236. Synthetic Biology-Enabled Biosystems Design and Construction of Radically Recoded Organisms

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Why it is important to radically modify microbial genomes? Four reasons are to develop multi-virus resistant cells, enable efficient use of novel amino acids, and enforce genetic and metabolic isolation. Recently, we successfully developed a biocontainable C321.ΔA strain that lacks the UAG stop codon and functionality, which cannot grow without human provision of a non-standard amino acid (NSAA) and exhibits only very minimal evolutionary escape rates in the absence of the NSAA. We our now extending upon this work to construct a radically recoded organism (RRO). Building upon our experience in removing the stop codon and functionality of UAG in the C321.ΔA E. coli, the testing of multicodon reassignment in E. coli essential genes, and our analysis of E. coli promoter/ribosome binding sites and promoter/N terminal codon sequence combinations, we developed computationally aided design tools for radical recoding to facilitate the in silico reassignment of 7 codons in all E. coli genes (62,733 codons or 5.4% of the genome), in order to free up 3 anticodons for non-standard amino acid usage and leave one codon unused. DNA synthesis of 99.7% of the 3,972,391 base pair radically recoded genome is complete. We have designed and optimized a method for using integrases to replace genomic segments with synthetic recoded sequence, and have made 2,358 codon substitutions (7 of 64 codon types) in 3 sequence verified E. coli strains. Our software package Millstone provides the first software platform that can fully automate the intense computational process required to analyze the genomes of highly modified strains, such as RROs. Once complete, our RROs will provide a safe organism that can incorporate multiple redundant genomic and metabolic safeguards that prevent release or deliberate misuse. Moreover, RROs can be combined with our in vivo sensor selector systems and pathway engineering, allowing us to both sense and select for strains that produce optimal quantities of a desired biomolecule, without interference from phage and within a biocontained organism.