Nature Reviews Genetics | Published online 12 Apr 2017; doi:10.1038/nrg.2017.30

SYNTHETIC BIOLOGY

Building a custom eukaryotic genome *de novo*

The Synthetic Yeast Project (Sc2.0) aims to create the first synthetic eukaryotic genome. It is based on synthesizing, from scratch, a reworked *Saccharomyces cerevisiae* genome that is optimized for genomic stability and includes various design features to make it an easily engineerable chassis for future applications. Following the synthesis of the first complete Sc2.0 chromosome in 2014, several new papers in *Science* describe the design of the complete Sc2.0 genome and the synthesis and characterization of five additional complete chromosomes, representing more than one-third of the entire genome.

In their overview paper, Richardson *et al.* outline the key design features of the Sc2.0 genome and the synthesis strategies. Relative to a wild-type *S. cerevisiae* genome the Sc2.0 genome is streamlined for genomic stability by removal of various classes of repetitive element and many introns. Other features have been recoded or added: all TAG stop codons are changed to TAA (to enable TAG codons to be added and repurposed in future, such as coding for custom amino acids); restriction enzyme sites are altered to facilitate genome assembly; stretches of synonymous (amino-acid conserving) changes are made, which serve as 'PCRTags' to distinguish synthetic from wild-type sequences; and *loxPsym* sites are introduced to enable intentional genome rearrangements by the inducible SCRaMbLE system.

Based on this *in silico* genome design, the practical task of synthesizing each chromosome was distributed across different institutions. Five of the new papers detail the complete assembly of individual chromosomes: Shen *et al.* for chromosome II, Xie *et al.* for chromosome V, Mitchell *et al.* for chromosome VI, Wu *et al.* for chromosome X and Zhang *et al.* for chromosome XII.

Although each team used minor variations in strategy, all teams followed the following general scheme. Initial *de novo* synthesized oligonucleotides of ~750 bp were hierarchically assembled *in vitro*, culminating in 'megachunks' of 30–50 kb. These megachunks were then introduced sequentially into yeast, where they segmentally replaced the wild-type chromosome sequence in a process called SwAP-In, which involves the endogenous homologous recombination machinery and selection on the basis of two alternating selectable markers. Replacement of the entire wild-type chromosome was achieved either completely by end-to-end SwAP-In or by using meiotic recombination to combine multi-megachunk chromosome segments.

All teams used genome sequencing and various phenotypic analyses (such as fitness assays, RNA sequencing and proteomics) to monitor the strains following SwAP-IN steps and the final strains harbouring a fully synthetic chromosome. The aim is for the synthetic chromosomes to be near-identical in sequence to the *in silico* design but to closely match the molecular and organismal phenotypes of wild-type *S. cerevisiae*.

Genome sequencing identified various types of unintended mutation in the synthetic strains; these were corrected if they were likely to be detrimental based on their size, location or phenotypic outcome, or left uncorrected if probably inconsequential. More interesting were the designed genome alterations that caused unwanted or unexpected phenotypic consequences, as these are informative about aspects of genome function, regulation and flexibility. For example, some synonymous PCRTags in genes led to fitness defects through diverse mechanisms, including altered transcription factor binding and reduced translation efficiency. Thus, although synonymous mutations are commonly known as 'silent' mutations, they can have numerous functional effects. Beyond PCRTags, Xie *et al.* and Mitchell *et al.* showed that telomere-proximal genes on synthetic chromosomes V and VI deviated from wild-type expression levels, indicating that the designed universal telomere cap might not confer the same gene regulatory properties as the native telomeric repeats.

Despite these challenges, a key strength of the modular nature of SwAP-In is that strains showing defects following a SwAP-In step can be 'debugged' by mapping the defect, then replacing or editing the problematic megachunk.

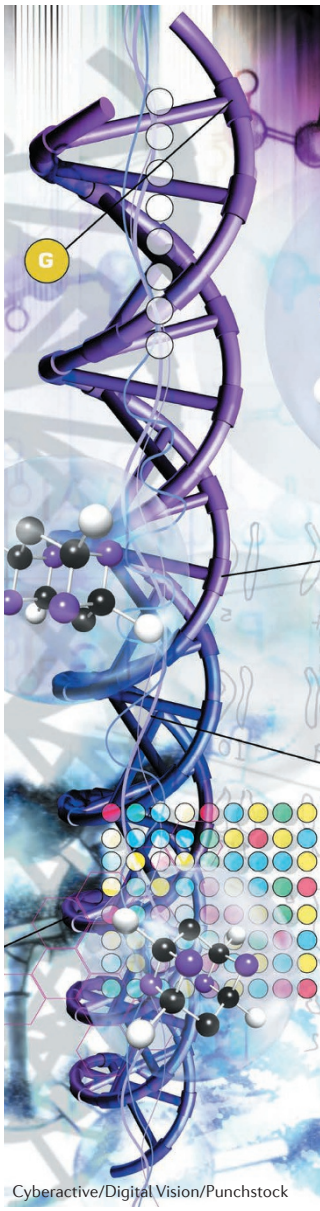
In a related study, Mercy *et al.* used Hi-C to analyse the 3D conformations of the synthetic chromosomes reported in the other papers, finding that the synthetic chromosomes largely matched wild-type chromosomes in intrachromosomal interactions, unless particular elements such as mating-type cassettes or rDNA repeats were intentionally moved to non-native locations.

Overall, following debugging, all resultant strains harbouring single synthetic chromosomes displayed near-wild-type phenotypes. As a step towards complete genome replacement, Mitchell *et al.* used an endoreduplication intercross to combine up to three synthetic chromosomes in a single strain. Although largely healthy, the triple-synthetic strain had mildly slower proliferation than strains containing pairs of synthetic chromosomes.

It will be interesting to see further progress towards the ultimate goal of fully synthetic yeast, including monitoring the fitness, molecular phenotypes and applications of the increasingly synthetic strains.

Darren J. Burgess

ORIGINAL ARTICLES Richardson, S. M. *et al.* Design of a synthetic yeast genome. *Science* **355**, 1040–1044 (2017) | Shen, Y. *et al.* Deep functional analysis of synII, a 770-kilobase synthetic yeast chromosome. *Science* **355**, eaaf4791 (2017) | Xie, Z. X. *et al.* "Perfect" designer chromosome V and behavior of a ring derivative. *Science* **355**, eaaf4704 (2017) | Mitchell, L. A. *et al.* Synthesis, debugging, and effects of synthetic chromosome consolidation: synVI and beyond. *Science* **355**, eaaf4831 (2017) | Wu, Y. *et al.* Bug mapping and fitness testing of chemically synthesized chromosome X. *Science* **355**, eaaf4706 (2017) | Zhang, W. *et al.* Engineering the ribosomal DNA in a megabase synthetic chromosome. *Science* **355**, eaaf3981 (2017) | Mercy, G. *et al.* 3D organization of synthetic and scrambled chromosomes. *Science* **355**, eaaf4597 (2017)



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