Engineering a reduced 57-codon genetic code in *Escherichia coli*

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Project Goals: Develop an *E. coli* strain that has been engineered to use only 57 codons, and demonstrate its ability to incorporate multiple non-standard amino acids, to enable tight biocontainment, and to resist phages.

We report progress towards assembly of a 3.97 Mb, 57-codon *Escherichia coli* genome in which seven codons were replaced with synonymous alternatives in all protein coding genes. In-house design software selected optimal synonymous alternatives for targeted codons, and the recoded genome was synthesized in 87 segments. We de-risked >3,000 recoded genes and established a rapid troubleshooting procedure for the 27 design exceptions identified. Here, we present our pipeline for the construction of a single recoded strain. We have developed an iterative process using CRISPR/Cas9-assisted λ-Red recombineering for scarless replacement of genomic sequences with recoded segments. Using this system, we constructed two parental strains (seg16 and seg25) and began parallelized, successive segment replacement. Importantly, this process is modular (in segment replacement order and choice of markers) and is compatible with hierarchical genome recoding. This work underscores the feasibility of rewriting genomes and construction of synthetic organisms.

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