2.3. 3' End Labeling of RNA

1. [5'-^{32}P] cytidine 3',5' *bis*(phosphate). 2000–4000 Ci/mmol (74–148 TBq/mmol). Store at –20°C. Take standard precautions for handling radioactive material.
2. T4 RNA ligase, RNase-free, 3–15 U/μL.
3. Dimethyl sulfoxide (DMSO), 99.9%, spectrophotometric grade. Handle in fume cupboard.
4. 0.5*M* HEPES, adjusted to pH 8.3 with KOH.
5. 0.1*M* $MgCl_2$.
6. 0.1*M* Dithiothreitol (DTT). Store at –20°C. Labile.
7. 0.2 m*M* ATP. Store at –20°C.
8. Sephadex G-50. Suspend in approx 50 vol TE (10 m*M* Tris-HCl, pH 7.5, 1 m*M* EDTA). Leave overnight. Autoclave.

2.4. Determination of 3'-Terminal Nucleotide

1. 1.0*M* NaOH.
2. Dye mix containing 0.05% (w/v) each of orange G, acid fuchsin, and xylene cyanol FF in water.
3. Whatman 3MM paper.
4. Paper electrophoresis buffer, pH 3.5, containing 5% (v/v) glacial acetic acid, 0.5% (v/v) pyridine, and 1 m*M* EDTA. **Caution:** All solutions containing pyridine should be handled in a fume cupboard and gloves should be worn at all times.
5. X-ray film.

3. Methods

3.1. Electrophoresis of RNA Through Formaldehyde-Containing Agarose Gels

1. Wash gel apparatus, comb, and spacers with detergent, and rinse well. Give a final rinse with distilled water. Dry with 70% (v/v) ethanol solution.
2. Set up apparatus on a level surface in a fume hood (*see* **Note 2**).
3. Melt 0.5 g of agarose in approx 30 mL sterile distilled water. Cool to about 55–60°C and add 5 mL 10X MOPS buffer and 3.75 mL 37% (v/v) formaldhyde solution. Make up to 50 mL with sterile dH_2O.
4. Pour gel into mold and leave to set for approx 30 min.
5. Mix RNA sample in ratio 1:3 (v/v) with solution containing 67 parts deionized formamide solution, 20 parts formaldehyde solution, and 13 parts 10X MOPS buffer and 100 μg/mL ethidium bromide. Heat to 60°C for 5 min in sealed Eppendorf tube. Immediately cool on ice. Spin solution to bottom of tube in a microcentrifuge. Add 0.2 vol loading dye.
6. Remove comb and end-spacers. Load whole of sample on gel. Run gel submerged in 1X MOPS buffer. (Minigels will take approx 45 min when run at a constantb 80 mA.)
7. At the end of the run, wash gel briefly in dH_2O. Observe under ultraviolet illumination (*see* **Notes 3** and **6**).