The Yeast Biodesign Library: leveraging DNA synthesis to assess and harness genes from diverse organisms

William G. Alexander*,1,2 (walexander@wisc.edu), Nikolay Rovinskiy1,2, Mariana Lopes2,3, David Peris1,2, Jin Kang1,2, Angela Tarver4, Miranda Harmon-Smith4, Jan-Fang Cheng4, Samuel Deutsch4, Jeff S. Piotrowski1, Trey K. Sato1, Audrey P. Gasch1,2, Chris Todd Hittinger1,2

1Department of Energy Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI; 2Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 3Universidade Federal de Minas Gerais, Belo Horizonte, Brasil; 4Joint Genome Institute, Walnut Creek, CA

http://www.glbrc.org/research/conversion

Project Goals: identify and characterize genes that provide novel phenotypes relevant to efficient cellulosic bioethanol production by *Saccharomyces cerevisiae* using synthesized DNA constructs.

Two major issues encountered by biofuel researchers in the optimization of *Saccharomyces cerevisiae* cellulosic ethanol strains are the tolerance to toxins in hydrolysate derived from the lignin in plant biomass and the conversion of pentoses, disaccharides, and other unfavored sugars. The latter is especially problematic as the vast majority of *S. cerevisiae* strains are completely unable to use the pentose xylose, the major component of hemicellulose, as a carbon source without genetic modification. However, many yeasts that belong to a group known as the “CUG clade” are capable of using xylose and cellobiose readily; unfortunately these yeasts also possess an alternate genetic code, complicating the evaluation of their genes in heterologous contexts. To address these difficulties, we designed 266 synthetic gene cassettes called the Yeast Biodesign Library. Each cassette is driven by a medium-strength constitutive *S. cerevisiae* promoter and contains an open reading frame that has been cleansed of problematic restriction enzyme sites and incompatible codons. To date, we have successfully used the Yeast Biodesign Library to generate artificial multi-gene pathways and to implicate novel genes in xylose fermentation.

This work was supported by the National Science Foundation under Grant No. DEB-1253634 and funded in part by the DOE Great Lakes Bioenergy Research Center (DOE Office of Science BER DE-FC02-07ER64494). CTH is a Pew Scholar in the Biomedical Sciences, supported by the Pew Charitable Trusts. The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported under Contract No. DE- AC02-05CH11231.