CAPTURING LOBLOLLY PINE CHROMOSOMES FOR GENOME SEQUENCING USING LASER CAPTURE MICRODISSECTION MICROSCOPY

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The loblolly pine (*Pinus taeda*) genome (23 Gb/1C; 2*n* = 2*x* = 24) has recently been sequenced, but the ~1.8 million scaffolds forming the most recent assembly are mostly unordered and unmapped on the known linkage groups. Genome assembly is especially challenging for such large and highly repetitive genomes as in conifers. Sequencing individual chromosomes, separately, obtained through Laser Capture Microdissection Microscopy (LCMM), promises to reduce the complexity of genome assembly and allow for the confident assignment of contigs and scaffolds to specific linkage groups. However, preparation of karyotype-quality slides (full complement of well-separated chromosomes) for LCMM is notoriously difficult in plants compared to animals, where chromosome-based sequencing has been done using LCMM (frog, *Xenopus tropicalis*, Seifertova et al. 2013; salamander, *Ambystoma mexicanum*, Keinath et al. 2015). Obtaining karyotype-quality mitotic spreads is the key for capturing of individually targeted chromosomes with LCMM. We have successfully developed a robust protocol for preparing high-quality pine chromosome spreads on pen-membrane slides for LCMM. As an initial test for individual chromosome sequencing, we selected loblolly pine chromosome 12 (Ch12) for its special characteristic as being the smallest and only sub-metacentric chromosome in pines, and thus it can be easily identified under a 40X objective. Loblolly pine Ch12 are currently being collected and will be amplified using a whole genome amplification technique and sequenced using next generation sequencing (NGS) platforms. We will report the progress of the project.

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