# MODULAR DESIGN OF HETEROLOGOUS PATHWAYS FOR PORTABILITY ACROSS DIVERSE MICROORGANISMS

Julie E. Chaves, Oak Ridge National Laboratory
chavesje@ornl.gov
Timkhite-Kulu Berhane, Oak Ridge National Laboratory
Joshua Elmore, Oak Ridge National Laboratory
Adam Guss, Oak Ridge National Laboratory
Joshua K. Michener, Oak Ridge National Laboratory

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We utilized a method for the high-throughput assembly of heterologous biochemical pathways, and an integration platform for these pathways into the genomes of diverse, non-model microorganisms. Carbon catabolic pathways have been chosen as the first test cases for the pathway design and assembly method. Catabolic pathway optimization has the inherent advantage that growth selections can be used to separate pathways with differing productivities. As such, it provides a tractable testbed for the development of modular engineering approaches. First, we selected pathway enzymes from a group of evolutionarily-divergent host organisms, to capture a wide diversity of possible enzyme structures and pathway configurations. The coding sequences of each pathway have been synthesized and assembled into individual plasmids with unique barcodes, to be combinatorially constructed into pathways using the CombiGEM DNA assembly method [1]. These pathways will be assembled in pooled reactions, resulting in the final generation of ~1M pathways. These pathways will be integrated into the recipient microbial host genome using a phage serine recombinase system [2]. A “Landing Pad” comprised of three attB sites will be integrated into the host genome using the Tn7 transposase [3]. Finally, the designed pathways will be integrated into the recipient genome, through recombination of the Landing Pad attB site, with the respective attP site on the target pathway’s destination vector. Modified hosts will be grown under the selection of the target carbohydrate substrate. The identification and fitness of each pathway will be measured by sequencing the barcodes of the final versus initial pooled populations. In-depth analysis including metabolomics, transcriptomics and proteomics can illuminate regulatory and metabolic changes that result from introduction of the heterologous pathway. Experimental evolution can also be used with poorly-functioning pathways to select for improved variants. Characterizing the resulting mutants can help to identify factors that were initially limiting activity. Comparing successful, unsuccessful, and evolved pathways will help to explain why species differ in their ability to functionally express various heterologous pathways of interest. Ultimately, the final pathway analysis can be used to design modular metabolic units that are highly active and portable across dissimilar microbes. Understanding the requirements for effective use of various heterologous pathways will allow selection of the best pathway for a particular host, based on its unique genetics and physiology.

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