Transient delivery of Cas9 using Agrobacterium for genome editing

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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 biofuels using CBP with cotreatment at high rates, titers and yield in combination with catalytic upgrading into drop-in hydrocarbon fuel blendstocks.

Improving our knowledge on plant genes is essential to achieve domestication of non-model plant species important for bioenergy. Great advances in this front have been done in recent years, but often genetic engineering and genome editing rely on stable integrations of the DNA of interest. These DNA fragments usually encode genome editing reagents, selectable markers, or novel traits and once integrated in the genome DNA can pose regulatory issues. As such, removal of the integrated DNA is required and achieved via downstream breeding. However, this is not a feasible option in the case of switchgrass and poplar, as downstream breeding and segregation cannot be performed without losing the genotype.

To overcome the limitations imposed by current methods for plant engineering, we generated a system in Agrobacterium, called pTrans, to transiently deliver genome editing reagents as proteins, thus preventing any stable DNA integrations. As preliminary work in tobacco showed promising results, the system has now been improved to target important bioenergy traits in switchgrass. A newer version of pTrans, designed to have higher cas9 expression and more efficient reagent delivery, has also been assembled. This system is currently being tested in switchgrass.

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