of starting template. Too many cycles will give smearing and too few will give low yields.

6. The addition of restriction sites is a useful application of PCR, but most restriction enzymes will not cleave sites close to the end of DNA fragments. Addition of several GC bases can reduce this problem for enzymes, such as *Eco*RI, *Bam*HI, *Kpn*I, *Pst*I, *Sma*I and *Xba*I, by clamping the end as dsDNA. Alternatively, the restriction site could be located internally in the primer, rather than at the 5' end.
7. A range of template concentrations (1 pg–10 ng) can be tested to find the optimum that gives satisfactory yields with the minimum number of cycles of amplification. Fewer cycles reduce the probability of misincorporation during DNA elongation, resulting in base changes to the final product (and subsequent clone).
8. The majority of heat-stable DNA polymerase enzymes have no proofreading activity, which can lead to PCR products containing sequence differences from the original template. If high fidelity is required during the reaction, it is best to use one of the more recently developed enzymes, which have a proofreading activity. In addition, it is advisable to minimize both the number of cycles performed and the length of template amplified. Even if a proofreading enzyme is used, it is worthwhile checking the fidelity by sequence comparison of the product and the original template. If the sequence of the template is unknown and it is being amplified by PCR to provide such data, it is advisable to sequence products from more than one reaction to obtain a consensus sequence.

References

1. Nicolas, O. and Laliberte, J.-F. (1992) The complete nucleotide sequence of turnip mosaic potyvirus RNA. *J. Gen. Virol.* **73,** 2785–2793.
2. Robertson N. L., French, R., and Gray S. M. (1991) Use of group-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. *J. Gen. Virol.* **72,** 1473–1477.
3. Frohman, M. A., Dush, M. K., and Martin, G. R. (1988) Rapid produciton of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad Sci USA* **85,** 8998–9002.
4. Foulds, I. J., Lea, V. J., Sidebottom, C., James, C. M., Boulton, R. E., Brears, T., Slabas, A. R., Jack, P. L., and Stratford, R. (1993) Cloning and sequence analysis of the coat protein gene of barley mild mosaic virus. *Virus Res.* **27,** 79–89.
5. Lutke, H. A., Chow, K. C., Mickel, F. S., Moss, K. A., Kern, H. F., and Scheele, G. A. (1987) Selection of AUG initiation codons differs in plants and animals. *EMBO J.* **6,** 43–48.