based on pBin19, or with a construct-specific primer if a second vector primer is not available.

Recently, Martineau et al. ***(4)*** have examined *Agrobacterium*-transferred DNA in a population of several hundred independent transformed plants representing several crop species. From this study it appears that DNA from beyond the border-repeats in the original bacterial plasmid are also integrated into the genomes of 20–30% of the transgenic plants. This high frequency of beyond-the-border DNA transfer has several implications. First, the use of a primer that is designed against DNA sequences flanking the borders for identifying *Agrobacterium* contamination on primary transformants might not be as clear cut as expected. Second, researchers using T-DNA tagging as a means of cloning genes may encounter problems when attempting to identify adjacent plant DNA sequences because the T-DNA is larger than expected.

Despite these findings, if care is taken when devising PCR experiments, the results can be informative. In the PCR experiment shown in **Fig. 1**, the primers were designed to identify an introduced β(1,3)-glucanase transgene ***(5)***. However, the native β(1,3)-glucanase gene is also recognized, but this gene can be distinguished from the transgene by the presence of an intron that increases the mol wt of the amplified product. The PCR amplification of the native β(1,3)-glucanase gene also served as a convenient positive control for each sample.

The PCR method presented here has some negative points, but it can be extremely useful for the preliminary screen of large numbers of regenerated putative transformed plants. However, it is important to remember that much more information about the number of copies and arrangement of the introduced DNA within the plant genome can be obtained by carrying out Southern analysis (*see* Chapters 41 and 43).

2. Materials

2.1. DNA Extraction

1. Disposable grinders/spatulas (Sarstedt, cat. no. 81970).
2. PCR DNA extraction buffer: 200 m*M*Tris-HCl, pH 7.5, 250 m*M*NaCl, 25 m*M* EDTA, 0.5% SDS. Store at room temperature.
3. Propan-2-ol.
4. TE: 10 m*M* Tris-HCl, pH 7.5, 0.1 m*M* EDTA.

2.2. PCR from Plant DNA

1. *Taq* DNA polymerase (Promega, Southampton, UK).
2. PCR buffer (10X): Supplied with the *Taq* DNA polymerase. Store at –20°C.
3. dNTP stock: 2 m*M* of each dNTP. Store at –20°C.
4. Thermal cycling machine (Cetus, Warrington, UK).