

Molecular Characterization of a Root-Specific Promoter of *Eucalyptus*

Ribeiro, C. L.¹, Sasaki, F.T.¹, Soprano, A.S.¹, Maia, I. G.¹

¹Departamento de Genética, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, SP, Brazil

The production of eucalyptus in Brazil, driven by advances in forestry technology, has achieved satisfactory rates at a worldwide level in recent years. The combination of conventional improvement techniques with modern biotechnology was essential to reach this benefit. Among the molecular tools necessary to ensure further biotechnological progress in *Eucalyptus*, the identification and characterization of organ/tissue-specific promoters is a priority. Recovery of highly active promoter sequences, especially those leading to tissue-specific expression, is of great interest to manage the perceived risks associated with transgene technology, and to restrict transgene expression to selected parts of the plant. In this context, an EST showing root-specific expression was recently identified by our group from *in silico* searches in the Brazilian *Eucalyptus* EST Database (FORESTs). In this study, the expected expression pattern of the mentioned EST was validated by RT-PCR. Subsequently, the DNA regions immediately upstream to the validated EST were amplified using a genome walking technique and fragments of 1.35 kb, 2.9 kb and 3.0 kb were obtained. These fragments were cloned into the pGEM-Teasy vector and sequenced. Among them, the fragment of smaller size (1.35 kb) was completely sequenced and its upstream (5') orientation to the corresponding EST confirmed by PCR using genomic DNA. This fragment was inserted into the binary vector pCambia-1381z in order to direct the expression of a GUS reporter gene. This expression cassette was inserted into the *Agrobacterium tumefaciens* for transformation of tobacco plants aiming functional analysis.

Key Words: *Eucalyptus*, gene expression, promoters, tissue-specificity

Financial Support: FAPESP and CNPq