Separation of RNA by electrophoresis

Method: Separation of RNA by electrophoresis.

A. Preparation of a 50mL 0.66M formaldehyde, 2% agarose gel:

Note- A 2% agarose gel is appropriate for RNA fragments less than 1000 nts in length. You should use the Low Range RNA ladder for RNA fragments of this size.

1. Add 1.0g agarose to 42.3mL sterile DEPC treated water.
2. Microwave 30-90secs until the agarose is melted.
3. Cool to ~55°C.
4. Add 2uL EtBr.
5. Add 10 mL 10X MOPS
6. Add 2.7mL deionized formaldehyde.
7. Pour gel. Be careful to remove any bubbles. Formaldehyde gels tend to form more bubbles and are thinner and "slimier" than typical agarose gels.

B. Preparing RNA samples to run in a 0.66M formaldehyde denaturing agarose gel:

Note- RNA samples should be at a concentration of up to 20ug/uL.

In an eppendorf tube, prepare the following –

<table>
<thead>
<tr>
<th>RNA (up to 20ug)</th>
<th>1 uL</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X MOPS</td>
<td>2 uL</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>4 uL</td>
</tr>
<tr>
<td>Formamide</td>
<td>10 uL</td>
</tr>
<tr>
<td>EtBr</td>
<td>1 uL</td>
</tr>
</tbody>
</table>

(Note – I have added up to 4 uL of RNA with comparable results)

1. Mix gently.
2. Incubate for 60 mins at 55°C.
3. Chill for 10 mins in ice water.
4. Centrifuge briefly.
5. Add 2 uL of 10X formaldehyde gel loading buffer and return to ice until ready to load into gel

C. Running the gel:

1. Cover the gel in 1X MOPS.
2. Pre-Run at 7-10V/cm for 5mins. (This corresponds to the 100V setting on our gel boxes).
3. Load your samples.
4. Run at 7-10V/cm until the bromophenol blue has migrated sufficiently.

Using a 2% agarose gel, the bromophenol blue is sufficient to mark migration of an RNA fragment ~30-40 nts in length. At 7-10V/cm, a run time of approximately 45mins is typically sufficient. For run times longer than one hour, the pH of the MOPS running buffer should be monitored to maintain pH.

D. Preparation of 10X Formaldehyde Gel Loading Buffer:

50% glycerol
10mM EDTA (pH 8.0)
0.25% bromophenol blue
0.25% xylene cyanol

E. Preparation of 10X MOPS (1L)
1. Dissolve 41.8g MOPS into 700mL of DEPC treated sterile water.
2. Adjust pH to 7.0 using NaOH.
3. Add 20mL Sodium Acetate (1M).
4. Add 20mL 0.5M EDTA (pH 8.0)
5. Adjust to a final volume of 1L using DEPC treated sterile water.
6. Filter sterilize.
7. Store at room temperature away from light. MOPS will turn a shade of yellow over time. If the buffer appears dark, straw yellow, discard and prepare fresh.