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Southern Analysis of Transgenic Tobacco Plants

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1. Introduction

Southern analysis ***(1)*** is routinely carried out to determine whether a plant regenerated from tissue-culture has been transformed with foreign DNA. The technique of Southern analysis begins with the extraction of genomic DNA from the plant, digestion of the DNA with diagnostic-restriction enzymes, and fractionation of the restricted DNA by agarose gel electrophoresis. Following the transfer of the fractionated DNA to a nylon membrane by capillary blotting (Southern blotting), a radioactively labeled fragment of the foreign DNA is used for the detection of homologous sequences within the plant genomic DNA. This technique allows not only the detection of foreign DNA, but also an estimation of the number of copies and the arrangement of the foreign gene(s) within the plant genome.

Polymerase chain reaction (PCR) techniques can also be used to demonstrate the presence of foreign DNA (*see* Chapters 41 and 42). However, although PCR gives results rapidly, it is necessary to carry out a range of control experiments to avoid false-positive results that may arise from contaminating DNA sequences. The PCR method can be useful when there are only limited amounts of tissue available for analysis. However, if there is sufficient material, Southern analysis should be performed in preference since much more information can be gained.

Detailed protocols are presented below for the extraction of genomic DNA, Southern blotting, radioactive labeling of DNA fragments, and for hybridization. A method is also included for the stripping and reprobing of DNA blots.

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