

From Gene to Network, Switchgrass TOP Line RNA-seq Data Analysis Pipeline at BESC

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) designing plant cell walls for rapid deconstruction and (2) developing multi-talented microbes or converting plant biomass into biofuels in a single step (consolidated bioprocessing). BESC biomass formation and modification research involves working directly with two potential bioenergy crops (switchgrass and *Populus*) to develop varieties that are easier to break down into fermentable sugars. We are testing large numbers of natural variants and generating specific and modified plant samples as well as developing genomics tools for detailed studies into poorly understood cell wall biosynthesis pathways.

Switchgrass (*Panicum virgatum*) is within one of the two plant genera (*Populus* the other) targeted in BESC for biomass improvement. For switchgrass, over 140 unique genes, promoters, promoter-genes, or stacked genes, represented by over 160 constructs, were targeted for modified expression through stable transformation to diminish recalcitrance. In addition, evaluation of natural variants for enhanced biofuel traits were carried out. After preliminary analysis to determine which lines had increased sugar release and normal or enhanced plant growth, twelve transgenic and natural variant lines were identified as switchgrass TOP Lines. These TOP Lines were compared in greenhouse and field to aid in selecting the best lines for enhanced biofuel production. Transcriptome analysis by RNA-seq is one of the characterization methods used to identify underlying pathways through which modification may further improve biofuel production.

TOP Line transcriptome and wall analyses were performed with greenhouse-grown plants at reproductive stage 1 (R1). RNA-seq was conducted at Joint Genome Institute (JGI) using Illumina TruSeq technology. For each sample, a total of 40–50 million paired-end (PE) reads of 150 bp was generated. From data quality control to differential gene expression analysis, each RNA-seq dataset was processed through a pipeline compiled from a set of publically available software. Unique mapping results and gene level assembly were generated with HISAT2 and related programs against switchgrass genome assembly v1.1. To facilitate data inspection at sample and gene levels, the RNA-seq data are also being displayed in BESC Jbrowser portal along with the switchgrass reference genome. For each TOP Line, genes whose expression was different from the control were selected through comparison between each TOP Line and its control, using differential analysis software such as DESeq, NOIseq and NOIseqbio as well as an array-like method. The selected gene list from each TOP Line was then analyzed by network analysis tools developed by BESC. The switchgrass genes were converted to *Populus* orthologs which were used to extract metabolites, genes and correlations from GWAS, co-evolution and co-expression networks developed for

Populus. These networks help to identify changes contributing to the cell wall modification in the RNAi TOP Lines, providing further gene targets for cell wall modifications. In addition, RNA-seq data will also be combined with data from other wall analyses to be analyzed at system biology level to understand cell wall structures and their impacts on recalcitrance.

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