# Transcriptome Analysis of Two *Populus trichocarpa* Genotypes with Contrasting Responses to In Vitro Regeneration Treatments

Xavier Tacker[1], Sara Jawdy[2], Michael Nagle[3], Cathleen Ma[4], Kate Permasolva[5], Haiwei Lu[6], Wellington Muchero[7], and Steven Strauss[8]

[1]Undergraduate Researcher, Oregon State University, Corvallis, OR, 97330 USA; [2]Technical Professional; Plant Systems Biology, Oak Ridge National Laboratory, Oak Ridge, TN, 37831 USA; [3]Post Doctoral Fellow, Oregon State University, Corvallis, OR, 97330 USA; [4]Senior Research Associate, Oregon State University, Corvallis, OR, 97330 USA; [5]Faculty Research Associate, Oregon State University, Corvallis, OR, 97330 USA; [6]Professor of Biology, Central Community College—Hastings, Hastings, NE 68902 USA; [7]Geneticist- Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, 37831 USA; [8]Distinguished Professor of Forestry, Oregon State University, Corvallis, OR, 97330 USA; *tackerx@oregonstate.edu

Advances in plant molecular biology, and the use of recombinant DNA-based biotechnologies, depend heavily on the ability of species to undergo genetic transformation and regeneration. However, for most species the rate of regeneration is a limiting factor, and is highly variable between plant species and genotypes. Widespread failure to regenerate (known as recalcitrance) can severely constrain the gene pool available to scientists for modifying clonal varieties using genetic engineering or gene editing (GE). To help understand why regeneration varies so widely among genotypes, we are using "transcriptome" analysis in the model tree black cottonwood (*Populus trichocarpa*). Changes in gene expression throughout the genome were characterized while explants (pieces of plant stem used for transformation) regenerated into plants under *in vitro* conditions similar to those of plants undergoing GE. We report on two genotypes selected because their rates of regeneration vary widely. RNA was collected from tissues every three days for about six weeks and subjected to high intensity DNA sequencing by the Department of Energy Joint Genome Institute in California. From raw RNAseq files, several terabytes of data were processed and organized for mapping to the *Populus trichocarpa* reference genome. HISAT2 and STRINGTIE programs were used to filter and map reads to a host genome, subsequently consolidating them into gene count matrices for differential gene expression analysis (DGE) within R. The expression patterns of known developmental regulator genes such as *WUSCHEL* and *SHOOT MERISTEMLESS* are being studied to help characterize tissue developmental states. PCA and cluster analysis is underway to analyze data quality. The identities of the genes that differ in expression among stages and genotypes, and the developmental processes that these genes take part in, should provide insights into recalcitrancy and ways to overcome it.