place in a Pyrex dish and squeeze any air bubbles.

5. Place the gel segment on top of the 20 x SSC moistened Whatman 3 mm paper and remove any bubbles trapped between gel and paper.

6. Place nitrocellulose paper on top of the gel. Remove any air bubbles that may be trapped between gel and nitrocellulose paper.

7. Place a few sheets of dry Whatman 3 mm paper cut just a little smaller than the gel segment on top of the nitrocellulose. Smooth out with a gloved hand to ensure efficient and thorough wicking. Place 1-2 inches of paper toweling cut a little smaller than the gel segment on top of the Whatman 3 mm.

8. Place a glass plate and a 200-500 g weight on top. Blot from 4 hours - overnight. Check the reservoir of 20 x SSC in the Pyrex dish to see that it is blotted upwards.

9. When blotting is complete, peel off the nitrocellulose paper from the gel, mark paper with an indelible pen or pencil for orientation and rinse briefly in 2 x SSC, gently swirl.

10. Remove nitrocellulose from 2 x SSC, let dry on paper towels for 2-4 hours at room temperature.

11. Wrap filter in aluminum foil, bake in vacuum oven for four hours at 70-80°C. Store filter in the cold room.

<u>Probing a Southern/Northern filter:</u>

1. Place filter in a heat sealing bag or a Zip-loc bag. Wet with 6 x SSC

2. Squeeze out the 6 x SSC. Replace with 0.2 ml prehybridization solution/cm^2 of filter. Squeeze as much air out as possible. Seal. Incubate at 65°C for 2-18 hours, or 42°C for RNA blots.

3. Remove pre-hybridization solution, squeeze out as much as possible.

4. Add hybridization solution using a pasteur pipette. (Use ~59 µl/cm^2 of filter)
Remove air bubbles and seal.
Incubate at 65°C (or 42°C for RNA blots) for 2-24 hours (depending on the conditions)

5. Remove filter, discard ^{32}P waste, submerge filter in 2xSSC 0.5% SDS at room temperature for 5-15'.

6. Transfer filter to fresh tray of 2x SSC, 0.1 SDS for 15' at room temperature.

7. Rinse in 0.1xSSC, 0.1% SDS at 65°C for 60' 2x.

8. Air dry, wrap in Saran.

Reference:

Southern, E. (1975) J. Mol. Biol. 98: 503.