Electrophoresis of RNA through Gels Containing Formaldehyde

This method is adapted from those of Lehrach et al. (1977), Goldberg (1980), and Seed (1982a).

Caution: Formaldehyde vapors are toxic. Solutions containing formaldehyde should be prepared in a chemical hood, and electrophoresis tanks containing formaldehyde solutions should be kept covered whenever possible.

1. Prepare 5 × formaldehyde gel-running buffer.

5 × Formaldehyde gel-running buffer

0.1 M MOPS (pH 7.0)
40 mM sodium acetate
5 mM EDTA (pH 8.0)

Dissolve 20.6 g of 3-(*N*-morpholino)propanesulfonic acid (MOPS) in 800 ml of diethyl pyrocarbonate (DEPC)-treated 50 mM sodium acetate (see page 7.4 for DEPC treatment of solutions). Adjust the pH to 7.0 with 2 N NaOH. Add 10 ml of DEPC-treated 0.5 M EDTA (pH 8.0). Adjust the volume of the solution to 1 liter with DEPC-treated water. Sterilize the solution by filtration through a 0.2-micron Millipore filter, and store it at room temperature protected from light. The buffer yellows with age if it is exposed to light or is autoclaved. Straw-colored buffer works well, but darker buffer does not.

Caution: DEPC is suspected to be a carcinogen and should be handled with care.

2. Prepare the gel by melting the appropriate amount of agarose in water, cooling it to 60°C, and adding 5 × formaldehyde gel-running buffer and formaldehyde to give final concentrations of 1 × and 2.2 M, respectively. (One part of a stock 12.3 M formaldehyde solution should be diluted with 3.5 parts of agarose in water and 1.1 parts of 5 × formaldehyde gel-running buffer.) Cast the gel in a chemical hood, and allow the gel to set for at least 30 minutes at room temperature.

The fractionation properties of gels containing formaldehyde and different concentrations of agarose have been measured by Lehrach et al. (1977) and Miller (1987).

Formaldehyde (M_r = 30.03) is usually obtained as a 37% solution (12.3 M) in water. Check that the pH of the concentrated solution is greater than 4.0.

3. Prepare the samples by mixing the following in a sterile microfuge tube:

RNA (up to 30 μg)	4.5 μl
5 × formaldehyde gel-running buffer	2.0 μl
formaldehyde	3.5 μl
formamide	10.0 μl

Incubate the samples for 15 minutes at 65°C, and then chill them on ice. Centrifuge the samples for 5 seconds to deposit all of the fluid in the bottom of the microfuge tubes.

Up to 30 μg of RNA may be analyzed in each lane of the gel. Abundant mRNAs (0.1% or more of the mRNA population) can usually be detected by northern analysis of 10–20 μg of total cellular RNA. For detection of rare RNAs, between 0.5 and 3.0 μg of poly(A)$^+$ RNA should be applied to each lane of the gel.

Many batches of reagent-grade formamide are sufficiently pure to be used without further treatment. However, if any yellow color is present, the formamide should be deionized by adding Dowex XG8 mixed-bed resin and stirring on a magnetic stirrer for 1 hour and filtering twice through Whatman No. 1 paper. Deionized formamide should be stored in small aliquots under nitrogen at −70°C.

Some workers add a small amount of ethidium bromide (1 μl of a 1 mg/ml solution in DEPC-treated water) to the samples before electrophoresis (Fourney et al. 1988). Although this solves the problem of staining RNA in the gel after electrophoresis, it can reduce the efficiency of northern hybridization, especially when the samples contain small amounts of poly(A)$^+$ RNA.

4. Add 2 μl of sterile, DEPC-treated formaldehyde gel-loading buffer.

Formaldehyde gel-loading buffer

50% glycerol
1 mM EDTA (pH 8.0)
0.25% bromophenol blue
0.25% xylene cyanol FF

5. Before loading the samples, prerun the gel for 5 minutes at 5 V/cm. Immediately load the samples into the lanes of the gel. As molecular-weight markers, use RNAs of known size, for example, 18S and 28S rRNAs or 9S rabbit β-globin mRNA. The sizes of these RNAs are 6333, 2366, and 710 nucleotides, respectively. Alternatively, mixtures of RNAs of known size can be purchased from BRL. The markers are usually loaded into the outside lanes of the gel so that they can be cut from the gel after electrophoresis and stained with ethidium bromide. If possible, leave an empty lane between the markers and the samples that are to be transferred to a nitrocellulose filter or nylon membrane.

DNA and RNA migrate at different rates through agarose gels containing formaldehyde: RNA migrates faster than DNA of equivalent size (Wicks 1986). Although DNA markers are preferable because they run as sharp bands, they cannot readily be used to measure the absolute size of unknown RNAs.

6. Run the gel submerged in 1 × formaldehyde gel-running buffer at 3–4 V/cm. Constant recirculation of the buffer is not necessary, but after 1–2 hours the buffer from each reservoir should be collected, mixed, and returned to the gel apparatus.

7. At the end of the run (when the bromophenol blue has migrated approximately 8 cm), the lanes containing the markers may be cut from the gel

and stained with ethidium bromide (0.5 μg/ml in 0.1 M ammonium acetate) for 30–45 minutes. Align a transparent ruler with the gel, and photograph the gel and ruler by ultraviolet illumination. Use the photograph to measure the distance from the loading well to each of the bands of RNA. Plot the $\log_{10}$ of the size of the fragments of RNA against the distance migrated. Use the resulting curve to calculate the sizes of the RNA species detected by hybridization after transfer from the gel to a solid support.

Cautions: Ethidium bromide is a powerful mutagen and is moderately toxic. Gloves should be worn when working with solutions that contain this dye. After use, these solutions should be decontaminated by one of the methods described in Appendix E.

Ultraviolet radiation is dangerous, especially to the eyes. To minimize exposure, make sure that the ultraviolet light source is adequately shielded and wear protective goggles or a full safety mask that efficiently blocks ultraviolet light.

For a description of alternative methods of staining RNA, see page 7.51.

Gels containing formaldehyde are less rigid than nondenaturing agarose gels, and care must be exercised in handling them.

8. Transfer the RNA from the gel to a nitrocellulose filter as described on the following pages or to a nylon membrane as described on pages 7.49–7.50.