3.2. Glyoxal Treatment of RNA and Electrophoresis Through Tris-Acetate Agarose Gels

1. Make 1.4% (w/v) solution of agarose in 1X TEAc containing 0.1% (w/v) SDS. Autoclave. (Stocks can be prepared in advance and will store well in an unopened container at room temperature.)
2. Prepare and set up apparatus as above. (No need to position in a fume hood.) Melt agarose in a microwave and pour into mold. Leave to set.
3. Denature 1 vol RNA sample with 9 vol GFP solution. Heat to 55°C for 15 min. Cool. Spin briefly.
4. Add 0.2 vol loading dye and load onto gel. Cover gel with 1X TEAc buffer and run at 3 V/cm for 2 h, or until dye reaches the end of the gel.
5. Soak for 15 min in 0.1*M* NaOH (*see* **Note 7**).
6. Stain with 0.15*M* NaOAc, pH 5.5, containing 4 μg/mL ethidium bromide.
7. Destain with dH_2O. Observe bands under UV illumination (*see* **Note 6**).

3.3. 3' End Labeling of RNA

1. Make up 20 μL of a reaction mixture containing 5 μg RNA and 50 μCi (1.85*M*Bq) [5'-^{32}P]pCp 50 m*M* HEPES-KOH, 10 m*M* $MgCl_2$, 3.3 m*M* DTT, 20 μ*M* ATP, 10% (v/v) DMSO.
2. Start ligation reaction by adding 5 U of T4 RNA ligase. Incubate reaction mixture overnight at 4°C.
3. Separate labeled RNA from unincorporated [5'-^{32}P]pCp by spinning through columns of Sephadex G-50.
4. To the first peak of radioactivity which comes off the column (excluded volume), add 0.1 vol 3*M* NaOAc, pH 5.5, 2.5 vol ethanol. Precipitate RNA overnight at –20°C.
5. Centrifuge to recover RNA, wash the pellet with 70% (v/v) ethanol, and dry in a vacuum desiccator.
6. Redissolve dried pellet in 20 μL of sterile dH_2O.

3.4. Determination of 3'-Terminal Nucleotide

1. To 2 μL of pCp-labeled RNA, add 2 μL of 1*M* NaOH and 6 μL of dH_2O. Incubate the reaction mixture overnight at 37°C.
2. Apply the sample as a 1-cm-wide strip 15 cm from one end of a 58-cm piece of Whatman 3MM paper. Spot 1–2 μL of dye mix on either side of the sample.
3. Wet the paper with pH 3.5 paper electrophoresis buffer. To ensure the sample does not move during the wetting process, allow the buffer to approach the origin from both sides simultaneously. Blot excess buffer off the paper.
4. Place paper in suitable electrophoresis tank (*see* **Note 9**) and electrophorese at 3000 V for 90 min. The negatively charged mononucleotides migrate toward the anode.
5. Remove paper from tank and allow to dry thoroughly in fume hood.
6. Mark the position of the dye markers with ink to which a small amount of ^{35}S has been added. The orange G (orange) and acid fuchsin (pink) dyes run with nearly