10. DNA in the gel is then denatured, neutralized, and transferred to nitrocellulose for conventional Southern blot hybridization (*see* Chapter 43).

4. Notes

1. In their original isolation method, Hull et al. ***(15)*** reported CaMV yields of 0.6–1 mg virus per 100 g tissue. Using a more rapid method of purification from relatively large amounts of leaves, in which virus inclusion bodies were pelleted before they were disrupted with urea and Triton X-100, Hull et al. ***(15)*** reported virus yields of 70% of their original method. Our standard method typically yields 1.5–2 mg virus per 100 g tissue. We have found that higher yields are generally obtained from younger leaves. Greater virus yields (2–4 mg/100 g tissue) have been reported for the rapid CaMV purification method of Gardner and Shepherd ***(20)*** suitable for use with small amounts of tissue. Our quick method yields only 0.1 mg virus/100 g tissue, but is most suitable for rapid preparation of viral DNA from large numbers of samples of small amounts of tissue for DNA restriction analysis, PCR, sequencing, or cloning. Further virion purification can be achieved by ultracentrifugation through a sucrose gradient, as described by Hull et al. ***(15)***.
2. Purification of all other caulimoviruses should be possible using the methods described herein, employing urea to disaggregate the inclusion bodies, which are a characteristic feature of the group.
3. CaMV particles have shown resistance to disruption by phenol, and this feature can be exploited to specifically isolate nonencapsidated viral DNA and RNA by phenol:chloroform extraction of whole-cell nucleic acid ***(19)***. The resistance to phenol might be explained in part by occlusion of virions in inclusion bodies, which are probably pelleted during centrifugation to separate the phenol:chloroform from the aqueous phase. We have heard that some CaMV strains do not show such resistance to phenol, possibly because they are not retained so tightly in inclusion bodies.
4. The CP of CaMV is glycosylated ***(23)*** and phosphorylated ***(24)***, and synthesis of the mature CP polypeptide proceeds via a processing step. However, analysis of the composition of CaMV CP has been hampered, because during virus purification, the CP can undergo degradation into specific fragments. It can also aggregate to form multimeric polypeptides, as resolved on denaturing PAGE ***(25)***.
5. CaMV virion DNA can be identified by its characteristic mobility during gel electrophoresis (**Fig. 2**). Under nondenaturing conditions, virion DNA separates as a genome-length (8 kbp) linear form produced by breakage of the circular DNA, together with more slowly migrating open-circular components. The most rapidly migrating open-circular form is of typical open conformation (**Fig. 2**, ND). However, a series of more slowly migrating forms are also observed, which comprise molecules that are twisted to varying degrees (not supercoiled, because the DNA has single-strand discontinuities). The single-stranded components of virion DNA can be revealed by denaturing the sample before electrophoresis (**Fig. 2D**).
6. 2D gel electrophoresis (**Fig. 3**) is an extremely powerful method of resolving complex populations of DNA. It allows separation of linear single-stranded and