3.5. Preparation of Northern Blots

3.5.1. RNA Gels

1. In a 100-mL flask, mix: 0.6 g agarose, 5 mL 10X MOPS buffer, and 34 mL sterile distilled water. These constituents should be melted, then cooled slightly before adding 11 mL formaldehyde (37%). Pour the gel and position the sample comb.
2. In a 1.5-mL microcentrifuge tube, mix: 1 μL 10X MOPS buffer, 3.5 μL formaldehyde (37%), 5.5 μL containing 10 μg of sample RNA in sterile distilled water, and 10 μL deionized formamide. Heat samples at 55°C for 15 min and then add 3 μL loading buffer.
3. Once the gel is set, place in the gel tank and use 1X MOPS as the electrophoresis buffer.
4. Load the samples, including a sample of RNA size marker (*see* **Note 7**), onto the gel and run for approx 2 h at 60 mA, or until the dye is about 3 cm from the bottom of the gel.

3.5.2. Transfer of RNA to Nylon Membrane

RNA can be transferred onto a membrane using a vacuum-transfer blotter or by using the following traditional procedure. The membrane must not be touched with bare hands.

1. Trim off excess agar from gel, including the loading wells. Remove right-hand corner to provide orientation.
2. Cut a nylon membrane and two squares of Whatman 3MM chromatography paper to the same size as the gel, and two long strips (cut to the width of the gel) to act as wicks.
3. Label, with pencil, the side of the membrane that will be in contact with the gel.
4. Place a piece of glass across a large dish containing 300 mL 20X SSC. Wet the two longest pieces of filter paper in 20X SSC and drape across the glass and into the SSC in the dish, to act as a wick.
5. Carefully place the gel on the wick and smooth out any air bubbles.
6. Cover all exposed areas of filter paper with cling film.
7. Wet the membrane in 10X SSC (*see* **Note 8**) and place on top of the gel, smoothing out any air bubbles and making sure that the corners are well-matched.
8. Wet the two squares of filter paper in 20X SSC, place on top of the membrane, and smooth down gently.
9. Place a 70-mm stack of absorbent paper towels on top, weigh down evenly with 500 g weights, and leave to transfer overnight.
10. Bind the RNA to the membrane by exposing to UV or by heating (*see* **Note 2**). The membrane should be irradiated on both sides before hybridization with DIG probes, as described in **Subheading 3.7.**

3.6. Preparation of RNA Dot Blots

1. Prepare serial dilutions of total RNA extracts from leaves in sterile dH_2O (typically from 5 μg/μL to 0.5 pg/μL). 2μL of each dilution should be spotted onto a piece of positively charged nylon membrane.