ent centrifugation, but separation may be unsatisfactory if there is turbulence during deceleration caused by even slight rotor imbalance.

9. (**Subheading 3.1., step 11**) Visualize virus-containing band after centrifugation by shining a light (like from a binocular microscope light source). You can remove this band directly with a Pasteur pipet, or fractionate it using absorbed light at 254 nm with a commercial fractionator.
10. (**Subheading 3.1., step 13**) We recommend that one determine absorbance, regardless of purification method used, at 260 and 280 nm spectrophotometrically. The 260/280 ratio for potyviruses should be around 1.2–1.3 (or up to 1.4 for rymoviruses). Readings that are well out of this range (more than ca. 0.1 U) indicate probable contamination and/or degraded virus. Store potyviruses by refrigeration at 4°C in the presence of 0.04% (w/v) NaN_3. EDTA can also be added as a preservative. We do not recommend freezing virus preparations and, although there appears to be wide variation in storage life of potyviruses, most should remain stable and useful for several months.

4.2. MDMV Purification

11. Titer of viruses such as MDMV does not seem to be as time-dependent as many dicot-infecting viruses, but yields will be greater if younger infected tissue is used, rather than older leaf tissue. Some workers remove leaf mid-rib tissue prior to grinding.
12. (**Subheading 3.2., step 1**) Try to keep the ratio of extraction buffer to tissue low, in the 1:1–1:1.5 range, if possible.
13. (**Subheading 3.2., step 1**) Perhaps the greatest loss of virus at this stage is caused by poor or incomplete grinding of tissue and failure to extract as much sap as possible from the cheesecloth.
14. (**Subheading 3.2., step 3**) Be sure to carefully decant the supernatant to remove all carbon tetrachloride. Failure to do so will require repeating **Subheading 3.2., step 3**.
15. (**Subheading 3.2., step 4**) Making a 25% stock solution will make it easier to pipet Triton X-100.
16. (**Subheading 3.2., step 4**) Do not stop at this point. Extended contact between virus and the detergent will significantly reduce yield.
17. (**Subheading 3.2., step 7**) The band containing virus should be clearly visible if the tube is illuminated from the bottom. Alternatively, one can simply remove the virus-containing fraction by pipeting gently with a U-shaped Pasteur pipet, or by inserting a 20-gage needle through the side of the tube. Most laboratories will use an ISCO density gradient fractionation system to retrieve the virus-containing band.

4.3. Purification of Legume-Infecting Potyviruses

18. (**Subheading 3.3., step 2**) The organic phase will come loose, but you do not want any of this mixed with supernatant. Pour off the rest into a separatory fun-