9. Precipitate the viral ssDNA by adding 2.5 vol of ice-cold ethanol. After at least 30 min at –20°C, pellet the precipitated nucleic acid in a microcentrifuge at 4°C. Wash the pellet with 70% ethanol, then air-dry at room temperature. Resuspend the ssDNA in sterile TE buffer.

3.2. Extraction of Total Nucleic Acids from Infected Plants

1. Grind 5–10 g of infected leaf material to a fine powder in liquid nitrogen with a mortar and pestle (*see* **Note 3**).
2. Suspend the frozen powdered leaf material in an equal volume of DNA extraction buffer and stir until the mixture reaches room temperature (*see* **Note 4**). If the homogenized plant material is too viscous, add a little more extraction buffer.
3. Centrifuge the mixture for 10 min at 4°C in 30-mL polypropylene tubes at 12,000*g* (in a Sorvall SS34 fixed angle rotor) to pellet the plant debris.
4. Transfer the supernatant to 30-mL polyproylene tubes containing 10 mL of Tris-buffered phenol and mix well. Centrifuge at 4°C for 10 min at 12,000*g*.
5. Transfer the aqueous phase into clean polypropylene tubes (*see* **Note 5**). Add an equal volume of chloroform, mix, and centrifuge again at 4°C for 10 min at 12,000*g*. Repeat this step.
6. Transfer the aqueous phase to clean polypropylene tubes. Add 0.7 vol of isopropanol and mix gently. A stringy precipitate of nucleic acids should appear in the tube.
7. Pellet the nucleic acids at 17,000*g* for 20 min, discard the supernatant, and stand the tubes upside down to drain off excess isopropanol.
8. Wash the pellet by adding about 10 mL of 70% ethanol, dislodge the pellet, and mix and centrifuge again at 17,000*g* for 5 min at 4°C (*see* **Note 6**).
9. Invert the centrifuge tube on absorbent paper and allow the pellet to air-dry for about 10 min at room temperature.
10. If only a crude nucleic acid preparation is required, resuspend the pellet in 0.5–1 mL of TE buffer (*see* **Note 7**).

3.3. Isolation of RF-DNA by Alkaline Denaturation and Anion-Exchange Chromatography

This procedure uses a plasmid isolation protocol based on alkaline denaturation of chromosomal DNA and purification of cccDNA by anion-exchange chromatography. The protocol is basically as described by the manufacturers of the kit that we use (the Qiagen-tip 20 kit, Qiagen GmbH and Qiagen), but can easily be adapted for the use of similar plasmid isolation kits.

1. Resuspend the total nucleic acid pellet from **Subheading 3.1., step 9** in 0.3–0.5 mL of solution P1 in a microcentrifuge tube (*see* **Note 8**).
2. Add the same volume of solution P2. Mix gently to avoid shearing chromosomal DNA and incubate at room temperature for 5 min.
3. Add the same volume (0.3–0.5 mL) of ice-cold solution P3. Mix well and place on ice for 10 min.