PREPARATION OF HOT cDNA FOR DIFFERENTIAL SCREENING

1. Denature 10 µg of total RNA at 70°C for 5' and transfer to ice.

2. Combine the following in order:

   H₂O to 50 µl
   5 x AMV-RT buffer 10 µl
   Oligo dT (250mg/ml) 2 µl
   2 mM dGTP, dTTP 2.5 µl
   10 mM spermidine-HCl 2.5 µl
   80 mM sodium pyrophosphate 1 µl
   RNAGuard 1 µl
   Total RNA 10 µg
   α³²PdCTP 50 µCi (5 µl)
   α³²PdATP 50 µCi (5 µl)
   AMV reverse transcriptase 10-20 units

3. Incubate 42°C 15'.

4. Add 5 µl chase: 10 mM dATP
   10 mM dCTP
   10 mM dGTP
   10 mM dTTP
   Incubate 42°C 20'.

5. Add 50 µl 0.3 M NaOH, 30 mM EDTA. Boil for 5'.

6. Add 7.5 µl 2 M Tris pH 8.0 and 100 µl 25:24:1 phenol:chloroform:IAA.

7. Spin 3' in microfuge.

8. Remove supernate and add:
1 µl 10mgml\(^{-1}\) salmon sperm DNA
50 µl 7.5 M NH\(_4\)OAc
200 µl ethanol

Precipitate on dry ice/ethanol.


10. Wash with 50 µl NH\(_4\)OAc and 100 µl ethanol.

11. Spin 10'. Wash with 200 µl ethanol.


13. Resuspend in 200 µl TE and count 2 µl in aqueous scintillant.

14. Assume maximum 200 ng of the 10 µg total RNA is used in 1\(^{st}\) strand synthesis - probes should be about 10\(^7\)cpmµg\(^{-1}\).

15. Adjust volumes to give equal number of counts per µg of each probe.

16. Denature probe by boiling before use.