3. Resuspend pellet in 0.8 mL DNase buffer and incubate with 10 μg/mL DNase I and 10 μg/mL pancreatic ribonuclease A at 37°C for 60 min. Add SDS to a final concentration of 0.5% (w/v) and incubate at 65°C for 10 min. Add proteinase K to 0.5 mg/mL and incubate at 65°C for a further 15 min.
4. Spin out debris in microcentrifuge for 3 min. Add magnesium chloride ($MgCl_2$) to 100 m*M* and spin out any precipitate that forms. Remove supernatant to a fresh tube and extract with 0.5 mL phenol/chloroform. Microcentrifuge for 5 min. Remove aqueous supernatant to a fresh tube.
5. To selectively precipitate intact viral DNA, excluding fragmented DNA, add $MgCl_2$ to 100 m*M* and 0.6 mL 30% PEG and incubate at room temperature for 10 min. Pellet precipitate in microcentrifuge for 5 min. Discard supernatant and take up pellet in 0.4 mL SDW and add $MgCl_2$ to 100 m*M*. Precipitate DNA by addition of an equal volume of isopropanol, mix, and pellet precipitate. Wash DNA precipitate two to three times with 70% ethanol to remove traces of PEG.

3.4. 2D Gel Electrophoresis of Caulimoviral DNA

1. For a 20 × 20 × 0.45 cm 1% agarose gel, melt 1.8 g agarose in 180 mL TA buffer and pour gel. The sample well is formed by placing a 2-mm diameter sealed Pasteur pipet or custom-made well former in a horizontal position about 1–2 cm in from each side of the gel at one of its corners. The well former should not quite touch the glass plate on which the gel is cast.
2. Assemble the set gel into its running apparatus. Pour on neutral buffer (TA) to submerge the gel totally. Set up a buffer recirculation system.
3. Prepare DNA sample in a total volume of less than 5 μL containing 0.5–1 μL of tracking dye. If the sample is purified virion DNA to be detected by hybridization with a radioactive probe, then less than 20 ng should be loaded. If the sample is from a total cellular DNA preparation from infected plants, about 10 μg is loaded.
4. Electrophoresis in the first dimension is at 1.25 V/cm for about 24 h.
5. To prepare the gel for the second (denaturing) dimension, carefully remove the gel from the running apparatus (it is best to keep the gel on the glass plate on which it was cast) and place in alkaline running medium for 15 min, with gentle agitation, then into fresh alkaline medium for a further 30 min to complete the denaturation.
6. Reassemble the gel into the electrophoresis apparatus, being careful to reorientate it at 90° relative to the first dimension. Pour denaturing medium into the apparatus and recirculate as before. Size markers can then be loaded into the sample well before denaturing electrophoresis.
7. Electrophoresis in the second (denaturing) dimension is at 1.25 V/cm for about 24 h.
8. After electrophoresis, in preparation for Southern blotting, the gel is washed for 5 min in distilled water.
9. DNA in the gel is depurinated to prevent snap-back of supercoiled or hairpin species. Soak the gel for exactly 10 min in 100 m*M* HCl. Wash briefly in distilled water.