High Molecular Weight RNA gel blot

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For 1% Agarose Gel:

Stock	
Agarose	1g
10 X FF buffer	10ml
water	88ml

1. Boil to dissolve agarose
2. Cool it to about 60C
3. Add formaldehyde to a final concentration of 7% (18ml of lab 40% stock) and mix well and pour (I use the thin 1mm well comb).
4. Run gel in 1 X FF buffer with 0.7% formaldehyde (18ml of lab 40% stock in 1L of 1 X FF buffer)

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10 X FF running buffer:

Final concentration
200mM HEPES sodium salt
10mM EDTA
pH buffer to 7.8

For 500mL 10 X FF: add 23.8g HEPES and 1.9g EDTA to 500mL with water (don't forget to pH to 7.8)

To prepare RNA for loading:
I usually load 5-15ug of RNA (depending on the concentration of my RNAs and the abundance of the mRNA I am trying to detect).

Master Mix:

Stock	
Deionized formamide	7ul
(40% by vol) Formaldehyde	2ul
10 X FF buffer	1ul
400ug/ml EtBr	1ul
0.5% Bromophenol Blue	1ul
	12ul

1. Calculate the volume of each RNA needed for Xug.
2. Add 12ul of Master Mix to each tube.
3. Add appropriate amount of RNA (the final volume of the sample, including master mix, should not exceed 16ul to be able to fit it into the well)
4. Fill each tube to a final volume of 16ul with water (final ratio of Master mix to RNA is 3 volumes to 1 volume)
5. Incubate at 85C for 2 minutes to denature RNA

6. Put RNA on ice and quickly spin down each sample (leave samples on ice while loading the gel
7. Run gel until BPB dye is about ¾ down the gel then take a picture.

Transfer to Nylon membrane (Genescreen Plus/NEN) by capillary action

1. Wash gel in water
2. Soak gel in 50mM NaOH, 10mM NaCl for 10 minutes at room temperature (with shaking)
3. Neutralize by shaking for 10 minutes in 2.5 X TBE at room temperature
4. Equilibrate the gel and the nylon membrane by soaking them in 10 X SSC at room temperature
5. Transfer RNA to nylon membrane in 10 X SSC overnight

To make transfer sandwich:

1. Place a support wrapped with 3mm Watman paper in a dish containing 10 X SSC (the SSC should not wash over the support)
2. Place gel upside down on support
3. Place parafilm around the gel to ensure that buffer only transfers through the gel
4. Place the membrane on top of the gel
5. Place two pieces of 3mm Watman paper on top of the membrane
6. Roll out any glass bubbles with a glass rod
7. Place a stack of paper towels on top of the sandwich
8. Put a glass plate on top of this and place a 500mg weight on top of this
9. Let the RNA transfer overnight.
10. Next morning, remove paper towels and Watman
11. Place membrane RNA side up, UV X-link with 100uJ of energy, and bake at 80C for 30 minutes to dry the membrane

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Prehybridize, Hybridize, and wash membrane in Ambion Ultrahyb buffer following manufacturers protocol.

In vitro transcribed RNA probes
(using Bartel lab T7 polymerase):
1-2ul DNA template (~100-500ng)
5ul 10 X T7 buffer
1ul 10mM ATP
1ul 10mM CTP
1ul 10mM GTP
5ul alpha P32 UTP (800Ci/mmol) NEN cat# : BLU007X
1ul 0.25M DTT
2.5ul T7 polymerase
to 50ul with water
Incubate at 37C for 1hour

Random primed DNA probes:

I use Amersham Megaprime labeling kit following manufactures protocol.
Label with alpha-P32 dCTP (6000Ci/mmol) NEN cat#: BLU013Z