

HYBRIDIZATION OF NORTHERN 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 10' in **2 x wash buffer**. Check filters with Geiger counter. If too hot, wash for 2 x 10' in **1 x wash buffer**.
4. Air dry filter and expose to film.

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 μgml^{-1} sheared ss DNA	2 ml 10mgml ⁻¹
	57 ml H ₂ O

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 μgml^{-1} sheared ss DNA	2 ml 10mgml ⁻¹
10% dextran sulphate	10 g
	up to 100 ml with H ₂ O

2 x wash buffer (2L)

2 x SSC	200 ml 20 x
1% SDS	20 g
0.1% sodium pyrophosphate	20 ml 10%

1 x wash buffer (2L)

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

100 × Denhardt's

10g Ficoll
10 g polyvinylpyrrolidone
10 g BSA
up to 500 ml with H₂O

Sheared salmon sperm DNA

Dissolve herring sperm or salmon sperm DNA at 10mgml⁻¹ in dH₂O.
Autoclave for 5' and rapidly cool. Store frozen in aliquots