ELECTROPHORESIS OF RNA THROUGH GELS CONTAINING FORMALDEHYDE

1. Prepare gel-running buffer and formaldehyde.

   a. 5 X Gel-running buffer:
   0.2 M morpholinopropanesulfonic acid (MOPS) (pH 7.0)
   50 mM sodium acetate
   5 mM EDTA (pH 8.0)

   This buffer yellows with age if exposed to light or autoclaved. Discoloration does not affect its performance appreciably.

   b. .Formaldehyde (F.W.=30.03) is usually obtained as a 37% solution in water (12.3 M). Check that the pH of the concentrated solution is greater than 4.0. The concentrated solution should be handled and stored in a chemical hood.

2. Prepare the gel by melting agarose in water, cooling to 60°C, and adding 5X gel buffer and formaldehyde to give 1X and 2.2 M final concentrations, respectively. (One part of stock formaldehyde solution should be diluted with 4.6 parts of agarose solution).
   Note: The fractionation properties of formaldehyde gels with different agarose concentrations have been determined by Lehrach et al. (1977).

3. Prepare the sample by mixing the following in a sterile Eppendorf tube:

RNA (up to 20 µg)	4.5 µl
5 X gel-running buffer	2.0 µl
formaldehyde	3.5 µl
formamide	10.0 µl

   Incubate at 55°C for 15 minutes.

   Note: Formamide oxidizes readily in air and should be deionized by passage through a mixed-bed resin (Bio-Rad AG 501-X8) until its pH is neutral. It is then recrystallized at 0°C and stored at -20°C in small aliquots in tightly capped tubes.

4. Add 2 µl of sterile loading buffer.
   Loading buffer:
   50% glycerol
   1 mM EDTA
   0.4% bromophenol blue
   0.4% xylene cyanol

5. Load the RNA samples onto the gel. Restriction fragments of DNA are

convenient molecular-weight markers. They should be treated and run exactly as the RNA samples. Labeled DNA markers or markers that will be detected by a labeled hybridization probe are preferred because the ethidium fluorescence of formaldehyde-denatured nucleic acids is weak. If such markers are impossible to obtain, the following protocol may be used:

a. Apply to the gel sufficient DNA to give at least 50-100 ng per band.

b. Cut the lanes containing the markers from the gel before the alkaline hydrolysis step (see below), and wash with four or five changes of water for 2 hours.

c. Wash with two changes of 0.1 M ammonium acetate for 1 hour.

d. Stain for 1 hour with 0.5 μg/ml of ethidium bromide in 0.1 M ammonium acetate, and 0.1 M ß-mercaptoethanol.

e. Destain for 45 minutes with a solution of 0.1 M ammonium acetate and 0.01 M ß-mercaptoethanol.

Transfer of Formaldehyde-denatured RNA to Nitrocellulose

1. After electrophoresis is complete, soak the gel for 5 minutes in several changes of water.

   *Note: Gels containing formaldehyde are less rigid than nondenaturing agarose gels. Care must be exercis*ed in handling them.

2. Soak the gel in an excess of 50 mM NaOH and 10 mM NaCl for 45 minutes at room temperature.

   Note: The partial alkaline hydrolysis improves the transfer of high-molecular-weight RNA.

3. Neutralize the gel by soaking for 45 minutes at room temperature in 0.1 M Tris Cl (pH 7.5).

4. Soak the gel for 1 hour in 20X SSC.

5. Transfer the RNA to nitrocellulose by the method described for Southern blots. The transfer is complete in 3-4 hours.

6. After transfer is complete, wash the filter in 3X SSC, dry in air for 1-2 hours, and bake for 3-4 hours at 80°C under vacuum.

Reference:

Procedure from Maniatis cloning manual.
Lehrach, H., D. Diamond, J.M. Wozney and H. Boedtker. 1977. Biochemistry 16, 4743.