

Genome walking in Sugarcane: an approach to clone unknown flanking sequences

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The isolation and characterization of unknown DNA sequences flanking known regions are critical, especially for the analysis of upstream and downstream non-coding regions. Promoters are segments of DNA that regulate the timing and location of gene expression. The study of genes involved in abiotic stress and cell wall modification is essential for adaptation of several crops to climatic changes and, also, as a biotechnological tool for second generation biofuels production. The use of new technologies associated to precision breeding via transgeny represents a huge impact on agro-industrial crops, especially for sugarcane, the most important crop for ethanol production in Brazil. One very powerful way of modifying the characteristics of plants is to target the expression of introduced genes to specific parts of the plant or at specific stages of the life cycle using promoters with known specificity. Very few sugarcane promoters are currently known. The goal of this study is demonstrate our success with cloning promoter sequences of sugarcane genes using the technique of genome walking. For this study, we have selected key genes involved in cell wall modification, leaf senescence, abiotic stress and induced by chemical molecules. RT-qPCR and *Northern* blot analyses were conducted in order to validate candidate genes differentially expressed under the previous related conditions. Thus, the seven genes were considered key to clone promoter regions. For the construction of Genome Walker libraries, 5 blunt-end cutting restriction enzymes (*DraI*, *SmaI*, *PvuII*, *EcoRV* and *StuI*) were used individually to digest this genomic DNA completely. Each batch of digested genomic DNA was purified and ligated to the adaptors provided in the Universal Genome Walker kit (Clontech, USA) according to the manufacturer's protocol. The primary PCR gives multiple bands in each lane with a general background smear. The secondary PCR using the internal primers and the diluted primary PCR product as the template, selectively amplifies the desired product in the subsequent nested PCR resulting in a single bright band. After primary and secondary PCRs, many fragments sizes were generated (ranging 150 and 3000 bp) and purified with the Wizard[®] SV PCR Clean-UP system kit (Promega). The fragments were cloned in pGem-T-Easy[™] vector and, then, used to transform *Escherichia coli* XL10-Gold. To verify the authenticity, these products were sequenced and compared for the 5'-end overlap. Also, the putative isolated 5'-flanking regions were fused to the *gus* gene for promoters tests. To summarize, the technique of Genome Walking has been standardized in sugarcane and can now be exploited to dissect out promoters of important genes for future research involving the understanding of transcriptional regulation and directed expression in this economically important energy crop.