HYBRIDIZATION OF SOUTHERN BLOTS.

1. Prehybridize filter for 1 – 2 hr at 65°C in **prehybridization buffer**.

2. Hybridize overnight at 65°C in **hybridization buffer**.

3. Wash filters at 65°C for 2 x 30' in **1 x wash buffer** and if necessary for a further wash in **0.1 x wash** buffer.

4. Air dry filter and expose to film.

Prehybridization buffer (100ml)

3 x SSC	15 ml 20 x
1% SDS	10 ml 10%
0.1% sodium pyrophosphate	1 ml 10%
5 x **Denhardt's**	5 ml 100 x
200 μgml-1 **sheared ss DNA**	2 ml 10mgml^{-1}
	67 ml H_2O

Hybridization buffer (100ml) (Don't use dextran for non-genomic blots)

3 x SSC	15 ml 20 x
1% SDS	10 ml 10%
0.1% sodium pyrophosphate	1 ml 10%
5 x **Denhardt's**	5 ml 100 x
200 μgml-1 **sheared ss DNA**	2 ml 10mgml^{-1}
10% dextran sulphate	10 g
	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%

0.1 x wash buffer (2L)

0.1 x SSC	10 ml 20 x

1% SDS	20 g
0.1% sodium pyrophosphate	20 ml 10%

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.

RECYCLING MEMBRANES

1. Place filter in 0.2M NaOH, 0.1%SDS for 30' at 37°C.

2. Rinse in 2 x SSC and then prehybridize as normal.