SYNTHETIC BIOLOGY

Yeast 2.0

Researchers synthesize chromosome arms to facilitate genetic studies in yeast.

Every geneticist has dreamed of the perfect genome. For many, the baker's yeast genome comes close, being compact and easy to manipulate. But a team led by Jef Boeke at Johns Hopkins University wants to make the microscopic fungus even better by giving it a genomic makeover with designer chromosomes.

The ambitious plan, which they have dubbed the Synthetic Yeast Genome Project or Sc2.0, is a collaboration with fellow Johns Hopkins University researchers Joel Bader and Srinivasan Chandrasegaran. The group recently reported the replacement of one 90-kilobase arm of chromosome IX (synIXR) and a 30-kilobase piece of chromosome VI (semi-synVIL); the ultimate goal is a fully synthetic streamlined genome that will aid experimentation. "We're already well on our way," says Boeke. "We have about 10% of the genome in pieces of various sizes."

Although this work used commercial synthesis for synIXR, that is too expensive for an entire genome. The scale has forced the participants to be creative, turning to undergraduates to drive progress in a 'build-a-genome' course they have offered for the past four years. Students learn synthetic biology and collaborate to produce ~750-base-pair 'building blocks' that are combined using native or synthetic rare restriction sites into 10-kilobase stretches. "This has turned out to be just a fantastic thing for the students," says Boeke. "They love it because they're actually doing something meaningful in a laboratory course environment."

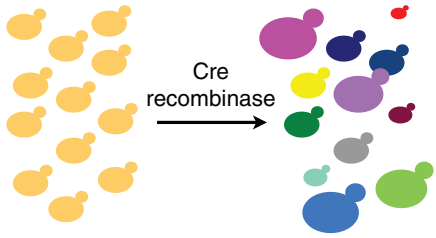
The building blocks can be assembled by designing oligos with complementary overlaps that are joined by PCR. Larger linear pieces can replace chromosome fragments using the yeast's natural ability to undergo homologous recombination. The authors introduced semi-synVIL this way, though they transformed synIXR as an artificial chromosome into a strain from which they deleted native chromosome IXR.

Engineering a useful organism from the bottom up requires strict design principles. They distilled three principles, starting with the rule that synthetic yeast should look and behave like fit wild-type strains. "We sometimes jokingly call that principle 'do no harm,'" says Boeke. The team assessed fitness by measuring colony size and morphology under different conditions and profiling gene expression. Only a handful of genes deviated from normal expression, despite changes every 500 base pairs or so in the synIXR sequence.

The second design principle stipulated that the genome should be stable and streamlined. Stability is a useful property for biotechnology. The group purged synthetic chromosomes of less stable repetitive sequences, including transposable elements and subterminal telomere repeats believed to be nonfunctional. They also moved all the tRNA genes, known hotspots of instability, to an artificial chromosome. "This is riskier in terms of the first design principle," notes Boeke, but adds that it has had little effect on yeast function so far.

What excites the researchers most is the third principle, encoding flexibility for genetic studies. "We don't just want to build a stripped down version of wild type but something that we can learn from," explains Boeke. For example, the team engineered TAG stop codon replacements with TAA, freeing up a tRNA that could encode a synthetic amino acid. Key to their strategy is the incorporation of symmetrical *loxP* sites next to nearly every gene. They show that these sites have an equal chance of creating deletions or inversions in the presence of an inducible Cre recombinase. This synthetic chromosome rearrangement and modification (SCRaMbLE) method yields a mutational spectrum from point deletions to large scale deletions and inversions.

To ease analysis, they monitor genome alterations via designer PCRTags: silent changes in exon sequence that allow gene-specific amplification for very rapid distinction between wild-type and synthetic sequence.



Expressing Cre recombinase in engineered yeast produces genomic and phenotypic diversity. Image courtesy of J. Boeke.

SCRaMbLE also provides a randomized way to streamline the genome by iteratively testing for fitness in strains that have gene deletions. "This type of genome minimization doesn't happen all at once," stresses Boeke. "Depending on which genes get deleted first, the organism is going to take a different trajectory to a minimal genome." The approach allows researches to rewind to a high fitness point. They hope to study possible routes that approach the basic toolset needed for eukaryotic life, what he calls the 'universe of minimal genomes'.

The researchers are constantly adding new innovations. "What we're moving toward now is reconfiguring the system to produce duplications because that's going to be a more effective system for generating gain-of-function phenotypes," says Boeke. Ultimately, the Sc2.0 yeast should greatly facilitate studies of genome evolution and structure, down to a quantitative analysis of what changes drive speciation. This is not your grandmother's baker's yeast.

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RESEARCH PAPERS

Dymond, J.S. *et al.* Synthetic chromosome arms function in yeast and generate phenotypic diversity by design. *Nature* **477**, 471–476 (2011).