3. Sterilized glass rod with tip flattened obliquely.
4. Sodium phosphate buffer, pH 7.2: Stock solutions, 0.5*M* Na_2HPO_4 (17.91 g/100 mL), 0.5*M* NaH_2PO_4 (7.8 g/100 mL). For 200 mL of buffer, mix 144 mL of 0.5*M* Na_2HPO_4 with 56 mL 0.5*M* NaH_2PO_4; cool to 4°C.
5. Solid sodium sulfite (0.75%): Weigh out 1.5 g per 200 mL extraction buffer.
6. Solid urea (1*M*): Weigh out 12 g for 200 mL buffer.
7. 10% Triton X-100 (stock solution).
8. DNase buffer: 50 m*M* Tris-HCl, pH 7.5, 5 m*M* $MgCl_2$.
9. Phenol:chloroform mixture: phenol:chloroform:isoamyl alcohol (25:24:1). **Caution:** Phenol:chloroform is extremely corrosive and toxic. It is best bought as a preprepared solution; handle in small volumes with great care.
10. 10 mg/mL Proteinase K (Boehringer) in TE with 1% SDS.
11. 2 mg/mL Deoxyribonuclease I (DNase I, Sigma) in DNase buffer.
12. 2 mg/mL Pancreatic ribonuclease A (RNase A) in TE (heat-treated by incubation at 95°C for 10 min).
13. 10% Sodium dodecyl sulfate (SDS).
14. TE solution.
15. 0.5*M* $MgCl_2$.
16. 30% Polyethylene glycol 6000 (PEG).
17. Centrifuge rotors cooled to 4°C: Sorvall GSA high speed rotor (6 × 500 mL); Sorvall TFT 65.38 (8 × 38 mL); Sorvall TFT 65.13 (12 × 13 mL) or equivalent rotors.
18. Bottom-drive blender cooled to 4°C.
19. Muslin (four layers), washed in distilled water and squeezed dry.
20. Rubber policeman (round-ended glass rod covered at one end with a rubber sleeve).

2.2. 2D Gel Electrophoresis

1. Agarose.
2. Neutral dimension buffer (TA): 25 m*M* Tris-acetate, pH 7.9.
3. Alkaline solution for second denaturing dimension: 30 m*M* NaOH, 2 m*M* EDTA.
4. Tracking dye: 1% orange G, 20% Ficoll, 5 m*M* EDTA in appropriate running buffer.
5. Depurination solution: 100 m*M* HCl.
6. Denaturing solution: 0.5*M* NaOH, 1.5*M* NaCl.
7. Neutralizing solution: 1*M* Tris-HCl, pH 7.6, 1.5*M* NaCl.
8. Transfer solution: 3*M* NaCl, 0.3*M* trisodium citrate.

3. Methods

3.1. Inoculation of Plants

1. Inoculum should contain one of the following in 10 μL of solution: infectious sap in water; 0.1–1.0 μg purified virions in water; 1–2 μg purified virion DNA in TE; 2–4 μg cloned virion DNA in TE treated with the appropriate restriction enzyme to liberate the viral DNA from the cloning vector. Cloned CaMV DNA is infectious when inoculated as linear molecules in a mixture with cloning vector DNA.
2. Add a trace of celite abrasive to the solution and apply 10 μL per plant on the second true leaf when plants are at the two-leaf stage, but with the inoculated leaf