

Supplemental Fig. 1. The 3 kb bands of "long" PCR lanes A, B, C were copied from cDNA.

As shown in panel a (a copy of Fig. 4c), a "long" PCR was carried out to detect the 3' half of completed cDNA. Deletion constructs A, B, C and undeleted Tto1 (Tto1) allow synthesis of this band. In contrast, the band is characteristically absent from panels D and E. indicative of the absence of full length cDNA from plants carrying constructs D and E (see text for further interpretation). In this experiment, the upward reading primer can bind to the genomic copy of the undeleted control construct, but not to the genomic copy of any deletion construct, because its binding site is part of the deleted region. "Long" PCR products of deletion constructs should therefore lack introns. As gel resolution does not allow to distinguish a 100 bp difference of the 3 kb sequence, an additional control reaction was carried out. The visible 3 kb bands of panel a, and material from the same gel position of lanes D and E, were excised and used for a "short" PCR (panels b, c; cf. Fig. 4). Panel c is identical to panel b, but a lower number of cycles was used to underscore the predominance of the lower (intron-less) band in lanes A to C. Both panels show that the abundant bands from deletion constructs A, B and C are almost completely devoid of introns, indicative of their generation from cDNA. In contrast, the intron-less bands for constructs D and E are much less intense and only visible in panel b, suggesting that they were derived from low amounts of largely aberrant reverse transcripts present in these transgenic lines. Intron-containing (250 kb) fragments are also present in small amounts for all deletion constructs. They vary much less in abundance between lanes A to C and D, E, and may be derived from a low background of genomic DNA present in the 3 kb region of the "long" PCR gel (panel a). Molecular weight marker positions are indicated to the right.

Supplemental Figure 1