Design, Synthesis, and Testing Toward a 57-Codon Genome

Nili Ostrov,1 Matthieu Landon,1,2,3 Marc Guell,1,4 Gleb Kuznetsov,1,5 Jun Teramoto,1,6 Natalie Cervantes,1 Minerva Zhou,7 Kerry Singh,7 Michael G. Napolitano,1,8 Mark Moosburner,1 Ellen Shrock,1 Benjamin W. Pruitt,4 Nicholas Conway,4 Daniel B. Goodman,1,4 Cameron L. Gardner,1 Gary Tyree,1 Alexandra Gonzales,1 Barry L. Wanner,1,9 Julie E. Norville,1 Marc J. Lajoie,10 George M. Church1,4,* (church@genetics.med.harvard.edu)

1Department of Genetics, Harvard Medical School, Boston, MA; 2Program in Systems Biology, Harvard University, Cambridge, MA; 3Ecole des Mines de Paris, Mines ParisTech, Paris 75272, France; 4Wyss Institute for Biologically Inspired Engineering, Boston; 5Program in Biophysics, Harvard University, Boston; 6Department of Biological Sciences, Purdue University, West Lafayette, IN; 7Department of Biological Engineering, MIT, Cambridge, MA; 8Program in Biological and Biomedical Sciences, Harvard University, Cambridge, MA; 9Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA.; 10University of Washington, Seattle, WA

http://arep.med.harvard.edu

Project Goals:

We report progress towards assembly of a 3.97 MB, 57-codon *Escherichia coli* genome in which seven codons were replaced with synonymous alternatives across all protein coding genes. Target codons AGA (Arg), AGG (Arg), AGC (Ser), AGU (Ser), UUG (Leu), UUA (Leu) and UAG (Stop) were chosen by in-house designed software, guided by experimentally validated synonymous recoding rules1,2. Thus far, we have completed computational design and de novo synthesis of the recoded genome in 87 ~50kb segments, as well as functional testing of 85% of all recoded genes. We developed a robust pipeline for design, assembly, testing and troubleshooting of recoded segments, with only 25 of 3031 tested genes showing lethal effects. Here, we present our pipeline for the final genome construction step, combining multiple recoded segments into a single strain in a hierarchical fashion. This work underscores the feasibility of rewriting genomes and establishes a framework for assembly of synthetic organisms.

Publications


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