tive-control lane (+) shows a product of the predicted size. Taken together, these data indicate the results of this experiment should be valid. It can be concluded that the plants assayed in lanes 3–5, which show fragments of the predicted size, contain at least one unrearranged copy of the GFP coding region (between bp 117 and 817). However, it is not clear whether the results from plants assayed in lanes 1 and 2 are valid. If these two DNA extracts could serve as substrates for PCR amplification, the negative result would be valid. In contrast, if they could not support amplification, they could be false negatives. Therefore, all samples were tested for the ability to act as substrate for PCR by amplifying an endogenous single-copy gene (triose phosphate isomerase), as shown in Panel B.

In Panel B, the negative (–) and positive (+) controls gave the expected results, indicating the experiment should be valid. However, the positive control (TPI plasmid) resulted in the amplification of additional, unexpected fragments. The very high-mol-wt fragments indicate the presence of rice DNA added to that reaction, and the smaller than predicted fragments are probably caused by nonspecific priming at other sites in the plasmid. This is likely because of the low annealing temperature used. The results in lanes 1–7 indicate that all the samples, importantly, samples 1 and 2, can serve as substrate for PCR. Taken together with those in Panel A, these data provide strong evidence that samples 1 and 2 lack an intact copy of the central portion of the GFP coding region. Be aware that this result does not necessarily indicate that genomic blot analysis of these plants (using GFP as a probe) would result in no hybridization, since rearranged or deleted portions of GFP could be present. Rather, it simply indicates that the two primer sites are not present or, if present, are not in the correct relative orientation.

The presence and absence of fragments in the positive (+) and negative (–, 5 and 6) control lanes, respectively, validate the results of the PCR shown in Panel C. Another reaction (data not shown) indicated all samples served as substrate for PCR. Therefore, it can be concluded that the plants assayed in lanes 1–3 contain at least one unrearranged copy of the GUS coding region (between bp 400 and 1599). An additional higher mol-wt fragment (lane 1) and lower mol-wt fragments (lanes 2 and 3) are also present, probably indicating the presence of additional rearranged copies of the GUS coding region in these plants. Furthermore, since the banding pattern is identical in lanes 2 and 3, it is likely that these two plants are siblings. The presence of a low-mol-wt fragment, but not a fragment of the expected size in lane 4, suggests this plant contains only a rearranged copy(ies) of the GUS coding region.