De novo synthesis of five yeast chromosomes

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"What I cannot create, I do not understand."

This sentence taken from Richard Feynman's board at the time of his death essentially captures, if applied to a biological frame, the holy grail of synthetic biology; the understanding of life to such an extent that living systems can be rationally designed to gain and perform specified functions. Synthetic biology is perceived by many as an extension of metabolic engineering, where organisms are modified with end goal to produce a product. This is however part of the story, and the real strength of synthetic biology is the understanding via synthesis, the decomposition of a system to its building blocks and recombination of parts from the bottom up to elucidate the biological complexity.

The construction of a bacterial minimal genome, namely the reduction of the genetic content of Mycoplasma genitalium to 473 genes, was hailed as a landmark about a year ago. Last week, a series of seven research articles were published, describing the advancements in the construction of yeast synthetic chromosomes—and as the articles were published in *Science*, I use this journal's citation format throughout this blog.

This work is a result of a large consortium, Synthetic Yeast 2.0 , which aims to design and implement a fully synthetic genome of Saccharomyces cerevisiae. The project has existed for several years, and started with the construction of a chromosome's arms, and the first reported synthetic yeast chromosome. The recent publications report the synthesis of five more yeast chromosomes, totaling the synthetic sequences to more than one third of the full genome. The workflow started from the generation of 700-2000 bp DNA blocks, which are in vitro or in yeast assembled into 10 kb 'chunks'. A number of chunks are chemically ligated to 30-60 kb 'megachunks', which are inserted sequentially into the genome.

The new chromosomes contain slight modifications from the native ones. The tRNA genes were removed from their original loci, and relocated to a specialized neochromosome. Repeating sequences were also removed, the TAG stop codon was replaced with TAA—leaving TAG open for repurposing, such as incorporation of a non-canonical amino acid. The design took place in BioStudio, an open-source computational framework that allows working in genome-scale.

Maybe the most important intervention is the inclusion of recombination sites every 10 kb and after every non-essential open reading frame. Consequently, one can implement SCRaMbLE (synthetic chromosome rearrangement and modification by loxP-mediated evolution) and produce numerous strains with rearranged genetic loci, thus acquiring a powerful tool for directed evolution and functional analysis experiments.

What comes next? According to Jeff Boeke, the consortium expects to have a fully synthesized genome by the end of the year. However, having obtained all synthetic chromosomes does not automatically mean that a fully 'synthetic' yeast strain will become immediately available; although strains incorporating one heterologous chromosome do not display a particular phenotype, a strain with three of its chromosomes replaced had a growth defect. Issues arising from chromosome interactions and telomere function already show, while more unknown challenges are sure to appear.

The long-term goal of this colossal undertaking is to allow research labs and organizations to routinely use custom-made organisms. This dream seems to come closer and closer, but I do not think it will happen within the next few years. The cost for synthesizing the whole S. cerevisiae genome is estimated to 1-1.5 M dollars, without taking into account salaries and maintenance. But I believe that the scientific insights obtained by this project will affect synthetic biology in multiple ways. And then, why not expand to more organisms? The recent proposal to synthesize a human genome gained a lot of publicity (and controversy). A minimal or synthetic plant genome [see Marchantia polymorpha, a small liverwort, and Physcomitrella patens, a moss where homologous recombination happens with the same efficiency as in yeast] will be leap forwards in understanding and harnessing photosynthesis…

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