Electrophoresis of RNA Through Gels Containing Formaldehyde

References:

- Sambrook, J., E. F. Fritch, and T. Maniatis, 1989. Molecular Cloning, a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.
- Farrell Jr., R. E., 1993. RNA Methodologies: A Laboratory Guide for Isolation and Characterization. Academic Press, Inc., San Diego.

Caution: Formaldehyde is toxic through skin contact and inhalation of vapors. All operations involving formaldehyde should be carried out in a fume hood.

Materials:

- DEPC- treated dH_2O
- Agarose
- 10 X MOPS: 0.2 M MOPS (sodium salt), 0.05 M NaOAc, 0.01 M EDTA in DEPC-treated dH_2O. Adjust the pH to 7.0 with NaOH. Store at room temperature in the dark. Do not autoclave. NOTE: 10 X MOPS is no longer useful if it develops a yellow color.
- Formaldehyde
- RNA Denaturing Premix (Prepare fresh) per RNA sample:
  5 µl 10 X MOPS
  8.75 µl 37 % formaldehyde
  25 µl deionized formamide
- 10 X RNA Loading Buffer: 50 % glycerol, 1 mM EDTA, 0.25 % (w/v) bromophenol blue, 0.25 % xylene cyanole

Preparation:

- Pre-warm two water baths. One to 60°C and the other to 65°C.
- Make the RNA Denaturing Premix.

Gel Preparation:

for a 11 X 14 cm (medium) gel

1. Add 1.0 gm of agarose to 84.4 mls of DEPC-treated dH_2O. Melt and cool to 60°C.
2. Mix 10 mls of 10 X MOPS and 5.6 mls of formaldehyde. Pre-warm to 60°C.
3. Add the pre-warmed MOPS and formaldehyde solution to the gel solution. Mix gently and quickly. Cast the gel.
4. Allow the gel to set for at least 30 minutes at room temperature.
5. Prepare 850 mls of 1 X MOPS running buffer.
6. Cover the gel with the running buffer until the level of the buffer is 2-3 mm above the gel.

NOTE: for a 20 X 25 cm (large) gel:
Prepare as in steps 1-6 above except:

1. Add 3.0 gm of agarose to 253.2 mls of dH_2O.

2. Prewarm 30 mls of 10 X MOPS and 16.8 mls of formaldehyde.
5. Prepare 2000 mls of 1 X MOPS running buffer.

Sample Preparation:

1. Prepare the RNA denaturing premix.
2. 10 to 20 µg of total RNA, or 0.5 to 5 µg of poly(A) RNA can be loaded per well.
   - Prepare a 11.25 µl RNA solution with the desired amount of RNA. The volume can be made up using DEPC-treated dH_2O.
   - To prepare the RNA marker, mix 3 µl of Life Technologies 0.24 - 9.5 Kb RNA Ladder with 8.25 µl of DEPC-treated dH_2O.
3. Add 38.75 µl RNA denaturing buffer to the 11.25 µl RNA solutions from step 2. Mix well.
4. Denature the RNA samples by heating to 65°C for 10 minutes.
5. Add 5 µl of 10 X RNA loading buffer to each RNA sample. Mix well then centrifuge at full speed for 10 seconds. Load the samples onto the gel immediately.

Electrophoresis:

1. Run the RNA samples into the gel for approximately 15 minutes. Electrophorese the gel at a maximum of 5 V per centimeter of gel length (70 V for a 14 cm gel).
2. Turn on the pump to recirculate the running buffer. Continue electrophoresis until the bromophenol blue has run halfway down the gel (approximately 2.5 hours).