FORMALDEHYDE RNA GELS

(Nelson protocol + modifications)

Always keep the RNA on ice.

1. Pretreat gel box, comb and tray with 0.1 M NaOH for 30' before use. **Rinse well with deionized water**.

2. Pour 1.5 % agarose gel in 1x Gel Buffer:

   a) add 17 ml formaldehyde solution (37%) to 33 ml dH_2O in a flask in the fume hood. Mix.

   b) rinse comb and gel tray well with deionized water and set up in the fume hood.

   c) add 1.5 g agarose to 10 ml **10x Gel Buffer** and 40 ml dH_2O in flask. Microwave for 2'. Make sure agarose is completely dissolved and add to formaldehyde solution in fume hood. Pour gel immediately.

3. While gel is setting, rinse gel box well. Dilute **10 x Running Buffer** to 1 x and and fill to the platform. The less buffer over the gel, the less diffusion of formaldehyde.

4. Prepare RNA samples by adding an appropriate amount of RNA and DEPC treated H_2O to a final volume of 7 µl. Add 23 µl **RNA Sample Buffer** to each RNA sample and mix well by pipetting up and down a few times. More or less RNA can be used but the 23:7 ratio must be maintained.

5. Heat samples to 65ºC for 5' and place on ice. Load samples into the gel. The sample is heavy enough to sink in the well, but add tracking dye + loading buffer to the last lane to keep track of how far the gel has run.

6. Run the gel overnight. Place a glass plate on top of the gel to keep the formaldehyde from diffusing out and run at 25 volts. If running.at high voltage (80-100v) keep the mA @ 75.

7. Photograph gel under UV and quantitate RNA loading using Biorad Multi-fluor S.

Solutions:

10 x Gel Buffer (100 ml)

200 mM MOPS pH 8.0	20 ml 1 M
50 mM NaOAC	5 ml 1 M
10 mM EDTA	2 ml 0.5 M

Final pH with added formaldehyde is 7. Autoclaving is OK but not necessary (the MOPS turns yellow when autoclaved, but it's fine). Wrap in foil.

10 x Running Buffer (2L)

200 mM MOPS pH 7.0	400 ml 1 M
50 mM NaOAC	100 ml 1 M
10 mM EDTA	40 ml 0.5 M

RNA Sample Buffer

300 µl 10x Gel Buffer
1500 µl formamide
14 µl EtBr [10 $mgml^{-1}$]
486 µl formaldehyde

Mix in 15 ml tube and aliquot into eppendorf tubes. Store at -80°C and keep away from light. Use a 23:7 buffer to RNA ratio