HYBRIDIZATION OF NORTHERN BLOTS WITH RIBOPROBES.

1. Prehybridize filter for 1 – 2 hr at 50°C in **prehybridization buffer**.

2. Hybridize overnight at 50°C in **hybridization buffer**.

3. Wash filters at 50°C for 2 x 30' in **1 x wash buffer**.

4. Air dry filter and expose to film.

5. If filter is dirty after exposure, can RNAase treat to get rid of background but you will not be able to use the filter again. Incubate in **RNAase buffer** for 1 hour at 37°C and then rinse with RNAase buffer minus enzyme for a further 30' at 37°C.

Prehybridization buffer (100ml)

5 x SSC	25 ml 20 x
1% SDS	10 ml 10%
0.1% sodium pyrophosphate	1 ml 10%
5 x **Denhardt's**	5 ml 100 x
200 $\mu g ml^{-1}$ **sheared ss DNA**	2 ml 10$mg ml^{-1}$
50% deionized formamide	50 ml
	7 ml H_2O

Hybridization buffer (100ml)

5 x SSC	25 ml 20 x
1% SDS	10 ml 10%
0.1% sodium pyrophosphate	1 ml 10%
5 x **Denhardt's**	5 ml 100 x
200 $\mu g ml^{-1}$ **sheared ss DNA**	2 ml 10$mg ml^{-1}$
10% dextran sulphate	10 g
50% deionized formamide	50 ml
	up to 100 ml with H_2O

1 x wash buffer (2L)

1 x SSC	100 ml 20 x
1% SDS	20 g
0.1% sodium pyrophosphate	20 ml 10%
50% formamide	1L

100 x Denhardt's
10g Ficoll
10 g polyvinylpyrollidone
10 g BSA
up to 500 ml with H_2O

Sheared salmon sperm DNA
Dissolve herring sperm or salmon sperm DNA at 10mgml^{-1} in dH_2O. Autoclave for 5' and rapidly cool. Store frozen in aliquots.

RNAase buffer (100ml)

20 μgml^{-1} RNAase A	200 μl 10 mgml^{-1}
0.5 M NaCl	10 ml 5 M
10 mM Tris pH 8.0	500 μl 2M
1 mM EDTA	200 μl 0.5 M