4. Pellet the denatured chromosomal DNA by centrifugation at 4°C in a microcentrifuge.
5. Decant the supernatant to a clean microcentrifuge tube.
6. Equilibrate the plasmid isolation column by passing 1 mL of solution QBT through the column.
7. Add the DNA supernatant to the equilibrated column (*see* **Note 9**).
8. Once the supernatant has passed through the column, wash the column twice with 1 mL of solution QC.
9. Elute RF-DNA with 0.5–0.8 mL of solution QF. Collect the eluate in a clean microcentrifuge tube.
10. Add 0.7 vol of isopropanol, mix gently, and pellet the DNA in a microcentrifuge for 20 min.
11. Discard the supernatant, and wash the pellet by adding 200 µL of cold 70% ethanol and centrifuging for 10 min at 4°C.
12. Discard the supernatant, invert the tube on absorbant paper, and allow the pellet to air-dry.
13. Resuspend the RF-DNA in 10–50 µL of TE buffer (*see* **Note 10**).

4. Notes

1. It is best to use young leaf material, infected within the previous 2–3 wk. We have found that older leaf material usually yields mainly single, not geminate, particles.
2. In our experience harvested material may also be stored at 4°C for 2–3 d with no deleterious effects on virus isolation.
3. A standard electric coffee grinder is a good alternative to the mortar and pestle: The plant material is frozen in liquid nitrogen, transferred to the grinding chamber, and processed with short grinding spurts (5–10 s). Before it thaws, transfer the ground material to a beaker containing the DNA-extraction buffer.
4. Oxidation of sap components can occur during the extraction procedure, resulting in the DNA pellet being colored yellow to brown. This may affect the quality of the DNA extract. If oxidation is found to be a problem, we suggest adding 2-mercaptoethanol to the extraction buffer to a final concentration of 10 m*M*. (**Caution:** mercaptoethanol is toxic and smells unpleasant, so should be confined to a fume hood.)
5. Take care not to disturb the interphase between the aqueous and phenol phases: It will be necessary to repeat the phenol extraction if any denatured protein matter contaminates the aqueous phase.
6. To clean the DNA preparation further, it is sometimes advisable to resuspend the DNA pellet at this stage in 2–4 mL of TE and to reprecipitate the DNA by adding 0.1 vol of 4*M* LiCl and 2 vol of ethanol, and pelleting the DNA again at 4°C. This step is not always necessary, and may be omitted if the pellet looks clean.
7. We have often found that DNA isolated from maize plants infected with severe isolates of MSV contains such high amounts of RF-DNA that further treatment to enrich for RF-DNA is unnecessary. However, plants showing milder symptoms will usually yield less RF-DNA.