234. A Platform E. coli Strain for Optimizing Biosynthetic Pathways via High-throughput Genome Engineering and Screening

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Project Goals: Efforts to optimize biofuel synthesis or other engineered functions in microbes is complicated by poor understanding of interactions between engineered components and host functions and among engineered components. Developments in regulated gene expression, genome engineering, high-throughput screening and selection have each made the tasks of understanding host context and engineering novel behaviors easier. However, these developments have not been integrated for use in one strain for an integrated approach to the design, build, test, and learn cycle. This project aims to develop a general-use host platform to perform high-throughput studies to improve biofuel production and engineer other DOE-relevant phenotypes. We plan to extend the strain engineering methodology to other organisms known to utilize unique feedstocks for growth and produce useful chemicals and polymers.

Precision genome engineering is an important tool used to discover the genetic determinants of phenotype and to engineer synthetic gene circuits. Major advances in phage-derived, short-homology recombination systems and high-throughput DNA synthesis have accelerated the integration rate of biosynthetic pathways and mutational diversity to bacterial chromosomes. However, the highest directed mutation rates are currently available in only persistent mutator strains, which could generate mutations on introduced biosynthetic pathways, thus limiting efforts towards multi-stage genome engineering and directed evolution. These strains also lack a collection of inducible regulators to concurrently control the expression of genes that mediate genome engineering along with integrated gene expression cassettes.

Here we engineer a platform strain of E. coli that includes multiple inducible regulators and a high-efficiency recombination system that can act as a chassis for engineering and evolving complex gene networks for biofuel production and other uses. We include engineered induction systems for lactose, cumate and arabinose to allow multi-input control of genome engineering functions and biosynthetic pathways. We have re-engineered the lambda Red recombination cassette to reduce toxicity and recombination cycle time. We have acheived single-cycle mutation rates of nearly 20% in a transient mutator background by introducing multiple mutations to increase single-stranded DNA half-life in cells and its accessibility to the chromosome. We also characterize gene expression, dsDNA recombination efficiencies and ssDNA recombination rates for a collection of context-neutral genomic loci, termed SafeSites. This strain will be a resource for engineering, characterizing and optimizing complex synthetic gene networks via iterative cycles of metabolic modeling and high-throughput screening and will be used to model the development of similar induction and recombination systems in other DOE-relevant target organisms.

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