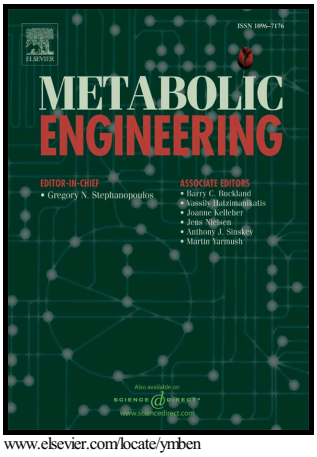

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PII: S1096-7176(17)30425-1
DOI: https://doi.org/10.1016/j.ymben.2018.04.007
Reference: YMBEN1382

To appear in: *Metabolic Engineering*

Received date: 17 November 2017
Revised date: 9 April 2018
Accepted date: 11 April 2018

Cite this article as: Rongming Liu, Liya Liang, Alaksh Choudhury, Marcelo C. Bassalo, Andrew D. Garst, Katia Tarasava and Ryan T. Gill, Iterative Genome Editing of *Escherichia coli* for 3-Hydroxypropionic Acid Production, *Metabolic Engineering,* https://doi.org/10.1016/j.ymben.2018.04.007

Iterative Genome Editing of *Escherichia coli* for 3-Hydroxypropionic Acid Production

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Abstract

Synthetic biology requires strategies for the targeted, efficient, and combinatorial engineering of biological sub-systems at the molecular level. Here, we report the use of the iterative CRISPR EnAbled Trackable genome Engineering (iCREATE) method for the rapid construction of combinatorially modified genomes. We coupled this genome engineering strategy with high-throughput phenotypic screening and selections to recursively engineer multiple traits in *Escherichia coli* for improved production of the platform chemical 3-hydroxypropionic acid (3HP). Specifically, we engineered i) central carbon metabolism, ii) 3HP synthesis, and (iii) 3HP tolerance through design, construction and testing of ~162,000 mutations across 115 genes spanning global regulators, transcription factors, and enzymes involved in 3HP synthesis and tolerance. The iCREATE process required ~1 month to perform 13 rounds of combinatorial genome modifications with targeted gene knockouts, expression modification by ribosomal binding site (RBS) engineering, and genome-level site-saturation mutagenesis. Specific mutants conferring increased 3HP titer, yield, and productivity were identified and then combined to produce 3HP at a yield and concentration ~60-fold higher than the wild-type strain.