

8. It may be difficult to resuspend large amounts of plant total nucleic acids in such a small volume of buffer P1. If larger volumes are required, scale up the amounts of P2 and P3 used, proportionally.
9. A high concentration of residual plant DNA after the alkaline lysis procedure may congest the column. To remedy this problem, precipitate the supernatant from **step 5** with 0.8 vol of isopropanol, pellet the DNA in a microcentrifuge and repeat the alkaline denaturation procedure (**steps 1–5**).
10. Trace amounts of contamination with genomic DNA are unavoidable; however, the RF-DNA preparation is usually clean enough for standard molecular manipulations like restriction mapping, cloning, and even direct DNA sequencing.

## References

1. Lazarowitz, S. G. (1992) Geminiviruses: genome structure and gene function. *Crit. Rev. Plant Sci.* **11**, 327–349.
2. Timmermans, M. C. P., Das, O. P., and Messing, J. (1994) Geminiviruses and their use as extrachromosomal replicons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 79–112.
3. Briddon, R. W. and Markham, P. G. (1995) Geminiviridae, in *Virus Taxonomy: Sixth Report of the International Committee on the Taxonomy of Viruses* (Murphy, F. A., Fauquet, C. M., Bishop, D. H. L., Ghabrial, S. A., Jarvis, A. W., Martelli, G. P., Mayo, M. A., and Summers, M. D., eds.), Springer-Verlag, Vienna, pp. 158–165.
4. Rybicki, E. P. (1994) A phylogenetic and evolutionary justification for three genera of Geminiviridae. *Arch. Virol.* **139**, 49–77.
5. Bisaro, D. M. (1996) Geminivirus replication, in *Eukaryotic DNA Replication* (De Pamphilis, M., ed.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
6. Laufs, J., Traut, W., Heyraud, F., Matzeit, V., Rogers, S. G., Schell, J., and Gronenborn, B. (1995) *In vitro* cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. *Proc. Natl. Acad. Sci. USA* **92**, 3879–3883.
7. Laufs, J., Jupin, I., David, C., Schumacher, S., Heyraud-Nitschke, F., and Gronenborn, B. (1995) Geminivirus replication: genetic and biochemical characterisation of Rep protein function, a review. *Biochimie* **77**, 765–773.
8. Stanley, J. (1995) Analysis of African cassava mosaic virus recombinants suggests strand nicking occurs within the conserved nonanucleotide motif during the initiation of rolling circle replication. *Virology* **206**, 707–712.
9. Orozco, B. M. and Hanley-Bowdoin, L. (1996) A DNA structure is required for geminivirus replication origin function. *J. Virol.* **70**, 148–158.
10. von Wechmar, M. B. and Milne, R. G. (1983) Purification and serology of a South African isolate of maize streak virus, in *Proceedings International Maize Virus Disease Colloquium and Workshop, 2-6 August 1992* (Gordon, D. T., Knoke, J. K., Nault, L. R., and Ritter, R. M., eds.), Ohio State University, Wooster, OH.
11. Hamilton, W. D. O., Saunders, R. C., Coutts, R. H. A., and Buck, K. W. (1981) Characterisation of tomato golden mosaic virus as a geminivirus. *FEMS Microbiol. Lett.* **11**, 263–267.