RUNNING AN RNA GEL

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Date: 2002

Pouring gel

1. Treat gel rig including combs either by soaking in 3% hydrogen peroxide for at least 15 minutes and rinsing with DEPC treated H2O or scrubbing with RNase Zap and rinsing with DEPC treated H2O). Wash gloved hands with RNase Zap when handling gel rig items.
2. Melt 0.45 g agarose in 28.12 ml H2O-DEPC treated (in RNase-away treated flask or a flask treated with 0.1% DEPC overnight and autoclaved).
3. Cool to 60° in waterbath.
4. Working quickly so agarose won't gel, add 8.04 ml formaldehyde
   8.84 ml 5x formaldehyde gel running buffer
5. Pour in gel rig. Let gel set for at least 30 minutes.
6. (Optional) Prerun the gel in 1x formaldehyde gel running buffer for at least 5 minutes before loading.

Preparing RNA samples for loading on gel

(also Gibco's RNA ladder- use 4.5 µl)

To 4.5 µl of RNA sample (use filter tips for all pipetting),

1. Add 2.0 µl 5x formaldehyde gel running buffer, 3.5 µl formaldehyde (37%), and 10.0 µl formamide. (Make master mix and add 15.5 µl/tube). **OR** To whatever sample volume you want, add an equal volume of Ambions 2X RNA loading buffer. Generally, 5 ug RNA is a good amount to load.
2. Heat at 65° for 15 minutes.
3. Chill on ice.
4. Add 2 µl loading buffer (=50% glycerol, 1 mM EDTA pH 8.0, 0.25% bromophenol blue, 0.25% xylene cyanol - 0.22 micron sterile filtered) + 1µl ethidium bromide 1 mg/ml. (You can make a master mix of these two and then add 3 µl to the samples.) **OR** If using Ambion's loading buffer just add 1 ul ethidium bromide.
5. Load all of the sample on gel.
6. Run at ~60 V for ~2-4 hours. View on UV transilluminator.

For other size gels:

25 ml	70 ml
0.25 g agarose	0.7 g agarose
15.6 ml water	44 ml water
4.5 ml formaldehyde	12.5 ml formaldehyde
4.9 ml formaldehyde gel buffer	13.75 ml formaldehyde gel buffer

5X Formaldehyde gel running buffer

0.1M MOPS, 40 mM sodium acetate, 5 mM EDTA

To make, first make 800 ml 50 mM sodium acetate. DEPC treat and autoclave in bottle with stir bar in it. Then add 20.6 g MOPS. Adjust the pH to 7.0 with 2 N NaOH. Add 10 ml 0.5 M EDTA pH 8.0 – DEPC treated. Adjust the volume to 1 L with DEPC treated water. Sterilize through 0.2 micron filter into DEPC treated bottle. Store in dark at room temp.