48

Detection and Quantification of Plant Viruses by PCR

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1. Introduction

The polymerase chain reaction (PCR) is a technique that enables the specific amplification and hence detection of target DNA sequences from complex mixtures of nucleic acid. A combination of short, specific primers and thermostable DNA polymerases are used to amplify the target sequence, through repeated cycles of denaturation, reannealing, and DNA synthesis at high temperatures, allowing an exponential increase in the amount of the DNA of interest. By addition of a reverse-transcription (RT) step, PCR can also be applied to cDNA generated from RNA templates. Its extreme sensitivity and high specificity make it an unparalleled technique for the detection and characterization of rare messages, including viral infections that are difficult to detect and diagnose by serology or electron microscopy.

Although PCR is now a routine technique in many laboratories, there are still a considerable number of problems in getting good, reproducible amplifications. With plant tissues, the vast majority of the problems have to do with the initial purification of the nucleic acid, to give samples pure enough to be used in the enzymatic reactions. The protocols described here are a combination of routine methods used in our laboratories, and hints and comments on the problems that might be encountered, with pointers to further reading for those working with particularly recalcitrant tissues.

2. Materials

2.1. General Equipment

1. Polythene bags: 10 × 15 cm, 500 gage (e.g., from Polybags, Greenford, UK).
2. Hand-held wallpaper seam roller: narrow roller ~3-cm width (available from hardware stores).

From: *Methods in Molecular Biology, Vol. 81: Plant Virology Protocols: From Virus Isolation to Transgenic Resistance*
Edited by: G. D. Foster and S. C. Taylor