

Mining the largest collection industrially-deployed *Clostridium* strains for highly-evolved gene variants related to improved productivity and industrial robustness

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Project Goals: Even with the advent of next generation sequencing, there are limited options for many key metabolic genes considered for next-generation biofuel and bioproduct synthesis, and most genes found in public repositories are derived from type-strain or environmental sequences with unproven performance. To expand the pool of available sequences that are likely to result in high performance, we have sequenced and mined the largest collection of industrially-deployed *Clostridium* strains, evolved over several decades of intense development.

To rapidly prototype the performance of identified genes and to develop improved, industrial-robust production strains for conversion of lignocellulosic biomass to next-generation biofuels and bioproducts, we are establishing a new interdisciplinary venture, the clostridia Foundry for Biosystems Design (cBioFAB) that combines advancements in cell-free and *Clostridium* engineering metabolic engineering.

Clostridia have been commercially deployed in large-scale industrial fermentations for more than 100 years. The acetone-butanol-ethanol (ABE) fermentation has historically been the second largest industrial fermentation process only behind ethanol fermentation¹. More recently, LanzaTech has commercialized a *Clostridium* based gas fermentation process for ethanol production, that utilizes CO/CO₂ containing gases such as syngas derived from lignocellulosic biomass as feedstock rather than sugar or starch that the ABE process requires as substrates².

High substrate costs eventually led to a decline of the ABE process in most Western countries in favor of petrochemical production, but development and commercial operation continued in politically isolated countries. LanzaTech owns the largest collection of industrially-deployed ABE strains. The collection dates back to 1944 and contains hundreds of highly evolved strains spanning several decades of development at commercial plants in South Africa and Taiwan as well as a number of research strains, including immunized strains¹.

In order to leverage the genetic diversity in this collection we have sequenced hundreds of strains from the collection using single-molecule, real-time (SMRT) sequencing, which we have

previously demonstrated to be effective in yielding high-quality genomes with a minimal number of contigs for complex *Clostridium* genomes.² To generate high-molecular weight, high-quality genomic DNA from all of the strains, we have developed a modified *Clostridium* growth medium and refined extraction protocols. Using this pipeline, we were able to generate more than 230 genomes with 1-6 contigs. To supplement this, we also sequenced over 100 genomes using Illumina chemistry.

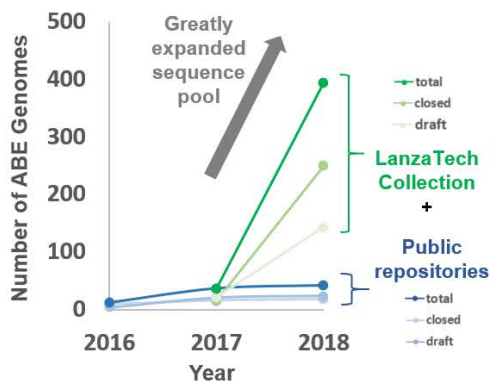
All genomes were annotated using several different pipelines and mined for differences in key metabolic genes. On an amino acid level between 33 and 13 unique new sequences were mined for the genes in the core ABE pathway, with thiolase having the most and butyrate kinase the least diversity. The analysis resulted in discovery of new gene variants for the genome editing machinery and other genetic elements.

Of the identified genes, we have selected over 200 genes for synthesis. To rapidly prototype the performance of identified genes and benchmark them against wild-type genes, we will leverage a cell-free protein synthesis approach.³ ABE products have successfully been engineered into gas-fermenting *Clostridia* and preliminary data shows a significant improvement in production from lignocellulosic biomass syngas using genes from the collection over wild-type genes.



NCP commercial ABE plant
1935-1982

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References

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