PROGRESS TOWARDS BETTER DNA MARKER SETS FOR SOUTHERN PINES

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Microsatellite DNA (SSR) markers are appropriate for small to mid-scale studies to establish parentage and sibships, clone identification, gene conservation, biogeographic history, or forensic provenance. An ideal set of SSR markers can identify every unique tree genotype without error on the first pass of laboratory analysis, preferably in fully automated fashion. We are not there yet, but are getting closer. Over the past few years at the Southern Institute of Forest Genetics, we have developed various sets of SSR markers to analyze of populations of loblolly, slash, shortleaf, and longleaf pines. We also have SSR sets for DNA fingerprinting of specific loblolly clones, like 7-76 and 20-1010. Although we strived to develop a handful of robust and unambiguously informative markers from thousands of candidates, the more we work with them, the more we become aware of their shortcomings. Truly good markers are hard to find.

This has led us to try a new approach: use the billions of bases of DNA sequence available from the Pine Reference Sequence Project to find special classes of SSR sequences, be very selective during marker evaluations, and develop a new generation of SSR markers for southern pines. In a proof-of-concept study, we used very stringent criteria to search a portion of the loblolly pine genome sequence for perfect, unique SSRs with 4, 5 or 6 bp motif lengths. This approach was in part inspired by new standards in DNA forensics to use only tetranucleotide SSRs (4 bp motifs), which largely avoid allele calling problems encountered with markers of shorter motif length. We have screened 147 of these new markers in loblolly, shortleaf, longleaf and Table Mountain pines. Progress and prospects will be reported.