

Building a suite of CRISPR/Cas9 tools for efficient switchgrass gene 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 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.

CRISPR/Cas9 technology has become the genome editing tool of choice in almost all kingdoms of life and reports of its use in organisms that range from protists, to fungi, animals and plants are widespread in the literature. By capitalizing the increasing knowledge on how this system works, we aim to develop an efficient and predictable gene editing system for switchgrass (*Panicum virgatum* L.). Switchgrass has been recognized as an important biomass resource of fermentable mixed sugars that can yield biofuels and other value-added chemicals and biomaterials. Being able to fine-tune switchgrass metabolic capabilities via CRISPR/Cas9 gene editing would be extremely useful to exploit its potential and well as to validate putative gene functions. However, switchgrass is an allotetraploid, whose two subgenomes show extensive gene duplication. This makes the identification of gene-specific targets very challenging.

Fortunately, since CRISPR/Cas9 system was first described and in parallel to its widespread use, a suite of Cas9/Cas12a proteins with diverse size, activity, recognition target site and trans-activating crRNA (scaffolds) have been characterized. Taking advantage of the wide range of target specificities that the available Cas9/Cas12a proteins offer, we are developing a platform that will allow editing any gene of interest in switchgrass. The ultimate goal is to ensure the editing of all copies in the switchgrass genome. As a first step, we have tested five different Cas9/Cas12a proteins (SpCas9, SaCas9, St1Cas9, Mb3Cas12a, and AsCas12a) in embryogenic rice calli from Taipei-309, aiming to transfer the knowledge acquired in the rice model system into switchgrass. Also, all protein combinations have been tested both at 37°C (optimal temperature for most Cas9/Cas12a proteins) and 27°C (optimal temperature for tissue culture) to assess rate of editing under standard tissue culture conditions. Our results have identified SpCas9 as the best protein in terms of editing efficiency at both 27°C and 37°C. Mb3Cas12a and SaCas9 yielded similar results to SpCas9 but only at 37°C; at 27°C Mb3Cas12a

efficiency dropped by 50% and SaCas9 didn't work at all. This suite of best performing nucleases and working conditions is being tested with gene targets of interest in switchgrass.

The Center for Bioenergy Innovation is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.