CRISPR Genome Editing in *Populus*

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https://cbi.ornl.gov

Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

The overarching goal of the Rapid Genetics team is to develop cutting-edge genetic toolkits to enable efficient and multiplexed genome editing for development of optimal feedstocks specifically Poplar. This requires the establishment of an efficient transformation system, the development and optimization of a robust CRISPR genome editing platform for knock-out (KO) and knock-in (KI) mutagenesis, and development of computational resources for genome editing in heterozygous plants. An improved Variant database (http://aspendb.uga.edu/s717) for *P. tremula* x *alba* INRA 717-1B4 has been deployed to facilitate variant-free guideRNA (gRNA) design. The database serves a global research community. By using a single gRNA, we are able to target individual, as well as duplicated genes, with 100% KO efficiencies for traits ranging from lignin biosynthesis to trichome formation. The use of one specific gRNA per target gene is preferred over multiple gRNAs per gene, especially for multiplex editing when several unrelated genes are targeted for KO simultaneously, as it simplifies vector design and reduces potential off-target effects. The lignin and trichome-less KO mutants are being exploited to develop a KI system based on MMEJ (microhomology-mediated end-joining) repair pathway, rather than the standard homology-directed repair, which requires extensive homology with poor efficiencies in eukaryotes. Successful repair of the previously generated KO alleles will restore gene functions, and in the case of trichome-less plants, will lead to visually identifiable phenotypes. This will alleviate lengthy molecular screening during various optimizations. The effects of homology length, template excision, and target gene choice on KI efficiency are currently being assessed. In collaboration with other CBI researchers, the KO system is being applied to target genes selected for improving growth, sustainability and bioprocessing characteristics of *Populus*.

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