'Synthetic' chromosome permits repid, on-demand 'evolution' of yeast

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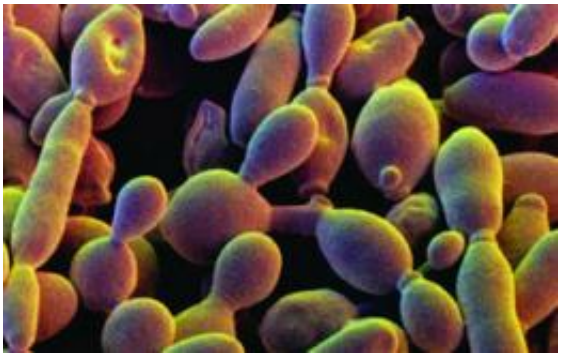

Frequently described as bow-tie-shaped, a yeast chromosome has two arms that are positioned similarly to the two sides of a bow-tie. Credit: Jef D. Boeke and Sarah Richardson, Johns Hopkins University

In the quest to understand genomes -- how they're built, how they're organized and what makes them work -- a team of Johns Hopkins researchers has engineered from scratch a computer-designed yeast chromosome and incorporated into their creation a new system that lets scientists intentionally rearrange the yeast's genetic material. A report of their work appears September 14 as an Advance Online Publication in the journal *Nature*.

"We have created a research tool that not only lets us learn more about yeast biology and genome biology, but also holds out the possibility of someday designing genomes for specific purposes, like making new

vaccines or medications," says Jef D. Boeke, Ph.D., Sc.D., professor of molecular biology and genetics, and director of the High Throughput Biology Center at the Johns Hopkins University School of Medicine.

Boeke notes that yeast is probably the best-studied organism with a nucleus on the planet and is "already used for everything from medicine to biofuel," making it a good candidate for his team's focus.

In designing the synthetic yeast chromosome, Boeke says, the goal was to make it maximally useful to researchers by laying down some ground rules: First, the product could not compromise yeast survival; second, it must be as streamlined as possible; and third, it had to contain the capacity for genetic flexibility and change.

Using the already known full genetic code—or DNA sequences—of the yeast genome as a starting point, Johns Hopkins graduate student Sarah Richardson wrote a software program for making a series of systematic changes to the DNA sequence. The changes were planned to subtly change the code and remove some of the repetitive and less used regions of DNA between genes, and to generate a mutated "version 2.0" of a yeast cell's original 9R chromosome. The smallest chromosome arm in the yeast genome, 9R contains about 100,000 base pairs of DNA and represents about one percent of the single-celled organism's genome.

Building the actual chromosome started with stringing individual bases of DNA together that were then assembled into longer segments. Large segments of about 10,000 base pairs were finally put into live yeast cells and essentially swapped for the native counterpart in the chromosome, a process for which yeast are naturally adept. In addition to 9R, the team also made a smaller piece of the chromosome 6L. Yeast cells containing the synthetic chromosomes were tested for their ability to grow on different nutrients and in different conditions, and in each case came out indistinguishable from natural yeast.

The Hopkins teams says what distinguishes this constructed chromosome from the native version — and sets it apart from other synthetic genome projects — is an "inducible evolution system" called SCRaMbLE, short for Synthetic Chromosome Rearrangement and Modification by Lox-P mediated Evolution.

"We developed SCRaMbLE to enable us to pull a mutation trigger — essentially causing the synthetic chromosome to rearrange itself and introducing changes similar to what might happen during evolution, but without the long wait," explains Boeke. Why build in the scrambling system? To change multiple things at once, says Boeke, which is anathema among experimental scientists who traditionally change only one variable at a time, Nature is never that well controlled, he says.

The team activated SCRaMbLE in yeast containing both the synthetic 9R and 6L chromosomes, then analyzed the DNA from the yeast cells. Testing this population of SCRaMbLEd yeast fed various nutrients they found some grew fast, some grew slowly and others really slowly, and some of the fast-growing ones had very specific defects resulting from specific gene loss, showing that SCRaMbLE does indeed introduce random variation. When the team analyzed the molecular structure of the synthetic 9R and 6L chromosomes from this SCRaMbLEd population, they found chromosomes with small deletions, rearrangements, and other alterations, at wildly varying locations.

"If you think of the yeast genome as a deck of cards, we now have a system by which we can shuffle it and/or remove different combinations of 5000 of those cards to get lots of different decks from the same starter deck," Boeke says. "While one derivative deck might yield good hands for poker, another might be better suited for pinochle. By shuffling the DNA according to our specifications, we hope to be able to custom design organisms that perhaps will grow better in adverse environments, or maybe make one percent more ethanol than native

yeast."

Boeke says the 9R and 6L experiments are "the beginning of a big project, whose ultimate goal is to synthesize the whole yeast genome (about 6000 genes) and SCRaMbLE the 5000 likely to be individually dispensable. And he wants to make the tool available to anyone who wants to use it, without intellectual property protection.

More information: Sc2.0 project: www.syntheticyeast.org

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