When geneticist Ronald Davis first suggested a decade ago that his colleagues try to create artificial yeast chromosomes and install them in a living cell, Jef Boeke didn’t think much of the idea. Davis, who is at the Stanford University School of Medicine in California, was known as a visionary. He proposed that a lab-made yeast would be the next step in the then-emerging field of synthetic biology. But Boeke couldn’t see the point of replicating what nature had already made, especially because designing and synthesizing a 12.5-million-base genome seemed onerous, or even impossible. As Boeke listened to Davis’ s talk at a major yeast genetics meeting in 2004 in Seattle, he says, “I remember thinking ‘Why on Earth would you want to do that?’”

How times have changed. Boeke, a geneticist who recently moved to New York University Langone Medical Center in New York City, and his colleagues have just finished the first complete synthetic yeast chromosome and are well on their way to putting together several more, thanks to technological advances in manufacturing DNA and a global army of collaborators, mainly undergraduate students.

Other researchers have synthesized a bacterium’s full genome, but the yeast job is orders of magnitude more complex. For starters, *Saccharomyces cerevisiae* has 16 chromosomes compared with the one in bacteria. Yet if the effort by Boeke and his army succeeds, it should offer broad benefits. “It gives us the ability to fully explore the yeast genome,” Davis says. “If you really want to understand an organism, you should be able to design or redesign one.”

Commonly called baker’s, brewer’s, or budding yeast, *S. cerevisiae* is vital for many food and drink industries, from beer to bread. Genetic engineers have already tweaked it in myriad ways for many uses, such as making ethanol. Recently it’s been put to work building an antimalarial drug, artemisinin, and its potential for churning out other key chemicals is slowly being realized.

Yeast is also a workhorse for biologists probing basic cellular and metabolic processes in eukaryotes. Back in 1996, it became the first eukaryote to have its genome deciphered, and since then yeast geneticists have knocked out every gene and done analyses of all the interactions among them and their encoded proteins. For a long time, it was the only organism in which biologists could readily mutate specific DNA bases, as they found it easily incorporates foreign DNA through a process called homologous recombination.

The synthetic genome under construction by Boeke’s army will be the ultimate modification. When it’s done, Sc2.0, as some call it, will not be just any ordinary yeast strain. In designing Sc2.0, Boeke and his colleagues streamlined the typical yeast genome and built in sites that will make it possible to reshuffle the genome at will, potentially yielding more desirable, properties and helping biologists figure out what each gene does. The endeavor “is bold, imaginative, and is going...
to teach us a lot about what the constraints are for synthesizing a whole genome and what constraints there might be relative to genes and chromosomes,” says Jasper Rine, a yeast geneticist at the University of California, Berkeley. “It will definitely serve as a landmark in the development of synthetic biology,” adds Chantal Shen, a collaborator at BGI in Shenzhen, China.

Until recently, however, no one knew if a truly synthetic chromosome could even sustain eukaryotic life. And when Boeke first attempted to construct a small chunk of yeast chromosome 9—a mere 90,000 bases—few had tried to work with a piece of DNA that big. During the project’s earliest days, the effort threatened to fizzle out.

But companies are now able to make ever bigger pieces of DNA, and labs from several different countries are now sharing the labor of synthesizing yeast chromosomes. Those developments, on top of his most recent success, make Boeke hopeful that live yeast containing the whole synthetic genome will be replicating in his lab within 4 years. “We are making history,” says Sc2.0 collaborator Junbiao Dai, a molecular biologist from Tsinghua University in Beijing.

**Rough beginning**

Boeke traces his change of heart about the synthetic yeast project to 2006, while he was still at Johns Hopkins University in Baltimore, Maryland. Over coffee, his Johns Hopkins colleague Srinivasan Chandrasegaran was trying to convince him to make a large number of zinc-finger nucleases. These DNA-modifying enzymes are Chandrasegaran’s specialty, and a toolkit full of them would make the yeast genome easier to manipulate. Boeke wasn’t that interested and, almost as a joke, suggested a more drastic way to take control of the yeast genome: synthesize the whole thing. To his surprise, Chandrasegaran jumped on the idea, and the pair brought Joel Bader, a computer scientist at Johns Hopkins, into the discussion.

Despite Boeke’s dismissal of Davis’s proposal just 2 years earlier, the trio quickly concluded that making an artificial yeast genome would, in fact, be worthwhile if it could be a testbed for learning about the genome itself. They decided to start with the 90,000-base “R” arm of chromosome 9—the shortest in the yeast’s genome—and spent a year arguing about how the synthetic sequence should differ from the natural one. The trio considered just including the genes they wanted, “but we quickly realized it would be very risky to eliminate whole bunches of genes” without really knowing in advance what the effect of that loss on the whole system would be, Boeke recalls.

So they started with the natural sequence of the chromosome 9 arm and instead added DNA to it that would enable them to induce changes at will. They inserted short sequences of DNA called loxP just after the end of each nonessential gene—those they knew could be knocked out or changed without killing the yeast. They also put loxP sites near significant landmarks, such as the telomeres at the tips of chromosomes and the centromeres at each one’s center. LoxP is a part of a standard molecular biology tool. When activated by a chemical added to cells, it kicks off a chromosomal version of musical chairs. The result: genomic rearrangements and new yeast strains with different properties. Boeke and his colleagues called this system SCRAMbLe, for synthetic chromosome rearrangement and modification by loxP-mediated evolution.

Although Boeke’s team wanted to make the genome unstable when they desired, they didn’t want the genome to undergo changes or rearrangements of its own accord, potentially disrupting the integrity of the synthetic strain. To increase the genome’s stability, they took out mobile DNA elements, such as retrotransposons, that in theory could jump to new spots at any time.

The design they pioneered with the chromosome 9 arm cuts out other noncoding DNA as well. The natural telomerases, the repetitive regions next to the ends of chromosomes that can also be unstable, are now gone. Shorter synthetic ones will cap each chromosome. The researchers also took out many of the introns, the noncoding sequences between coding regions of a gene.

The quest for stability also prompted some radical steps. The team is taking out the DNA coding for the yeast’s 275 transfer RNAs, which shuttle amino acids to the ribosome for stitching together into a protein. The transfer RNA genes are essential, but because their sequences can sometimes cause a cell’s DNA copying machinery to stall out, they are “DNA damage hotspots,” says Patrick Yizhi Cai of the University of Edinburgh in the United Kingdom. Cai is building a “neochromosome” that will have all those genes relocated onto it. Collected into one place, these genes will still be available to the modified organism, but will do less damage if they become unstable. (The neochromosome will not increase the total number of chromosomes in Sc2.0, because Boeke plans to do away with chromosome 1. It naturally has just 230,000 bases, and given the design rules of Sc2.0, its synthetic counterpart would be as much as 70,000 bases shorter. Worried that this diminished chromosome would be unstable, the team plans to append that DNA to another synthetic chromosome.)

Boeke and his colleagues added two more modifications to the design. Throughout the genome, they are inserting short, specific DNA sequences, detectable by the polymerase chain reaction, that distinguish each synthetic chromosome segment from its natural counterpart. Finally, they tinkered with some of the natural stop signs in eukaryotic genomes—the “stop” codons that tell a cell when to cease making an RNA. On the chromosome 9 arm, the researchers turned every single instance of one stop codon, “TAG,” into another, “TAA,” by switching in the base adenine (A) for a guanine (G). In the complete synthetic genome, they will make more than 1000 such substitutions all together. The “stop” sign is therefore still there, but in a different form. But this frees up “TAG” as a codon for an artificial amino acid, if they ever decide to introduce one into the makeup of their enhanced yeast.

With the aid of a software program developed by computer scientist Bader, Boeke designed a version of the chromosome 9 arm incorporating all of these changes and carefully checked as much as possible that the added bases would not interfere with the expression of any remaining yeast genes. He then contracted with a biotech company called Codon Devices to synthesize the chromosomal arm.

Eleven months went by with no word from the company, which had never attempted to make such a long piece of DNA. “That was a tense time,” Boeke recalls.

But that dark period proved inspirational as well. Boeke wondered whether he could speed up and decrease the cost of the work, as well as help others learn molecular biology,
if he set up a course dedicated to building a synthetic yeast genome (see sidebar, p. 1429). He launched the idea as a summer school offering in 2007, even before he knew that the 9R synthetic arm he had ordered would work. Now, 6 years later, the thrice-weekly course at Hopkins is packed, even on Friday nights. “We were overwhelmed with interest” from biology, engineering, computer science, public health, and other majors, says Boeke, who, despite his move to New York, will keep the Hopkins course going with colleagues there.

Scrambled DNA
Once Codon Devices finally delivered a 90,000-base circular chromosome, it took Boeke and colleagues months more to successfully stick it into the yeast, cut out the natural 9R, and then test the effects. The synthetic chromosome arm performed without a hitch, yielding healthy yeast and reasonable gene expression, Boeke’s team reported in 2011 in *Nature*.

The SCRaMBLE system worked as well. The yeast carry a piece of DNA that codes for a highly modified version of an enzyme called Cre recombinase, which randomly deletes or inverts the DNA lying between any pair of loxP sites. This enzyme is usually stuck in an unfolded form in the cell’s cytoplasm, but when the researchers add the chemical estradiol to yeast, it folds up and can sneak into the nucleus. LoxP activation leads to the random removal of different genes, sometimes killing the yeast but other times simply changing the yeast’s properties, rendering it incapable of making certain amino acids, for example. “We knew our design had panned out,” Boeke says. “Our hope then was to scale up.”

Meanwhile, the students from that initial summer course and subsequent ones had taken on chromosome 3. From an initial class goal of 1500 bases per student, productivity had ramped up the point where each student promised to turn in at least 30,000 bases by the semester’s end. It took 49 students and 1.5 years to make the synthetic chromosome’s 272,871 bases—the native version has 316,667 bases—but under the guidance of Boeke’s postdocs Narayana Annaluru and Hélène Muller, their effort has paid off with a publication online this week in *Science* (http://sicom.ag/NAnnaluru). Yeast carrying the shortened, modified chromosome grew normally and looked little different from their natural counterparts under almost all the growing conditions tested, the researchers report.

“They made some pretty dramatic changes,” says Mike Tyers, a yeast systems biologist at the University of Montreal in Canada. “But on the other hand, they were conservative enough that the experiment would have a chance of working. I think they hit the sweet spot.”

As Boeke and his colleagues entered the homestretch with chromosome 3 and began to work on other yeast chromosomes, a chance meeting between Cai, then Boeke’s postdoc, and Ying-Jin Yuan of Tianjin University in China at a synthetic biology competition led to the globalization of the effort. “An international project wasn’t on our mind at all,” Boeke recalls. Yet Yuan was so excited about Sc2.0 that he got Huaming Yang of the sequencing powerhouse BGI involved. Yang helped organize the first synthetic yeast genome meeting, held in Beijing in 2012, and partnerships emerged. Yuan set up his own “Build A Genome” course and in the summer of 2012, 60 Chinese students assembled all the bases needed for chromosome 5.

Momentum built elsewhere as well. Tom Ellis, a synthetic biologist at Imperial College London, became hooked on the ambitious venture after attending the Beijing meeting. He helped organize a second Sc2.0 meeting in July 2013, following a larger synthetic biology conference at which the British government announced it would commit £1 million toward the synthetic yeast genome project. Other countries are getting involved as well, Boeke says (see chart).

The hope is that in 2 years, the various partners will have stitched together each of the

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**Genome remake.** The successful reinvention of yeast chromosome 3 involved the removal of many elements and multiple additions to its DNA (upper diagram). This chromosome now serves as a model for the international effort to synthesize all the other chromosomes (lower chart).
Custom yeast

Once the synthetic organism is in hand, yeast biologists will be eager to tinker with it. With 5000 loxP sites, the synthetic genome will be ripe for mutations, opening the way for researchers to take a systems approach toward understanding which genes matter when during the yeast life cycle.

The longer they expose a yeast strain to estradiol, the greater the number of genetic changes that the SCRaMBLE system will induce. Leslie Mitchell, a postdoc in Boeke’s lab, plans to grow the mutated strains under different conditions, document their behavior and other characteristics, and sequence their genomes. From the data, she hopes to piece together how different genes interact.

SCRaMBLE should also make it easier to harness yeast for biotechnology. Right now, finding a yeast strain that makes enough of a product to be commercially viable “takes many steps and many person years,” Boeke says. SCRaMBLE should speed that search up by providing an “unbelievable number of mutants that would be much more time-consuming to get one at a time.” With more strains to evaluate, researchers are more likely to find the best one for the job.

Success at synthesizing versions of yeast’s native chromosomes will also open the way to adding entirely novel chromosomes to the organism—ones that endow it with brand-new properties or enable it to model human diseases. Boeke, for example, would like to install in yeast all of the genes of the molecular pathway that, in humans, is defective in Lesch-Nyhan syndrome, a rare disorder characterized by gout and kidney defects. SCRaMBLE should also make it easier to harness yeast for biotechnology. Right now, finding a yeast strain that makes enough of a product to be commercially viable “takes many steps and many person years,” Boeke says. SCRaMBLE should speed that search up by providing an “unbelievable number of mutants that would be much more time-consuming to get one at a time.” With more strains to evaluate, researchers are more likely to find the best one for the job.

Researchers who have watched the steady progress made by Boeke’s army say the project will be a boon for basic science, promising “deeper mechanistic understanding of biological processes in yeast,” says Huimin Zhao, a synthetic biologist at the University of Illinois, Urbana-Champaign.

But Kirsten Benjamin, a synthetic biologist at Amyris Inc., the Emeryville, California, company that harnesses yeast for producing artemisinin and other chemicals, expects that problems loom for the project: The more synthetic DNA Boeke tries to incorporate in a yeast, the sicker the strain will likely be, she predicts. “We’re going to find a bunch of ways where it doesn’t work,” she says. But she agrees that the problems could be revealing, saying they “will allow us as a scientific commun-ity to discover all these unappreciated phenomena.”

She and others are not sure how useful synthetic genomes will be for commercial applications. Less drastic approaches to improving yeast’s manufacturing prowess may work better. “From a practical viewpoint, it is too costly to [make synthetic genomes] for most engineering applications,” Zhao says.

But maybe it’s enough to just build a eukaryotic genome once, Boeke suggests. “In a way, you can put it as a kind of milestone, like the first human genome was a milestone for genomics,” he says. “When we finish it, we can really plant a flag in it.”

—ELIZABETH PENNISI

Student Assembly Drives Yeast Project

The homework that James Chuang and Katrina Caravelli turned in to Jef Boeke consisted of just four letters: A, C, G, and T, representing DNA’s four bases, each repeated thousands of times. But there was nothing tedious about their assignment: “Build A Genome,” as the undergraduate course’s name put it. Boeke conceived the course at Johns Hopkins University in Baltimore, Maryland, 6 years ago while struggling to figure out how to do all the work required to build a synthetic yeast genome (see main story, p. 1426).

For Boeke, the course supplies labor—scores of undergraduates have taken it so far. For students, it offers intensive training in synthetic biology and the thrill of taking part in frontline research. “I was really fascinated by the potential and by my ability to have an impact,” recalls Caravelli, who signed up for the course in 2008 and is now Boeke’s senior lab coordinator at New York University. During the semester-long course, students learned basic molecular biology procedures such as performing polymerase chain reactions and cloning DNA in bacteria.

Each student committed to completing 10,000 DNA bases during the course. “My building blocks went to chromosome 8,” Chuang, now a graduate student in biomedical engineering at Boston University, says proudly. After being supplied with the DNA sequence he needed to synthesize, he started out with 16 pieces of DNA about 75 bases long, ordered from a commercial DNA synthesis firm. The ends of some pieces overlapped, so when he mixed the pieces together with enzymes, they self-assembled into 750-base spans dubbed building blocks. After making sure the building blocks had the correct sequence, he put them into bacteria to generate many copies of the newly assembled DNA. Now, Johns Hopkins graduate student Jing-chuan Luo is putting those bigger DNA sections into yeast and making “minichunks” about 3000 bases long. If the yeast stays healthy, then she adds the next chunk in line to it, and so on. Her goal: to make a yeast strain with a totally new chromosome 8.

Caravelli recalls that her own bacteria sometimes wouldn’t grow with the yeast segment in their midst. She enjoyed figuring out why. “The trial-and-error process challenges you to think properly like a scientist.”

Boeke’s course has proved such a success that three other U.S. universities are hosting or will soon host their own. Because it’s now cheaper to buy 750-base segments than to have students make them, current students start with such DNA blocks and turn them into 3000-base spans, and, ultimately, 10,000-base chunks. Class productivity has soared. Each student’s target is now 30,000 to 50,000 bases.

The students have high hopes for their work. “I want to see synthetic biology do really useful things for society,” Chuang says. “The synthetic yeast genome provides a template for doing [that].”

—E. P.