A spring collection of designer yeast chromosomes

Synthetic biology has made progress in creating synthetic replicas of prokaryotic genomes. Now it is moving on to eukaryotes. In a set of seven papers published on March 10 in *Science*, members of the international Sc2.0 Consortium provide an update on the effort to redesign and synthesize the genome of the baker's yeast *Saccharomyces cerevisiae*. Working in 'design-build-assemble-test-learn' cycles, the scientists constructed five synthetic chromosomes and made progress toward creating 'designer' organisms that could be useful to basic scientists and engineers alike.

The first *de novo*–synthesized viral and prokaryotic genomes were modified from reference sequences to tackle questions about the identity of the essential genes and regulatory regions, and the character or the overall amount of modifications an organism can tolerate. Similar goals have guided the Sc2.0 project. The scientists reported the creation of the first synthetic yeast chromosome arm in 2011 and the first fully synthetic chromosome in 2014.

The first paper by Richardson *et al.*[1] outlines the main design principles adopted by the consortium to engineer designer chromosomes. The yeast genome is simplified by deleting most of the known non-essential features (including repetitive sequences, introns and transposons) and recoding all the TAG stop codons to TAA. Selected open reading frames are also recoded to create synonymous sequences unique to the synthetic genome and distinct from the wild type. The resultant designer chromosomes are also seeded with recombination sites, providing a means for further controlled rearrangement of the synthetic genome.

The process relies on the combination of synthesized oligonucleotides into ~750-bp "building blocks" or 2- to 4-kb "mini-chunks," which are then assembled into 10-kb "chunks," followed by further assembly to create 30- to 60-kb synthetic "megachunks," which are inserted sequentially *in vivo* into the yeast chromosome of interest and validated by PCR or sequencing. By testing the fitness of the resultant semi-synthetic yeast under numerous culture conditions and then sequentially targeting an adjacent part of the chromosome for incorporation of the next megachunk, the researchers recoded the entire chromosome. In addition to the previously published artificial chromosomes III and IXR (a semisynthetic version of chromosome IX in which the right arm is synthetic), the new publications report artificial chromosomes II, V, VI, X and XII[2–6]. Ultimately, the plan is to combine all of the Sc2.0 chromosomes in the same strain by a process termed endoreduplication intercross, which is described in ref. 1.

The construction of each synthetic chromosome provided immediate insights into genome biology. In some instances, incorporation of a new megachunk resulted in loss of strain viability or fitness. In most such cases, after identifying the culprit locus, the researchers reverted the synthetic sequence (and the strain's fitness) back to the wild type, noting what they've learned. For example, the designed universal telomere caps on chromosomes V and VI proved insufficient to protect the adjacent genes from silencing[2,3]. "Biology always has ways of surprising us, so I'm eager to see what this work will reveal that we might not have anticipated about chromosomal function," says Annie Tsong of Amyris (Emeryville, CA, USA). "The patterns that emerge can be used to help us learn about the natural organism, too."

Other instances revealed the practical potential of Sc2.0. During engineering of synthetic chromosome II, a recoded 3-ketosphinganine reductase gene (encoding a key enzyme in sphingolipid synthesis) caused a defect in growth on glycerol at 37 °C[4]. After rearrangement of the synthetic chromosome via transient expression of Cre recombinase, analysis of new colonies capable of growth in glycerol revealed that rescue of phenotype was achieved, not through restoration of function in the synthetic 3-ketosphinganine reductase gene, but rather by altered expression of regulatory proteins associated with the same pathway.

The project also provides a path to a new engineerable yeast chassis. "Engineers like the idea of a clean chassis, a frame on which we can build our own designs," says Peter Carr of the Massachusetts Institute of Technology's Lincoln Laboratory (Lexington, MA, USA). He thinks that pruning the genome, deleting features that can cause unpredictable behavior may produce such a platform, adding, "Of all these whole-genome engineering projects so far, yeast is arguably the most useful organism." Tsong sees this work as an experiment to evaluate the yeast genome's hidden functionalities, which can help establish what will actually make a more stable organism.

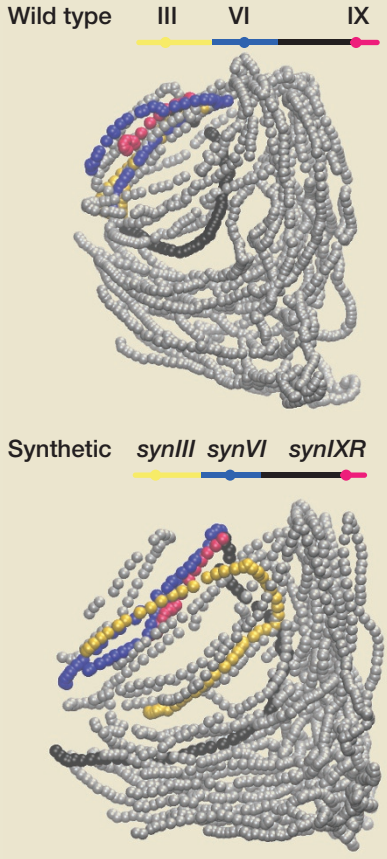

Image adapted with permission from ref. 7.

Currently a synthetic equivalent of a third of the yeast genome exists, so the Sc2.0 project still has some way to go. The consortium hopes to synthesize *de novo* all 16 yeast chromosomes by the end of 2017. "They've done the first step," points out Tsong. "There is a technology now to iterate."

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1. Richardson, S.M. *et al. Science* **355**, 1040–1044 (2017).
2. Mitchell, L.A. *et al. Science* **355**, eaaf4831 (2017).
3. Xie, Z.-X. *et al. Science* **355**, eaaf4704 (2017).
4. Shen, Y. *et al. Science* **355**, eaaf4791 (2017).
5. Wu, Y. *et al. Science* **355**, eaaf4706 (2017).
6. Zhang, W. *et al. Science* **355**, eaaf3981 (2017).
7. Mercy, G. *et al. Science* **355**, eaaf4597 (2017).