

Development and demonstration of CRISPR/Cas9 platform for *Aspergillus niger*

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Project Goals: JBEI's long-term vision is that bioenergy crops can be converted into economically-viable, carbon-neutral, specialty biofuels, all of the organic chemicals currently derived from petroleum, and many other bioproducts that cannot be efficiently produced from petroleum. This vision will only be possible when we have sustainable bioenergy crops, biorefinery technologies capable of converting as much carbon in biomass into biofuels as possible, and a vast array of bioproducts that will make biorefineries economically viable. JBEI's mission is to establish the scientific knowledge and new technologies in feedstock development, deconstruction and separation, and conversion needed to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts. When fully scaled, JBEI's technologies will enable the production of replacements for petroleum derived gasoline, diesel, jet fuel, and bioproducts. In doing so, JBEI will reduce the nation's dependence on fossil fuels, significantly reduce the amount of carbon added to the atmosphere, reduce contamination of the environment, and provide the scientific tools and knowledge required to transform the bioenergy marketplace.

Aspergillus niger and other filamentous fungi are widely used in industry, but efficient genetic engineering of these hosts remains nascent. For example, while molecular genetic tools have been developed, including CRISPR/Cas9, facile genome engineering of *A. niger* remains challenging. To address these challenges, we have developed a simple Cas9-based gene targeting method that provides selectable, iterative, and ultimately marker-free generation of genomic deletions and insertions.¹ This method leverages locus-specific "pop-out" recombination to suppress off-target integrations. We demonstrated the effectiveness of this method by targeting the phenotypic marker *alba* and validated it by targeting the *glaA* and *mstC* loci. After two

selection steps, we observed 100% gene editing efficiency across all three loci. This method greatly reduces the effort required to engineer the *A. niger* genome and overcomes low Cas9 transformations efficiency by eliminating the need for extensive screening. This method represents a significant addition to the *A. niger* genome engineering toolbox and could be adapted for use in other organisms. It is expected that this method will impact several areas of industrial biotechnology, such as the development of new industrially relevant fungal strains for the secretion of heterologous enzymes and the discovery and optimization of metabolic pathways.

References

1. Leynaud-Kieffer LMC, Curran SC, Kim I, Magnuson JK, Gladden JM, Baker SE, Simmons BA (2019) A new approach to Cas9-based genome editing in *Aspergillus niger* that is precise, efficient and selectable. PLoS ONE 14(1): e0210243. <https://doi.org/10.1371/journal.pone.0210243>

The Joint BioEnergy Institute 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.