GEL ELECTROPHORESIS OF RNA

The two systems most frequently used to measure the molecular weight of RNA and to separate RNAs of different sizes for "Northern" transfer or in vitro translation are:

1. Electrophoresis through agarose gels after denaturation of the RNA with glyoxal and dimethylsulfoxide.

2. Electrophoresis through agarose gels containing methylmercuric hydroxide or formaldehyde.

In each case, the RNA is fully denatured, and its rate of migration through the gel is in linear proportion to the $\log_{10}$ of its molecular weight.

Electrophoresis of RNA after Denaturation with Glyoxal and Dimethylsulfoxide[3]

1. Mix in a sterile Eppendorf tube:

6 M glyoxal	2.7 μl
dimethylsulfoxide (DMSO)	8.0 μl
0.1 M NaH_2PO_4 (pH 7.0)	1.6 μl
RNA (up to 20 μg)	3.7 μl

Glyoxal is usually obtained as a 40% solution (6 M). Because it readily oxidizes in air, the glyoxal solution must be deionized before use by passage through a mixed-bed resin (Bio-Rad AG 501-X8) until its pH is neutral. It is then stored at −20°C in small aliquots in tightly capped tubes.

The sodium phosphate solution is made up as follows:

a. Dissolve 0.1 moles of NaH_2PO_4 in a minimum volume of water.

b. Adjust the pH to 7.0 with concentrated phosphoric acid.

c. Adjust volume to 1 liter and autoclave.

2. Incubate the RNA solution at 50°C for 60 minutes in a tightly closed tube.

3. While the RNA is incubating, pour a horizontal agarose gel. For RNAs up to 1 kb in length, use 1.4% agarose; for larger RNAs, use 1.0% agarose. The gels are poured and run in 0.01 M NaH_2PO_4 (pH 7.0).

Note. Because glyoxal reacts with ethidium bromide, the gels are poured and run in the absence of the dye.

4. Cool the RNA sample to 20°C and add 4 μl of *sterile* loading buffer and load the sample immediately. As molecular-weight markers, use glyoxylated RNAs (e.g., 18S and 28S ribosomal RNAs; globin 9S mRNA).

Loading buffer

50% glycerol
0.01 M NaH_2PO_4 (pH 7.0)
0.4% bromophenol blue

5. The gel is run submerged in buffer at 3–4 V/cm. Constant recirculation of the buffer (see Fig. 6.1) is required in order to maintain the pH within acceptable limits (glyoxal dissociates from RNA at pH >8.0). Alternatively, the buffer may be changed every 30 minutes during the run.

6. At the end of the run, the gel may be stained with ethidium bromide (0.5 μg/ml in H_2O) and photographed as described on page 162.

[3] McMaster and Carmichael (1977).

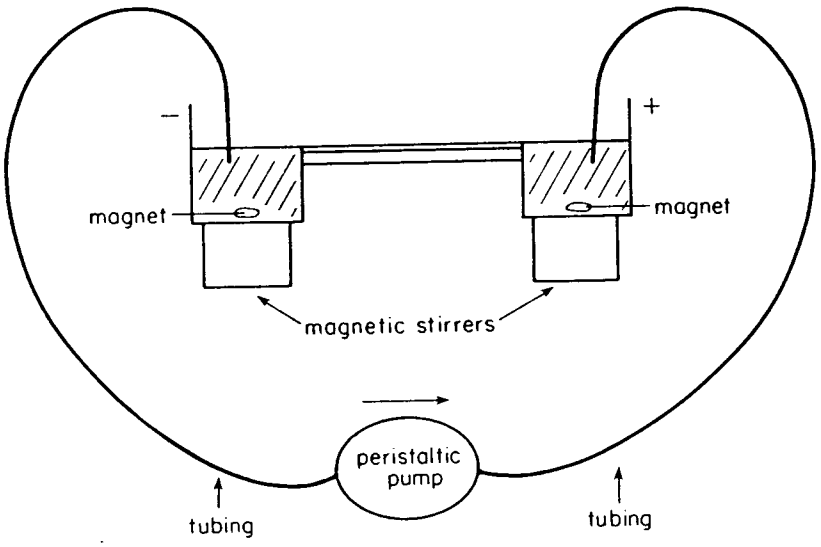

Figure 6.1

Transfer of Glyoxylated RNA to Nitrocellulose Filters

Glyoxylated RNA may be transferred immediately after electrophoresis from agarose gels to nitrocellulose filters; no further treatment of the gel is necessary (Thomas 1980). The gel is placed in contact with the nitrocellulose filter and blotted essentially as described on pages 382ff.

Notes

i. The blotting buffer is 20× SSC (3 M NaCl and 0.3 M trisodium citrate).

ii. The nitrocellulose filter should be wetted in water and then soaked for 5 minutes in 20× SSC just before use.

iii. Transfer of RNA is less efficient if the gel is presoaked in 20× SSC or if the RNA has been stained with ethidium bromide.

iv. Transfer is complete in 15-24 hours.

v. Do not wash the blot with more dilute salt solutions before baking, otherwise most of the RNA will be lost.

vi. The blots are dried at room temperature and baked in a vacuum oven at 80°C for 2 hours.

Electrophoresis of RNA through Gels Containing Formaldehyde[4]

1. Prepare gel-running buffer and formaldehyde.

   5× 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.

   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 5× gel buffer and formaldehyde to give 1× 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× 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

[4]Lehrach et al. (1977); Goldberg (1980); B. Seed and D.A. Goldberg (unpubl.).

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 β-mercaptoethanol.

   e. Destain for 45 minutes with a solution of 0.1 M ammonium acetate and 0.01 M β-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 exercised 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 20× SSC.

5. Transfer the RNA to nitrocellulose by the method described on page 382ff. The transfer is complete in 3–4 hours.

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

Electrophoresis of RNA through Gels Containing Methylmercuric Hydroxide[5]

Methylmercuric hydroxide reacts primarily with the amino bonds of uridine and guanosine in RNA. Because these bonds are normally involved in Watson-Crick pairing, methylmercuric hydroxide is an effective denaturing agent that disrupts all secondary structure in the RNA.

Methylmercuric hydroxide also reacts reversibly with various small molecules; for example:

$$CH_3HgOH + RSH \rightarrow CH_3HgSR + H_2O$$

Thus, sulfhydryl compounds can be used to reverse the binding of CH_3Hg^+ to nucleic acids.

Caution

Methylmercuric hydroxide is extremely toxic. It is also volatile. Therefore all manipulations of pouring, running, cutting, and processing of gel slices should be carried out in a chemical hood. All solid and liquid wastes should be treated as toxic materials and disposed of accordingly.

1. Prepare the gel (1–1.5% agarose, depending on the size of RNA to be analyzed) in gel-running buffer. No methylmercuric hydroxide is added to the running buffer. The compound is uncharged and does not migrate rapidly out of the gel.

   Gel-running buffer

   50 mM boric acid
   5 mM sodium borate
   10 mM sodium sulfate

   Note. Buffers that contain nitrogen bases, EDTA, or chloride ions should not be used because these compounds form complexes with methyl hydroxide.

2. Mix equal volumes of the RNA solution (up to 10 μg may be loaded per standard 0.6-cm slot) and 2× loading buffer.

   2× Loading buffer

methylmercuric hydroxide	25 μl
4× running buffer	500 μl
100% glycerol	200 μl
H_2O	275 μl
bromophenol blue	0.2% w/v

[5] Bailey and Davidson (1976).

3. Load the samples and run the gel at 1.5 V/cm for 12–16 hours. Recirculate the buffer to avoid generating a pH gradient in the gel.

4. After electrophoresis, RNA may be stained by incubating the gel for 30 minutes in 0.5 M ammonium acetate and 0.5 μg/ml of ethidium bromide. The ammonium salt complexes methylmercury and enhances binding of the dye to RNA.

5. The RNA may be transferred from methylmercury gels to nitrocellulose filters. The transfer of RNA is inefficient if the gel is presoaked in 20× SSC or if the RNA has been stained with ethidium bromide.

Recovery of RNA from Agarose Gels Containing Methylmercuric Hydroxide[6]

1. The gel is prepared and run as described above, except that low-melting-temperature agarose is used (Wieslander 1979).

2. After electrophoresis, soak the gel in 0.1 M dithiothreitol for 30–40 minutes.

3. Cut the gel into slices approximately 3 mm in width. Stained tracks containing 18S and 28S ribosomal RNAs and 9S globin RNAs may be used as rough molecular-weight guides.

4. To each gel slice, add approximately 4 volumes of 0.5 M ammonium acetate preheated to 65°C. Be sure to use a large volume of extraction buffer so that the gel is completely melted. Otherwise, agarose will be carried over into the aqueous phase during subsequent extraction with phenol and chloroform.

5. Heat at 65°C until the gel is melted. Vortex well.

6. Extract with phenol at room temperature. Centrifuge at 2000*g* for 10 minutes at 4°C. During extraction with phenol and chloroform, agarose becomes a powder and forms a layer at the interface upon centrifugation.

7. Reextract the aqueous phase twice more with chloroform. Repeated chloroform extractions are required to remove the agarose.

8. Precipitate the RNA with ethanol and wash the precipitate with 70% ethanol and 0.05 M ammonium acetate.

Note

The extracted RNA translates well in in vitro protein-synthesizing systems and is an efficient template for cDNA synthesis using reverse transcriptase.

[6]See, for example, Lemischka et al. (1981).