2. Materials

2.1. Genomic DNA Extraction

1. Liquid nitrogen.
2. Pestles and mortars.
3. 50-mL Polypropylene tubes.
4. 2X cetyl triethylammonium bromide (CTAB) solution: 2% (w/v) CTAB, 100 m*M* Tris-HCl, pH 8.0, 1.4*M* NaCl, 20 m*M* EDTA.
5. Chloroform:octanol: mix the two solutions in the ratio 24:1.
6. 10% CTAB solution: 10% (w/v) CTAB, 0.7*M* NaCl.
7. CTAB precipitation buffer: 1% (w/v) CTAB, 50 m*M* Tris-HCl, pH 8.0, 10 m*M* EDTA.
8. 1*M* NaCl.
9. Absolute ethanol.
10. 70% (v/v) Ethanol.
11. TE: 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 7.2.
12. RNase A (Sigma, Poole, UK): Prepared by dissolving in sterile dH_2O to a concentration of 10 mg/mL and heat-treating by boiling for 10 min to destroy any contaminating DNases. Store at –20°C.
13. Phenol:chloroform:isoamyl alcohol solution: Mix the solutions 25:24:1. The phenol can be purchased ready-equilibrated in Tris buffer from Sigma. This solution can be stored in the dark at 4°C for up to 6 wk. **Caution:** Phenol is toxic.
14. 5*M* NaCl.

2.2. Southern Blotting

2.2.1. Restriction Enzyme Digestion of Genomic DNA and Agarose Gel Electrophoresis

1. High-gelling temperature agarose (molecular biology grade, e.g., Seakem™, Flowgen, Lichfield, UK).
2. Electrophoresis buffer (10X TAE): 4*M* Tris-acetate, pH 8.0, 10 m*M* EDTA.
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). Store this solution at 4°C.
5. DNA size markers: 1-kb ladder (BRL, Paisley, UK).
6. Electrophoresis apparatus (e.g., Bio-Rad, Hemel, Hempstead, UK).
7. UV transilluminator (e.g., Flowgen) and an orange G filter.

2.2.2. Preparation of Gel for DNA Transfer

1. Depurinating solution: 0.25*M* HCl.
2. Denaturing solution: 0.5*M* NaOH, 1.5*M* NaCl.
3. Neutralizing solution: 3.0*M* NaCl, 0.5*M* Tris-HCl, pH 7.4.