

5. Load the PCR samples into the gel and also include in one well approx 1  $\mu\text{g}$ , 1-kb ladder mol-wt markers.
6. Run the gel at 60–100 V until the bromophenol blue visual dye marker has migrated to approx 4 cm from the bottom of the gel.
7. Visualize the PCR products on a UV transilluminator and photograph using Polaroid Land Camera or video camera (Flowgen) with an orange G filter to maximize the contrast (*see Note 10*).

## 4. Notes

### 4.1. DNA Extraction

1. If the plant material is to be processed directly, store at room temperature. Alternatively, store on dry ice or at  $-80^{\circ}\text{C}$ . Leaf disks are the usual source of material for this extraction procedure. However, the technique has also proved to be successful with small amounts of tissue from hairy root cultures.
2. Samples do not appear to deteriorate after 1 h of storage under these conditions.
3. At this stage, a stringy DNA precipitate may be visible.
4. DNA pellets are often green or brown in color; this is expected from a crude extraction procedure.
5. The pellet may not resuspend completely, but the small amount of debris remaining does not appear to inhibit the PCR amplification.

### 4.2. PCR from Plant DNA

6. Initially, it is advisable to try several different volumes of the sample DNA to determine the optimum.
7. Although PCR buffer is usually supplied with *Taq* DNA polymerase, a recipe is provided below for those who wish to assemble their own reagents. It is useful to have a recipe, so that components can be varied, if necessary. For example, certain primer sets work more efficiently with different magnesium concentrations. 1X PCR buffer: 44 mM Tris-HCl, pH 8.8, 11 mM  $\text{NH}_4\text{SO}_4$ , 4.5 mM  $\text{MgCl}_2$ , 7 mM  $\beta$ -mercaptoethanol, 113  $\mu\text{g}/\text{mL}$  BSA, 4.5 mM EDTA.
8. To speed up the process and to minimize errors, make a stock solution containing enough buffer, primers, *Taq* DNA polymerase, dNTPs, and water for all the reactions, then aliquot into the PCR tubes before adding the DNA sample. Adding the DNA template last minimizes the risk of contamination between samples. It is generally good practice to include a water negative control and a plasmid positive control (1 ng plasmid DNA is sufficient) among the plant DNA samples.
9. The annealing temperature can be determined from the sequence of the primers. The melting temperature for duplex DNA of less than 20 bp can be calculated as follows:  $2^{\circ}\text{C}$  for every AT pair, plus  $4^{\circ}\text{C}$  for every GC pair. To obtain the annealing temperature,  $5^{\circ}\text{C}$  is subtracted from the melting temperature.

### 4.3. Detection and Analysis of PCR Products

10. The product should appear as a sharp band at the expected size. Small DNA products (primer dimers) are sometimes visible as diffuse bands close to the leading