

## New Loblolly Pine Microsatellite Markers for Mapping and Population Genetics

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Select microsatellite (SSR) markers are being developed from enriched genomic libraries, EST databases, and published sources for uses in breeding programs, population genetic studies, enhancing pine genome maps, and DNA fingerprint kits. We tested 1264 primer pairs in loblolly pine (*Pinus taeda*) and selected 137 suitable markers, of which 111 are new. Primer sequences were obtained de novo and from external sources. For de novo primer development we used 18,498 *P. pinaster* EST sequences obtained from Christophe Plomion (INRA-UMR BIOGECO, France) and 179,433 *P. taeda* EST sequences from various public databases, combined to obtain 869 'sifg' markers. For a portion of the *P. taeda* EST primers we used assembled contig sequences generously provided by the Laboratory for Genomics and Bioinformatics at the University of Georgia. We also used *P. taeda* genomic sequences from SSR-enriched libraries to obtain 118 'ript' markers under a prior cooperative agreement with International Paper Company. Primers that we evaluated from extant external sources were those of Auckland et al. 2002 for 164 'PtTX' markers, Chagné et al. 2004 for 38 'SsrPt' and 5 'RPtest' markers, and Phil Wilcox (Scion LLC, New Zealand) for 70 'NZPR' *P. radiata* markers.

Our criteria for marker selection were, in order of precedence, strong amplification of single loci with a one-size-fits-all PCR protocol, easily interpretable chromatographic allelic profiles obtained from capillary electrophoresis, and gene diversity values above 0.30. Gene diversity was estimated from 14 *P. taeda* genotypes whose provenances were distributed across the species' natural geographic range. The proportions of SSR motif classes comprising the select set were 62% dinucleotide, 27% trinucleotide, with the rest being tetra-, penta-, or hexanucleotide repeats. EST-SSR markers made up 40% of the select set.

Ninety six percent of select markers were heterozygous in at least one of two sets of parents of the publicly distributed 'Base2' and 'Qtl2' reference mapping populations, with 72% heterozygous in both pedigrees. Genome mapping is underway and progress on the integrated framework reference maps will be reported.

Population genetic analyses comparing EST to genomic DNA origins of select marker loci in the 14 screening genotypes revealed gene diversities of 0.57 vs. 0.77, numbers of alleles/locus of 4.2 vs. 8.1, and inbreeding coefficients of 0.07 vs. 0.15, respectively. Although the EST-SSR markers were on average less diverse and more homozygous than the genomic markers they should work in a wider range of pine species because of the greater sequence conservation generally found in expressed genes. The EST-SSR markers generally had simpler allelic profiles than the genomic-SSR markers as characterized by fewer stutter patterns and shoulder peaks. Together these characteristics suggest that select EST-SSR markers would be well suited for construction of multi-species pine DNA fingerprinting kits.