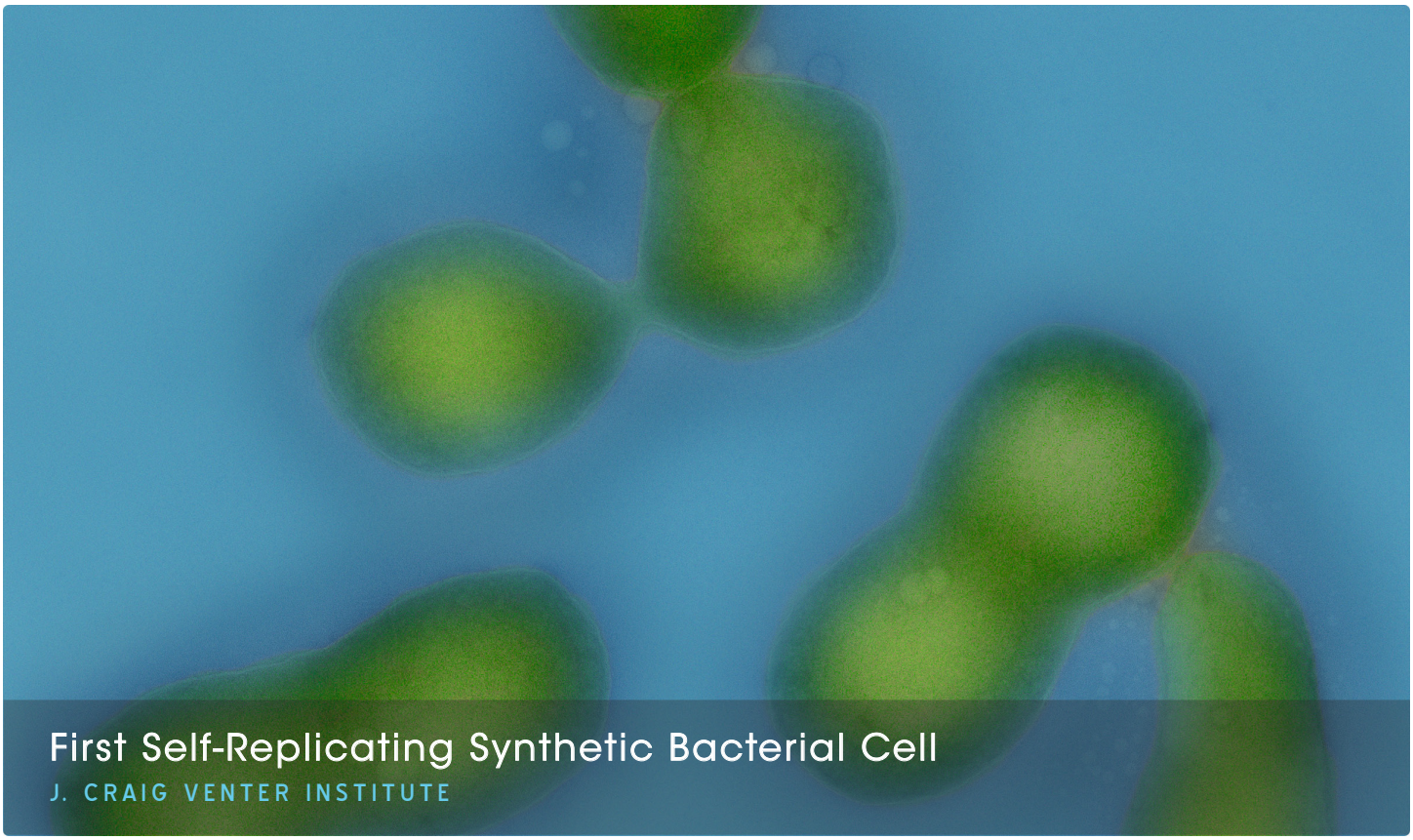

First Self-Replicating Synthetic Bacterial Cell

J. CRAIG VENTER INSTITUTE

Fact Sheet: JCVI's Synthetic Genomics Research

Background/Rationale for Creation of a Synthetic Bacterial Cell ▸

The ability to sequence or "read" an organism's entire genome—the full repertoire of genes in that organism—has been possible for several decades and is now quite routine. Much can be learned about an organism by sequencing its genome. However, learning to write genetic code is crucial to truly understanding some of the most fundamental aspects of life. If scientists can write genetic code then it becomes possible to optimize certain functions in organisms that would be beneficial for society. With these ideas in mind Drs. J. Craig Venter, Hamilton Smith, and Clyde Hutchison set out to create a synthetic bacterial cell. The work has its roots in the 1995 and 1999 publications on *Mycoplasma genitalium*, but the quest to develop the first synthetic bacterial cell began in earnest in 2003.

May 21, 2010 Science Publication

- The JCVI synthetic genomics team of nearly 25 researchers, led by J. Craig Venter, Hamilton Smith, Clyde Hutchison, John Glass, and Dan Gibson are publishing results today detailing the first cell constructed in the lab using only synthetic DNA.
- Today's announcement is the culmination of more than a decade of work by scientists at JCVI.
- Today's work is being published online in the journal Science and details the work to chemically synthesize the 1.08 million base pair genome of the bacterium *Mycoplasma mycoides*.
- This and previous breakthrough work by the JCVI was funded by Synthetic Genomics Inc. The US Department of Energy also funded early work in this area particularly the work to create the synthetic phiX174 published in 2003.
- Using previously published techniques and breakthroughs with the genetic system of yeast and of genome transplantation, the team put chemically synthesized pieces of the *M. mycoides* DNA into yeast which assembled the

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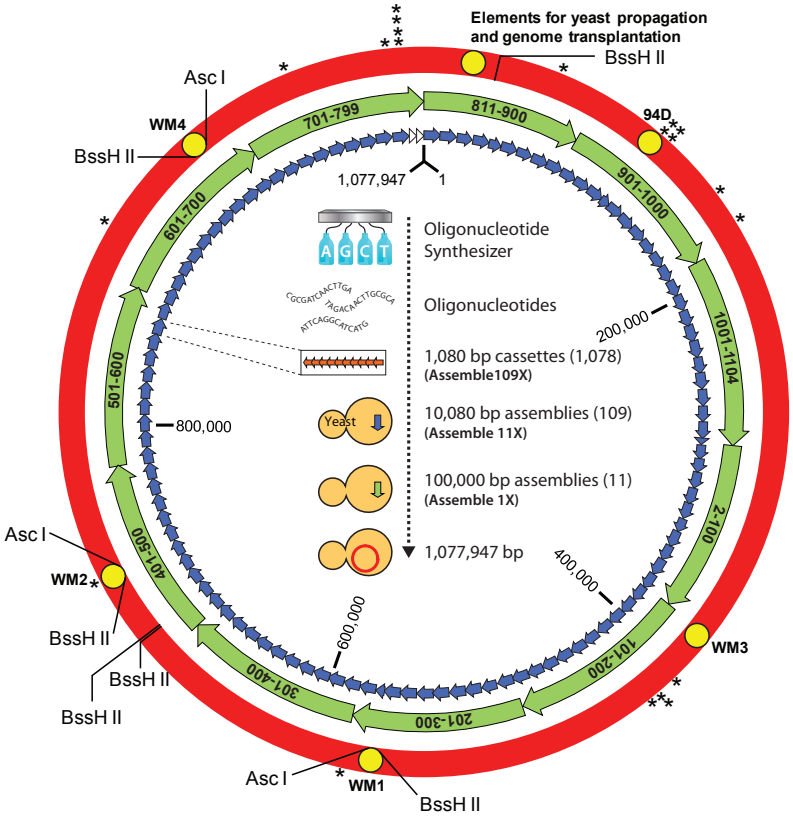
bacteria's genome. Then, the *M. mycoides* genome was transplanted into *Mycoplasma capricolum* and "booted up" to create a new synthetic version of *M. mycoides*.

- Steps involved in building the synthetic *M. mycoides* are as follows:

1. The JCVI team designed specific cassettes of DNA that were 1,080 base pairs long with overlaps of 80 base pairs (bp) at their ends to aid in building the longer stretches of DNA. These were made according to JCVI's specifications by the DNA synthesis company, Blue Heron Biotechnology.
2. Then the team employed a three stage process using yeast to build the genome using 1,078 cassettes that are 1,080 bp in length. The first stage involves taking 10 cassettes of DNA at a time to build 10,000 bp long segments. In the second stage, these 10,000 bp segments are taken 10 at a time to produce eleven 100,000 bp long segments. Finally, all 11 segments are assembled into a complete synthetic genome as an extra chromosome in a yeast cell, by using yeast genetic systems.
3. The complete synthetic *M. mycoides* genome is then released from the yeast cell and transplanted into *M. capricolum* recipient cells that have had the gene for a restriction enzyme removed. Following incubation, viable *M. mycoides* cells are produced in which the only DNA present is the synthetic genome. These cells are controlled only by that synthetic genome.



Scientific Milestones on the Quest to Create the First Synthetic Bacterial Cell ▸

1995

After sequencing the *M. genitalium* genome (published in 1995), Dr. Venter and colleagues began work on the minimal genome project. This area of research, trying to understand the minimal genetic components necessary to sustain life, started with *M. genitalium* because it is a bacterium with the smallest genome known that can be grown in pure culture. This work was published in the journal Science in 1999.

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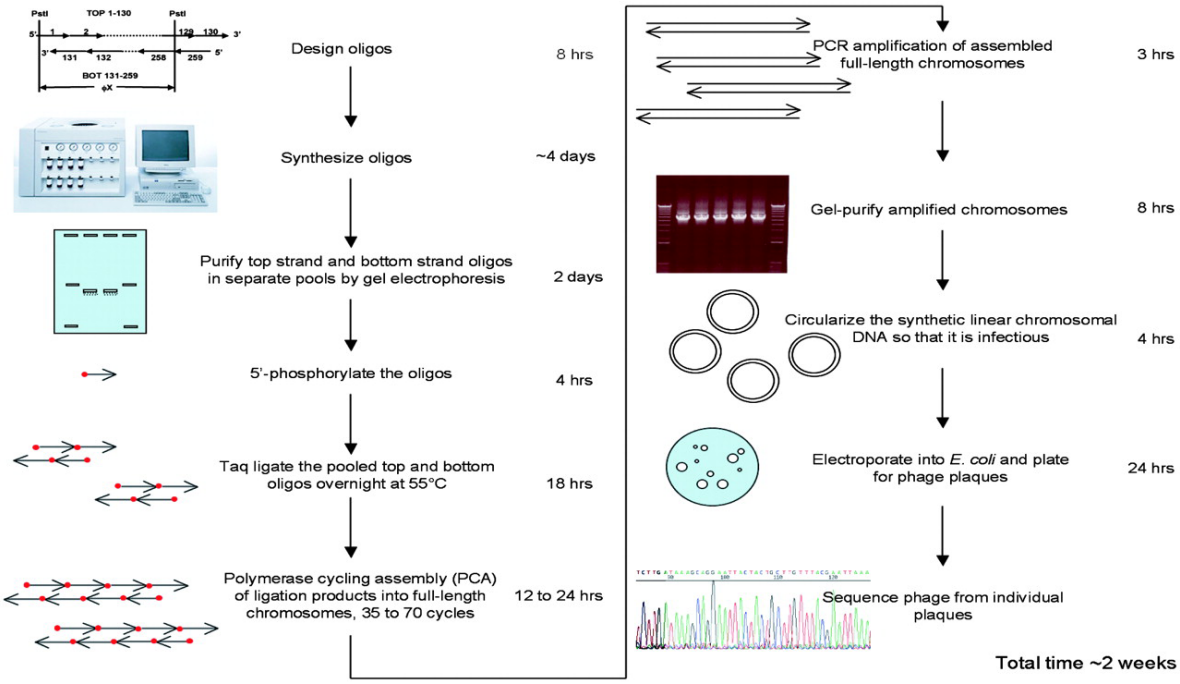
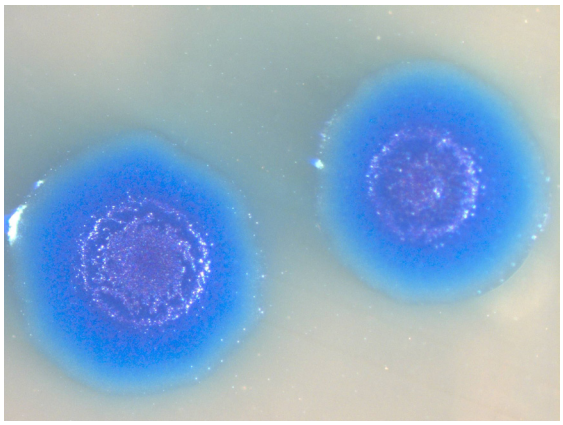
2003

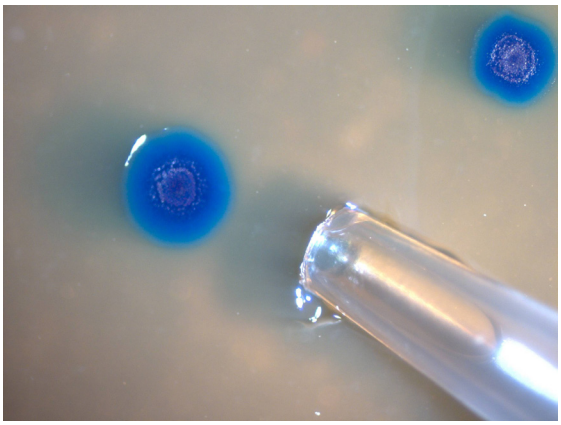
Drs. Venter, Smith, and Hutchison (along with JCVI's Cynthia Andrews-Pfannkoch) made the first significant strides in the development of a synthetic genome by assembling the 5,386 base pair genome of bacteriophage phiX 174. They did so using short, single strands of synthetically produced, commercially available DNA (known as oligonucleotides) and using an adaptation of polymerase chain reaction (PCR), known as polymerase cycle assembly (PCA), to build the phiX genome. The team developed methods that allowed the synthetic phiX to be produced in just 14 days. This work was published in the *Proceedings of the National Academy of Sciences* (PNAS).



2007

JCVI researchers led by Carole Lartigue, Ph.D., announced the results of work published in the journal *Science*, which outlined the methods and techniques used to change one bacterial species, *M. capricolum*, into another, *M. mycoides* , by replacing one organism's genome with the other one's genome. Genome transplantation was the first essential enabling step in the field of synthetic genomics as it is a key mechanism by which chemically synthesized chromosomes can be activated into viable living cells.





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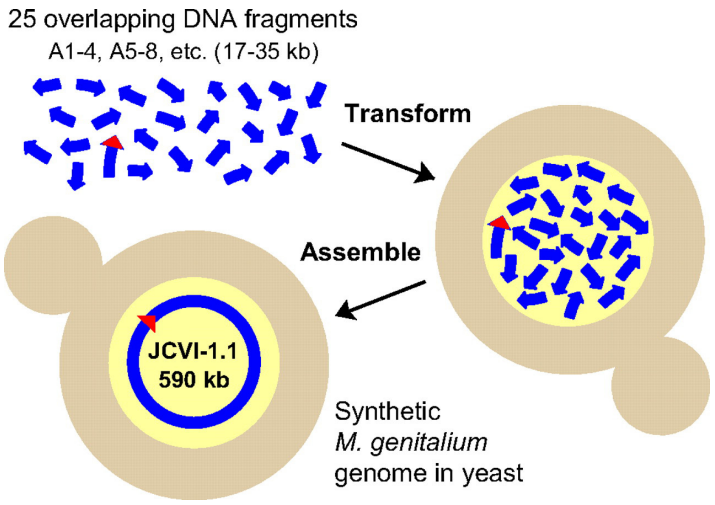
January 2008

The second successful step in the JCVI team's journey to create a cell controlled by synthetic DNA was completed when Gibson *et al.* published in the journal *Science*, the synthetic *M. genitalium* genome.

December 2008

Gibson *et al.* published a paper in *Proceedings of the National Academy of Sciences* (PNAS) describing a significant advance in genome assembly in which the team was able to assemble in yeast the whole bacterial genome, *M. genitalium*, in one step from 25 fragments of DNA. The work was funded by the company Synthetic Genomics Inc. (SGI). The team is still working to boot up the *M. genitalium* synthetic cell using all the knowledge gleaned from their previous work.



2009

JCVI researchers published results describing new methods in which the entire bacterial genome from *M. mycoides* was cloned in a yeast cell by adding yeast centromeric plasmid sequence to the bacterial chromosome and altered it in yeast using yeast genetic systems. This altered bacterial chromosome was then isolated from yeast and transplanted into a related species of bacteria, *M. capricolum*, to create a new type of *M. mycoides* cell. This was the first time that genomes were transferred between branches of life—from a prokaryote to eukaryote and back to a prokaryote. The research was published by Lartigue *et al.* in *Science*.

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