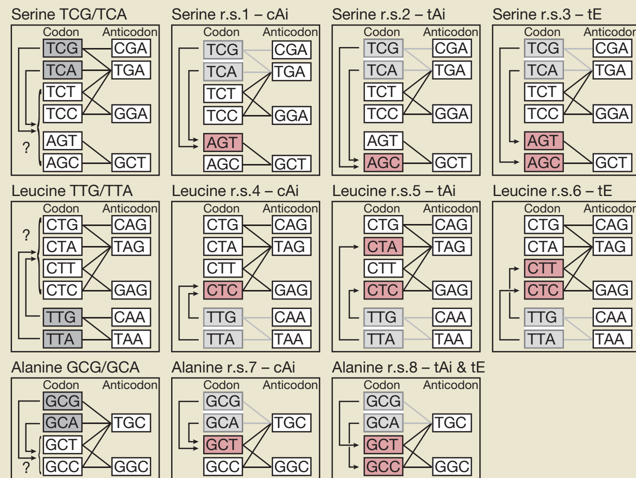
Hacking rules for *E. coli*



Changing the codons used in the genetic code promises to enable the creation of new types of organisms, endow life with new functions and result in industrial strains capable of enhanced protein production. But the rules for swapping out codons—those changes that are allowed and those that are not—remain unclear. With a clever and efficient approach, Jason Chin at the Medical Research Council Laboratory of Molecular Biology in Cambridge, UK, and his colleagues[1] have created a system that enables researchers to decipher the codon rules in *Escherichia coli* by recoding large genomic regions and validating whether the resulting organisms remain viable and functional. Using lambda-red-mediated recombination and CRISPR–Cas9, they swap large regions of the *E. coli* genome in one step and examine how recoding schemes work.

Chin's group set out to repurpose some codons to incorporate unnatural chemical building blocks with an orthogonal translation system. First, they came up with several different schemes assigning different combinations of alternative codons to encode the same amino acid. "Then, we can delete from the genome the cognate tRNAs for the codons we eliminate and reassign those codons to unnatural chemical blocks," Chin explains.

But recoding the entire genome requires the iterative replacement of large regions of DNA. Although *E. coli* has undeniably served as an amenable cellular model for synthetic biologists, swapping large regions of DNA remains a non-trivial task. For example, the low efficiency of lambda-red-mediated homologous recombination and the difficulty of delivering large dsDNA molecules to *E. coli* cells limits most efforts to targeting a few kilobases at a time.

Chin and colleagues' solution is to deliver the recoded DNA in a bacterial artificial chromosome (BAC) and increase the efficiency of lambda-red-mediated recombination by excising the DNA region of interest, from both the BAC and the genome, using CRISPR–Cas9. Using antibiotic markers, and positive and negative selection for insertion of the synthetic DNA and loss of the endogenous locus, they were able to identify clones containing recoded regions of interest. As the approach is iterative, the recoded genome created from one round of recombination can serve as a template for the next round.

With the ability to swap out 100 kilobases in a single step, Chin's team asked "what is the best synonymous codon choice for a particular set of codons we want to remove?" Their systematic approach consisted of testing eight recoding schemes for serine, leucine and alanine simultaneously in the 15 genes of the *E. coli* cell division operon. Following recombination of the rationally designed DNA, they sequenced resulting clones, which were chimeras between the wild-type and recoded DNA. The degree of chimerism they observed enabled the researchers to deduce which recoding schemes—and at which positions—are allowed.

Chin intends to apply these rules across the entire *E. coli* genome and use the resulting organisms to synthesize unnatural polymers, such as Kevlar, which is used in tires and bullet-proof vests. "We imagine you'd be able to discover many new materials with interesting properties," Chin says. "Not only would they be synthesized in a scalable way, but you would also have the opportunity to evolve the sequences of the polymers to do what you would like them to do."

Whether the rules for codon swapping in *E. coli* and the specific components of the lambda-red-mediated recombination technique can be applied across other organisms is yet not clear, Chin says. But the general strategy will likely be transferable to other operons and applications, including insertion of biosynthetic pathways into specific loci. Recoding the genome and repurposing synonymous codons is now in sight.

Irene Jarchum, Associate Editor

1. Wang, K. *et al.* Defining synonymous codon compression schemes by genome recoding. *Nature* **539**, 59–64 (2016).

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