RNA (Northern) Blot Analysis

Procedure:

(1)	Wet membrane in 2X SSC		
(2)	Pre-pre hyb	60°C	~1h
(3)	Pre-hyb	42 °C	4h - O/N
(4)	Hyb	42 °C	O/N
(5)	Low stringency washes (3)	RT	~15min each
(6)	High stringency washes (3 or 4)	55 °C	~60min each
(7)	Expose film to filter		
(8)	Strip membrane (2 washes)		10-15min each
	(Boil stripping solution then add to filter)		

Hybridization Solution Preparation:

		Pre-pre (20ml)	Pre-hyb (10ml)	Hyb (5ml)
(1)	Formamide	-	5.0ml	2.5ml
(2)	25X SSC	0.8ml	2.0ml	1.0ml
(3)	2M PB	-	0.25ml	0.05ml
(4)	Denhardt's	-	1.0ml	0.1ml
(5)	10% SDS	1.0ml	0.2ml	0.05ml
(6)	Dextran Sulfate	-	-	1.0ml
(7)	10mg/ml Fish DNA	-	0.2ml	0.05ml
(8)	dH2O	38.2ml	1.35ml	-
(9)	Probe	-	-	0.5-1.0ml

Stock Solution Preparation:

(1) **Formamide**: stock solution is deionized by stirring with AG 501-X8 (D) ion exchange resin (BIORAD) for 30 min and subsequently filtering/decanting.

(2) **25X SSC**

	Stock		Conc
1.	NaCl	43.8g	3.75M
2.	Na-Citrate	22.1g	0.375M
3.	pH w/HCl to	7.0	
4.	ddH_2O to	250ml	
5.	Filter through a 0.45µM filter, Autoclave		

(3) **2M PB**

1.	Na_2HPO_4	28.3g
2.	ddH_2O	160ml
3.	Mix until dissolved (heat ok)	
4.	NaH_2PO_4	27.5g
5.	Mix until thoroughly dissolved	
6.	Bring vol to	200ml

7. Filter through a 0.45µM filter, Autoclave

(4) **100X Denhardt's**

1.	BSA	0.5g
2.	PVP	0.5g
3.	Ficoll	0.5g
4.	ddH_2O to	25.0ml
5.	Filter through cinder-glass using Millipore apparatus	
6.	Store at -20^oC	

(5) **10% SDS**

1.	SDS	10g
2	ddH_2O to	100ml
3.	Sterile filter	

(6) **50X Dextran sulfate**

1.	Dextran sulfate	25g
2.	Sterile H_2O to	50ml
3.	Heat/Vortex to dissolve	
4.	Store -20^oC	

(7) **10 mg/ml Fish DNA**

1. Dissolve 0.1g of Fish DNA in 10ml sterile ddH_2O. Shear by drawing the DNA up into a syringe and passing it out though a 22 g needle. Repeat ~ 20x.
2. Store at -20^oC.
3. Boil for 10 minutes before using.

(8) **5% PPi**

1.	Sodium pyrophosphate	7.5g
2.	ddH_2O to	150ml
3.	Filter through a 0.45µM filter, Autoclave	

Wash Buffers:

Stock	Low stringency Vol	Conc	High stringency Vol	Conc
(1) 20X SSC	25ml	[2X]	2.5ml	[0.2X]
(2) 10%SDS	1.25ml	[0.05%]	1.25ml	[0.05%]
(3) 5% PP_i	1.0ml	[0.02%]	0.5ml	[0.01%]
(4) dH_2O	222.75ml		245.75ml	

Strip Buffer:

(1) dH_2O	500ml
(2) BOIL	
(3) 10% SDS	5ml

Note: the above Hyb volumes are for a 10 X 15 cm filter.