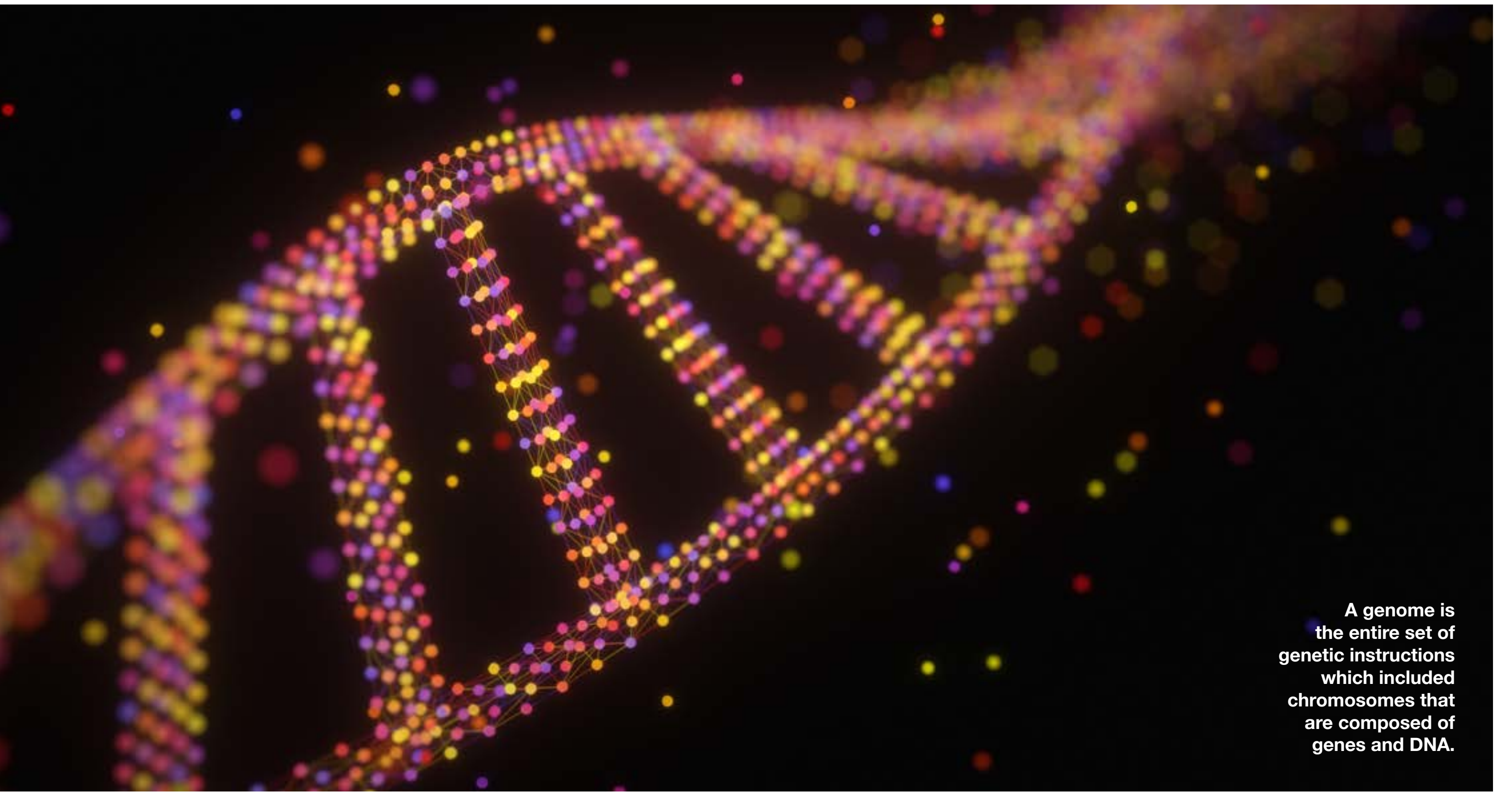
Innovation

Synthetic biology application

In this, the third of four articles, the application of Synthetic Biology is considered. The learnings gained from research and its application continue to contribute to our understanding of wine production and vine cultivation. Furthermore, the precision with which Synthetic Biology may be applied offers environmental benefits, and allows for application according to regulatory requirements, as **Simone Madden-Grey** writes.



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Synthetic yeast Sc2.0

An important part of research and learning in the wine industry has been based on the yeast *Saccharomyces cerevisiae*. The *Synthetic Yeast 2.0* project (Sc2.0) was established on the premise that to fully understand how an organism works, one should be able to design and redesign one.

Beginning in 2013, an international consortium of researchers and scientists set a goal of synthesising all 16 chromosomes in the *S. cerevisiae* yeast strain. The learnings from this work have enriched understanding of how eukaryotic cells, that is cells containing a clearly defined nucleus, function. Fundamental to this research is a conscious governance infrastructure incorporating safety, ethics and regulatory requirements.

In the paper *Mini-Review: Yeast 2.0—connecting the dots in the construction of the world's first functional synthetic eukaryotic genome*, authors Sakkie Pretorius and Jef Boeke highlight the role yeast plays in research connecting numerous scientific questions with practical application in the areas of environment and health. "In this context, *Saccharomyces cerevisiae* has, over time, emerged as the most successful dot-connecting yeast species and eukaryotic model organism—a lighthouse navigation beacon that illuminates and guides scientific ingenuity from the laboratory to inventive innovations in the field."

Several characteristics single out *S. cerevisiae* as a "broad shouldered study model", including a relatively short reproduction time, simple and inexpensive cultivation, and stability in various research protocols. *S. cerevisiae* was the first host of a genetically modified (GM) vaccine, the first GM food enzyme and the first genome to be fully sequenced. A genome is the entire set of genetic instructions, including chromosomes which are composed of genes and DNA. As of 2023 the Sc2.0 project has completed synthesising the genome of *S. cerevisiae*, meaning all 16 chromosomes and their constituent parts have been synthesised. All but one of the synthetic chromosomes are under review or in the process of being revised as per review feedback. The final chromosome is expected to be presented for peer review by the end of April 2023.

Design and construction

As part of the project a computer-generated design blueprint was created for the Sc2.0 genome in order to simplify assembly of the synthetic chromosomes, enhance stability and facilitate research goals. The design included PCRtags, which work as 'watermarks' for all synthetic DNA, making it possible to distinguish between synthetic and native DNA.

Chromosome synthesis began with small building blocks of DNA that included specific markers to facilitate joining those blocks together to create larger blocks of synthetic DNA. Consecutive rounds of testing monitored any changes to the overall genome. If changes were detected, the synthetic DNA segment was rolled back for defect identification and rectification.

Application

During the Sc2.0 project several side projects were developed by members of the consortium and here in Australia those projects have mostly focussed on work with aromas and flavours in wine. The first project was at the AWRI where Drs Anthony Borneman and Darek Kutyna developed a semi-synthetic genome with a specific pathway for raspberry ketones. Other projects have worked on developing synthetic yeasts that express aromas and flavours such as vanillin, esters, terpenes, thiols and rotundone.

Further application of the lessons learned from the Sc2.0 project may include the development of sentinel plants to help manage plant health, environmental stress, and disease pressure. That, says Professor Pretorius, is a long way into the future: "What we do with yeast is complex but to do that with plants is significantly more complex". Where he does see more immediate use is in the benefits gained from research applications of DNA-edited yeast strains that create stable combinations of desired aromas and flavours. When the research model yeast has been proven the team then searches for natural strains that can be genetically crossed with a view to commercial release.

Biologics

Biological products, or biologics, are products that are isolated from natural sources and that may be produced using Synthetic Biology technologies. "Synthetic Biology is about engineering biology to a desired function and there are many ways to apply this technology", says Sydney-based Natalie Curach of Ginkgo Bioworks Inc.

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>
> Professor Isak Pretorius

"If you know what is in nature already and how it functions, it is a matter of tweaking and optimising it in order to best fit the needs for commercial application," she explained.

Furthermore, classifying engineering levels enables precise application according to regulatory requirements, without necessarily releasing GMOs.

Root growth and soil microbiome

Two projects currently underway at Ginkgo Bioworks involve researching and developing biologics for plant root growth and soil microbiome. Biologics can change the chemical properties of soil to increase nutrient uptake through plant roots. This is achieved by creating more soluble forms of key nutrients related to plant growth and disease resilience.

A database containing more than 200,000 isolates from soil was created using high throughput automation to test thousands of soil samples at a time. The richness of the data generated makes it easier for scientists to identify microorganisms with useful functions in the soil, and it makes it easier to engineer biology. The facilities at Ginkgo Bioworks in California are sufficiently large that engineered strains can be tested in both greenhouse and field conditions. The impact of this extends beyond plant health, says Curach, to include environmental and operational gains. These gains can be seen through reduced fertiliser use and augmented soil carbon content as a result of increased biomass and root growth.

Engineering levels

The way products of gene technologies are developed has matured to align with parameters set by application and by extension, regulation. In the case of biologics, Curach says the simplest form is the identification of a useful organism, which is then grown and applied. Improvements might include natural mutation processes or adjusting growth conditions in order to increase production of the desired feature. These are not classified as genetically modified

Biologics can change the chemical properties of soil to increase nutrient uptake through plant roots.

organisms in most jurisdictions and therefore not subject to GMO regulation.

Increasingly complex engineering techniques may be used to augment and optimise a desired functionality, and these can include the gene editing technique CRISPR or the transfer of DNA from like or closely related strains. For a full transgenic cell, "you can use all the tools in the shed". This is when it is possible to modify a particular function to be the strongest and most economically viable organism, such that when it is being grown at commercial scale it is being done so with the highest efficiencies. With increased complexity of engineering, the cell may be classified as GMO and subject to regulation. The diversity of techniques makes it possible to select the appropriate level of engineering, from working with a molecule derived from genetic modification but without a GM component, through to working with a completely transgenic cell.

Environmental benefits

Synthetic Biology techniques can include the use of living cells rather than petrochemicals. "That's the beauty of SynBio", says Curach, "it can be an alternative to petrochemical derived molecules, which has been the traditional method of deriving molecules of interest."

Synthetic Biology also offers an alternative source for the extraction of the molecule of interest from plants and animals, through the process of biomanufacturing in the lab, which is arguably more sustainable than growing crops for the same purpose. Efficiencies are also gained from the precise and targeted functionality available with these techniques, which enables the end goal to be structured in the context of regulatory, environmental, intellectual property and commercial operations.

For the wine industry to benefit from the breadth and depth of research and development in Synthetic Biology, education about the technologies and their application is essential at all levels within industry. Advocates and critics must proactively come together for rational, well-informed discussion if a meaningful and practical industry strategy is to be generated.

Simone Madden-Grey is a writer based in Melbourne, Australia writing about the people, places and stories she has discovered on her travels. Her portfolio can be found at happywinewoman.com including articles on climate and sustainability, new tech and the latest in scientific research supporting the wine industry in Australia and her home country, New Zealand.

Interviews

Curach, Natalie – Senior Director of Business Development, Ginkgo Bioworks Inc., Sydney

Pretorius, Isak S. Professor – Deputy Vice-Chancellor, Research, Macquarie University, Sydney

Further information

Gingko Bioworks Inc.:
www.ginkgobioworks.com

Synthetic Yeast 2.0:
https://syntheticyeast.github.io

Sources

'*Mini-Review: Yeast 2.0—connecting the dots in the construction of the world's first functional synthetic eukaryotic genome*'. I.S. Pretorius and J.D. Boeke (2018) GW