RNA Gels
(Adapted from the Red Book i.e., Ausubel)

Gel (One 1.2% Agarose, 10 X 15cm, 165ml gel):

(1) Agarose	2g
(2) ddH_2O	143.55ml
(3) Boil	
(4) Cool	to 60^oC
(5) 10X MOPS	16.5ml
(6) Formaldehyde	4.95ml

Sample Prep:

	Per Sample (adjust volumes according to well volume)
(1) 10X MOPS	5µL
(2) 37% (stock) Formaldehyde	8.75µL
(3) Formamide (Deionized)	25µL
(4) 10mg/ml Ethidium Bromide	0.05µL

(5) Mix:

(a) RNA (2-10µg*)	___µL
(b) Sample Prep Sol'n	38.75µL
(c) DEPC treated dH_2O	to 50µL

(6) Incubate @ 55-68^oC	15min
(7) Formaldehyde loading buffer	10µl

(8) Load samples onto the gel.

(9) Run ~100V ~2-3h (200 -300 V-h).

(10) Photograph the gel

(11) Rinse the gel by incubating it in ddH_2O for 30min with gentle shaking.

(12) Discard the water.

(13) Repeat the wash step with a second 30 min incubation.

(14) Set up a transfer blot. See the procedure "Genomic(Plant)DNARestrictionDigestionForSoutherns" on how to set up a blot. Note that RNA is already denatured and so there is no need to run through the denaturation/neutralization steps as with Southerns. Simply follow the steps for *Transfer Membrane*, *Gel Blot Paper* and *Assembling the Blot*, and blot gel onto Hybond N nylon membrane overnight using 10X SSC.

(15) Disassemble the gel blot and remove the filter from the gel.

(16) Invert the filter so the DNA side is facing up and lay it on a piece of plastic wrap.

(17) Cross-link the RNA to the filter using the UV Stratalinker 2400:
Turn on the UV Stratalinker 2400
Place filter, DNA side up, into the chamber
Close the door
Press Auto Cross Link
Press Start

Solutions and Stuff:

	Stock	Final concentration
(1) **Formaldehyde loading buffer**		
(a) 0.5M EDTA	2µL	1mM
(b) Bromophenol Blue	Dab	~0.25%
(c) Xylene Cyanol	Dab	~0.25%
(d) 80% Glycerol	625µL	50%
(e) dH_2O	373µL	
(2) **10X MOPS Buffer**		
(a) MOPS	41.8g	
(b) 3M DEPC treated NaOAc	16.6ml	
(c) 0.5M EDTA	20.0ml	
(d) ddH_2O	to 1L	
(e) DEPC	1ml	
(f) Filter sterilize		
(g) Store at RT in a brown bottle		

Give the gel box, casting tray and comb a good wash with detergent before pouring the gel.
*We have found that a range of total RNA concentrations work for hybridization analyses. Typically we load 8µg barley, 5µg *Medicago spp*., 8µg Solanaceous *spp*., and 8-10 for *Arabidopsis thaliana*.