## Electrophoresis of RNA in Formaldehyde Gels

### 1. 5X Formaldehyde Gel-Running Buffer:

0.1 M MOPS (pH 7.0)
40 mM Sodium Acetate
5 mM EDTA (pH 8.0)

- 20.6 g 3-(N-morpholino)propranesulfonice acid (MOPS)
- 800 mL Diethyl pyrocarbonate (DEPC) treated 50 mM sodium acetate
- adjust pH 7.0 with 2 N NaOH
- Add 10 mL DEPC treated 0.5 M EDTA, pH 8.0

**Autoclave: Solution should be light straw color when done.**

### 2. Formaldehyde Gel

For 1% gel (Agar and water will change if % changes all else stays the same

- 700 mg Agar into 70 mL of DEPC-treated water
- let cool to 60 C
- add 22 mL MOPS running buffer
- add 20 mL 37% formaldehyde

(112 mL of gel mix ~1% agarose)

### 3. Denaturation of RNA Samples:

| | |
|---|---|
| RNA (up to 30 μg) | 4.5 μL |
| 5 X formaldehyde gel running buffer | 2.0 μL |
| Formaldehyde | 3.5 μL |
| formamide | 10.0 μL |

**Incubate samples for 15 minutes at 65 C then chill on ice.**

### 4. Add 2 μL of formaldehyde gel loading buffer

50% sterile glycerol
1 mM EDTA, pH 8.0
0.25 % bromophenol blue
0.25 % xylene cyanol FF

**Prerun gel for 15 minutes at 5 V/cm then load samples**