enough to prevent annealing at related sequences. Repetition of this cycle 20–40 times results in amplification of target sequences between the primers, thereby providing a very sensitive method of detection. However, the high sensitivity of this reaction makes false positives caused by contamination a serious problem (*see* **Note 1**).

1.2. Genomic Blot Analysis

Genomic DNA blot analysis, originally described by Southern ***(1)***, can provide extensive information about the transformation event being studied. Important controls include the use of DNA from a nontransformed plant to show if sequences homologous to those of the probe being used are present in the plant genome, and large (>50 kb) DNA not treated with any endonuclease to confirm integration into chromosomal DNA. Detection of hybridizing bands of a size corresponding to the plasmid used for transformation provides cautionary information regarding the putative transformation. The choice of restriction digests that will yield a predicted gene fragment are important for determining if rearrangement has occurred, and also provide insight to copy number. The use of an endonuclease that cuts only once within the gene of interest is valuable, since this permits determination of copy number and distinction of independent transformants. Hybridization patterns are most simple to interpret if the probe used corresponds to a sequence that lies entirely to one side of the single restriction site.

The DNA isolation protocol presented is a slightly modified version of the method developed by Taylor et al. ***(2)***. Plant tissue is frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. The powder is added to an extraction buffer composed of CTAB detergent and a high concentration of salt, in which the nucleic acids (NAs) are solubilized and subsequently separated from cell walls and other particulates by centrifugation. Extraction of the solution with an organic solvent removes many carbohydrates and proteins. Upon addition of the precipitation buffer that dilutes the salt concentration, an insoluble NA:CTAB complex forms. After centrifugation, the NA:CTAB precipitate is solubilized under high-salt conditions and the NAs are selectively precipitated with isopropanol. RNA is removed by RNase digestion, and, after additional alcohol precipitations, DNA of sufficient purity to be digested with restriction enzymes has been isolated.

Hundreds of variations of genomic blot analysis are currently used with success. The method described below relies on a minimum amount of equipment that might be available in all laboratories. Genomic DNA is size fractionated by agarose gel electrophoresis. The DNA is made single-stranded by base denaturation and is transferred to a membrane by capillary action. Heat treatment under vacuum is then used to bind the DNA to a mem-