

Next-generation Synthetic Biology Technologies for Controlling Metabolism in Clostridia

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Project Goals: We are harnessing next-generation Synthetic Biology technologies for rationally controlling metabolic flows in *Clostridium autoethanogenum*, an organism capable of converting syngas feedstock into valuable chemical products.

Clostridium autoethanogenum is non-model organism capable of converting low-cost C1 feedstock (e.g. carbon dioxide and carbon monoxide from waste gas streams) into valuable chemical products. Here, we leverage recently developed Synthetic Biology technologies to improve our ability to rationally control metabolic flows in *Clostridium autoethanogenum* with the overall goal of reducing byproduct formation, diversifying the bioprocess' product portfolio, and improving production titers. These technologies include: (1) 4600 highly non-repetitive promoters with well-characterized transcription rates (across a 1.4-million range) that can all be used simultaneously without triggering genetic instability; (2) rationally designed operons expressing multi-enzyme pathways with optimized expression levels for maximal productivities; and (3) Extra Long sgRNA Arrays co-expressing 20+ sgRNAs to knock-down the expression of enzymes responsible for byproduct formation. In each case, we previously developed and experimentally validated automated algorithms to design these genetic parts and systems to achieve desired functionalities, while minimizing sources of genetic instability (e.g. the Operon Calculator, ELSA Calculator, and the Non-Repetitive Parts Calculator). The first application of these technologies is the design and characterization of over 10000 highly non-repetitive AT-rich promoters for use in *Clostridia* and other organisms with AT-rich genomes, leveraging oligopool synthesis and massively parallel reporter assays.

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