**Title:**

CRISPR EnAbled Trackable Genome Engineering: A Technology for High Throughput Genetic Prototyping of Large DNA constructs.


## Authors:

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**Project Goals:**

The goal of this project was to develop a generalizable genome editing technology with the following capabilities:

1. editing anywhere in the genome
2. at efficiencies >90% at many loci
3. using a broad range of mutations (ranging from single nucleotide changes to indels of varying size)
4. allow tracking of each mutation in the population to enable relative fitness comparisons
5. perform such editing at tens of thousands of edits in parallel.

As a result of this work we have developed a simple and unique solution that can simultaneously address all of these engineering objectives.


**Abstract text.**

The declining cost and increasing throughput of DNA sequencing has revolutionized modern biological workflows and enabled increasingly sophisticated approaches to understanding the effects of genotypic variation on molecular processes involved in metabolic function, regulatory control and disease. Although similar improvements in DNA synthesis have occurred in parallel, technologies that can effectively implement synthetic DNA to systematically manipulate and study living systems have lagged behind. Strategies that span the design-build-test forward-engineering cycle therefore offer the promise of closing this gap by enabling simplified and systematic end-to-end read/write workflows. Here we describe CRISPR

EnAbled Trackable genome Engineering (CREATE), a strategy that couples the high efficiency of CRISPR editing with massively parallel oligomer synthesis to enable trackable precision editing on a genome wide scale. In this work we employed CREATE it to a variety of application areas that are of general importance to the biological engineering community. For example we applied CREATE to survey > 4000 mutations in the AcrB one of the central efflux pumps in *E. coli* that is implicated in tolerance to a broad range of solvents and a key component of multiple antibiotic resistance phenotypes. We identified multiple mutations to a loop-helix motif adjacent the central funnel that appear to improve efflux activity against multiple substrates, suggesting that this region may serve as an ideal engineering target. Furthermore we show that CREATE can scale to the whole genome level and serve as a tool to reconstruct mutations identified from long term adaptive laboratory evolution (ALE) studies or perform genome scale searches with up to single nucleotide resolution that improve industrially relevant antibiotic resistance and solvent tolerance phenotypes.

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