

## **Multiplex Genome Engineering for Bioproduction of 3-Hydroxypropionic Acid and 1,3-Propanediol from Waste Gases**

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**Project Goals:** Gas fermentation is a commercially scalable platform for the sustainable biomanufacturing of valuable chemicals from abundant, low cost C1 feedstocks (1,2). In this project, we aim to engineer gas-utilizing *Clostridia* strains to produce 3-hydroxypropionic acid (3-HP), which is an ideal bio-renewable precursor to acrylates and polymers (acrylonitrile, acrylamide, acrylic acid and acrylate esters) with a global market estimated as 3.6 million tons per year. Through combinatorial analysis, we compared the levels of 3-HP production from different biosynthesis routes. In order to systematically redirect metabolic flux towards 3-HP biosynthesis, we developed and employed Extra-Long sgRNA Arrays (ELSAs) (3) to knockdown gene expression levels & reduce undesired reaction fluxes, guided by genome-scale metabolic modeling (GSM). The expected outcome of this project is the development of sophisticated multiplex genome editing tools for a non-model microorganism and a bioprocess that efficiently converts industrial waste gas into 3-HP at commercially relevant productivity and selectivity.

Gas fermentation has emerged as a promising biorenewable platform for manufacturing valuable chemicals from gaseous, non-food feedstocks that would normally be considered pollutants or waste. These gases include carbon dioxide (CO<sub>2</sub>; a greenhouse gas) and carbon monoxide (CO; a harmful pollutant that will be oxidized to CO<sub>2</sub> when released in the atmosphere). LanzaTech is a worldwide leader in gas fermentation having commercialized and scaled up the production of ethanol from CO/CO<sub>2</sub> gas mixtures using *Clostridium autoethanogenum* as the whole-cell biocatalyst. Gas feedstocks are sourced from lignin-derived syngas, steel mill waste gas, and biorefinery waste gas, providing ample commercial opportunities for upgrading negative value pollutants into valuable co-products (1,2).

In this project, we are engineering industrial *C. autoethanogenum* strains to manufacture 3-hydroxypropionic acid (3-HP) with commercially relevant metrics (selectivity, titer, yield, and productivity) from a syngas feedstock. Following evaluation of 25 different 3-HP biosynthesis pathways using our customized GSM, we focused our effort on two metabolic routes towards 3-HP. A highly efficient, modular cell-free expression plasmid assembly system (4) was employed to build two combinatorial libraries (up to 3780 permutations per library), which were subjected to plasmid sequencing to investigate promoter-gene diversity. These libraries were then transformed into *C. autoethanogenum* generating >1000 strains.

Screening of these combinatorial strains in our high-throughput, automated anaerobic biofoundry (1) showed strains that produced 3-HP and its downstream product, 1,3-propanediol (1,3-PDO) at various levels. 1,3-PDO is an important chemical with market size of \$490 million and we previously showed that conversion of 3-HP to 1,3-PDO in *C. autoethanogenum* occurs via

aldehyde::ferredoxin oxidoreductase (AOR) enzymes (5). A LC-MS method was developed to confirm the identity of 3-HP and measure pathway intermediates to aid metabolic engineering efforts. In continuously stirred tank reactor (CSTR) using synthetic gas blend as feedstock, these recombinant strains produced 3-HP and 1,3-PDO at high titer and productivity. To further enhance the production of 3-HP, we are employing Extra-Long sgRNA Arrays (ELSA) (3) to simultaneously knockdown multiple gene expression levels to reduce competing metabolic fluxes, guided by our GSM (6).

## References/Publications

1. Köpke, M., and Simpson, S.D. (2020). Pollution to products: recycling of ‘above ground’ carbon by gas fermentation. *Current Opinion in Biotechnology* **65**: 180-189.
2. Fackler, N., Heijstra, B., Rasor, B., *et al.* Stepping on the Gas to a Circular Economy: Accelerating Development of Carbon-Negative Chemical Production from Gas Fermentation. *Annual Review of Chemical and Biomolecular Engineering*, (in review).
3. Reis, A.C., Halper, S.M., Vezeaus, G.E. *et al.* (2019). Simultaneous repression of multiple bacterial genes using nonrepetitive extra-long sgRNA arrays. *Nature Biotechnology* **37**(11): 1294-1301.
4. Karim, A.S., Liew, F., Garg, S. *et al.* (2020). Modular cell-free expression plasmids to accelerate biological design in cells. *Synthetic Biology* **5**(1): ysaa019.
5. Liew, F., Henstra, A.M., Köpke, M. *et al.* (2017). Metabolic engineering of *Clostridium autoethanogenum* for selective alcohol production. *Metabolic Engineering* **40**: 104-114.
6. Marcellin, E., Behrendorff, J.B., Nagaraju, S. (2016). Low carbon fuels and commodity chemicals from waste gases – systematic approach to understand energy metabolism in a model acetogen. *Green Chemistry* **18**: 3020-28.

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