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Molecular Analysis of Transgenic Rice

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1. Introduction

It makes little sense to invest time and effort in assaying biological activities of transgenic plants if the plants do not contain a functional gene of interest. Although this appears to be self-evident, many investigators attempt to use biological assays for the presence of the gene of interest prior to conducting informative molecular characterization of transgenic plants. Where facile tests are available, such as those for β-glucuronidase (GUS) activity, this may be feasible. However, low levels of activity frequently make simple biochemical screening approaches unreliable. Of the several analytical techniques available, we have chosen to describe methods for polymerase chain reaction (PCR) and genomic DNA blot analysis: methods that are fundamental for confirming and characterizing gene insertion.

1.1. PCR

PCR is a powerful and extremely sensitive technique for detecting DNA sequences in transgenic plants. PCR uses the activity of a (thermostable) polymerase, combined with oligonucleotide primers, substrates (DNA template, dNTPs, and so on), and cofactor, to preferentially synthesize and, hence, amplify a specific fragment of target DNA. One cycle of a reaction generally consists of sequential incubation at high temperature (e.g., 94°C, 45 s) to denature the double stranded DNA substrate, incubation at a lower temperature (e.g., 50–-65°C, 45–60 s) to allow the oligonucleotide primers to anneal, and incubation at 72°C (e.g., 2 min) to allow chain elongation. A major variable in the reaction is the annealing temperature; it must be low enough to allow the primers to anneal to the complementary sequences in the target DNA, but high

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