not yet fully expanded. Gently stroke the glass rod about six times, pressing the liquid lightly over the leaf surface.
3. Propagate plants at 16–20°C in a 16-h photoperiod. For maximal virus yields, harvest between about 15–25 d pi.

3.2. Large-Scale Virus Purification

1. Harvest 100 g turnip leaves, systemically infected with CaMV. Select younger leaves up to ca. 10 cm in length. Wash and lightly dry leaves (we use a salad centrifuge for this).
2. Place leaves in the blender with 200 mL 0.5*M* sodium phosphate buffer, pH 7.2, and 0.75 g sodium sulphite per 100 mL homogenate. Blend to fine fragments in the cold.
3. Pour homogenate into a beaker, adding 6 g urea and 25 mL of 10% Triton X-100, both per 100 mL homogenate. Stir with magnetic stirrer at 4°C overnight.
4. Centrifuge extracted homogenate at 4000*g* for 10 min at 4°C in an appropriate high-speed rotor.
5. Gently pour the supernatant through four layers of muslin and then distribute the green liquid into tubes for pelleting virus by ultracentrifugation at 70,000*g* for 2 h at 4°C in an ultracentrifuge.
6. Pour off supernatant and resuspend pellets (pellets might be slightly green and also contain starch as well as virus) by dispersing in 1 mL sterile distilled water (SDW) per tube for 1–2 h using a rubber policeman occasionally.
7. Pool resuspended pellets and centrifuge twice in a microcentrifuge to remove particulate matter.
8. Pellet virions from the supernatant (volumes can be made up with SDW) by ultracentrifugation at 136,000*g* at 4°C for 1 h.
9. Resuspend pellets each in 1 mL of DNase buffer. Virion yield can be assessed at this point by UV spectrophotometry. A suspension of CaMV virions of 1 mg/mL has an OD_{260} of 7 (adjusted for light scattering). Virions can be stored at 4°C or –20°C.
10. To isolate virion DNA, the purified virions are first treated with DNase I (10 μg/mL for 10 min at 37°C; reaction stopped by addition of EDTA to 1 m*M*) to remove fragments of plant DNA. Virions are disrupted by adding stock proteinase K to a final concentration of 0.5 mg/mL with 1% SDS in TE and incubating for 15 min at 37°C. DNA is purified from the lysed mixture by phenol:chloroform extraction (minimum of twice). DNA is concentrated by ethanol-precipitation, collected, and quantified by absorbance at 260 nm.

3.3. Quick Method

This method is suitable for isolating CaMV DNA very rapidly from small quantities of tissue when yield is not of major importance and is suitable for PCR analysis, cloning, or sequencing.

1. Grind a single infected leaf in a mortar with 1 mL SDW, add Triton X-100 to 2%, and vortex thoroughly.
2. Pellet inclusion bodies containing virus by spinning in a microcentrifuge for 2 min. Resuspend pellet in 1 mL SDW and pellet again. Repeat spin and wash a total of three times to remove all traces of Triton X-100.