Evolution machine: Genetic engineering on fast forward

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Automated genetic tinkering is just the start – this machine could be used to rewrite the language of life and create new species of humans

IT IS a strange combination of clumsiness and beauty. Sitting on a cheap-looking worktop is a motley ensemble of flasks, trays and tubes squeezed onto a home-made frame. Arrays of empty pipette tips wait expectantly. Bunches of black and grey wires adorn its corners. On the top, robotic arms slide purposefully back and forth along metal tracks, dropping liquids from one compartment to another in an intricately choreographed dance. Inside, bacteria are shunted through slim plastic tubes, and alternately coddled, chilled and electrocuted. The whole assembly is about a metre and a half across, and controlled by an ordinary computer.

Say hello to the evolution machine. It can achieve in days what takes genetic engineers years. So far it is just a prototype, but if its proponents are to be believed, future versions could revolutionise biology, allowing us to evolve new organisms or rewrite whole genomes with ease. It might even transform humanity itself.

These days everything from your food and clothes to the medicines you take may well come from genetically modified plants or bacteria. The first generation of engineered organisms has been a huge hit with farmers and manufacturers - if not consumers. And this is just the start. So far organisms have only been changed in relatively crude and simple ways, often involving just one or two genes. To achieve their grander ambitions, such as creating algae capable of churning out fuel for cars, genetic engineers are now trying to make far more sweeping changes.

Grand ambitions

Yet changing even a handful of genes takes huge amounts of time and money. For instance, a yeast engineered to churn out the antimalarial drug artemisinin has been hailed as one of the great success stories of synthetic biology. However, it took 150 person-years and cost $25 million to add or tweak around a dozen genes - and commercial production has yet to begin.

The task is so difficult and time-consuming because biological systems are so complex. Even simple traits usually involve networks of many different genes, which can behave in unpredictable ways. Changes often do not have the desired effect, and tweaking one gene after another to get things working can be a very slow and painstaking process. Many biologists think the answer is to try to eliminate the guesswork. They are creating libraries of ready-made "plug-and-play" components that should behave in a reliable way when put together to create biologicial circuits. But George Church, a geneticist at Harvard Medical School in Boston, thinks there is a far quicker way: let evolution do all the hard work for us. Instead of trying to design every aspect of the genetic circuitry involved in a particular trait down to the last DNA letter, his idea is to come up with a relatively rough design, create lots of variants on this design and select the ones that work best.

The basic idea is hardly original; various forms of directed evolution are already used to design things as diverse as proteins and boats. Church's group, however, has developed a machine for "evolving" entire organisms - and it works at an unprecedented scale and speed. The system has the potential to add, change or switch off thousands of genes at a time - Church calls this "multiplexing" - and it can generate billions of new strains in days.

Of course, there are already plenty of ways to generate mutations in cells, from zapping them with radiation to exposing them to dangerous chemicals. What's different about Church's machine is that it can target the genes that affect a particular characteristic and alter them in specific ways. That greatly increases the odds of success. Effectively, rather than spending years introducing one set of specific changes, bioengineers can try out thousands of combinations at once. Peter Carr, a bioengineer at MIT Media Lab who is part of the group developing the technology, describes it as "highly directed evolution". The first "evolution machine" was built by Harris Wang, a graduate student in Church's lab. To prove it worked, he started with a strain of the *E. coli*bacterium that produced small quantities of lycopene, the pigment that makes tomatoes red. The strain was also modified to produce some viral enzymes. Next, he synthesised 50,000 DNA strands with sequences that almost matched parts of the 24 genes involved in lycopene production, but with a range of variations that he hoped would affect the amount of lycopene produced. The DNA and the bacteria were then put into the evolution machine.

The machine let the *E. coli* multiply, mixed them with the DNA strands, and applied an electric shock to open up the bacterial cells and let the DNA get inside. There, some of the added DNA was swapped with the matching target sequences in the cells' genomes. This process, called homologous recombination, is usually very rare, which is where the viral enzymes come in. They trick cells into treating the added DNA as its own, greatly increasing the chance of homologous recombination.

The effect was to create new variants of the targeted genes while leaving the rest of the genome untouched. It was unlikely that all 24 genes would be altered simultaneously in any one bacterium, so the cycle was repeated over and over to increase the proportion of cells with mutations in all 24 genes.

Repeating the cycle 35 times generated an estimated 15 billion new strains, each with a different combination of changes in the target genes. Some made five times as much lycopene as the original strain, Wang's team reported in 2009 (*Nature*, vol 460, p 894). It took Wang just three days to do better than the biosynthesis industry has managed in years. And it was no one-off - he has since repeated the trick for the textile dye indigo. Church calls this bold approach multiplex automated genome engineering, or MAGE. In essence, he has applied the key principles that have led to the astonishing advances in DNA sequencing - parallel processing and automation - to genetic engineering. And since Church was one of the founders of the human genome project and helped develop modern sequencing methods, he knows what he is doing.

Just as labs all over the world now buy thousands of automated DNA sequencing machines, so Church envisions them buying automated evolution machines. He hopes to sell them relatively cheaply, at around $90,000 apiece. "We're dedicated to bringing the price down for everybody, rather than doing some really big project that nobody can repeat," Church says.

He hopes the machines will greatly accelerate the process of producing novel microbes. LS9, a biofuels company based near San Francisco that was co-founded by Church, has said it hopes to use MAGE to engineer *E. coli* that can produce renewable fuels. Church and colleagues are also adapting the approach for use with other useful bacteria, including *Shewanella*, which can convert toxic metals such as uranium into an insoluble form, and cyanobacteria which can extract energy from light using photosynthesis.

A big revolution

In principle, the technique should work with plant and animal cells as well as microbes. New methods will have to be developed for coaxing cells to swap in tailored DNA for each type of organism, but Church and his colleagues say that progress has already been made in yeast and mammalian cells.

"I think it is a big revolution in genome engineering," says Kristala Jones Prather, a bioengineer at the Massachusetts Institute of Technology who is not part of Church's

collaboration. "You don't have to already know what the answer is. You can manipulate multiple things at a time, and let the cell find a solution for you."

Because biological systems are so complex, it is a huge advantage to be able to tweak lots of genes simultaneously, rather than one at a time, she says. "In almost every case you'll get a different solution that's a better solution."

The disadvantage of Church's approach is that the "better solution" is mixed up with millions of poorer solutions. Prather points out that the technique is limited by how easy it is to screen for the characteristics that you want. Wang selected good lycopene producers by growing 100,000 of the strains he had created in culture dishes and simply picking out the brightest red colonies. "Essentially nothing that we use in my lab can be screened so easily," Prather says.

By automating selection and using a few tricks, though, it should be practical to screen for far more subtle characteristics. For instance, biosensors that light up when a particular substance is produced could be built into the starting strain. "The power going forward will have to do with clever selections and screens," says Church.

As revolutionary as this approach is, Church thinks MAGE's most far-reaching potential lies elsewhere. He reckons it will be possible to use the evolution machine to make many thousands of specific changes to a cell's DNA: essentially, to rewrite genomes. At the moment, making extensive changes to even the smallest genome is extremely costly and laborious. Last year, the biologist and entrepreneur Craig Venter announced that his team had replaced a bacterium's genome with a custom-written one (*Science*, vol 329, p 52). His team synthesised small pieces of DNA with a specific sequence, and then joined them together to create an entire genome. It was an awesome achievement, but it took 400 person-years of labour and cost around $40 million.

MAGE can do the same job far more cheaply and efficiently by rewriting existing genomes, Church thinks. The idea is that instead of putting DNA strands into the machine with a range of different mutations, you add only DNA with the specific changes you want. Even if you are trying to change hundreds or thousands of genes at once, after a few cycles in the machine, a good proportion of the cells should have all the desired changes. This can be checked by sequencing.

If the idea works it would make feasible some visionary projects that are currently impossibly difficult. Church, needless to say, has something suitably ambitious in mind. In fact, it is the reason he devised MAGE in the first place.
In 2004 he had joined forces with Joseph Jacobson, an engineer at the MIT Media Lab, best known as inventor of the e-ink technology used in e-readers. Searching for a "grand goal" in bioengineering, the pair hit upon the idea of altering life's genetic code. Rather than just alter the sequence of DNA, they want to change the very language in which the instructions for life are written(see diagram).

This is not as alarming as it might sound. Because all existing life uses essentially the same genetic code, organisms that translate DNA using a different code would be behind a "genetic firewall", unable to swap DNA with any normal living thing. If they escaped into the wild, they would not be able to spread any engineered components. Nor would they be able to receive any genes from natural bacteria that would endow them with antibiotic resistance or the ability to make toxins. "Any new DNA coming in or any DNA coming out doesn't work," says Church. "We're hoping that people who are concerned, including us, about escape from industrial processes, will find these safer."
There is another huge advantage: organisms with an altered genetic code would be immune to viruses, which rely on the protein-making machinery of the cells they infect to make copies of themselves. In a cell that uses a different genetic code, the viral blueprints will be mistranslated, and any resulting proteins will be garbled and unable to form new viruses.

Doing this in bacteria or cell lines used for growing chemicals would be of huge importance to industry, where viral infections can shut down entire production lines. And the approach is not necessarily limited to single cells. "It's conceivable that it could be done in animals," says Carr.

Completely virus-proof

Carr and his colleagues have already begun eliminating redundant codons from the genome of *E. coli*. They are starting with the rarest, the stop codon TAG, which appears 314 times. Each instance will be replaced by a different stop codon, TAA. So far they have used MAGE to create 32 *E. coli* strains that each have around 10 of the necessary changes, and are now combining them to create a single strain with all the changes. Carr says this should be completed within the next few months, after which he hopes to start replacing another 12 redundant codons. To make a bacterium completely virus-proof will probably require replacing tens of thousands of redundant codons, he says, as well as modifying the protein-making factories so they no longer recognise these codons.

To ensure novel genes cannot be translated if they get passed on to other organisms, the team would have to go a step further and reassign the freed-up codons so a different amino acid to normal is added to a protein when they occur. This could include amino acids that do not exist in nature, opening the door to new types of chemistry in living cells. Artificial amino acids could be used to create proteins that do not degrade as easily, for example, which could be useful in industry and medicine.

There are potential dangers in making organisms virus-proof, though. Most obviously, they might have an advantage over competing species if they escaped into the wild, allowing them to dominate environments with potentially destructive effects. In the case of *E. coli*, those environments could include our guts.

"We want to be very careful. The goal is to isolate these organisms from part of the natural sphere with which they normally interact," says Carr. "We shouldn't pretend that we understand all possible ramifications, and we need to study these modified organisms carefully." But he points out that we deal with similar issues already, such as invasive species running riot in countries where they have no natural predators. Additional safeguards could be built in, such as making modified organisms dependent on nutrients they can get only in a lab or factory. And if the worst came to the worst, biologists could create viruses capable of killing their errant organisms. Such viruses would not be able to infect normal cells.

Church argues that with proper safety and regulatory controls, there is no reason why the approach shouldn't be used widely. "I think that to some extent you'd like every organism to be multi-virus resistant," he says. "Or at least industrial microbes, agricultural species and humans."

Yes, humans. Church is already adapting MAGE for genetically modifying human stem cell lines. The work, funded by the US National Human Genome Research Institute, aims to create human cell lines with subtly different genomes in order to test ideas about which mutations cause disease and how. "Sequencing is now a million times cheaper, and there are a million times as many hypotheses being generated," he says. "We'd like to develop the resources so that people can quickly test hypotheses about the human genome by synthesising new versions."

As the technology improves and becomes routine, says Church, it could also be used to alter the cells used for cell-based therapies. Tissue-engineered livers grown from stem cells, say, could have their genetic code altered so that they would be immune to liver-destroying viruses such as hepatitis C.

"Everybody getting stem cell therapies will be given a choice of doing ordinary stem cell therapy - either with their cells or donor cells - or doing stem cells that are resistant to viruses," he says. "There will have to be all kinds of safety checks and FDA approval

and so forth, but most people faced with two fairly safe choices, one of which is virus-sensitive and one of which is virus-resistant, are going to take the virus-resistant one."

Of course, there would be enormous experimental and safety hurdles to overcome. Not least the fact that gene targeting using homologous recombination or any other method is not perfect - the added DNA is sometimes inserted into the wrong place in the genome, and the process can trigger other kinds of mutations too. Such off-target changes might be a big problem when making hundreds of targeted changes at a time. So not surprisingly, Carr describes the move to humans as "fraught with peril". But if we do get to a point where there are lots of people walking around with virus-resistant tissues or organs, and lots of farm animals that are completely virus-resistant, Church thinks it is only a matter of time before clinics create virus-resistant babies. "If it works really well, somebody somewhere will decide to try it in the next generation."

Making changes to the genomes of humans that will get passed on to their children has long been seen as taboo. But Church points out that there was strong resistance to techniques such as in vitro fertilisation and organ transplants when they were new; yet as soon as they were shown to work, they were quickly accepted. "Many technologies start out that way," he says. "But once they work really well, everybody says it's unethical not to use them."

Arthur Caplan, a bioethicist at the University of Pennsylvania in Philadelphia who advises the US government on reproductive technologies, is sceptical about the idea of making virus-resistant people, because anyone modified in this way would only be able to conceive children naturally with a partner whose genome had been altered in exactly the same way. "You would be denying a hugely important choice to a future modified human."

But, he says, if MAGE really can be used to edit the genome of human cells, it would provide a way to fix the mutations that cause inherited disease. It could be the technology that opens the door to the genetic engineering of humans. We should start debating now how best to use it, Caplan says. Should it be limited to preventing disease, or used for enhancement too? What sort of regulation is needed? Who should be eligible?

This prospect might seem a long way off, but Caplan argues that if the technique works well in other species, it could become feasible to attempt to engineer humans in as little as 10 years. "If you learn to do this in microbes and then in animals, you'll find yourself wondering how we got to humans so fast," he says. "You've got to pay attention to what's going on in lower creatures because that's the steady march to people."
If all this sounds wildly implausible, bear in mind that the idea of sequencing an entire human genome in days seemed nigh on impossible just a few years ago. Now it's fast becoming routine. Most biologists would probably agree that it is just a matter of time before we develop the technology needed to rewrite the DNA of living creatures at will. If Church succeeds, this future will happen faster than any imagined.

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