A Cas9-Based Toolkit to Program Expression Context in *Saccharomyces cerevisiae*

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Project Goals: The Joint BioEnergy Institute (JBEI) is focused on providing clean, renewable transportation fuels identical to gasoline, diesel and jet fuel. In order to produce fuels at high titers, we need to have well-characterized expression "parts" so that we can easily and quickly modulate pathway genes. Additionally, for this process to be renewable, our engineered microbes must also be able to grow and produce fuels from biomass hydrolysates. To address these problems, we employed an integrated research approach, which is illustrated below.

Engineering of biological systems can be difficult to predict due to the vast complexity of living cells. In order to control chemical production, it is necessary to regulate a number of variables including DNA copy number, transcriptional timing, transcript stability, protein solubility and localization. *Saccharomyces cerevisiae* is an excellent organism for industrial production of biological molecules, and has many well-characterized tools available for genetic engineering. While the potential for the use of these technologies is immense, specific applications are often hampered by slow development times and unpredictability. Here we report on developing a Cas9-based toolkit to quickly institute genetic changes in yeast and to program heterologous gene expression. We characterize gene expression "parts" using a fluorescent reporter protein, exploring how chromosomal integration locus and promoter affect expression levels and dynamics. We further develop protein tags to control gene expression level, solubility, and sub-cellular localization. In parallel, we develop the organism as a microbial production platform for chemicals with potential uses as biofuels. We focused on the fatty acid biosynthetic pathway that produces large, hydrophobic molecules similar to modern fuels. Titers of biofuels produced by our engineered yeasts approach the g/L range from simple sugars. Our yeast strains

are also capable of high-level production in biomass hydrolysates, a renewable production platform.

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