How the first synthetic Bacterium was made

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What has Craig Venter actually produced, and what might he be planning to do with it?

For the first time, scientists have created life from scratch – well, sort of. Craig Venter's team at the J. Craig Venter Institute in Rockville, Maryland, and San Diego, California, has made a bacterial genome from smaller DNA subunits and then transplanted the whole thing into another cell. So what exactly is the science behind the first synthetic cell, and what is its broader significance?

What are the basics?

Craig Venter's team at the J. Craig Venter Institute (JCVI) in Rockville, Maryland, and San Diego, California, made a synthetic cell by stitching together the genome of a goat pathogen called Mycoplasma mycoides from smaller stretches of DNA synthesized in the lab. They then inserted the genome into the empty cytoplasm of a related bacterium, Mycoplasma capricolum. The transplanted genome booted up in its host cell, and then divided over and over to make billions of M. mycoides cells. The new strain has been named JCVI-syn1.0.

Cool. But it sounds familiar.

Venter and his team, which includes geneticists Hamilton Smith and Clyde Hutchison, have previously accomplished both feats - creating a synthetic genome and transplanting a genome from one bacterium into another - but this time they have combined the two.

To trick the M. capricolum host into accepting an artificial genome from another species, the team added chemical markers called methyl groups to the synthetic DNA - making it appear to be natural - and knocked out an "anti-invader" enzyme in the host cell. Achieving this trick was the breakthrough - and Venter has not published all the details on how it was achieved.

Why not - do they want to patent the technique?

Yes. JCVI's main funder, a company also headed by Venter called Synthetic Genomics, has exclusive access to all the technology JCVI produces, and has applied for 13 patents on unique synthetic genomes invented by the JCVI team. The JCVI applied in 2006 for a patent on the "minimal bacterial genome" that Venter now hopes to assemble. Entire, customized synthetic genomes with industrially useful capabilities may be easier than natural genes to patent as they do not face the objections raised by attempts at "patenting nature".

Can Venter expect to become mega-rich?

Very likely. The JCVI is a not-for-profit foundation but Venter is hoping that the huge range of potentially useful applications of customized bugs will eventually produce rich dividends for him and for society. Venter is collaborating with Exxon Mobil to produce biofuels from algae and with Novartis to create vaccines. "As soon as next year, the flu vaccine you get could be made synthetically," he says.

What are the pure science applications?

Synthetic cells have potential as a scientific tool. For example, bacteria could be created that produce new amino acids, the chemical units that make up proteins. Geneticists could then see how these "cyborg" bacteria evolve, compared with bacteria that produce the usual suite of amino acids.

How can they be sure that the new bacteria are what they intended?

The bugs' genomes are "watermarked" with distinctive markers, all of which were found in the synthetic cell when it was sequenced. The watermarks contain the names of 46 scientists on the project, several quotations written out in a secret code, and a website address. As a hint to the code, Venter has revealed the quotations, which include: "To live, to err, to fall, to triumph, to recreate life out of life," from A Portrait of the Artist as a Young Man by James Joyce.

Does this mean they created life?

No. The team made the new genome out of DNA sequences that had initially been made by a machine, but bacteria and yeast cells were used to stitch it together and duplicate it.

The cell into which the synthetic genome was then transplanted contained its own proteins, lipids and other molecules. Until the host cell is itself built artificially from scratch it cannot be said that life has been created.

Bioterror, kill switches and hara-kiri

Now that synthetic life has been made in the lab, how do we make sure it stays there?

For Venter and his team, bio-containment was simple: the cells they created require a broth of nutrients unlikely to be found outside the lab. Their genome also lacks the harmful genes from the goat pathogen on which it was based. "We don't work with goats, so we think we have pretty good containment systems," Venter says.

Future synthetic cells, though, will require extra measures. One approach would be to make cells that incorporate a synthetic amino acid into their proteins, so no proteins could be made without the supplement. James Collins at Boston University envisions a killer genetic circuit that is shut off by a lab chemical, and switched on outside the lab. "If they are not in their happy lab environment they would commit cellular hara-kiri," he says. Bacteria could also be programmed to stop dividing after a certain number of generations.

George Church of Harvard Medical School has called for all synthetic biology labs and their suppliers to be registered, an idea the US National Institutes of Health is looking into. "Everybody in the synthetic biology ecosystem should be licensed," Church says.

Some companies that make stretches of DNA to order have begun scanning requests to see if they match genes for known toxins, but these measures are only voluntary, and therefore patchy.

Andy Ellington at the University of Texas in Austin says fears of synthetic bioterrorism are in any case overhyped, and probably unrealistic given the $40 million and thousands of person- hours it took Venter's team. "It's not a real threat," he says.

Venter: The implications of our synthetic cell

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ON 14 December 1967, Arthur Kornberg at Stanford University and colleagues announced that they had copied the DNA of the Phi X174 virus, producing an entity with the same infectivity as the wild virus. Though the DNA sequence in question was not known, Kornberg hoped the achievement would aid studies of genetics and the search for cures for diseases, and reveal the most basic processes of life itself. President Lyndon B. Johnson cited Kornberg's claim to have come the "closest yet" to creating life and hailed it as "a very spectacular breakthrough".

In 2003 we were able to go a step further, using DNA made from a sequence in a computer rather than a copy made by an enzyme. By 2008 we could synthesize a small bacterial chromosome over 20 times as big as that of Phi X174, though we were unable to activate it in a cell. With our publication in *Science* last week, we have now achieved this step with the 1.08-million-base-pair genome of *Mycoplasma mycoides*.

We did not create life from scratch: we transformed existing life into new life. Nor did we design and build a new chromosome from scratch. Rather, using only digitized information, we synthesized a modified version, a copy of the *M. mycoides* genome with 14 of its genes deleted and a "watermark" written in another 5000-plus base pairs. The result is not an "artificial" life form; it is a living, self-replicating cell that most microbiologists would find hard to distinguish from the progenitor cell, unless they sequenced its DNA.

It took 15 years to get to this proof-of-concept experiment. And it is just that: proof that it is possible to use a computer and four chemical bases to create a cell with no biological ancestor. Of course, we began by modifying an existing genome. Where else to start? Had we tried a new genome design it wouldn't have worked. Even so, we had 99 failures for every success.

Our synthetic cell is a small but highly significant step in synthetic genomics. Without this success, there would be no future for what has been, until now, a theoretical field. We now have a new set of tools to begin to understand cellular life, to test combinations of the 40 million sequenced genes in our computers, few of which have well-defined functions.

Nor is there any cell - and certainly not our synthetic cell - where the function of every gene is understood. We don't know yet which genes are essential for life and why. It will be interesting to see how few components are needed to boot up a synthetic chromosome. Perhaps all it will take is a lipid vesicle and the ability to make messenger RNA and ribosomes - but we don't know.

We now have the means to design and build a cell that will define the minimal set of instructions necessary for life, and to begin the design of cells with commercial potential, such as fuel production from carbon dioxide. We can assemble genome-sized stretches of DNA that can also be used to mix and match natural and synthetic pieces to make genomes with new capabilities.

Synthesizing DNA in this way is still expensive, but we expect the cost to fall dramatically. This may make the complete synthesis of genomes competitive with the alteration of natural genomes to add new capabilities to bacterial cells. It should also be practical to synthesize simple eukaryotes, such as yeast, to which it is already possible to add extra chromosomes. The construction of large pieces of synthetic DNA and their introduction into a receptive cytoplasm is no longer a barrier. The limits to progress in synthetic biology are now set by our ability to design genomes with particular properties.

All potential applications of this science depend on review, discussion and debate, to ensure that the technology is used for positive purposes and that society understands the science and the issues. We intend to be part of this process. In this way, our first synthetic cell represents a new beginning.

J. Craig Venter, Clyde Hutchison III and Hamilton Smith.

Where next for synthetic life?

by **Ewen Callaway** and **Andy Coghlan**

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MAKE a genome - check. Transplant it into an emptied cell to create the world's first synthetic life form - check. Frenzied media coverage accusing the researchers concerned of "playing God" - check.

Craig Venter and his teams at the J. Craig Venter Institute in Rockville, Maryland, and San Diego, California, have shown themselves to be technical wizards by synthesizing a genome from code contained on a computer, and using it to start a cell line of the resulting synthetic organism . If demonstration was needed that there is no such thing as the "mystery of life", they have provided it in stunning style. The new life form they have made is derived from information, pure and simple.

Other synthetic biology researchers, while impressed by Venter's technical achievement, are restrained about its implications, both practical and philosophical. They were already well aware that there is no magical Wizard of Oz behind life's curtain, and they feel the first fruits of synthetic biology - organisms designed to make clean fuels and cheap pharmaceuticals, for example - are more likely to come through less ambitious approaches.

"It's cool and has taken a lot of effort," says Alistair Elfick at the University of Edinburgh, UK. "But it doesn't take us that much further scientifically." He and many other researchers in the field say they are unlikely to synthesize whole bacterial genomes themselves.

"This is a marvelous advance, but it doesn't immediately open up or enable new studies for the broad community," says James Collins of Boston University, who notes that Venter's team spent about $40 million to create the synthetic cell. "We don't have that kind of money in academic research."

The costs of making long stretches of DNA - currently about $1 per letter - will almost certainly fall. But even if synthetic genomes become dramatically cheaper to make, there is still the question of how to write one. "We have a long way to go to really develop sufficient understanding to build an operational genome from scratch," Collins says.

Genomes are too much of a black box for deliberate and predictable tinkering, says Gos Micklem at the University of Cambridge. "It's like trying to build a car engine when you don't understand what the individual parts do." Even if biologists learn how to write novel genomes fluently, they face another huge hurdle: getting the enormous molecules to "boot up" in a foreign cell.

Venter's genome was modeled on that of a mycobacterium, and was implanted into the cytoplasm of a closely related species. It remains to be seen whether these vessels will accept the genome of drug-making *Escherichia coli* or, more difficult still, a biofuel-producing alga. "It will be very challenging to jump between very different species," Collins says.

These criticisms may be unfair to Venter and his team, as their stated goal was to synthesize a bacterial genome that existed as data and implant it into a cell. As Venter is fond of saying: "This is the first self-replicating species that we've had on the planet whose parent is a computer."

More than anything, the guarded reception from Venter's peers demonstrates how far synthetic biology has come via other routes. In recent years, it has yielded the once costly anti-malarial drug artemisinin, a valuable polymer, and even biofuels. "Those didn't involve millions of genetic changes, those involved a dozen," says George Church at Harvard Medical School in Boston.

The chemical company DuPont has spent the better part of a decade and hundreds of millions of dollars identifying about 20 genetic changes that enable *E. coli* to produce a polymer called 1,3-propanediol. Church and his team have come up with a way of introducing multiple genetic changes into bacteria more quickly and cheaply, called multiplex automated genome engineering or MAGE.

Church is now working on improving the technique. "It's an order of magnitude less expensive to do partial genomes than to do the whole ones, and there are really amazing things that can be done," he says.

For now the preferred approach - and one that is acknowledged by Venter - is to create a "toolbox" of genetic components or "BioBricks" that act in a predictable way, ready for assembly into combinations with whatever properties are desired. These genes or circuits of genes are kept ready and available for assembly into bio-devices that actually have a function.

The Massachusetts Institute of Technology keeps a registry of 2500 BioBricks. Many of these have come from students competing in an annual event called the International Genetically Engineered Machine competition, or iGEM, but according to Richard Kitney at Imperial College London, only about 10 per cent work properly.

So Kitney, in collaboration with the University of California, Berkeley, and Stanford University in California, is creating a professional BioBrick registry. "There are now about 300 parts that are fully understood and characterised," he says. "You can use them to make professionally engineered biological devices."

In contrast to Venter's latest achievement, which demonstrates a proof of principle but has no immediate practical use, everyone involved in BioBrick projects is using biological tools to try and solve practical problems, Kitney says. "All of us are focused on applications... producing devices and systems that spawn new industries."

Kitney and his colleagues have made a biological sensor which detects a protein from bacteria that cause urinary tract infections. The device has three BioBrick components: a detector; an amplifier that increases the signal; and an indicator. The three components form a bio-device which is then placed into *E. coli*.

Going one step further, the team is developing a version that doesn't need an *E. coli* cell. Instead, the three genes are added to a broth and produce a response equivalent to that of a live cell. "We're working on a new version that detects the superbug MRSA, with a red fluorescent protein," Kitney says.

Elfick and his colleagues are tinkering with six enzymes that together can break down cellulose, the normally indigestible polymer in waste plant matter, with the aim of turning plant waste into biofuel.

Venter has the same goals. He just envisions a different way of achieving them, and perhaps it is this ambition that sets him apart from his peers. "There's zero doubt in my mind that being able to control the whole thing from scratch is orders of magnitude more powerful than changing a genome," Venter says. "The unknown is how long it will take us."