Northern Blots

(based on protocol from Mitch Smith Lab)
Use gel apparatus designated for RNA. Wipe apparatus with "RNase Away".

1% Agarose gel (100ml)
73 ml sterile water
1 g agarose
Boil in microwave and cool a little
Add 10 ml 10X MOPS and 17 ml 37% Formaldehyde
Pour.

10X MOPS

0.2 M MOPS	23.1 g/500ml
50 mM Sodium Acetate	8.3 ml of 3M/500 ml
10mM EDTA	10ml of 0.5M/500ml
adjust pH 7.0	

Sample preparation and electrophoresis
Add 20 μg each RNA to eppendorf tube, dry samples in speed-vac. Add 17 μl Sample Buffer, heat samples at 65 C for 10 min, add 3 μl 10X dye, and then load gel and run in 1X MOPS
Run gel 1-2 hrs at 90 V
Photograph gel and then transfer

Sample Buffer

50% Formamide	500 μl/ml
2.2 M Formaldehyde (37%)	170 μl/ml
1X MOPS	100 μl of 10X/ml

10X Dye Solution

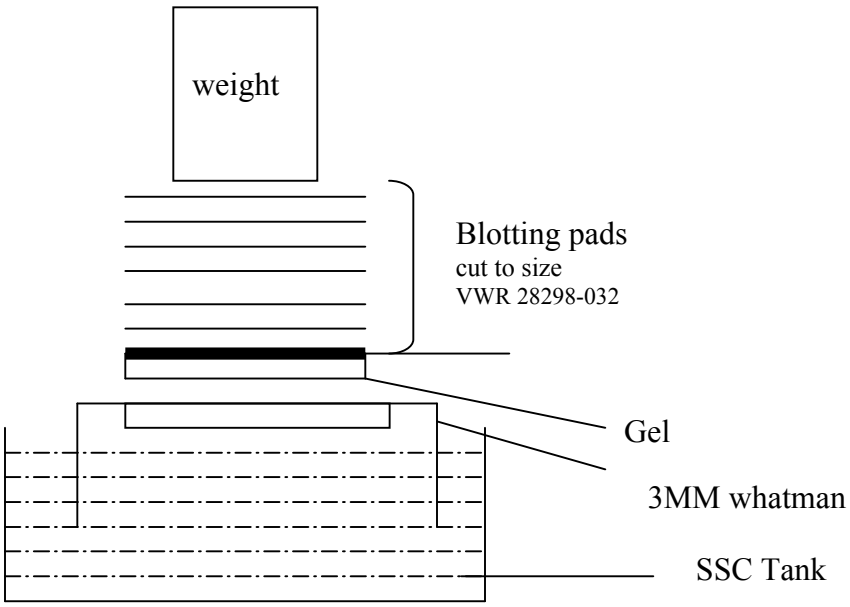
50% glycerol	100 μl
0.3% Bromophenol Blue	6 μl (10%)
Ethidium Bromide	25 μl 4mg/ml
Sterile water	75 μl

Transfer

Rinse gel twice for 15 min each in sterile water.
Cut nitrocellulose (Schleicher & Schuell Protran BA85, #10402580) to size of gel. Float nitrocellulose in water to wet and then soak in 20X SSC.

20X SSC
3.0M NaCl 175g/L
0.3 M sodium citrate 88g/L
Autoclave using liquid cycle for at least 40 minutes

Blot gel by capillary method using a reservoir of 20X SSC (see figure), usually overnight.



Place blot on plastic wrap, then UV crosslink RNA to blot using Stratalinker on automode.

Hybridization

Prehybridize blot at 42 C for 1-2 hrs in Hybridization Solution (plus freshly boiled herring sperm DNA (100 μg/ml final) and 1 ml of 50% dextran sulfate per 5 ml of Hybridization Solution) Use roller bottle in hybridization oven.

Hybridization Solution (100ml, store at 4 C)
50 ml formamide
1 ml 10% SDS
25 ml 20X SSC
5 ml 1M sodium phosphate, pH 6.8
10 ml 50X Denhardt's solution
9 ml H2O

Before use:
Add boiled SS DNA to 0.1 mg/ml
Add 20 ml 50% dextran sulfate per 100 ml

50% Dextran sulfate: 25 g/50 ml water, mix overnight and store at 4 C

Fish Sperm DNA (10 mg/ml, store at –20C)
5030 µl H2O
2942 µl 20XSSC
500 µl 1M Tris-Cl 7.5
352 5 M NaOH
100 mg DNA (Sigma D-1626)
Dissolve with shaking, heat at 80 C for 10 min. Then neutralize by adding 588 µl 2M Tris-Cl (pH 7.4) sonicate at max setting for 1 minute.
Autoclave and aliquot.

50X Denhardt's Solution (100 ml, store at –20 C)
1 g Ficoll (Sigma F4375)
1 g Polyvinlypyrrolidonesigma (Sigma PVP360)
1 g BSA
Filter solution through 0.45 µ filter unit. Aliquot and freeze.

Sodium phosphate, pH 6.8 (100 ml, store at room temp)
46.3 ml 1M Na_2HPO_4 (14.2 g/100ml)
53.7 ml 1M NaH_2PO_4 (13.8 g/100ml)

DNA probe preparation by random labeling

Use Invitrogen kit, #18187-013.
Denature 25 ng DNA in 5-20 ul water by boiling then place tube on ice. (Use gel isolated fragment from PCR or restriction digest of plasmid)
Add 2 µl each of dATP, dGTP and dTTP.
Add 15 µl random primers buffer mixture.
Add 5 µl (50 µCi) of α^{32}P dCTP (3000 Ci/mmol)
Add 1 µl Klenow enzyme, mix gently and centrifuge briefly.
Incubate at room temperature for 1 hour.
Add 5 µl stop buffer
Purify labeled DNA using Amersham Probe Quant G-50 Micro Column (#27-5335-01) exactly as described by the manufacturer.

Boil probe, cool on ice, then add 5-25 ng to prehyb.
Incubate blot at 42 C overnight in roller bottle.

Blot work-up

Wash twice in 0.1X SSC, 0.1% SDS for 10 min at 42 C in hyb bottle.

Wrap blot in plastic wrap and expose to film or phosphorimager screen. Do not let the blot dry out if you plan to re-probe it.

Strip blot for re-probing

Bring 1 liter 0.1 % SDS just to boiling.
Remove from heat and add blot. Allow solution to cool. Discard solution in radioactive waste. Repeat. Store blots at –20 C.