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DEVELOPMENT OF EST-PCR MARKERS AND SNPs DISCOVERY IN NORWAY SPRUCE (PICEA ABIES (L.) KARST.)

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A non-normalized cDNA library was constructed from mRNA of 1 month-old bare root Norway spruce seedlings germinated from a population of open-pollinated seeds. cDNA products were cloned into pGEM-3Z (Promega P2151) and 4007 sequences from either the 5' end or the 3' of each clone were obtained. A total of 408 contigs were formed after assembly of the 4007 ESTs. The clusters contained a total of 1324 sequences, whereas 2684 remained as singleton ESTs (67%), not identical to any other EST in the dataset. The clusters represent either independent clones of the same transcript, allelic sequences, or different members of multigene families. The largest cluster contained 36 sequences while 233 clusters contained only 2 sequences. Sequences produced within this project were analysed for homology with already available conifers databases and then partitioned into functional categories. New primers were designed for the amplification of ESTs originating from the cDNA library of Norway spruce and tested together with an additional set of EST primers derived from other conifers (*Pseudotsuga menziesii* and *Pinus taeda*). Parental trees of mapping populations of some conifer species were analysed. In total, 351 EST primers were tested in Norway spruce, among which 54% amplified. Only ESTs showing homology at least between two conifers were selected. Twenty-three Norway spruce ESTs originating from the cDNA library, 8 from *Pseudotsuga menziesii* and 7 from *Pinus taeda* met this requirement. EST polymorphism between the two parental trees of an additional Norway spruce mapping population was detected through sequencing. All amplified fragments of the two parental trees were sequenced from both ends using a capillary MegaBace *Amersham* automatic sequencer. About 50% of the ESTs displayed nucleotide variation between the parental trees, with at least one SNP (Single Nucleotide Polymorphism). The benefits of these newly developed EST markers are outlined with respect to population genetics.