

Rapid determination of transgene copy number in tobacco by competitive PCR using a pair of SSR primers

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ABSTRACT. We developed a straightforward, rapid, and inexpensive method to determine transgene copy number in tobacco. The plasmid (pSSRCopy) used for tobacco transformation contains a simple sequence repeat (SSR) locus, PT1199, which was partially deleted in the middle, a homogenous SSR locus in tobacco K326. A 168-bp segment of the cloned PT1199 was shortened to 95 bp by deleting a 73-bp internal fragment. Using a pair of SSR primers, competitive PCR was amplified from genomic DNA from transgenic tobacco harboring pSSRCopy, and the two expected bands were found. The 168-bp band (SSR-168) corresponds to endogenous PT1199 and the 95-bp band (SSR-95) comes from the integrated pSSRCopy. A single

copy of a transgene can be easily distinguished from multiple copies by comparing band densities.

Key words: Copy number; Transgene; Competitive PCR; SSR; Tobacco