MINIMAL CELL

JCVI-SYN3.0 FIRST MINIMAL SYNTHETIC BACTERIAL CELL

First Minimal Synthetic Bacterial Cell Designed and Constructed by Scientists at Venter Institute and Synthetic Genomics, Inc.

Cell, JCVI-syn3.0, was minimized to just 473 genes

(LA JOLLA, CA)—March 24, 2016—Researchers from the J. Craig Venter Institute (JCVI) and Synthetic Genomics, Inc. (SGI) announced today the design and construction of the first minimal synthetic bacterial cell, JCVI-syn3.0.

Using the first synthetic cell, Mycoplasma mycoides JCVI-syn1.0 (created by this same team in 2010), JCVI-syn3.0 was developed through a design, build, and test process using genes from JCVI-syn1.0. The new minimal synthetic cell contains 531,560 base pairs and just 473 genes, making it the smallest genome of any organism that can be grown in laboratory media. Of these genes 149 are of unknown biological function. By comparison the first synthetic cell, M. mycoides JCVI-syn1.0 has 1.08 million base pairs and 901 genes.

A paper describing this research is being published in the March 25th print version of the journal Science by lead authors Clyde A. Hutchison, III, Ph.D. and Ray-Yuan Chuang, Ph.D., senior author J. Craig Venter, Ph.D., and senior team of Hamilton O. Smith, MD, Daniel G. Gibson, Ph.D., and John I. Glass, Ph.D.

“Our attempt to design and create a new species, while ultimately successful, revealed that 32% of the genes essential for life in this cell are of unknown function, and showed that many are highly conserved in numerous species. All the bioinformatics studies over the past 20 years have underestimated the number of essential genes by focusing only on the known world. This is an important observation that we are carrying forward into the study of the human genome,” said Dr. Venter, Founder, Executive Chairman, and CEO, JCVI.

The research to construct the first minimal synthetic cell at JCVI was the culmination of 20 years of research that began in 1995 after the genome sequencing of the first free-living organism, Haemophilus influenza, followed by the sequencing of Mycoplasma genitalium. A comparison of these two genomes revealed a common set of 256 genes which the team thought could be a minimal set of genes needed for viability. In 1999 Dr. Hutchison led a team who published a paper describing the use of global transposon mutagenesis techniques to identify the nonessential genes in M. genitalium.

Over the last 50 years more than 2,000 publications have contemplated minimal cells and their use in elucidating first principals of biology. From the start, the goal of the JCVI team was similar—build a minimal operating system of a cell to understand biology but to also have a desirable chassis for use in industrial applications. The creation of the first synthetic cell in 2010 did not in-

form new genome design principles since the M. mycoides genome was mostly recapitulated as in nature. Rather, it established a work flow for building and testing whole genome designs, including a minimal cell, from the bottom up starting from a genome sequence.

To create JCVI-syn3.0, the team used an approach of whole genome design and chemical synthesis followed by genome transplantation to test if the cell was viable. Their first attempt to minimize the genome began with a simple approach using information in the biochemical literature and some limited transposon mutagenesis work, but this did not result in a viable genome. After improving transposon methods, they discovered a set of quasi-essential genes that are necessary for robust growth which explained the failure of their first attempt.

To facilitate debugging of non-functional reduced genome segments, the team built the genome in eight segments at a time so that each could be tested separately before combining them to generate a minimal genome. The team also explored gene order and how that affects cell growth and viability, noting that gene content was more critical to cell viability than gene order. They went through three cycles of designing, building, and testing ensuring that the quasi-essential genes remained, which in the end resulted in a viable, self-replicating minimal synthetic cell that contained just 473 genes, 35 of which are RNA-coding. In addition, the cell contains a unique 16S gene sequence.

The team was able to assign biological function to the majority of the genes with 41% of them responsible for genome expression information, 18% related to cell membrane structure and function, 17% related to cytosolic metabolism, and 7% preservation of genome information. However, a surprising 149 genes could not be assigned a specific biological function despite intensive study. This remains an area of continued work for the researchers.

“This paper represents more than five years of work by an amazingly talented group of people. Our goal is to have a cell for which the precise biological function of every gene is known,” said Dr. Hutchison, Distinguished Professor, JCVI.

The team concludes that a major outcome of this minimal cell program are new tools and semi-automated processes for whole genome synthesis. Many of these synthetic biology tools and services are commercially available through SGI and SGI-DNA including a synthetic DNA construction service specializing in building large and complex DNA fragments including combinatorial gene libraries, Archetype® genomics software, Gibson Assembly® kits, and the BioXp™, which is a benchtop instrument for producing accurate synthetic DNA fragments.

“This paper signifies a major step toward our ability to design and build synthetic organisms from the bottom up with predictable outcomes. The tools and knowledge gained from this work will be essential to producing next generation production platforms for a wide range of disciplines,” said Dr. Gibson, Vice President, DNA Technologies, SGI; Associate Professor, JCVI.

The other researchers on this paper have been integral to this work for much of the last decade. Current and former JCVI and SGI scientists are: Chuck Merryman, Ph.D., Ray-Yuan Chuang, Ph.D., Vladimir Noskov, Ph.D., Nacyra Assad-Garcia, John Gill, Krishna Kannan, Ph.D., Bogumil Karas, Ph.D., Li Ma, Zhi-Qing Qi, Ph.D., R. Alexander Richter, Ph.D., Lijie Sun, Ph.D., Yo Suzuki, Ph.D., Billyana Tsvetanova, Ph.D. and Kim Wise, Ph.D.

Other authors on the paper are: Thomas J. Deerinck and Mark H. Ellisman, Ph.D., University of California, San Diego National Center for Microscopy and Imaging Research; James F. Pelletier, Center for Bits and Atoms and Department of Physics, Massachusetts Institute of Technology; Elizabeth A. Strychalski, National Institute of Standards and Technology.

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About J. Craig Venter Institute

The JCVI is a not-for-profit research institute in Rockville, MD and La Jolla, CA dedicated to the advancement of the science of genomics; the understanding of its implications for society; and communication of those results to the scientific community, the public, and policymakers. Founded by J. Craig Venter, Ph.D., the JCVI is home to approximately 200 scientists and staff with expertise in human and evolutionary biology, genetics, bioinformatics/informatics, information technology, high-throughput DNA sequencing, genomic and environmental policy research, and public education in science and science policy. The JCVI is a 501 (c)(3) organization. For additional information, please visit http://www.JCVI.org.

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