endonuclease that leaves the insert downstream of the RNA polymerase promoter. If possible, select a restriction enzyme that generates a 5' overhang end, otherwise, longer-than-unit transcripts may be obtained. Following digestion, the linearized plasmid is extracted with phenol:chloroform before using the DNA for transcription reactions. For DNA probes, the complementary strand is synthesized by using a mixture of random hexanucleotides to prime DNA template. Although the linearization of the plasmid is not necessary, it is recommended because it increases the efficiency of the labeling. When no previous information about the viral primary structure is available, a digoxigenin-labeled cDNA probe can be synthesized from viral RNA by priming with random hexamers and using reverse transcriptase by standard protocols ***(10)***.

3.1.1. cRNA Probes

1. To a microcentrifuge tube containing 1 μg of linearized DNA template, add the following: 2 μL of 10X transcription buffer, 2 μL of 10X DIG-RNA labeling mixture, RNase inhibitor (40 U), SP6, T7, or T3 RNA polymerase (40 U), and make up to a final volume of 20 μL with sterile water. All the components must be added at room temperature, since DNA can precipitate at 4°C in the presence of spermidine.
2. Centrifuge briefly and incubate for 2 h at 37°C.
3. (Optional) Add DNase I, RNase-free (20 U), and incubate for 15 min at 37°C. This treatment is not usually necessary, because the DIG-labeled RNA transcript is 10-fold in excess of the template DNA.
4. Add 2 μL 0.2*M* EDTA, pH 8.0, to stop the reaction.
5. Precipitate the labeled RNA with 2.5 μL 4*M* LiCl and 2.5 vol of ethanol.
6. Centrifuge at 12,000*g* and resuspend the RNA sample in TE buffer or sterile water (*see* **Notes 1** and **2**).

3.1.2. DNA Probes

1. Denature the DNA by heating it at 95°C for 10 min. Rapidly submerge the tube in an ice bath.
2. Add to a microcentrifuge tube the following reagents: 10 ng–3 μg freshly denatured DNA, 2 μL of 10X hexanucleotide mixture, 2 μL of 10X DIG-DNA labeling mixture, Klenow enzyme (2 U), and make up to a final volume of 20 μL with sterile water.
3. Mix gently, centrifuge briefly, and incubate for at least 60 min at 37°C.
4. Add 2 μL of 0.2*M* EDTA, pH 8.0, to stop the reaction.
5. Precipitate, and recover the labeled DNA, as described above.

3.2. Preparation of the Samples and Hybridization

1. Homogenize the leaf tissue in a prechilled mortar with 2 vol 50 m*M* sodium citrate, pH 8.5. Alternatively, the tissue can be homogenized in resistant plastic bags (*see* **Note 7**).