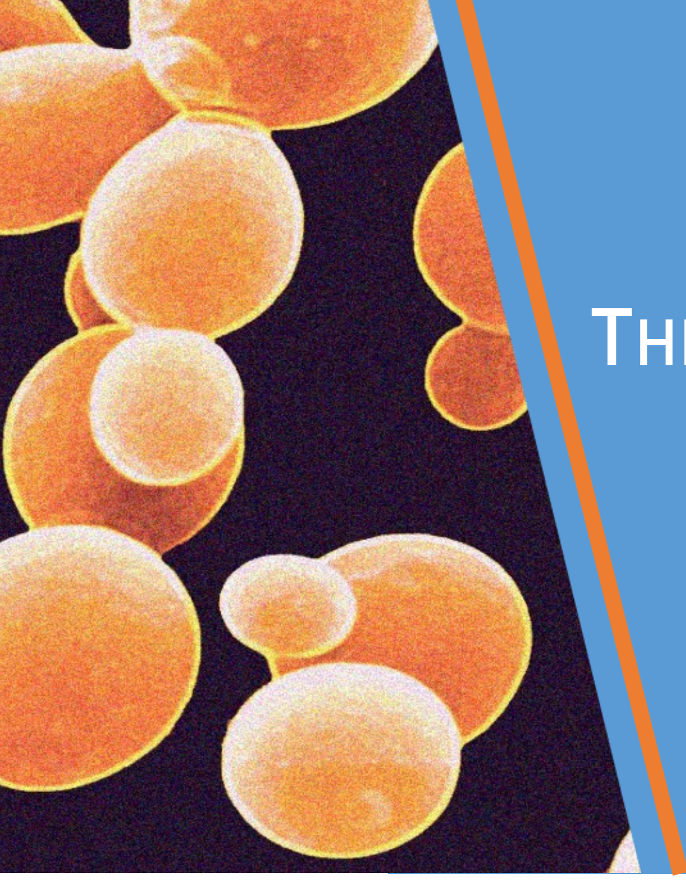


Franken-Yeast:

The Man Made Chromosome

The milestone in synthetic DNA could 'Revolutionize' medicine

In March 2014, an article published in science magazine stated that scientists at John Hopkins University created the first synthetic chromosome for brewers yeast, a remarkable step for Genomic Engineering. With the use of dozens of undergraduate students they successfully built a customized 'designer' yeast chromosome from scratch. This landmark research has the potential to revolutionize medicine.[1]

Where Did It Start?

This story starts 10 years ago when Jef Boeke, a geneticist at John Hopkins, and his colleague Srinivasan Chandrasegaran decide to undertake the project of their lifetime. And so they hatched a plan to build Synthetic Yeast 2.0. They began with meticulously building one arm of chromosome 9 of the humble bakers yeast (*Saccharomycerevisiae*). After completing 90,000 pairs of letters that make up the DNA, they realized that their effort proved too slow and too expensive. "I realized I would be dead long before the project could ever be completed" Boeke said.[2]

> **"You can put it as a kind of milestone, like sequencing the first human genome"**
>
> **—Jef Boeke, co-author and geneticist**

This led Boeke to the idea to enlist what he called "an army of undergrads' to follow a 'Build–A-Genome' course. The 60 undergraduates underwent painstaking work to construct long strings of DNA that would eventually become Chromosome III of their synthetic yeast in exchange for a great research opportunity and lectures regarding genetic engineering. "You can put it as a kind of milestone" says Jef Boeke, "like sequencing the first human genome."[2]

Why Yeast?

Brewers yeast is a primary ingredient in lots of our basic foods such as bread and beer. The Single-celled yeast organism was first used in baking and fermentation by ancient Egyptians 4,000 years ago. Later, French microbiologist Louis Pasteur was first to discover that alcohol was a product of yeast fermentation. The close relationship humans have had with yeast makes it a true model organism. Today, we know all the ins and outs when it comes to yeast. Yeast was one of the first organisms that had its genome sequenced. Yeast cells and human cells have a lot in common. They're both eukaryotes, have their DNA surrounded by a nucleus. Furthermore, yeast can reproduce sexually similar to human cells. In terms of genes, yeast has 12 Million Base pairs. Therefore, using yeast for this experiment provides the opportunity to learn a lot about our own genome and how it functions.[3]

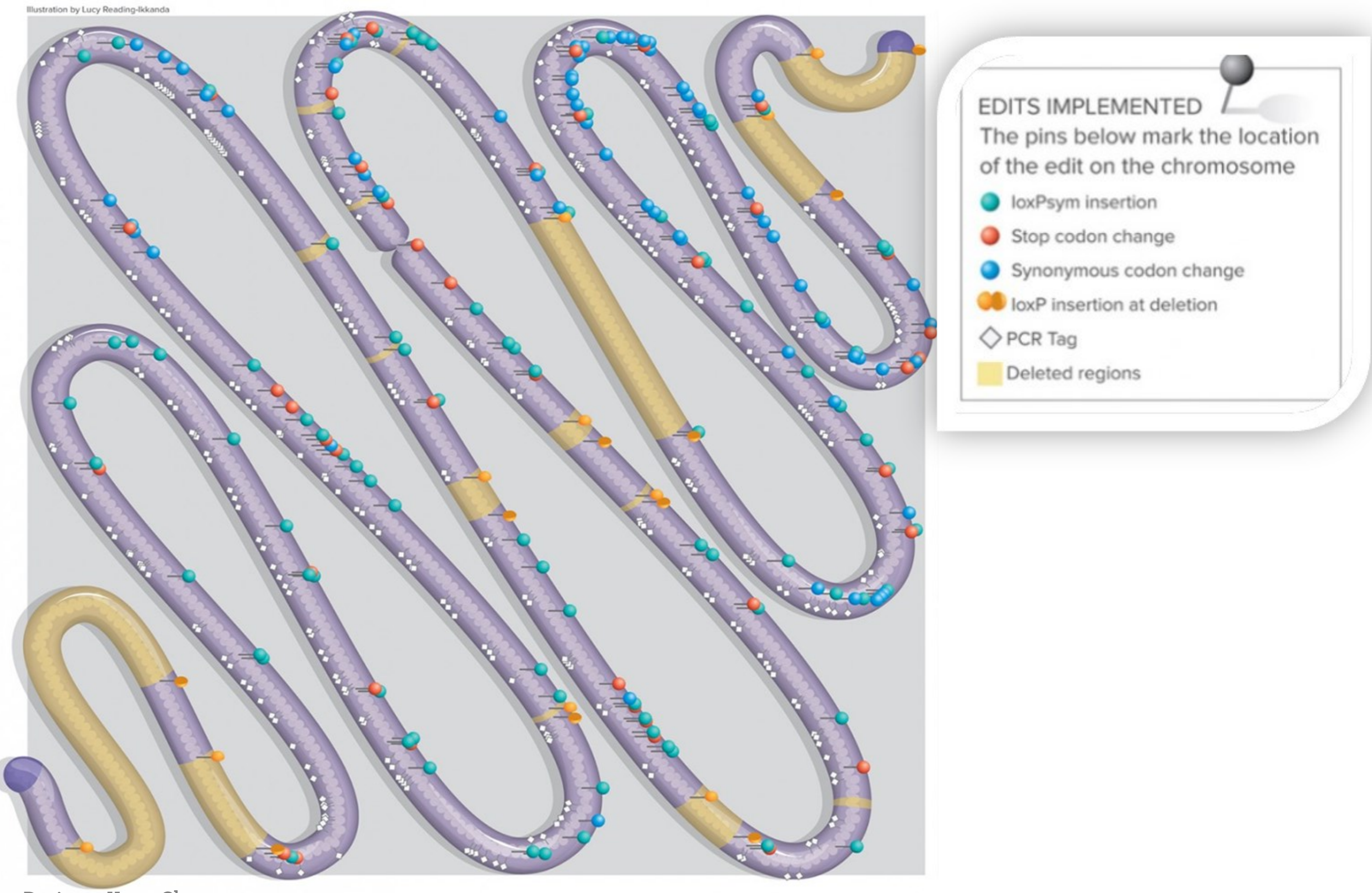
Yeast has all in all 16 chromosomes. Chromosome III was selected for it's short and convenient length. This chromosome contains approximately 6000 genes (functional units of chromosomal DNA that encode for proteins), of which a third are shared with humans. Furthermore genes on this chromosome also determine the reproductive type of yeast. [3]

How Did They Do It?

The process of building the chromosome is rather simple, but expensive and time consuming. The undergraduates would start by writing the DNA sequence on the computer. A DNA-synthesis machine would then create these short sequences. The next step was to stich up these bases into strands 750 base pairs long. DNA is built up of letter-designated base macromolecules that together form a code. 'A' stands for adenine, which is paired with 'T' for thymine; and 'C' represents cysteine, which is paired with 'g' for guanine. Together they form the double helix structure we are all familiar with. The 750bp were weaved together into chunks of 2,000 to 4,000bp long. Finally, these chunks were recombined into the original yeast chromosome until it was completely synthesized.[4]

The Result

Naturally Chromosome III consists of 272,871 base pairs. The team was successful at synthesizing the chromosome with approximately 50,000 base pairs deleted, inserted or changed. The illustration bellow shows all of the changes implemented. The areas shaded yellow are regions deleted from the chromosome. The red pins indicate stop codon base replacements



Designer Yeast Chromosome 3

from TAG to TAA. This makes the TAG code available for the researchers in the future. The blue pins represent other synonymous base changes. Instead of deleting nonessential genes, because they didn't know what the effect would be on the whole organism, they inserted loxPsyms to border these regions (indicated by the green pins). LoxPsym are short sequences of DNA that can be chemically activated to delete or change these nonessential genes. This provides the researchers a means to manipulate the yeast genome at will and observe the effects.[1]

Once inserted back into the cell, the team exposed the cell to numerous surroundings to see how it would function compared to the original cell. Even with all the changes, the synthetic yeast showed no alteration in fitness or replication.[1]

Sounds Familiar

Although an impressive technical achievement, in 2010 geneticist J. Craig Venter from the institute in Rockville, Maryland, had assembled his own synthetic genome for the parasitic bacteria Mycoplasma mycides. However, what took Venter 15 years and $40 million dollars to accomplish, it took Boeke's team 5 years and a team of eager undergraduate students.. [4]

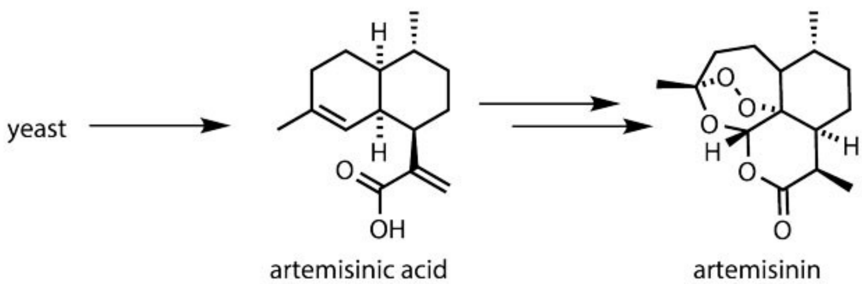
Furthermore, the yeast organism, a eukaryote, is far more complex than Venter's parasite, a prokaryote. Venter's group synthesized short strands of DNA mostly identical to that of the original cell, with only a few coded watermarks coded into the genome sequence, which would spell out the members of the team that conducted the experiment. In contrast Boeke's team altered many features of the genome; stripping the chromosome from many of its genes to test their importance. Boeke's experiment can justify the enormous cost and effort because it can provide so much more information about the way genes function in both yeast and humans.[5]

Its Potential

In addition to serving as a genome that researchers can learn a lot about, brewers yeast is commonly used to make foods such as bread and beer, and also used in other areas of industry as well, such as biofuel. What is less known is that yeast is playing a growing role in making and manufacturing medicines.

Malaria

Almost half a billion people in the world suffer from malaria, of which 2.7 million die a year. Therefore there is still an urgent need to find new antimalarial drugs for treatment and prophylaxis. The commonly used antimalarial drug artemisinin is normally a byproduct of natural sweet wormwood plant. Unfortunately to produce such a drug is time consuming and seasonal because of the plants involved and thus also expensive. Recently scientists have genetically engineered bakers yeast to come up with a short cut to produce artemisinin. The engineered cells are able to produce high concentrations of artemisinic acid, which through a chemical process can be turned into artemisinin.. This process in affect makes making the drug on demand and cheap, revolutionizing how we combat malaria. With the research done by Boeke's team it is expected to improve and enhance this process even more.[6]



Artemisinic acid produced by yeast and turned into artemisinin[7]

Hepatitis B

Hepatitis B virus is a serious infection affecting the liver. In 2009, almost 40,000 people were infected with hepatitis B. A chronic infection can even lead to liver cancer or death. Thanks to yeast, there is now a vaccine available. Brewers yeast was genetically engineered to produce the same surface proteins as that of the virus. The yeast cell cannot infect the individual but can reproduce these surface proteins. Subsequently these proteins are used to make a vaccine, building the immune response and thus making people immune to the hepatitis B virus. Vaccines are a great potential of yeast now that we have the knowledge and the means to genetically modify it even more.[8]

Premenstrual Syndrome

Another fascinating use of brewer's yeast is to reduce the symptoms of premenstrual syndrome. Participants of a randomized clinical trial were either given a placebo or a brewer's yeast based supplement. After 6 months of monitoring the participants did a Menstrual Distress Questionnaire, measuring the symptoms they experienced. Interestingly the yeast treatment group showed a significant 82% reduction in symptoms compared to those taking the placebo. Although more research is required, brewer's yeast has the potential to be genetically engineered to show enhanced features and products that were associated with reducing symptoms of Premenstrual Syndrome.[9]

Cancer

Probably one of the most interesting research done on the potential of yeast cells, is in treatment of cancer. Researchers explored how the invitro use of brewer's yeast when incorporated into breast cancer cultures showed escalated rates of apoptosis (self-induced cell death). These metastatic breast cancer cells are known to be extremely phagocytic, engulfing and breaking down surrounding cells including the added yeast cells. After only 30 minutes, already 13% of the cells had died. This rate was increased to 38% after four hours. All in all, the breast-cancer cells that were actively metastatic were most vulnerable to the yeast cells. They showed a 629% increase in apoptosis compared with the normal cells. The nonmetastic breast cancer cells showed a 178% increase in apoptosis. Although far from actual application, the brewer's yeast is showing means of a healthier way of combating breast cancer, instead of damaging and life-threatening chemo- and radiotherapy.[10]

> **"Our goal is to use it [synthetic yeast] to produce new antibiotics as resistance arises to existing ones"**
>
> **—Dr. Tom Ellis, geneticist at Imperial College London**

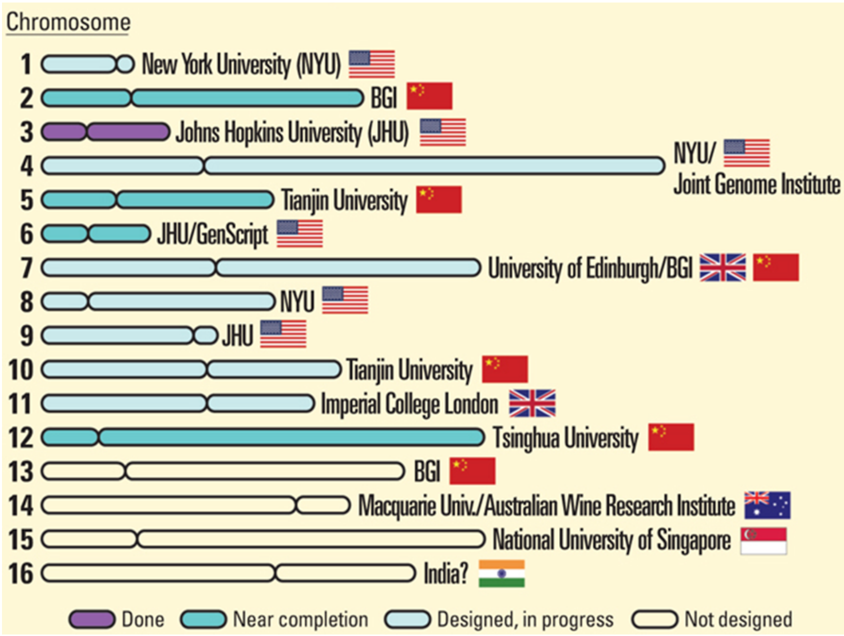
Synthetic Biology

Boeke's research truly is groundbreaking; it pushes the boundaries of synthetic biology. According to Dr. Tom Ellis, a professor in synthetic Biology at Imperial College London, "A synthetic genome will allow us to reprogram yeast and our goal is to use it to produce new antibiotics as resistance arises to existing ones." The Yeast cells can also be genetically engineered to model human disease and defective molecular pathways. This could teach us more about how these diseases work and how we should combat them.[3]

Furthermore, the synthetic biology developed in this research can be applied to a diverse number of situations. One of which is the production of human antibodies from animals. Certain animal cells can be genetically modified to also produce human antibodies specific for certain disease. A repertoire of human antibody genes can be transferred to these animals. Subsequently the antibodies produced here can be given to individuals with immune deficiency or be used to produce prophylactic vaccines against deadly diseases.[11]

The Future

Research laboratories all around the world have currently come together to completely build all but one of the 16 yeast chromosomes from scratch. This would lead genetic research one step closer to constructing an entire genome and would also provide an entire genome to tinker with. They are also planning studies on chromosome III where they scramble it, removing and duplicating certain genes and their order. Truly, this growing field in synthesizing genes is yielding endless possibilities.[12]



Status of Synthetic Yeast Genome[12]

Sources

1. Jef D. Boeke, Srinivasan Chandrasegaran et al.; Total Synthesis of a Functional Designer Eukaryotic Chromosome; 27 March 2014 Science Magazine

2. Richard Harris; Custom Chromo: First Yeast Chromosome Built From Scratch; National Public Radio; 27 March 2014; accessed March 31 2014

3. Kyang; NYU Langone Medical Center Press Release; syntheticyeast.org; March 27 2014

4. Ewen Callaway; First synthetic yeast chromosome revealed; Nature news; 27 March 2014

5. J. Craig Vemter Institute; First Self-Replicating Synthetic Bacterial Cell; www.jvci.org; 20 May 2010

6. Dae-Kyun Ro et al.; Production of the antimalarial drug precursor artemisinic acid in engineered yeast;13 April 2006; Nature

7. Haley Birch; Yeast to make malaria drug on demand; ChemistryWorld; 10 April 2013

8. Paul A. Offit, MD; A look at Each Vaccine: Hepatitis B Vaccine; the Children's Hospital of Philadelphia; April 2013;

9. Facchinetti F, Nappi RE, Sances MG, et al. Effects of a yeast-based dietary supplementation on premenstrual syndrome. A double-blind placebo-controlled study. Gynecol Obstet Invest. 1997;

10. Ghoneum M, Gollapudi S. Induction of apoptosis in breast cancer cells by Saccharomyces cerevisiae, the baker's yeast, in vitro. Anticancer Res. 2004;

11. Londberg N. Human antibodies from transgenic animals; Nature biotechnology; Sep 2005

12. Meegan Phelan; Science: Researchers Build First Synthetic Eukaryotic Chromosome; American Association for the Advancement of Science 27 March 2014