NEWS & ANALYSIS

GENOME WATCH

It's alive!

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This month's Genome Watch discusses the recent production of a bacterium under the control of a synthetic genome.

Researchers at the J. Craig Venter Institute (JCVI) in Rockville, Maryland, USA, recently described the construction of a bacterium controlled by a synthetic genome¹. Whether they have made the first synthetic organism or merely the first organism to be completely under the control of a synthetic bacterial genome is debated. The more sober commentators have pointed out that the advance falls short of creating an entirely synthetic organism and that only the first and arguably most trivial step has been achieved: that of generating a synthetic genome and using it to 'reboot' a pre-existing cell. This can be thought of as the equivalent to ditching Windows and installing Linux in its place. Gibson *et al.*¹ would perhaps reject this metaphor, pointing out that "the DNA software builds its own hardware". They argue that after several generations of existence cells of *Mycoplasma mycoides* JCVI-syn1.0 were rebuilt based on a genome that started off inside a computer.

Irrespective of any disagreement about how to define the resulting organism, this work is an important milestone in the journey towards the creation of uncontested artificial life. It was not a simple task and was necessarily preceded by other breakthroughs. JCVI researchers were responsible for two of the key steps that lead directly to their recent paper. The first of these was the synthesis and assembly of the *Mycoplasma genitalium* genome (*M. genitalium* JCVI-1.0) based on sequence data alone². The *de novo* generation of an *M. genitalium* genome was an order-of-magnitude increase on previous DNA synthesis experiments, which were limited to complete viral genomes and partial bacterial genomes. The 583 kb genome was initially synthesized as 101 cassettes of 5–7 kb overlapping by ~80 bp. These cassettes were

assembled into four pieces of ~144 kb each in three stages using *in vitro* recombination. To produce a single contiguous DNA molecule representing the entire genome, the researchers used *in vivo* recombination in yeast.

The second breakthrough was to successfully replace one bacterial genome with another. Lartigue *et al.*³ cloned the genome of *M. mycoides* into a yeast centromeric plasmid that was then transplanted into a *Mycoplasma capricolum* cell. A key stumbling block was the restriction system, which protects the bacterial cell by digesting unmethylated foreign DNA at particular sites. As the donor *M. mycoides* genome was not methylated when it was in yeast, it became a target for the *M. capricolum* restriction system. To circumvent this defence system, the donor genome could be treated with purified methyltransferases.

The techniques developed in these studies were then combined in the most recent work to generate a synthetic *M. mycoides* genome and transfer it to an *M. capricolum* recipient cell¹. The genome of *M. mycoides* is substantially larger than that of *M. genitalium* (0.6 Mb compared with 1.1 Mb), so the researchers had to synthesize 1078 cassettes of 1080 bp each, again with 80 bp overlaps. Groups of these cassettes were recombined in yeast, producing 109 assemblies of 10 kb each. This process was repeated with the 10 kb assemblies to produce 11 assemblies of 100 kb and then, eventually, a single molecule. The assembled synthetic genome was then transplanted from yeast into the recipient *M. capricolum* cell. Despite extensive checking and correction of errors, several unintended mutations were introduced during the procedure, including several single-nucleotide polymorphisms, as well as a transposon insertion from *E. coli* and an 85 bp duplication caused by non-homologous end joining that, together, disrupted two non-essential genes. To distinguish their genome from that

of natural isolates, the researchers also incorporated several short nucleotide 'watermark' sequences placed so as to cause minimal disruption to functional parts of the genome. The resulting cells contained only the synthetic genome and were able to replicate. On the basis of growth assays and proteomic analysis, the cells seemed to be essentially indistinguishable from *M. mycoides*.

This work shows that current genome-sequencing techniques produce data of sufficient quality to reconstitute a functional bacterial genome from an *in silico* copy. Furthermore, it provides proof of principle for producing genome sequences with any number of alterations without the use of extensive, laborious genetic manipulation. Applications may be far off, however, as it has proved difficult to design novel functional modules with more than a couple of genes. Nonetheless, the JCVI researchers already have plans. Perhaps the most ambitious and potentially beneficial of these is to produce a photosynthetic bacterium that can use water to generate hydrogen gas for use as a multipurpose fuel.

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1. Gibson, D. G. *et al.* Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 20 May 2010 (doi: 10.1126/science.1190719).
2. Gibson, D. G. *et al.* Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. *Science* 319, 1215–1220 (2008).
3. Lartigue, C. *et al.* Creating bacterial strains from genomes that have been cloned and engineered in yeast. *Science* 325, 1693–1696 (2009).

Competing interests statement
The author declares no competing financial interests.

DATABASES

Entrez Genome Project: <http://www.ncbi.nlm.nih.gov/genomeprj>
Mycoplasma capricolum | *M. genitalium* JCVI-1.0 |
Mycoplasma mycoides JCVI-syn1.0
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