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:

H_2O to 50 μl	
5 x AMV-RT buffer	10 μl
Oligo dT (250mgml^{-1})	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	10μg
α^{32}PdCTP	50μCi (5μl)
α^{32}PdATP	50μCi (5μl)
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_4OAc
200 μl ethanol

Precipitate on dry ice/ethanol.

9. Spin down 15' in microfuge.

10. Wash with 50 μl NH_4OAC and 100 μl ethanol.

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

12. Spin 10'. Dry in Speedvac.

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 1st strand
synthesis - probes should be about 10^7cpmμg^{-1}.

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

16. Denature probe by boiling before use.