Systems Biology of Isobutanol Production in *Saccharomyces Cerevisiae*

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Project Goals:

The overall goal of the project is to carry out a comprehensive systems biology study of branched-chain higher alcohol (BCHA) production and tolerance in yeast. We will leverage the genetically encoded biosensor of BCHA production described in this presentation to screen yeast genomic libraries to measure the effects of genetic perturbations on BCHA production and tolerance. Introducing this biosensor in strains engineered with optogenetic circuits that control BCHA production with light will enable us to establish a closed-loop control system to study these metabolic pathways. This includes measuring transcriptomic changes in steady state or dynamic production systems. Ultimately, we will use these genomic and transcriptomic data to discover the key cellular networks involved in BCHA production and tolerance, which will be instrumental in developing better producing strains.

Branched-chain higher alcohols (BCHAs), including isobutanol and isopentanol, have been identified as key biofuels by the Office of Energy Efficiency & Renewable Energy of the U.S. Department of Energy[1]. These alcohols have better fuel properties than bioethanol, including higher energy density and better compatibility with current gasoline infrastructure. The yeast *Saccharomyces cerevisiae* is a preferred host organism for BCHA production because of its relatively high tolerance to their toxicity, and the potential to retrofit existing bioethanol plants (most of which use this yeast) with strains engineered to produce these advanced biofuels.

Existing efforts to commercialize these types of biofuels are challenged by limited productivities, as well as the toxicity that these alcohols have on strains engineered to produce them. Significant progress has been made in boosting yields and titers, particularly of isobutanol, through extensive metabolic and enzyme engineering based on detailed knowledge of branched chain amino acid metabolism and the structure and function of the enzymes involved[2,3]. In contrast, virtually nothing is known about the interplay between different cellular networks and BCHA production and tolerance, leaving a basic question unexplored: What are the key cellular networks that influence BCHA production?

Using our recently described genetically encoded biosensor for isobutanol production[4], we are currently carrying out a genome-wide analysis of isobutanol production in yeast. We have previously shown that genomic screens are invaluable to discover the role of cellular networks in complex traits, such as tolerance to isobutanol[5]. Our biosensor reports on the activity of isobutanol biosynthesis by expressing a fluorescence reporter (GFP), allowing us to quantify this metabolic activity. By optimizing a protocol to transform yeast genomic libraries[6], we have completed the construction of a new library, in which we introduced this isobutanol biosensor into the entire yeast gene deletion collection[7]. We kept this library arrayed in a 96-well plate format, which allows us

to measure how each gene deletion affects isobutanol production and gives us maximum flexibility to develop high-throughput assays.

We have validated this library by verifying the correlation between the biosensor output signal and isobutanol production in a subset of strains. We have also confirmed the effect of gene deletions known to affect isobutanol production[8,9]. We will present the results of initial screens, which revealed previously unknown gene deletions that increase production. The effects of some of these deletions are consistent with what is known about yeast metabolism and physiology. However, the effects of other deletions cannot be explained by known yeast biology, constituting new scientific discoveries and areas of future inquiry. We are currently in the process of completing our screens of the entire collection, which will provide quantitative measurements of the effect of each non-essential gene on isobutanol biosynthesis. This will allow us to build a full genetic interaction map relating cellular networks involved in isobutanol production. Altogether, this project will produce invaluable knowledge towards understanding and developing strains for improved isobutanol production.

References

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