Glyoxal RNA Gel

- Note: wear washed and rinsed gloves. Dry gloves with white (not brown) paper towels. Re-wash gloves after touching contaminated surfaces.

- Use a fresh box of pipette tips and a fresh box of tubes. Label for “RNA use only.”

- Prepare ahead of time, DEPC-treated solutions: 5 X 1 L of ddH\(_2\)O; 1 L of 10 mM NaPi, pH 7.0. To DEPC treat, add 0.025% DEPC (250 µl/L), incubate at 37°C for >2 hours, or overnight at room temperature, and autoclave.

Treat electrophoresis apparatus:
1. Set up gel apparatus using pump and tubing for RNA gel. GIBCO/BRL apparatus is for RNA gels only, and should be stored clean. Pharmacia apparatus is for DNA and RNA gels. To use it, wash tank with mild detergent and rinse with ddH\(_2\)O prior to H\(_2\)O\(_2\) treatment.

2. Pre-rinse apparatus with ddH\(_2\)O. Then pump 1 liter of 3% H\(_2\)O\(_2\) in ddH\(_2\)O through apparatus (with comb and dams completely submerged) for 10 min. Dilute 30% H\(_2\)O\(_2\) to 3%. Check that pump tubing is locked down and moving liquid. Pour out H\(_2\)O\(_2\) solution. Tilt apparatus to remove liquid from each side.

3. Pump 1 liter DEPC-treated ddH\(_2\)O through apparatus (again with comb and dams completely submerged) for 10 min. Repeat this rinse two more times. Pour out all remaining fluid by tilting apparatus. Small amounts of residual H\(_2\)O\(_2\) will degrade RNA.

Pour and run gel:
1. Pour a 0.75-1.25% agarose gel in 10 mM NaPi, pH 7.0 (+ DEPC). Do not use ethidium bromide in gel. Treat agarose with ~10 mM sodium iodoacetate (0.20 g for 100 ml gel). Add sodium iodoacetate after melting and cooling agarose to ~70°C (flask too hot to hold). Pour gel when flask is at 50°C (can be held). After gel is cast, completely submerge in 10 mM NaPi, pH 7.0 (+ DEPC).

   GIBCO/BRL: 100 ml gel 14 tooth comb 20 µl per well ~250 V•hrs
   75 ml gel 10 tooth comb 25 µl per well
   Pharmacia: 75 ml gel 11 tooth comb 20 µl per well ~150 V•hrs
   50 ml gel 11 tooth comb 15 µl per well
   Pharmacia: 200 ml gel 14 tooth comb 50 µl per well ~500 V•hrs

2. Combine up to 10 µg RNA in 3 µl with 15 µl glyoxal/DMSO (G/D) solution (aliquots stored at -70°C). For GIBCO/BRL RNA standards, use 1-3 µl.

3. Incubate at 50°C for 1 hour. Chill on ice. Spin 5 seconds.

4. Add 2 µl of loading solution, mix completely, and load all of sample (20 µl).

5. Run gel at ~40-50 V maximum. After 4-5 hours dye is ~75% down gel. Recirculate buffer at setting 8.

6. If markers are to be stained, cut lane from gel and stain for 60 minutes with 1 µg/ml EtBr in 0.1 M ammonium acetate (250 mls ddH\(_2\)O + 20 g NH\(_4\)OAc + 500 µl 1000 X EtBr). Gently agitate. Take photo with fluorescent ruler.