DIGGING DESIGNER GENOMES

Techniques such as Gibson and Golden Gate assembly and homologous recombination in yeast allow researchers to assemble DNA sequences millions of base pairs in length. Sarah Webb investigates the challenges of constructing designer chromosomes and genomes, and their potential applications.

Image courtesy of Nathan Blow.

As early as 1995, Craig Venter's research team was thinking about a pie-in-the-sky proposition. They had just finished sequencing the genome of *Mycoplasma genitalium* and were now thinking about how to rebuild the genome from scratch and construct a synthetic cell. But at the time, the tools simply weren't available, notes Daniel Gibson, who is now an associate professor at the J. Craig Venter Institute (JCVI) and vice president for DNA technology at the company Synthetic Genomics, Inc. (SGI).

Gibson was not part of that original *M. genitalium* sequencing team. He arrived at JCVI as a postdoctoral fellow in 2004, joining a team that was attempting to devise new methods to build the whole bacterial genome. At the time, sequences of 5000 base pairs were amongst the largest DNA chunks being assembled routinely in labs. To build up a genome of nearly 600,000 base pairs, the team initially hoped to take advantage of cell extracts from *Deinococcus radiodurans*, harnessing that organism's ability to stitch together DNA fragments. But after 2 years, they hadn't made much progress. That was when Gibson decided to explore the molecular biology of commercially available enzymes, focusing on the essential functions needed to put DNA fragments together.

His efforts led to a method that combined a DNA exonuclease, a DNA polymerase, and a DNA ligase into a cocktail Gibson initially called one-step isothermal DNA assembly. After publishing the technique and giving a few talks, his new assembly method caught on in the molecu-

lar and synthetic biology communities, quickly becoming known as "Gibson Assembly." It was this technique that JCVI researchers used to overcome that core genome synthesis challenge, leading to the assembly of the *M. genitalium* genome in 2008 [1] and the construction of the first synthetic bacterial cell in 2010 [2].

Even with these early successes and the recent publication of the first synthetic yeast chromosome, many synthetic biologists have not yet embraced the challenge of building artificial chromosomes or genomes. But as de novo DNA assembly costs become cheaper and better design tools come on line, biologists may soon have the means to study the basic playbook of cells and even to start producing designer sequences that could pump out useful compounds.

Re-imagining yeast

It took a while to convince Jef Boeke, now a professor at New York University School of Medicine, that synthesizing a yeast chromosome was in fact a good idea. When he first heard geneticist Ron Davis call the construction of a yeast chromosome the next phase of gene synthesis, he thought, "Why would anyone ever want to do that?"

But the idea percolated subconsciously, says Boeke, who was then at Johns Hopkins University. And a conversation with his colleague Srinivasan Chandrasegran years later suddenly changed his mind. They then teamed

up with Joel Bader and designed a modified *Saccharomyces cerevisiae* chromosome and even devised an unusual strategy to assemble it—creating building blocks with the help of undergraduate student labor and, eventually, an international team.

Assembly challenges shaped how Boeke and his colleagues approached chromosome synthesis. They initially planned to order assembled sequences from a synthesis company. When it became clear that the DNA chunks they requested were beyond the size limits of what the company could assemble at that time, Boeke wondered if they should consider a different construction pipeline, one that they could oversee themselves.

The answer came in the development of a special course for undergraduates at Johns Hopkins University: the Build-A-Genome course. Armed with thermocyclers purchased off of eBay and led by a graduate student who walked students through vetted assembly protocols, eager undergraduates quickly learned how to synthesize 1500 base-pair pieces. Over the years, as assembly methods became more robust and efficient, the chunks of DNA assigned to each student

grew to 10,000 base pairs and now to 30,000–50,000 base pairs. In addition, teams from the United Kingdom, Australia, China, and Singapore have now joined the project as they continue to work on building a whole synthetic yeast genome.

The initial yeast chromosome construction effort was published in *Science* in May 2014 [3]. Boeke says they expect to complete the synthesis of all of the chromosomes by the end of 2017 and hope to have the whole genome assembled into a new strain within the next 2 to 3 years.

One notable feature of the Synthetic Yeast 2.0 project [4] is that none of the participants own the primary intellectual property to the initial sequences or the resulting strains. Everyone involved has agreed upfront to share the strains with anyone in academia or industry who wants to use them. “We view this as a platform that we want to see used as much as possible,” Boeke says. However, teams are free to develop and patent spin-off technologies—such as new ways to synthesize DNA or new commercial applications.

“We like it because it’s a chance to try out genome-scale design construction and verification,” says Tom Ellis of Imperial College London in the United Kingdom, who is one of the international Synthetic Yeast collaborators. “It allows you to work out the best ways to tackle big DNA in the future.”

Design challenges

Synthesis technologies such as Gibson and Golden Gate assembly are starting to make large-scale

The BioXp system is a workstation for ordering constructs and stitching together DNA fragments.
Image courtesy of Dan Gibson.

Dan Gibson joined JCVI in 2004, where he worked on developing new techniques for large-scale DNA assembly. Image courtesy of Dan Gibson.

Schematic representation of the Gibson assembly process. Image courtesy of Dan Gibson.

DNA synthesis tractable, but it is definitely not trivial. “We still don’t have the next-generation tools and technology that are going to make large genome engineering accessible and affordable,” says Andrew Hessel, Distinguished Research Scientist at Autodesk, a company focused on complex design problems. “We need to keep driving this technology and making it faster, better, cheaper.”

There are new instruments emerging now that could help researchers quickly assemble the initial building blocks of their sequences. Gibson and his colleagues at SGI have developed the BioXp system, a workstation that allows researchers to order up to 32 constructs and seamlessly stitch together DNA fragments up to 1800 base pairs for further assembly or other applications. Eventually, they hope to add oligonucleotide synthesis to the system so that researchers can simply dial in a sequence, Gibson says.

Synthetic biologists face a bigger challenge, though, before chromosome- and genome-scale projects become more routine. Researchers need better tools for *planning* the bottom-up design of chromosomes and genomes before they assemble them, experts say.

“There are no [software] programs to manage a project of this size,” notes Christopher Voigt of the Massachusetts Institute of Technology (MIT). Voigt is part of a MIT-Broad Institute team known as the Foundry that received \$32 million in Defense Advanced Research Projects Agency (DARPA) funding, some of which is directed towards these types of software and industrial design challenges in building large DNA sequences.

Building chromosomes and genomes is both a computer-aided design problem and a computer-aided manufacturing problem, says Hessel. As he explains it, right now most tools and editors cater to the individual academic scientist thinking about a small number of manual changes in a DNA sequence, rather than the large-scale, multi-researcher challenges involved in the construction of genomes and chromosomes.

At the moment, genome editing, particularly with the emergence of CRISPR/Cas9 technology, fills the needs of many researchers—those who can accomplish their goals with a relatively small number of genetic tweaks. “At present, editing techniques are suitable for the great majority of applications,” notes James Collins of MIT.

But Collins also expects that more researchers will embrace chromosome and genome assembly as synthesis becomes cheaper and more efficient. One key advantage of constructing a genome or chromosome from scratch is the ability to build in and control multiple features of the engineered cell. That’s difficult to do right now with genome editing, Collins says.

What’s next?

At the annual meeting of the Synthetic Yeast 2.0 project in July, researchers discussed the future for synthetic genomes and which organism might be next. According to

Chris Voigt, from the Massachusetts Institute of Technology, is part of a team working on tools for the management of large-scale DNA assembly projects. Image courtesy of Martha Bruce Photography.

Jef Boeke, from New York University, wasn't initially convinced that synthesizing a yeast chromosome was a good idea. He is now one of the leaders of the Synthetic Yeast 2.0 project. Image courtesy of Jef Boeke.

Boeke, *Drosophila* and *Caenorhabditis elegans* were the most popular picks. “We know a lot about them, their genes are well-annotated, they have interesting biology, and they are models for pathogens and pests.” But both of these genomes are more than 100 million base pairs long, nearly an order of magnitude larger than *S. cerevisiae*. And unlike yeast, these organisms do not carry out homologous recombination. Researchers would have to figure out how to stitch the new genome into the organism, making the expense and scope of such a project daunting.

“While it’s really cool to synthesize a whole genome, it’s also frankly a bit of a slog,” Boeke says. He’ll cheer on anyone who wants to take on the project, but he’s looking toward new designs of functional chromosomes, such as those that could manufacture therapeutic molecules, potentially even protein-based drugs.

Others are thinking along the same lines. Ellis is interested in designing chromosomes that synthesize secondary metabolites in yeast, medicinal molecules such as antibiotics, and novel materials. Voigt sees a variety of important applications on the sub-genome scale, such as creating pathways for nitrogen fixation or building designer mitochondrial or chloroplast DNA.

Designer genomes and chromosomes could also soon help biologists better understand cellular organization and processing, as well as provide fundamental tools for new biological designs. Scientists at JCVI and SGI continue to build a stripped-down version of their synthetic bacterial cell with a genome that only includes the bare essential genes for life, Gibson says. Synthetic chromosomes could also allow researchers to examine genetic

architecture, allowing scientists to group genes for related processes—genes for DNA transcription, translation, or even developmental processes clustered on an engineered chromosome.

Even at the chromosome scale, many scientists haven’t necessarily wrapped their heads around the potential of large-scale DNA synthesis. “It’s almost a technology that’s ahead of its time,” Boeke says. When he gives seminars, he often asks this question: “I can make anything up to a million bases, what should I make and why?” Surprisingly few people want to jump in with proposals, he says. “It’s just outside people’s comfort zones to think like that.”

References

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