nucleic acid, which are detected using an alkaline phosphatase-conjugated anti-digoxigenin antibody and a chemiluminescent substrate. Chemiluminescent substrates can be visualized by exposure to X-ray film, thus providing a permanent record that is directly comparable to results that would be obtained using radioactive probes. Luminescent detection is fast and sensitive, and membranes can easily be stripped and reprobed.

DIG probes have several advantages over radioactive probes. For example, using chemiluminescent substrates, detection can be completed with a 15- to 30-min exposure to X-ray film, compared to 1–3 d required for most ^{32}P-labeled probes. Another advantage is that probes can be stored at 4°C or –20°C for many years with no apparent loss in sensitivity. For example, radioactive and nonradioactive cDNA probes to tobacco rattle virus RNA were recently prepared from the same plasmid used to make a DIG probe nearly 3 yr earlier, and which had been stored at –20°C. In a comparative test, the stored DIG-labeled probe was as sensitive for detection as the freshly prepared DIG and radioactive probes. Many of the probe preparation, hybridization, and washing protocols are the same or similar for the DIG and radioactive systems. The following are techniques that we use for detection and quantification of RNA transcript in transgenic plants. The DIG system has many uses and variations; a more comprehensive guide and instructions can be found in **ref. *1***.

2. Materials

2.1. Boehringer Reagents

1. PCR DIG labeling kit: This contains PCR buffer, 25 m*M* magnesium chloride, *Taq* DNA polymerase, and dNTP mixture containing DIG-11-dUTP.
2. DIG-High Prime (random priming labeling) kit: Contains an optimized reaction mixture of buffer, random nucleotides, Klenow enzyme, dNTPs, and DIG-11-dUTP in 50% glycerol.
3. Positively charged nylon membrane.
4. DIG-labeled control DNA.
5. Blocking reagent.
6. DIG Easy Hyb (hybridization) buffer.
7. Anti-DIG-alkaline phosphatase, Fab fragments.
8. CSPD (chemiluminescent) substrate.
9. DIG-labeled RNA mol-wt markers.

2.2. Stock Solutions

Unless stated otherwise, autoclave and store at room temperature (*see* **Note 1**).

1. TE buffer: 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.0.
2. Buffer 1: 100 m*M* maleic acid, 150 m*M* sodium chloride, pH 7.5, with NaOH.