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PCR Cloning of Coat Protein Genes

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1. Introduction

The polymerase chain reaction (PCR) is used to amplify DNA between two oligonucleotide primers, of which one is complimentary to a sequence on the (+)-strand and the other to a downstream sequence on the (–)-strand. Amplification between the two primers is achieved by reiterative cycles of template denaturation, primer annealing, and primer extension by a heat-stable DNA polymerase enzyme, which is able to withstand the repeated high temperatures required for DNA denaturation. The products of each reaction cycle serve as templates for subsequent cycles, and so, theoretically, the amount of product doubles after each cycle. PCR is therefore a very sensitive technique that can be used to generate microgram quantities of DNA from as little as a single DNA molecule (*see also* Chapter 48).

PCR reactions can also be used to amplify products from RNA templates (RT-PCR; *see also* Chapter 48). The RNA is reverse-transcribed to form a single complementary strand of DNA, which is then amplified by PCR, using primers on either side of the target gene. RT-PCR, using either purified viral RNA or total RNA from infected plants, can be used for cloning genes or for diagnostic purposes ***(1,2)***. RT-PCR requires sequence information from both sides of the target gene, but a modification of the method can be used to clone genes from which only limited sequence data is available. The RACE-PCR technique developed by Frohman et al. ***(3)*** enables amplification using a single gene-specific primer combined with a generic primer. First-strand cDNA is synthesized using an oligo dT-adaptor primer, which anneals to the poly A tail present at the 3' end of all eukaryotic mRNAs. The subsequent PCR reaction uses the single gene-specific primer and a second primer corresponding to the adaptor sequence of the cDNA primer (**Fig. 1B**). Consequently, it is possible to clone

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