

Summary/Abstract for Public Release

Developing multi-gene CRISPRa/i programs to accelerate DBTL cycles in ABF hosts engineered for chemical production

Prof. James M. Carothers (Lead), Department of Chemical Engineering, Molecular Engineering & Sciences Institute and Center for Synthetic Biology, University of Washington, Seattle, WA 98195. Ph: 206-221-4902, email: jcaroth@uw.edu

For industrially-promising microorganisms in early stages of development, creating technologies for rapidly engineering complex multi-gene programs could be transformative for accelerating data-driven strain design. We will develop CRISPR-Cas expression technologies to create new abilities to activate bacterial gene expression, and to create platforms for multi-gene expression tuning. To enable accelerated Design-Build-Test-Learn (DBTL) cycles, we will combine these technologies with advanced Agile BioFoundry (ABF) capabilities for multi-omics analysis (Pacific Northwest National Laboratory) and machine learning (Lawrence Berkeley National Laboratory). We will demonstrate the usefulness of these tools by rapidly improving the production of bio-based industrial aromatics in multiple microbes currently under development by the ABF as hosts for industrial chemical production. Specifically, the goals are to 1) develop effective CRISPR gene activation (CRISPRa) tools for two ABF host organisms, 2) engineer multi-gene, mixed CRISPRa and CRISPR inhibition (CRISPRi) programs targeting as many as 5 genes simultaneously, 3) integrate machine learning and computational evolutionary strategies to infer mechanistic models to drive the design of CRISPRa/i programs for efficient sampling of multi-gene expression spaces, and 4) optimize industrial aromatic production from biomass intermediates in two ABF hosts through the construction of 5 gene CRISPRa/i programs in DBTL cycles that are at least 30% more efficient than the current state of the art. By supporting the development of new CRISPR-Cas gene expression tools and accelerated data-driven workflows, this EERE-funded project will dramatically improve the ability to engineer bacteria to produce industrial chemicals from lignocellulosic feedstocks.