SYNTHETIC BIOLOGY

Mission possible: Rewriting the genetic code

A research team is making steady progress at overhauling a bacterium's genome

By **John Bohannon**

The term "life hacking" usually refers to clever tweaks that make your life more productive. But on p. 819, a team of scientists comes a step closer to the literal meaning: hacking the machinery of life itself. They have designed—though not completely assembled—a synthetic *Escherichia coli* genome that could use a protein-coding scheme different from the one employed by all known life. Requiring a staggering 62,000 DNA changes, the finished genome would be the most complicated genetic engineering feat so far. *E. coli* running this rewritten genome could become a new workhorse for laboratory experiments and a factory for new industrial chemicals, its creators predict.

Such a large-scale genomic hack once seemed impossible, but no longer, says Peter Carr, a bioengineer at the Massachusetts Institute of Technology Lincoln Laboratory in Lexington who is not involved with the project. "It's not easy, but we *can* engineer life at profound scales, even something as fundamental as the genetic code."

The genome hacking is underway in the lab of George Church at Harvard University, the DNA-sequencing pioneer who has become the most high-profile, and at times controversial, name in synthetic biology (*Science*, 2 September 2011, p. 1236). The work takes advantage of the redundancy of life's genetic code, the language that DNA uses to instruct the cell's protein-synthesizing machinery. To produce proteins, cells "read" DNA's four-letter alphabet in clusters of three called codons. The 64 possible triplets are more than enough to encode the 20 amino acids that exist in nature, as well as the "stop" codons that mark the ends of genes. As a result, the genetic code has multiple codons for the same amino acid: the codons CCC and CCG both encode the amino acid proline, for example.

Church and others hypothesized that redundant codons could be eliminated—by swapping out every CCC for a CCG in every gene, for instance—without harming the cell. The gene that enables CCC to be translated into proline could then be deleted entirely. "There are a number of 'killer apps'" of such a "recoded" cell, says Farren Isaacs, a bioengineer at Yale University, who, with Church and colleagues, showed a stop codon can be swapped out entirely from *E. coli* (*Science*, 18 October 2013, p. 357).

The cells could be immune to viruses that impair bioreactors, for example, if crucial viral genes include now untranslatable codons. The changes could also allow synthetic biologists to repurpose the freed redundant codons for an entirely different function, such as coding for a new, synthetic amino acid.

Biologists are transforming the proteinmaking instructions of *Escherichia coli*.

ILLUSTRATION: C. BICKEL/*SCIENCE*

For this study, Church's team decided to eliminate seven of the microbe's 64 codons. That target seemed like "a good balance" between the number of changes that appeared technically achievable and the number that might be too many for a cell to survive, says Matthieu Landon, one of Church's Ph.D. students. And the seven spare codons could eventually be repurposed to code up to four different unnatural amino acids.

But making so many changes, even with the latest DNA editing techniques such as CRISPR, still appeared impossible. Luckily, the cost of synthesizing DNA has plummeted over the past decade. So instead of editing the genome one site at a time, Church's team used machines to synthesize long stretches of the recoded genome from scratch, each chunk containing multiple changes.

The team has now turned to the laborious job of inserting these chunks into *E. coli* one by one and making sure that none of the genomic changes is lethal to the cells. The researchers have only tested 63% of the recoded genes so far, but remarkably few of the changes have caused trouble, they say.

Does this progress report from Church's lab put biologists on the doorstep of a new era of virus-free bioengineered cells? "More likely on the driveway than the doorstep," Isaacs says. Carr agrees. "The upcoming phases of synthesis, testing, and assembly are likely to take several years," he says. "The toughest 5% of the design may end up requiring 95% of the effort."

In the meantime, another issue is likely to dominate discussions: safety. One concern is that many of the "unnatural" proteins that the recoded *E. coli* could be engineered to produce may be toxic, and the cells' resistance to viruses would give them a competitive edge if they escaped into the environment—or into our own guts. "As we get closer to full multivirus resistance, this becomes more critical," Church acknowledges.

The failsafe that Church plans to build into the microbes is superficially similar to the one used to control the bioengineered dinosaurs in the film *Jurassic Park*. Those resurrected creatures couldn't survive without a special nutrient supplied by their human masters—that is, until they found a source of the nutrient in the wild. In a study published in *Nature* last year, Church demonstrated a failsafe system for engineered microbes that should be far more robust. Not only does the required nutrient not occur naturally, but it appears to be virtually impossible for the cells to overcome the barrier through mutation or mating with normal cells in the wild.

Whether others will agree with Church that his failsafe is unbeatable remains to be seen. "The term 'safe' needs a lot more scrutiny," Carr says. "Instead of the all-or-nothing connotations of 'safe' or 'not safe,' it is more useful to describe degrees of risk." ■

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Editor's Summary