RNA Gel

20X Borate Buffer: 0.4 M Boric Acid, 4 mM EDTA, pH adjusted to 8.3 with NaOH.
Sample buffer: 900 ul deionized formamide, 294 formaldehyde, 90 ul 20X buffer, 90 ul dye mix.
Dye Mix: 0.1% each bromophenol blue and Xylene cyanol in 0.1 M EDTA.
Gel boxes and plastic material should be treated with 3% hydrogen peroxide to ensure absence of ribonucleases. All glassware and solutions should be autoclaved.

Pouring the Gel

To pour a standard 11X14 cm gel you will need 75 ml of gel mix.

Agarose	0.75-1.1 grams
20X Buffer	3.75 ml
Water	65 ml Melt agarose and cool to 50^{o}C. Then add:
Formaldehyde	6.25 ml

Pour the gel in the hood!!!
Allow the gel to set during which time make 1 liter 1X Borate Buffer.

Sample Preparation
Mix 7 ul RNA, containing as much as 10 ug, and 23 ul sample buffer.
Heat at 65^{o}C for 5 minutes. Cool on ice, add 3 ul dye mix and load gel.
Place the gel in tank and add buffer such that the gel is 1-2 mm below the surface. Load samples.
Run gel for 15 minutes at 20 volts then 80-90 volts for 3 hours.
This can be done at your bench since there is such a small amount of formaldehyde.

Transferring the Gel

Immediately transfer the gel to nitrocellulose or nylon membrane using 20X SSC or 20X SSPE. Prewet membrane in water, then soak in 20X SSC or 20X SSPE for 5-10 minutes. Transfer as for Southern but leave for overnight, i.e. >16 hours. Bake filter as per Southern for 2 or more hours.