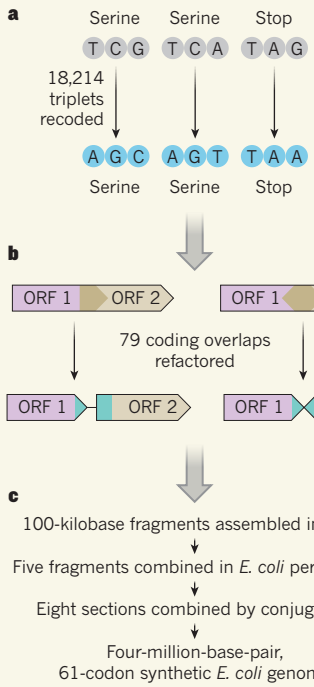

Figure 1 | Design and construction of a recoded genome. **a**, Fredens *et al.*[3] recoded three base triplets (codons) — TCG and TCA, which encode the amino acid serine, and TAG, a stop codon that marks the end of a protein-coding sequence — to alternatives that have the same functions (AGC, AGT and TAA respectively) in the genome of the bacterium *Escherichia coli*. **b**, In some genomic locations, open reading frames (ORFs; protein-coding regions) overlap, and a change in the codons of one ORF might produce an unwanted change in the overlapping region. Fredens *et al.* 'refactored' these ORFs to separate them, as illustrated for ORF1 and ORF2 (the two ORFs on the left are 'read' in the same direction; the two on the right are read in opposite directions). **c**, Redesigned DNA was synthesized and assembled into 100-kilobase fragments in the yeast *Saccharomyces cerevisiae*; fragments were then combined into sections and integrated into the *E. coli* genome. The sections were brought together to generate the complete functional synthetic genome.

E. coli genome by using large-scale DNA-assembly and genome-integration methods that they had developed previously[6] to probe the limits of codon changes in *E. coli*. In their approach (Fig. 1), DNA is computationally designed, chemically synthesized and assembled in 100-kilobase fragments in vectors in *S. cerevisiae*; these vectors are then taken up by *E. coli* and integrated into the genome in the direct place of the equivalent natural region. Iterating this process five times resulted in 500-kilobase sections of DNA being replaced by synthetic versions. Eight strains of *E. coli* were produced in this way, each harbouring synthetic DNA sections that covered a different region of the genome. These sections were then combined using conjugation methods to make the complete synthetic genome.

The large-scale construction was impressively successful, with very low off-target mutation rates, but was not without its challenges. Many genes in the *E. coli* genome partially overlap with others, and in 91 cases the overlapping regions contained codons that needed to be changed. This is complex because synonymous alterations in one protein-coding sequence might alter the amino acids encoded by the overlapping one. To tackle this, the team 'refactored' 79 locations in the genome, duplicating the sequence to separate out overlapped coding sequences into individual recoded ones (Fig. 1). Although this approach was generally successful, it did require careful debugging in a few cases in which refactoring also altered gene regulation.

The final strain proved viable and was able to grow in a range of typical laboratory conditions, albeit a little less vigorously than its natural counterpart. It no longer uses the stop codon TAG or the two serine codons TCG and TCA, so the cellular machinery that recognizes these can now be either deleted or reassigned to recruit 'non-canonical' amino acids beyond the usual 20 used by most living cells. Such recruitment has already been shown to be useful in the 63-codon *E. coli*, both for biotechnology projects, in which non-canonical amino acids are encoded into desired sequence positions to provide residues that can take part in chemical reactions that natural proteins can't; and for biosafety reasons, in that the natural transfer of readable DNA-encoded information in and out of the synthetic *E. coli* is limited because the cell operates with a slightly different genetic code from the rest of the natural world[5]. Expect all of these applications to be expanded in the new 61-codon *E. coli*, which has the potential to encode the use of more than one non-canonical amino acid, and to generate a more stringent genetic firewall (because 3 of the 64 codons are no longer recognized).

Synthesis of a 4-million-base-pair genome and reduction of the genetic code to 61 codons are new records for synthetic genomics, but might not be for much longer. The international Sc2.0 consortium is closing in on synthesizing all 16 chromosomes of the 12-million-base-pair *S. cerevisiae* genome — the first synthetic genome of a eukaryotic organism, the group that includes plants, animals and fungi — and the synthesis of a 57-codon *E. coli* genome is also under way[1,7]. A genome of the bacterium *Salmonella* Typhimurium that has two fewer codons than the natural organism is also being constructed[8]. This could one day enable bacteria with synthetic genomes to be used as cell-based technologies in the human gut.

From a technological standpoint, the most interesting aspect of all these different projects is that the workflows for synthetic-genome construction are remarkably similar, with kilobase sections of synthesized DNA being assembled (by the process of homologous

50 Years Ago

More than a hundred hearts have been transplanted in the 18 months since Dr C. N. Barnard first undertook the operation. The largest single group — fifteen operations — has been performed at the Texas Heart Institute by Dr Denton A. Cooley and his colleagues. The summary of their experience is that … a heart transplant improves the quality of, but does not greatly prolong, the life of the average recipient. But length and quality of survival are directly related to closeness of histocompatibility between donor and recipient, a factor on which future operations should be made to depend. Cooley and colleagues report that the mean survival time of their fifteen transplant patients is 111 days compared with the 74 days lived by patients marked as potential recipients but for whom no donor became available.
From *Nature* 24 May 1969

100 Years Ago

A correspondent forwards us a newspaper cutting from South Africa directing attention to the possibilities of the prickly pear (*Opuntia* spp.) as a source of industrial alcohol … The plant in question covers thousands of acres of good soil in South Africa, and is a pest to farmers … It may be remarked that the question of producing alcohol from the prickly pear has been carefully studied in Australia; the conclusion drawn, however, was unfavourable … Distillation experiments yielded alcohol equivalent to only 0.5 per cent of the weight of the plant used, so that the manufacture was considered unprofitable, and, indeed, scarcely practicable. But the South African prickly pear is said to be much richer in sugar … and this, of course, may make all the difference between success and failure in utilising the plant.
From *Nature* 23 May 1919