7. Incubate the membrane for 30 min in 5 mL of antibody solution at room temperature in a sealed plastic bag.
8. Discard the antibody solution and wash the membrane 2 × 15 min with 50 mL of wash buffer in a dish at room temperature.
9. Equilibrate the membrane in 10 mL of buffer 3 for 2 min in a dish.
10. Dilute chemiluminescent substrate CSPD (25 m*M*) 1:100 in buffer 3. Place the membrane (DNA side up) on one half of a plasic bag, and gently dispense the CSPD substrate onto the surface of the membrane scattering the drops across the surface. Approximately 0.5 mL of diluted CSPD is sufficient for a 60 × 30-mm blot. Rock the membrane gently to disperse the liquid before lowering a top sheet of plastic over the membrane gently and disperse any bubbles. Incubate the filter for 5 min at room temperature without shaking.
11. Move the membrane into a fresh plastic bag, allowing excess substrate to drip off during the transfer, seal the bag, and incubate at 37°C for 15 min. Do not allow the membrane to dry out during transfer.
12. Retain the membrane in the bag and expose to X-ray film for 2–5 min, or as necessary.
13. By comparing the intensity of the spots given by the control DNA and the labeled probe, the concentration of the new probe can be estimated (*see* **Note 6**).

3.4. Extraction of RNA from Plant Tissues

This method is based on a protocol described in *(2)*. **Caution:** It is essential that gloves are worn at all times.

1. Place 0.5 g leaf material (midribs removed) in a 4-mL polyethylene tube (or use less material in a microcentrifuge tube) into liquid nitrogen, and leave until the leaf material is completely frozen before grinding to give a fine powder, using a plastic or glass rod.
2. Add 1 mL extraction buffer and 1 mL TE saturated phenol. Heat at 80°C for 1 min, mix vigorously for 30 s, incubate at 80°C for 2 min, and mix again.
3. Add 1 mL of a 24:1 mixture of chloroform:isoamyl alcohol. Because chloroform boils at 55°C, it is essential to cool the tubes before adding it. Shake vigorously for 30 s.
4. Centrifuge tubes at 16,000*g* for 3 min, remove the upper aqueous phase, and add 1 vol of 4*M* LiCl. Mix well and leave overnight at 4°C.
5. Centrifuge tubes at 16,000*g* for 3 min and discard aqueous phase. Add 200 µL sterile dH_2O to resuspend the pellet, and transfer to a fresh microcentrifuge tube. Keep on ice and add 0.1 vol 3*M* sodium acetate and 2 vol of absolute ethanol. Mix well and store in the freezer at –20°C overnight, or at –70°C for 30 min.
6. Centrifuge at 16,000*g* for 5 min and discard solution. Rinse the pellet in 400 µL 70% ethanol, centrifuge at 16,000*g* for 2 min, and discard solution.
7. Remove remaining ethanol from pellets under vacuum, until dry. Resuspend and dissolve the pellet in 100 µL Tris-SDS, or more if required. Estimate RNA concentration in a spectrophotometer and store at –20°C, after addition of 2 vol of absolute ethanol and 0.1 vol 3*M* sodium acetate, pH 5.8, until ready for use.