nel, allow to separate, and add the remainder of aqueous phase to rest of supernatant. Discard pellet and organic solvents in proper containers.

19. (**Subheading 3.3., step 3**) Alternatively, add PEG to 4% (w/v) and NaCl to 0.25*M*. We have done this both ways and occasionally some viruses or virus strains appear to yield better with this method, rather than with 6% PEG and no NaCl.
20. (**Subheading 3.3., step 4**) We have done this for less time with no apparent loss in yield. Probably as little as 2 h is adequate.
21. (**Subheading 3.3., step 9**) One may use thick-wall polycarbonate tubes and the Ti 70 rotor at 125,000*g*. Fill these tubes no more than half full. If your ultracentrifuge will calculate $\omega^2 t$, use ≈12,000. Some potyviruses are unstable in CsCl. This will be apparent if most of the virus is lost during ultracentrifugation to remove the CsCl. If this should happen, use 35% (w/v) Cs_2SO_4, rather than CsCl.

4.4. Purification of Rymoviruses

22. (**Subheading 3.4., step 1**) Brakke and Ball *(2)* report that the best yields are obtained from younger leaves, but not until these leaves had well-developed symptoms throughout.
23. (**Subheading 3.4., step 1**) Take care not to heat up blender excessively.
24. (**Subheading 3.4., step 3**) It is best to keep track of temperature during this period of time.
25. (**Subheading 3.4., step 7**) When resuspending the first high-speed pellet, use about one-thirtieth vol of the initial extract. This step can be performed overnight at 4°C.
26. (**Subheading 3.4., step 12**) Our average yield for WSMV is about 1.2 A_{254} units, or about 0.4 mg/100 g tissue, using 3.0 as the extinction coefficient. The $A_{260/280}$ should be ≈1.37.

4.5. RNA Isolation from Virions

27. Do not use buffers that have over 50 m*M* potassium in them. **Caution:** Do not inhale SDS and avoid contact with skin, eyes, and clothing. Phenol is toxic if in contact with skin or swallowed and causes burns. Always use with gloves and protective eye wear. DEPC is toxic if in contact with skin or swallowed and causes burns. Always use with gloves and protective eye wear.
28. Use virus at concentrations greater than 250 μg/mL whenever possible. Lower starting concentrations of virus will adversely affect RNA yields.
29. Nucleic acid content of potyviruses is about 5%. One milligram of virus should yield close to 50 μg of RNA.
30. Although isolation of viral RNA is necessary for some experimental procedures involving viruses, it is not always necessary to isolate viral RNA to clone viruses via reverse transcription. Recent work has shown that a simple procedure can be used to obtain high-quality cDNA directly from potyviral virions *(7)*. This procedure circumvents the sometimes difficult isolation of high-quality, pure RNA from virions. Full-length or near-full-length cDNA from potyviruses can be syn-