

3. DNA loading buffer (10X): 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol, 25% (w/v) Ficoll (type 400).
4. Ethidium bromide solution: 5 mg/mL ethidium bromide dissolved in water (**Caution:** Ethidium bromide is a mutagen and irritant).
5. DNA size markers: 1-kb ladder (BRL, Paisley, UK).
6. Electrophoresis apparatus (e.g., Bio-Rad, Hemel, Hempstead, UK).
7. UV transilluminator plus orange G filter.

### 3. Methods

#### 3.1. DNA Extraction

1. Samples for PCR analysis can be taken from the plant by pinching out a disk of material (usually leaf) using the lid of an Eppendorf (*see Note 1*).
2. Grind up the plant tissue, using a disposable grinder, for approx 15 s.
3. Add 400  $\mu\text{L}$  PCR DNA extraction buffer and vortex for 15 s.
4. Leave at room temperature until all of the samples have been processed (*see Note 2*).
5. Centrifuge at 15,000g in a microcentrifuge for 1 min.
6. Remove 300  $\mu\text{L}$  of supernatant to a fresh tube and add an equal volume of propan-2-ol.
7. Incubate at room temperature for 2 min (*see Note 3*), before centrifuging at 15,000g for 10 min.
8. Completely remove supernatant and vacuum- or air-dry the pellet (*see Note 4*).
9. Resuspend the pellet in 100  $\mu\text{L}$  TE (*see Note 5*).
10. Typically, 2  $\mu\text{L}$  of this DNA solution can be used for PCR analysis.

#### 3.2. PCR from Plant DNA

1. For each DNA sample, set up a PCR reaction. A typical reaction: 2  $\mu\text{L}$  DNA solution (*see Note 6*), 2  $\mu\text{L}$  10X PCR buffer (*see Note 7*), 0.1 mM dNTPs, 1.5 U *Taq* DNA polymerase, 1  $\mu\text{M}$  of each primer; make volume of reaction up to 20  $\mu\text{L}$  with sterile water (*see Note 8*).
2. Overlay the reactions with a drop of mineral oil.
3. Carry out 30 cycles of denaturation (95°C for 1 min), annealing (50–60°C [*see Note 9*] for 1 min), and extension (72°C for 1 min/kb of predicted DNA product).
4. Check the PCR products by electrophoresis on an agarose gel.

#### 3.3. Detection and Analysis of PCR Products

1. Prepare a 1% agarose gel in 1X TAE buffer. Before pouring into casting tray, add ethidium bromide solution (to a final concentration 0.05 mg/mL).
2. Add 0.1 vol DNA loading buffer to the PCR reaction through the oil.
3. Place the gel in electrophoresis apparatus, and cover with 1X TAE buffer.
4. Add ethidium bromide solution to the 1X TAE buffer (to a final concentration of 0.05 mg/mL).