Engineering CRISPR/Cas9 platform to industrialize lignin modifying enzymes (LMEs) using *Aspergillus niger*

Laure Leynaud-Kieffer[1,2,3*], Vassily Hatzimanikatis[3], and **Blake Simmons [1,2]**

[1]Joint BioEnergy Institute, Emeryville, CA; [2]Biological Systems and Engineering, Lawrence Berkeley National Laboratory, Berkeley, CA; [3]Swiss Federal Institute of Technology, Lausanne, Switzerland.

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Project Goals: Short statement of goals. (Limit to 1000 characters)

The conversion of the biomass into advanced biofuels faces many challenges, one of which is finding the right organism for the job. The filamentous fungus *Aspergillus niger* has been chosen as a biocatalyst for cellulose, hemicellulose, and lignin degradation because it could secrete numerous hydrolytic enzymes, such as lignin modifying enzymes (LMEs), it is genetically tractable, and its genome sequence is available.

However, we currently lack efficient tools for editing and augmenting the *A. niger* genome. While genome editing techniques such as CRISPR/Cas9 editing function in *A. niger*, we are limited by the difficulty of making multiple mutations, restricted selection of markers, and inefficient, expensive and time-consuming methodologies for genome engineering.

Here I present progress towards developing a method for efficiently making multiple genomic mutations via Cas9/gRNAs without the use of permanent selective markers. This technique utilizes several approaches; 1) pyrG positive and negative selection by a transient donor DNA, 2) a sgRNA transcript in vitro for a more user-friendly method. Once complete, this strategy should (1) remove the need for laborious screening of colonies to identify mutants, (2) permit the rapid engineering of strains with multiple mutations without the need for multiple selection markers. Our objective is to first establish this method for genome engineering. Next, we will build a library of *A. niger* strains housing pathways for lignin degradation. Then we will optimize the technique of fermentation for *A. niger* in submerged bioreactor. The overall objective is to define the best strains and conditions for the productivity of LMEs in bioreactors at a pilot scale for industries.

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