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PCR Analysis of Transgenic Tobacco Plants

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1. Introduction

Although the display of antibiotic resistance can be a good indication that a regenerated plant is transformed with introduced DNA, escapes that represent partial or no transfer may be present within the population of primary transformants. The polymerase chain reaction (PCR) provides us with a technique for rapidly analyzing large numbers of putative transformants for the presence of a transgene DNA sequence.

Frequently, plant DNA extraction protocols are time-consuming and not applicable to very small amounts of tissue. The use of PCR to analyze large numbers of samples has therefore been unfeasible. However, since the publication of a small-scale DNA extraction method by Edwards et al. ***(1)***, screening of transgenic plants by PCR has become a routine procedure. The method includes few steps and does not involve the use of phenol or chloroform. Many samples can therefore be processed in a short period of time without exposure to toxic chemicals.

PCR can provide us with a useful tool to demonstrate the presence of specific DNA sequences within the genome; however, care must be taken to include the appropriate control PCR experiments. When *Agrobacterium* has been used as the transformation vector, it is important to be able to distinguish between *Agrobacterium* contamination and genuine PCR positive results. The presence of *Agrobacterium* can be tested by carrying out a control PCR experiment, which involves a primer that is specific to the binary vector sequence from beyond the classically defined T-DNA border region. Included in **Table 1** is the sequence of a non–T-DNA right border (RB) primer ***(2)*** designed against the pBin19 binary vector ***(3)***. This primer can be used in conjunction with *npt*-II 5' primer (also shown in **Table 1**), if the binary vector was

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