



Agile Biofoundry CRADA Project - Lygos

Title:

Implementing a Design, Build, Test, Learn *P. kudriavzevii* Engineering Cycle for Production of an Organic Acid Product

Project Partners:

Sandia National Laboratories, Lawrence Berkeley National Laboratory, Pacific Northwest National Laboratory & Lygos

Relevant ABF Capability(ies):

Design: [DIVA BioCAD](#)

Build: [DNA Sequence Validation](#)

Build: [DNA Construction, Synthetic Biology and Bioprocess Development](#)

Test: [Targeted Proteomics](#)

Test: [Targeted Metabolomics](#)

Test: [Global Metabolomics](#)

Test: [Experimental Data Depot](#)

Description:

Lygos is an industrial biotechnology company spun out of the U.S. Department of Energy-funded Joint BioEnergy Institute. Lygos uses *Pichia kudriavzevii*, an acid tolerant BSL-1 yeast, to produce organic acids, chemicals that are generally expensive to manufacture petrochemically but that can be produced at high yields and for low cost biologically. While *P. kudriavzevii*'s high tolerance for organic acids provides an advantage over *E. coli*, *S. cerevisiae*, and many other microbes, working with *P. kudriavzevii* presents unique challenges. For example, there is little published transcript and protein expression data for this organism, which could be used to guide strain engineering or to build new genetic tools. While Lygos has developed protocols and tools for designing, building, and testing *P. kudriavzevii* strains, there remains room to improve the engineering cycle, providing an opportunity to partner with the Agile BioFoundry to further advance this microorganism.

This ABF Directed Funding Opportunity was established with the primary aim of conducting an early assessment of the ABF capabilities by allowing Lygos to utilize core ABF DBTL capabilities to demonstrate and optimize the production of a commercial product. The project has at least two high-level goals. The first is to apply the Agile BioFoundry DBTL tools and technologies to establish and optimize production of a new, valuable, organic acid product in *P. kudriavzevii*. The second goal is to onboard *P. kudriavzevii* as a production host within the Agile BioFoundry. The organic acid selected for development is a commercial product that is difficult and costly to make petrochemically. As an added benefit, this



organic acid is also a metabolic intermediate useful for the production of other commercially important chemicals, making it an ideal candidate to demonstrate commercial applicability of Agile BioFoundry DBTL technologies.

Contact(s): Jeffrey Dietrich (Lygos), John Gladden (SNL), Jon Magnuson (PNNL), Taek Soon Lee (LBNL)

Performance period: 04/2019 to 03/2021

Resulting publication(s)/patent(s): None to date.