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

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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.

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