

## High-throughput Reductive Catalytic Fractionation for Lignin Characterization in the Genome Wide Association Study of Poplar

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**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 addresses 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, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Reductive catalytic fractionation (RCF) has emerged as a promising technique to extract and depolymerize lignin, an oxygenated aromatic polymer making up 15-30% of all biomass and the largest natural source of aromatic hydrocarbons.<sup>1</sup> RCF is a 2-step process, extracting lignin from whole biomass with a polar-protic solvent, and then selectively cleaving C-O ether bonds using a hydrogen donor and a heterogeneous catalyst.<sup>2</sup> When operating at complete ether bond cleavage at the catalyst, the lignin yields and product distributions are determined by the distribution of C-O and C-C linkages between monolignols present in native lignin in the plant.<sup>3</sup> Thus, to improve overall lignin utilization we must optimize the lignin structure in the plant to decrease heterogeneity in the product distribution. Unfortunately, targeted genetic improvements are not yet possible as the causal relationships between genes and lignin structure are not well understood at present.

Genome wide association mapping studies (GWAS) are a powerful tool to leverage the species-wide variability contained in a collection of individual organisms to draw links between naturally occurring genetic mutations and phenotypes.<sup>4</sup> We have designed and tested a 24-well reactor plate along with work-up protocols capable of performing high-throughput RCF – which we will use to analyze over 1000 poplar trees (*Populus trichocarpa*) with unique genotypes. Using LC-MS/MS, NMR and GC-FID, we can obtain high-quality quantitative data on lignin structure at a fine chemical scale to elucidate 1) which genotypes lead to exceptional lignin extraction and depolymerization and 2) whether certain genotypes contain unique and advantageous lignin chemistry that has yet to be discovered. Applying the GWAS analysis technique, we can relate these unique traits to the specific genes that drive favorable lignin biosynthesis. This presentation will demonstrate the proof-of-concept experimental design for our high throughput system, described above, and the reliability of the data which can be generated and fed into the GWAS pipeline.

### References

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