# Analysis of Tail DNAs by Southern Blots

I Restriction enzyme digests

1. 5 µg DNA; total reaction volume of 40 µl.
2. Usually 25-30 units of enzyme (5-6 fold excess of restriction enzyme).
3. (Optional) 2 µl of 0.1 M spermidine in reaction.
4. Digest overnight.
5. Add 6 µl of tracking dye (20% w/v ficoll, 0.2% bromphenol blue, 0.2% zylene cyanol, 0.1 M EDTA, pH 7.4).

II Running the Gel:

1. BRL large horizontal submarine gels with 20 tooth (2 mm) comb. 300 ml 0.6%- 1% agarose poured containing 10 µl of 10 mg/ml EtBr. Gel buffer is 1X TPE (2.5 L made for each gel; includes 300 ml needed for gel itself).

2. Load samples with a pipetman, run at 45 mA overnight for half gels or 90 mA overnight for full-length gels.

III Setting up the Blot

1. Photograph the gel, and then irradiate with short-wave UV light for 3-5 min (optional), depending on the strength of the UV source.

2. Cut gel to desired size and soak in Alkali solution (0.5 M NaOH, 1.5 M NaCl) for 45 minutes on an orbital shaker. Rinse gel briefly in dd $H_2O$.

3. Soak gel 45 min in neutralizing solution (1 M Tris-HCl, 3 M NaCl) on an orbital shaker.

4. Meanwhile, cut Nylon membrane (Hybond, Zetabind, Nytran) to size of gel; soak in dd$H_2O$ for 10 min and blotting buffer (10X SSC) for 30 min.

5. Set up blot. Let blot overnight in 10X SSC. Blot: plexiglass plate, S&S wick 004, gel, Nylon membrane, one sheet Whatman #1, 5 sheets S&S blot wick 004, paper towels, plexiglass plate, weight.

6. The next day, peal off membrane from gel, rinse in 2X SSC, UV cross-link filters, let dry, and bake at 80°C under vacuum for 1-2 hrs.

7. Wash filters in 0.1X SSC, 1% SDS (Biorad) for 1-2 hrs at 65°C to remove EtBr.

8. Add 50 ml of prehybridization buffer to each bag (see below).

## IV Making Probe

1. Prepare probe by Nick Translation or random priming. Following labeling reaction, separate incorporated from unincorporated label using a Sephadex spin column.

2. Prepare spin column: Mix bottle containing Sephadex G-50 (in TE buffer), and fill spin column (almost to top) with Sephadex. Let Sephadex settle to bottom, then spin 4 minutes (put column inside of a 15 ml tube) at 1K in IEC centrifuge. Dump TE that is inside 15 ml tube. Add contents of labeling reaction to the top of the column and spin again for 4 minutes. Put column in solid radioactive waste. Probe (the flow through) is now ready to add to hybridization solution.

## V Prehybridization.

Prehybridization buffer:
1 Liter:
618 ml dd$H_2O$
200 ml 20X SSCP
50 ml 20X Denhardt's solution
100 ml of 10% SDS
34 ml of sonicated salmon sperm DNA (3 mg/ml) that has been boiled for 10 minutes. (no need to sterilize)
Aliquot prehybridization solution in 50 ml tubes & store in freezer.

Put up to 4 filters into a heat sealable bag & seal. Add 50 ml of prehybridization solution and seal bag. Bags can be stored at 4°C if not setting up hybridizations immediately. Prehybridize for 2 hrs at 65°C.

## VI Hybridization

Hyb-1 mix:
0.4 ml salmon sperm DNA (3 mg/ml)
4.6 ml dd$H_2O$
1.2 ml 10% SDS (Biorad)
Add probe & boil for 10 minutes.
After 10 min, place tube in ice bucket.

Hyb-2 mix:
3 ml 40% dextran sulfate
0.6 ml 20X Denhardt's solution
2.4 ml 20X SSCP

Add Hyb-2 mix to Hyb-1 mix. Pour prehybridization mix out of bag containing filters & add Hyb1/Hyb2 mix.

Hybridize overnight at 65°C (18-22 hrs, 2 days is OK) in Tupperware with water covering bags.

## VII Washes

Prepare 2 L of 1X SSCP, 0.1% SDS (wash A):
100 ml of SSCP
20 ml of 10% SDS
1.9 L of dd$H_2O$

Pour hybridization solution into liquid radioactive waste container and pour water in Tupperware into radioactive sink. Add ~100 ml of wash A to Tupperware. Remove filters from bag & place in Tupperware. Be sure to completely separate filters from one another. Pour solution into radioactive sink & flush with copious amounts of water. Add ~ 1L of wash A solution. Wash for 30 minutes, pour wash into sink. Add remainder of wash A and wash for 30 minutes. After each wash, monitor filter to see how hot it is. Depending on the probe, it may also be necessary to wash at a higher stringency using wash B. Some probes may require a different washing scheme from the one described here.

Wash B (1 L):
10 ml of 20X SSCP
10 ml of 10% SDS
1 L of dd$H_2O$

VIII Reagents:

20X Denhardt's solution:
2 g polyvinyl-pyrrolidine
2 g ficoll
2 g BSA (add after other 2 components are in solution)
dd$H_2O$ to 500 ml. Filter sterilize and aliquot into 50 ml tubes & store in freezer.

Alkali Solution:
87 g NaCl
20 g NaOH
water to 1L

Neutralization Solution:
121.1 g Tris base
174 g NaCl
66 ml HCl
water to 1L

20X SSCP
140.25 g NaCl
88.2 g sodium citrate
43.7 g $Na_2HPO_4$ (dibasic)
12.7 g $NaH_2PO_4$ (monobasic)
water to 1L

Spin columns: Fisher cat. # 11-387-50