nature biotechnology

Unbottling the genes

The ability to plug and play synthetic genes into minimized genomes promises to transform biological engineering.

As the first decade of the 'century of biology' draws to a close, our attempts to engineer novel traits into living cells remain remarkably primitive. Most genetic modification is constrained to tinkering with a handful of genes in a handful of laboratory-adapted natural organisms. We can get cells to express new genes, as long as it's only one or a few genes, and as long as we don't want to control expression too precisely. Try to go much beyond that and our efforts flounder. If biological engineering were aviation, it would be at the birdman stage: some observation and some understanding, but largely naive mimicry. For the field to really take flight, it needs the machinery of synthetic biology.

There are many views on what synthetic biology is, and what it should be, but one aspect that differentiates this field from previous genetic/metabolic engineering is that everything proceeds from the computer: the necessary starting materials are digital code and four bottles of chemicals (A, G, T and C). A DNA synthesizer converts these precursors into oligonucleotide (oligo) sequences *in vitro*. The oligos are assembled into larger pieces (genes, gene circuits and even artificial chromosomes) and, after error checking, plied into use.

The simplest application of this approach, gene synthesis, already thrives commercially. More and more laboratories are requesting genes from oligo companies rather than using laborious recombinant DNA cloning techniques and PCR. Some gene synthesis providers recode sequences to improve protein properties, such as solubility, toxicity, efficiency of translation and ease of purification.

But the approach offers many more possibilities than simply cutting costs and time; it can create artificial products beyond the reach of nature's own evolved art. For example, nuclease-resistant sequences can be fabricated from nucleoside triphosphate analogs (e.g., for aptamers, RNAi or antisense for RNA, and gene therapies for DNA). Similarly, sequences can be redesigned with expanded codon usage that can be read by systems (e.g., *Escherichia coli* or yeast) engineered with corresponding orthogonal suppressor tRNA and aminoacyl-tRNA synthetase pairs to generate proteins containing unnatural amino acids with useful properties.

In more complex manifestations, synthetic biology aims to design gene circuits analogous to electrical components and circuits. Over the past decade, such efforts have led to all manner of synthetic gene switches, oscillators, digital logic evaluators, filters, sensors and communicators (see p. 1139). Even though these circuits remain relatively crude, falling short of the most complex gene manipulation achieved using traditional metabolic engineering (e.g., Jay Keasling's *tour de force* engineering of the pathway for precursors of the malaria drug artemisinin), they ultimately promise exquisite control of outputs. Rather than constructing the biological equivalent of a radio that receives one station and outputs at a set volume, engineers will be able to spin the dial and choose a listening comfort zone. Responsive elements will allow biological devices to adapt to their environment, a useful characteristic in, say, synthetic islet cells or gene therapy systems. Cell systems could even be designed to learn.

For synthetic biology to truly transcend the current limitations, however, it will be necessary to move away from laboratory-adapted versions of 'natural' organisms—organisms that bring with them the genetic and metabolic 'baggage' of millions of years of evolution. This baggage has been essential in the face of environmental instability and insult, reproductive fitness, invaders and predators, and the other rigors of survival. But it is redundant in the context of the constant, cosseted, aseptic and substrate-rich environments of man-made production systems. Natural organisms' metabolic flexibility almost invariably limits their metabolic flux in culture and thus industrial productivity. The ideal industrial bug is not a utility player but an extreme specialist honed to metabolic perfection.

This is where another concept in the field—the minimal genome capable of supporting a self-replicating organism—becomes important. Theoretically, an organism with a genome stripped of superfluous functions that drain away carbon, nitrogen or energy could serve as a 'shell' or 'chassis' into which interchangeable cassettes of genes encoding traits of interest could be placed.

Chassis organisms can be generated by serially deleting parts of an existing organism's genome or identifying nonessential genes and then synthesizing/assembling a minimized artificial chromosome from scratch (see p. 1121). The final step of 'rebooting' the synthetic minimal genome has not yet been attained, but we may not be so far from the goal of creating a 'chassis' organism—the blank canvas onto which the bold and efficient metabolic brushstrokes of synthetic biology can be made.

By applying the principles of engineering to living systems and allowing us to move away from mimicry and optimization of natural cells, synthetic biology thus opens up the possibility of design in completely artificial systems. One day, these systems may provide insights into existing living organisms—life as it already is—enhancing our understanding of basic biology and disease. But that goal still seems some way off.

In contrast, it is not too hard to imagine a future where, with relatively little effort, we can create alternative life forms—minimal-genome chassis organisms with interchangeable standardized gene circuits—that will enable genetic engineers to rapidly move from one industrial project to another. The technology is disruptive, with the potential to transform biological engineering, which until now has been limited to tinkering with natural organisms, and relies on a good deal of serendipity for success.

At the turn of the last century, the Wright brothers achieved manned flight not by mimicking natural systems, but by applying the principles of engineering and aerodynamics. Similarly, synthetic biology allows us to dispense with biological mimicry and design life forms uniquely tailored to our needs. In doing so, it will offer not only fundamental insights into questions of life and vitality but also the type of exquisite precision and efficiency in creating complex traits that genetic engineers could previously only dream of.