NORTHERN BLOT

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RNA formaldehyde gel

The gel

	For 80ml gel
Agarose	0.64g
10x Mops buffer	8ml
dH_2O	57.6ml

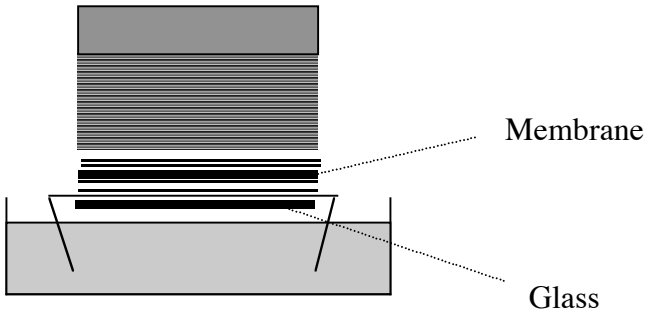
After cooled to 50°C add:
Formalin (=37% formaldehyde solution) 14.4ml

Running buffer (1%Mops): 700ml dH_2O containing 70ml 10x Mops

(1) Pre-run the gel for 1/2 hour before loading the samples
(2) Measure 10μg of RNA per sample (per lane).
(3) Dry RNA samples in speedvac and resuspend in 15μl of RNA loading dye*
(4) Heat to 70°C for 10min, quench on ice, centrifuge if necessary.
(5) Run gel until dyes are symmetrically placed in gel.

Transfer to Nylon membrane

(1) Rinse the gel in several changes of **DEPC-dH_2O**
(2) Soak the gel for 20mins in **7.5mN NaOH**
(3) Rinse the gel in **DEPC-dH_2O** and soak in **20x SSC** for 45mins
(4) Cut 3M paper (6 pieces) and nylon membrane to about the size of the gel (slightly larger). Place the papers in **2x SSC** for 5 mins.
(5) Place the gel in the apparatus for transferring. Running buffer=**20x SSC**



(6) Transfer overnight

(7) Peel the membrane from the gel. Soak the membrane in **6x SSC** for 5mins at room temperature. Place the filter on a paper towel to dry at least 30mins at room temperature.
Place the membrane between two 3MM paper and bake in 80°C vacuum oven for 2hrs. Alternatively, use UV Crosslinker (254nm) for 30sec.
(8) Store the membrane in a cool and dry place or proceed to the hybridization.

Reusing the membrane

After the membrane has been used, you can reuse the membrane by de-hybridize the filter as followed:

De-hybridization buffer:

1ml 20x SSC
1ml 10%SDS
add H_2O to 1l

Boil the solution, pour to a tank containing the filter, then incubate at 42°C, 1-2hr.

Hybridization

(1) Boil 100ml pre-hyb* solution for 10mins. Cool it down on ice, when SDS starts to precipitate, pour the solution to a seal bag with the baked membrane. Place the bag in 65°C for 2 hr.
(2) Make probe:

Random primed DNA labeling kit*
PCR the desired fragment by adding Hot ATP (frozen one worked better)

(3) Dump the pre-hyb solution if baking over 2hr, add new pre-hyb (boil and cool down as above).
(4) Add boiled probe into the bag and seal it. Put it into another bag and seal it. Place the doubled bag into a container and then on a rocking device in 65°C oven. Incubate overnight.

Hybridization-Wash the filter

Use the solutions in the following order, each wash is 15mins with gentle agitation, this follows Frohlich's lab procedure.

Low stringency

Wash number	Ingredient	For 3 liter
1-Rm temp	2x SSPE	300ml stock 20x SSPE
	0.5%SDS	75ml 20%SDS
	H_2O	2625ml H_2O
2-Rm temp	2x SSPE	300ml stock 20x SSPE
	0.1%SDS	15ml 20%SDS
	H_2O	2685ml H_2O
3-Rm temp	0.3x SSPE	45ml stock 20x SSPE
	0.5%SDS	75ml 20%SDS
	H_2O	2880ml H_2O
4-**55°C**	2x SSPE	300ml stock 20x SSPE
	0.5%SDS	75ml 20%SDS
	H_2O	2625ml H_2O
5-Rm temp	0.3x SSPE	45ml stock 20x SSPE
	No SDS	-
	H_2O	2955ml H_2O

High stringency

4-**65°C**	0.1x SSPE	15ml stock 20x SSPE
	0.5%SDS	75ml 20%SDS
	H_2O	2910ml H_2O
	NOTE: 500ml is pretty enough for one membrane. Pre-heat the solution before use!	

After the 5th wash, place the membrane in two layers of Sarahwrap, try to fold to edges so that the membrane won't dry out. The membrane is now ready to put into cassette. Put the cassette (with x-ray film) in -80°C freezer. Defrost before developing. If the isotope is hot (or quite fresh), then develop the film after 48hrs.

*__RNA loading dye__ formula

10x MOPS	650μl
Formamide	3550μl
Formalin	1250μl
EtBr (3mg/ml)	39μl
Ficol	300mg
1N NaOH as needed to bring to pH7	
Bromophenol blue	30μl
Xylene cyanol	50μl

Both bromophenol blue and xylene cyanol are saturated solution in 1M Tris, pH8.
Store loading dye wrapped in foil in -20°C freezer.

__Pre-Hybridization__ solution

250ml	20x SSPE
100ml	Denhart's reagent (50x)
25ml	20% SDS
10ml	Fragmented Hering sperm DNA
615ml	dH_2O

Store in 4°C

Making probe:
If using random primed DNA labeling kit.
For double reaction:

- 1.5μl template
- 14.5μl H_2O
- 4μl reagent #6
- 8μl CTG
- 10μl HOT A
- 2μl Klenow DNA polymerase I

99°C: 30sec, 37°C: 1hr