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To cite this article: Isak S. Pretorius (2017) Synthetic genome engineering forging new frontiers for wine yeast, Critical Reviews in Biotechnology, 37:1, 112-136, DOI: 10.1080/07388551.2016.1214945

To link to this article: http://dx.doi.org/10.1080/07388551.2016.1214945

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Published online: 18 Aug 2016.

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Synthetic genome engineering forging new frontiers for wine yeast

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ABSTRACT
Over the past 15 years, the seismic shifts caused by the convergence of biomolecular, chemical, physical, mathematical, and computational sciences alongside cutting-edge developments in information technology and engineering have erupted into a new field of scientific endeavor dubbed Synthetic Biology. Recent rapid advances in high-throughput DNA sequencing and DNA synthesis techniques are enabling the design and construction of new biological parts (genes), devices (gene networks) and modules (biosynthetic pathways), and the redesign of biological systems (cells and organisms) for useful purposes. In 2014, the budding yeast Saccharomyces cerevisiae became the first eukaryotic cell to be equipped with a fully functional synthetic chromosome. This was achieved following the synthesis of the first viral (poliovirus in 2002 and bacteriophage Phi-X174 in 2003) and bacterial (Mycoplasma genitalium in 2008 and Mycoplasma mycoides in 2010) genomes, and less than two decades after revealing the full genome sequence of a laboratory (S288c in 1996) and wine (AWRI1631 in 2008) yeast strain. A large international project – the Synthetic Yeast Genome (Sc2.0) Project – is now underway to synthesize all 16 chromosomes (~12 Mb carrying ~6000 genes) of the sequenced S288c laboratory strain by 2018. If successful, S. cerevisiae will become the first eukaryote to cross the horizon of in silico design of complex cells through de novo synthesis, reshuffling, and editing of genomes. In the meantime, yeasts are being used as cell factories for the semi-synthetic production of high-value compounds, such as the potent antimalarial artemisinin, and food ingredients, such as resveratrol, vanillin, stevia, nootkatone, and saffron. As a continuum of previously genetically engineered industrially important yeast strains, precision genome engineering is bound to also impact the study and development of wine yeast strains supercharged with synthetic DNA. The first taste of what the future holds is the de novo production of the raspberry ketone aroma compound, 4-[4-hydroxyphenyl]butan-2-one, in a wine yeast strain (AWRI1631), which was recently achieved via metabolic pathway engineering and synthetic enzyme fusion. A peek over the horizon is revealing that the future of “Wine Yeast 2.0” is already here. Therefore, this article seeks to help prepare the wine industry – an industry rich in history and tradition on the one hand, and innovation on the other – for the inevitable intersection of the ancient art practiced by winemakers and the inventive science of pioneering “synthetic genonomics”. It would be prudent to proactively engage all stakeholders – researchers, industry practitioners, policymakers, regulators, commentators, and consumers – in a meaningful dialog about the potential challenges and opportunities emanating from Synthetic Biology. To capitalize on the new vistas of synthetic yeast genomics, this paper presents wine yeast research in a fresh context, raises important questions and proposes new directions.

Synthesizing unscripted futures

Imagine, for a moment, it is 2050 and you are watching a television show called Who wants to be a Billionaire? A contestant – who happens to be a young winemaker – is one question away from the bonanza. The final question is a particularly intriguing and complex one: “What breakthrough-technology in biology emerged at the start of the twenty-first century that has had the most profound impact on the cost-effective production of things such as energy-rich molecules for renewable biofuels and sustainable industrial chemicals; compounds for the bioremediation of polluted environments; novel antibiotics, vaccines and personalized medicines; and adequate nutritious and safe food supplies to meet the demands of today’s global population of 9 billion people of which two-thirds are over the age of 60?” The contestant anxiously considers the four options as they come up. A: Molecular Biology; B: Systems Biology;
**C:** *Regenerative Biology; D:** *Synthetic Biology.* As a professional winemaker with formal training in science, the contestant discounts options *A* and *B* because those two disciplines originated in the previous century. However, the stakes are high and to be absolutely sure, the contestant uses the last lifeline by asking the audience to vote. The audience responds unanimously with option *D.* The contestant instantly locks in *D: Synthetic Biology.* Under loud applause the host hands the contestant a $1-billion holographic e-coin voucher and the world has yet another billionaire. Pure fantasy or not?

Without the benefit of 20/20 hindsight we cannot accurately predict an unscripted future. We know all too well that even familiar things can play out in very unfamiliar ways. However, one certainty is that tomorrow is shaped by, amongst other things, the ground-breaking scientific achievements, and technological developments of yesterday and today. Our individual and collective fascinations and concerns regarding emerging sciences such as Synthetic Biology, and our present choices and actions, will sculpt the world inherited by future generations. The art of turning *hindsight* into *foresight* gives us the necessary *insight* to learn from the past, prepare for the future and to conduct our research in new emerging sciences in the present so that the *past* and the *future* are ever *present* when we frame our research questions, conceptualize our experiments and develop the necessary policy and regulatory frameworks.

Today’s fast-paced world craves *Eureka! moments in science and “something-for-nothing” turning-point discoveries and big-picture technological advances that reveal new horizons at a stroke. Such headline-grabbing *Eureka!* breakthroughs are rare in the world of science, which is becoming increasingly specialized but, at the same time, also multi-disciplinary and interconnected. The gradual seismic forces build as researchers collaborate around the globe and across the boundaries of various disciplines to stack data point atop data point, and layer nano-insight on nano-insight – which is often too slow for society to feel the subtle tremors of an approaching tipping point during which shifting tectonic plates suddenly collide and irreversibly change the world. Such are the expected future-shaping impacts of Synthetic Biology on the world that today’s newborns will be living in by 2050.

During the first 15 years of this millennium, the magnitude of the early tremors from Synthetic Biology have become more pronounced as contributions from disciplines such as biomolecular, chemical, physical, mathematical and, computational sciences, information technologies, and engineering are closing the gaps from different directions and angles among themselves to give rise to this new trailblazing interdisciplinary

**Figure 1.** The emerging science of Synthetic Biology originates from the recent convergence of several “wet” biomolecular, chemical and physical sciences, and “dry” mathematical and computational sciences, information technologies, and engineering. This is a twenty-first-century transdisciplinary science, which is barely 15 years old. This futurist and transformative technology holds the potential to improve quality of life and sustain the planet in very significant ways.
I. S. PRETORIUS

briefly reviews the state-of-play in Synthetic Biology, illu-
controversial and disruptive new science, this paper
sensible debate over the complexities surrounding this
by outcome so this debate. In fact, the use of
research model organism in the advancement of
the development of improved wine yeast strains.[4]
minimizes the critically important role of the global
Synthetic Yeast Genome (Sc2.0) Project (more detail
available from www.syntheticyeast.org) in advancing the
frontiers of synthetic genomics and proposes future direc-
tions and guiding principles for future wine yeast
research.

Teetering on the edge of a new scientific frontier
The past and future of DNA science are ever present in Synthetic Biology
The discovery of the double-helical structure of DNA, more than 60 years ago, has captivated our imagina-
tions and continues to shape our endeavors to under-
stand and harness the programming of life’s genetic “software”. The complementary nature of perfectly
matching deoxyribonucleic acid strands of the double
helix and the simplicity of the digital code of DNA’s
software – consisting of only four letters: adenine (A),
cytosine (C), guanine (G), and thymine (T) – continue to
fascinate the world (Figure 2). From the beginning,
scientists were intrigued by how, and under what
circumstances, sequences of nucleotide triplets (codons)
of genes are transcribed into messenger ribonucleic
acid (mRNA) molecules, which in turn, are translated by
the ribosomal machinery in cells into sequences of
amino acids to form proteins (Figure 3).

The late 1950s saw the uncovering of the mysteries of
the universal genetic code, which was followed in the
1960s with the unraveling of the workings of regulatory
genetic circuits and programmed gene expression. Building on these new insights, the 1970s were marked
by the development of recombinant DNA and molecular
cloning techniques. With the improvement of transform-
ation procedures and the arrival of polymerase chain reac-
tion (PCR) techniques in the 1980s, genetic engineering of
microorganisms became fully entrenched in mainstream
research. This was followed by the advent of automated
DNA sequencing, high-throughput ‘omics technologies,
bioinformatics, and improved computational tools, which,
together, paved the way for the decoding of complete
genomes in the 1990s and beyond.

These developments expanded the discipline of
Biology – defined as “the study of the morphology,
physiology, anatomy, behavior, origin, and distribution
of living organisms” – and branched into Molecular
Biology and Systems Biology during the latter half of the
previous century (Figure 4). With Molecular Biology,
researchers sought to understand interactions between
the various components of a cell, including interactions
between the different types of DNA, RNA, and protein
biosynthesis, and to learn how these interactions are
regulated. Systems Biology extended this “reductionist”

science (Figure 1). The forces of these merging disci-
plines are shifting our bioengineering capabilities from
high-throughput “reading DNA” (i.e. DNA sequencing)
to affordable “writing and editing DNA” (i.e. DNA syn-
thesizing) paradigms. With the convergence of the lat-
est developments in “wet” biomolecular sciences and
“dry” information and engineering technologies, it is
now possible to design and construct new biological
parts (genes), devices (gene networks) and modules
(biosynthetic pathways), and to redesign natural
biological systems (cells and organisms) for useful pur-
oposes.[1] It does not only mean that we can now do the
same things differently; it also means that we can do
totally different things. That is why a growing number of
imaginative scientists, futurists, and commentators are
coming to believe that Synthetic Biology will erupt into
novel biological solutions to some of the most pressing
challenges facing humanity and the planet this century.

Unsurprisingly, the commercial world is responding
swiftly. The global value of the Synthetic Biology market
grew 10-fold between 2010 and 2016 to an estimated fig-
ure of more than $10 billion.[2] Some of the world’s lead-
ing politicians and entrepreneurial business leaders are
already referring to Synthetic Biology as one of the great
trendsetting technologies that holds the promise to “heal
us, feed us, fuel us” – a futurist technology that will
improve our well-being, protect our environment, and
power our economy.[1] This promise, however, also poses
great ethical challenges and risks, thereby placing great
responsibility on researchers, policymakers, regulatory
bodies, industry, and all citizen stakeholders to engage in
a meaningful dialog about how to capitalize on the poten-
tial of Synthetic Biology to improve quality of life and sus-
tain the planet while minimizing the risk of harm.[3]

We can expect that the “hype-horror-hope” nature of
a debate like this will intensify and polarize opinions in
every sector and industry in the years to come. Thus,
there is an urgency that well-informed opinion leaders
in the agricultural, food and beverage industries also
“lean in” to constructively contribute to this debate.

Given the pivotal role that yeast plays as a workhorse
in the fermentation industry as well as an experimental
research model organism in the advancement of
Synthetic Biology, the wine industry, too, will be impacted
by the outcomes of this debate. In fact, the use of
Synthetic Biology techniques are already spilling over into
the development of improved wine yeast strains.[4]

To engage all stakeholders of the wine industry in a
sensible debate over the complexities surrounding this
controversial and disruptive new science, this paper
briefly reviews the state-of-play in Synthetic Biology, illu-
minates the critically important role of the global
Synthetic Yeast Genome (Sc2.0) Project (more detail
Figure 2. The structure of the deoxyribonucleic acid (DNA) double helix consists of two complementary strands, which are held together by two hydrogen bonds between adenine (A) and thymine (T) and three hydrogen bonds between guanine (G) and cytosine (C). The sequence of the A-T and G-C base pairs (bp) comprises the genetic code of genes which reside on chromosomes. Each gene is transcribed into a messenger ribonucleic acid (mRNA) – a polymer with a ribose and phosphate backbone and four different nitrogenous bases, adenine (A), guanine (G), cytosine (C) and uracil (U). Nucleotide triplets, called codons, specify which amino acid will be added next during protein synthesis.

Figure 3. The process of protein biosynthesis starts with the transcription of a gene’s DNA to messenger RNA (mRNA) in the nucleus. Once the mRNA molecules are processed (e.g. the removal of non-coding introns separating the exons), they are exported through the pores of the nuclear membrane to the ribosomes where they are translated into proteins.
approach to a more “holistic” one by using computational and mathematical modeling of complex biological systems to “reverse-engineer” cellular networks. Building on the conceptual and technological advances in Molecular and Systems Biology, the new millennium welcomed the green sprouts of a new offshoot of Biology. Synthetic Biology combines molecular approaches with engineering principles to “forward-engineer” genetic systems by constructing collections of modular parts to design, build, and fine-tune gene regulatory networks (e.g. “quorum-sensing” circuitry for multicellular partnering) analogous to controllable electrical circuits.[5] In short, Synthetic Biology is on a continuum with Molecular Biology and Systems Biology and entails the design and construction of novel biological systems, including complex “synthetic” organisms.

**Genetic engineering upgrades to genome engineering**

With the arrival of next-generation “genetic engineering” tools at the beginning of this century, synthetic biologists started to acquire the equipment to rewrite, reshuffle and edit genomes. These twenty-first-century molecular tools reinvented “genetic engineering” and raised the bar to the level of “genome engineering”.

The synthesis of a DNA copy of the 7458-nucleotide poliovirus genome [6] and the 5386-bp genome of the bacteriophage Phi-X174 [7] gave the first glimpses of whole-genome synthesis. When the chemically synthesized genome of Phi-X174 was incorporated into Escherichia coli host cells, the transduced bacteria self-destructively produced new phage particles just as with natural Phi-X174 infections.

However, the size of viral genomes is miniscule when compared to the genomes of even the tiniest bacteria such as Mycoplasma. So, to synthesize an entire bacterial genome required a step-change; fortunately, this was made possible by rapid advances in lower-cost and improved DNA synthesis techniques.

The first bacterial genome – the 583-kb genome of Mycoplasma genitalium – was chemically synthesized in 2008.[8] This was a watershed moment; however, in 2010, whole-genome engineering became much more...
of a reality with the creation of a cell of one bacterial species controlled by a chemically synthesized genome of another species.\textsuperscript{[9]} Synthesized DNA cassettes were assembled via \textit{in vivo} recombination in yeast to recreate the 1.1 Mb \textit{Mycoplasma mycoides} genome, which was then transplanted into \textit{Mycoplasma capricolum}, resulting in viable bacteria that contained only the synthesized genome (Figure 5). To make the “synthetic” version of the bacteria recognizable different and detectable, 14 non-essential \textit{M. mycoides} genes were deleted and some novel “watermarking signature DNA” were added and inscribed into the synthetic genome. The “secret” watermark branded the synthetic bacteria with a unique serial number of the J. Craig Venter Institute (JCVI-syn1.0) and an encryption that contains the URL of a website and three quotations. The “synthetic” version of the bacteria recognizes different and detectable, 14 non-essential \textit{M. mycoides} genes were deleted and some novel “watermarking signature DNA” were added and inscribed into the synthetic genome. The “secret” watermark branded the synthetic bacteria with a unique serial number of the J. Craig Venter Institute (JCVI-syn1.0) and an encryption that contains the URL of a website and three quotations. This was a world-first and, in subsequent commentary, the JCVI team eloquently explained their groundbreaking achievement by likening a biological cell to a computer with the genome as the software that encodes the cell’s instructions.\textsuperscript{[10]} They referred to the cellular machinery as the hardware that interprets and runs the software. They further explained that advances in DNA technology have made it possible for scientists to act as biological “software engineers”, programming new biological “operating systems” into cells. Commentators in the popular press responded by reporting that “a Rubicon was crossed” with this “synthetic” bacterium containing “an artificial genome, which was designed on a computer and manufactured in a laboratory” – “a living creature with no ancestor”. These commentaries sparked an ongoing debate.

This evolving debate has not deterred the JCVI research team. This year, they succeeded in reducing the 1079-kb synthetic genome of \textit{M. mycoides} JCVI-syn1.0 to a bare minimum of 531 kb, carrying 473 genes.\textsuperscript{[11]} By retaining essential and quasi-essential genes through three cycles of design, synthesis, and testing, they produced strain JCVI-syn3.0 with a genome smaller than that of any autonomously replicating cell found in nature. This minimalist JCVI-syn3.0 genome contains almost all genes involved in the synthesis and processing of macromolecules, as well as 149 genes with unknown biological functions. It is widely believed that the use of this JCVI-syn3.0 versatile platform will boost further investigations into the core functions of life and exploration of whole-genome design.\textsuperscript{[11]}

This remarkable scientific achievement is likely to raise the temperature of the broader debate regarding the ethical and regulatory concerns of society at large because, suddenly, it has become possible to conceive of a future world in which new bacteria and, eventually, new complex microorganisms, plants, and animals are designed and built from scratch to predetermined specifications.

While the debate about the pros and cons of “whole-genome engineering” is intensifying, the focus of the scientific world is turning toward further improvements of DNA synthesis and genome editing techniques, the genetic recoding of bacteria such as \textit{E. coli},\textsuperscript{[12,13]} and a well-studied unicellular eukaryote with a remarkable track-record in bridging scientific successes achieved with viruses and bacteria to more complex multicellular organisms – the yeast, \textit{Saccharomyces cerevisiae}.\textsuperscript{[14]}

\textbf{Raising the “synthetic” bar with yeast}

\textit{Yeast is a workhorse with sound academic and industrial credentials}

Metaphorically speaking, of the \textasciitilde{}150 described yeast genera and 1500 known yeast species,\textsuperscript{[15]} the “sugar fungus”, \textit{S. cerevisiae}, can be described as the most revered, “graduating” with top marks in both majors of
“fundamental research” and “practical applications”. *S. cerevisiae* is the most versatile yeast and excels equally well in laboratory and industrial settings.[16] In the world of microbes, its unequaled versatility has earned *S. cerevisiae* the reputation as the “Swiss Army Knife” yeast (Figure 6).

In the research laboratory, this single-celled eukaryote is “well qualified” to answer basic biological questions about cellular, genetic, and metabolic processes asked by state-of-the-art science. It is one of the most intensively studied biological model systems (for a review see [43] and references therein). The round to ovoid cells of *S. cerevisiae* are 5–10 μm in diameter and compartmentalized (including an encapsulated nucleus) like most other eukaryotic cells (Figure 7). The three basic cell types of this budding yeast – α, α, and a/α cells – are easy and relatively inexpensive to grow in the laboratory and, under optimal nutritional and cultural conditions, they double their mass every 90 minutes (Figure 8). Through an asexual mitotic budding process, heterothallic strains can multiply as stable a or α haploids while both heterothallic and homothallic strains can reproduce in the a/α diploid state or in a state of higher ploidy (Figure 9). Most laboratory strains are haploid or diploid, whereas industrial wine strains are predominantly diploid or aneuploid, and occasionally polyploid. Two haploid cells of opposite mating types, a and α, can mate and produce a/α diploids, which, in turn, can undergo meiosis to generate four haploid ascospores, two of each mating type according to Mendelian segregation laws. Homothallic haploids can switch their mating-type from a to α and vice versa through the *HO*-controlled MATα, MATα, HML, and HMR loci on Chromosome 3, and conjugate with cells from the same single colony (Figure 10).[17]

This accessible genetic system, which can be followed through asexual and sexual cycles, together with the ease of yeast transformation procedures, make *S. cerevisiae* amenable to almost any modification (breeding, mutagenesis, and cloning) that biomolecular science can throw at a eukaryote with GRAS (Generally Regarded As Safe) status. Due to its tractability, *S. cerevisiae* provided the “nuts and bolts” that shaped the foundations of Genetics, Molecular Biology, Systems Biology, and Synthetic Biology and it remains the main-stay eukaryotic chassis of choice.[18]

In the context of industry application, the GRAS status of *Saccharomyces* yeast has been hard earned through its parallel development with civilization and human activity.[19] Yeast fermentation was one of the primary forces to move the ancient world beyond hunting and gathering. That is why yeast became the first microorganism to be “domesticated”: for several millennia, this yeast has been used to raise dough, brew beer, and sparkle wine. Today, this industry workhorse drives multi-billion-dollar fermentations – churning out, on a daily basis, a wide spectrum of fermented foods and beverages, on the one hand, and biofuel and pharmaceutical products on the other.[20]

The remarkable academic and industrial track-record of *S. cerevisiae* is highlighted by many world-firsts (Figure 11). Not only was this yeast the first microorganism to be domesticated for the production of fermented food and beverages in ancient times, it was also the first microbe to be observed under the microscope and described as a living biochemical agent of transformation.
In more recent times, *S. cerevisiae* was the host for the production of the first recombinant vaccine (against hepatitis B) and the first recombinant food enzyme (the milk coagulation enzyme, chymosin, for cheesemaking).[16] In 1996, the ∼12 Mb (non-redundant) to ∼14 Mb (total) genome of a haploid laboratory strain (S288c) of *S. cerevisiae* became the first eukaryotic genome to be fully sequenced, revealing that its 16 chromosomes encode ∼6000 genes of which ∼5000 are individually non-essential.[21,22] This publicly...
available genome sequence paved the way for building the world’s first systematic collection of bar-coded gene deletion mutants enabling high-throughput functional genomics experiments.[23] This Yeast Deletion Library comprising yeasts with specific genes removed, together with the freely available Saccharomyces Genome Database (SGD; available from http://www.yeastgenome.org), resourced yeast biologists with unparalleled laboratory tools to shift scientific frontiers forward at a more rapid pace. In 2014, S. cerevisiae became the first eukaryotic cell to be equipped with a fully functional synthetic chromosome.[24] A large international project – the Synthetic Yeast Genome (Sc2.0) Project – is now underway to synthesize all 16 linear chromosomes (ranging between 200 and 2000 kb in length) thereby positioning S. cerevisiae S288c to become the first eukaryote with a synthetic genome (see http://www.syntheticyeast.org).

Yeast 2.0 is breaking through the “synthetic” sound barrier

Designing and building the world’s first synthetic yeast genome is set to deliver a significant “step change” in “synthetic genomics” as a sub-field of Synthetic Biology. A consortium of a dozen laboratories from five countries – the USA, UK, Australia, China, and Singapore – has embarked on a collaborative project aimed at the synthesis of the entire genome of a haploid laboratory strain (S288c) of S. cerevisiae by 2018 (Figure 12). The goals of the Sc2.0 project include answering a broad spectrum of “profound questions about fundamental properties of chromosomes, genome organization, gene content, function of RNA splicing, the extent to which small RNAs play a role in yeast biology, the distinction between prokaryotes and eukaryotes, and questions relating to genome structure and evolution” while recognizing that “the eventual ‘synthetic yeast’ being designed and refined could ultimately play an important practical role” (see www.syntheticyeast.org).

The first step toward building the ultimate yeast genome, as envisioned with the Sc2.0 project, was taken in 2011 with the construction of synthetic chromosome arms.[25] This was followed up in 2014 with the synthesis of the first of the 16 chromosomes, namely Chromosome 3.[24] Interestingly, this is the chromosome that carries the MATa, MATa, HML, and HMR loci, which determine the mating-type of S. cerevisiae cells (Figure 10). Chromosome 3 is also the third smallest chromosome and it was the first S. cerevisiae chromosome that was fully sequenced almost two and a half decades ago.

The publicly available 316,617-bp DNA sequence of the native Chromosome 3 was used to redesign this chromosome on a computer according to a set of Sc2.0 principles relating to genome stability, genetic flexibility, and cell fitness (Figure 13). These in silico “software edits” included the deletion of dispensable DNA sequences; the incorporation of uniquely recognizable sequences that could be used to differentiate between the native and synthetic DNA; the replacement of particular stop codons with other codons that terminate gene transcription equally well; and the flanking of non-essential genes with DNA sequences designed to catalyze their deletion on a specific signal.[24] More specifically, subtelomeric regions, introns, transfer RNA (tRNA) genes, transposons, and silent mating-type loci (HML and HMR) were deleted; TAG stop codons were recoded to TAA stop codons, and loxPsym sites were added to enable genome scrambling when needed.

The computer-designed sequence was used to construct a synthetic version of Chromosome 3.[24] Overlapping stretches of 60–79 nucleotides were chemically synthesized in parallel and then assembled – by using standard PCR techniques and in vivo homologous recombination processes – into overlapping “minichunks” of approximately 3 kb in length. Together
with the use of alternating selectable markers in each experiment, an average of 12 of these overlapping 3-kb synthetic DNA fragments were utilized to systematically replace the corresponding native sequences in 11 successive rounds of integrative transformation. The end-result was the successful replacement of the 316,617-bp native Chromosome 3 with a 272,871-bp synthetic version (labeled syn3). Despite the more than 50,000 alterations made in syn3 and the fact that the synthetic chromosome was 14% shorter than the native chromosome, the transformed yeast was not less fit than the original S288c S. cerevisiae strain.[10] This result provides indicative answers to several fundamental biological questions. For example, from this work it is clear that the deletion of superfluous DNA among genes (introns), transposons (Ty elements) and tRNA genes, and the modification of unnecessary genetic code in syn3 did not negatively affect cell fitness.[10] Also, with 5000 loxP sites, further tinkering with syn3 is now possible.

Building on the foundations of this pioneering work, and assisted by improved technologies that enable the commercial synthesis of much larger pieces of DNA at ever-decreasing costs, it is expected that the synthesis of at least another five of the 16 S. cerevisiae chromosomes will be reported during the course of this year. Thus, with approximately two-thirds of the genome synthesized (hosted in multiple cells) at this point in time, the Sc2.0 project is on track to produce the world’s first synthetic yeast genome by 2018.

Figure 10. Chromosome 3 carries the two MAT genes (MATa and MATα) that determine the mating-type of Saccharomyces cerevisiae. The Yα and Yα regions are unique regions within these two MAT loci, each encoding two divergently transcribed mRNA molecules. In haploids, the two transcripts of the MATa locus code for the α1 and α2 polypeptides while the transcripts of MATα code for α1 and α2 polypeptides. MATα1 and MATα2, as well as all other α-specific genes, are constitutively expressed in the absence of the MATα2-encoded repressor, whereas all α-specific genes can only be transcribed in heterothallic haploids that express both MATα1 and MATα2. While expression of MATα1 and MATα2 is not required for a-mating, expression of MATα1 is required, along with the expression of MATα2, to repress haploid-specific gene expression in MATα/MATα2 diploids. Such heterothallic diploids can neither mate nor express other haploid-specific genes because of the action of the MATα1/MATα2-encoded co-repressor. Interestingly, homothallic haploid cells of S. cerevisiae can switch their mating-type from α to α and vice versa through the HO-controlled cassette model and conjugate with cells from the same single-spore colony. For example, an α cell can switch to an α cell by replacing the MATα allele with the MATα allele. The switching of the allele in the MAT locus is possible because homothallic haploids express the HO gene as well as an additional silenced copy of both the MATα and MATα alleles: the HML (Hidden MAT Left) locus carries a silenced copy of the MATα allele, and the HMR (Hidden MAT Right) locus carries a silenced copy of the MATα allele. The gene conversion event required for mating-type switching is initiated by the haploid-specific HO gene, which is tightly regulated and only activated in haploid cells during the G1 phase of the cell cycle. The HO-encoded DNA endonuclease cleaves a specific sequence at the MAT locus so that either the HML or HMR information can be duplicated and swapped into the active MAT locus. Thus, the silenced alleles of MATα and MATα present at HML and HMR serve as a reservoir of genetic information to repair the HO-induced DNA cleavage at the active MAT locus.
Advances such as these are of interest to the entire field of Synthetic Biology because each technological innovation and new insight gained in understanding biological fundamentals creates new and stronger platforms from which we can launch into the design of entirely novel genes, chromosomes, genomes, and metabolic pathways for the biosynthesis of useful products. The first steps have already been taken to endow yeast, including wine yeast strains, with brand-new properties.
Figure 12. The international Synthetic Yeast Genome (Sc2.0) Project aims to chemically synthesize all 16 linear chromosomes of the haploid S288c laboratory strain of *Saccharomyces cerevisiae* and use them to replace the entire native genome. In doing so, the goals are to answer some profound biological questions that will inform future fundamental research into the functioning of eukaryotic cells, as well as to benefit yeast strain development programs.

Figure 13. The synthesis of a functional 272,871-bp designer version (syn3) of *Saccharomyces cerevisiae*’s 316,617-bp native Chromosome 3. Changes to syn3 include the deletions of subtelomeric regions, introns, transfer RNA (tRNA) genes, transposons, and silent mating-type loci (*HML* and *HMR*), TAG/TAA stop-codons replacements, as well as the insertion of loxPsym sites to enable genome scrambling.[24]
Equipping yeast with novel “synthetic” properties

Bioengineered yeast put synthetic DNA to work in industry

A laboratory strain of *S. cerevisiae* was first transformed in 1978.[26] Since then, numerous genes were cloned into various strains, including industrial strains. Industrial yeasts have been genetically engineered for a wide variety of purposes over the past three decades. In the biotechnology-based industries, genes encoding enzymes, interferon, insulin, growth hormones, and vaccines were expressed in yeast. Patents were filed and several bioproducts generated by these genetically modified (GM) yeast strains were commercialized with varying degrees of success.[27] The fiercest resistance from the general public was aimed against food products containing ingredients produced by genetically modified organisms (GMOs), such as the yeast-derived milk-clotting enzyme, chymosin. However, much less resistance was encountered with the commercialization of the yeast-derived recombinant vaccine against hepatitis B.

With the advent of Synthetic Biology, GM technologies have become much more powerful, precise, and sophisticated.[28–30] In addition to the production of yeast-derived ethanol in the fermented beverage and biofuel industries, this mainstay eukaryotic chassis with GRAS status is increasingly being harnessed as “cell factories” for the production of high-value–low-volume products that could, for example, improve health, wellness, and nutrition.[31] Commercial firms use a combination of genetic and genome engineering (including CRISPR editing technologies; Figure 14) approaches to (re)design, (re)construct, and analyze specific *Saccharomyces* strains by employing three major strategies: rational engineering; inverse metabolic engineering; and evolutionary strategies.[32] Currently, the most common approach is to re-engineer the metabolism of existing cells and to retrofit existing strains. However, there are efforts underway to eventually create synthetic “minimal” yeast cells that could be used to generate valuable bioproducts.

The list of products generated by bioengineered *S. cerevisiae* cell factories is long, ranging from aliphatic alcohols, β-amyrin, β-carotene, casbene, cinnamoyl anthranilates, cubebol, propanediol, and ribitol to acetic, adipic, artemisinic, and eicosapentaenoic acids. As an example, a company such as Evolva (see www.evolva.com) that specializes in the use of yeast cell factories for the production of high-value–low-volume ingredients has recently brought several bioproducts onto the market. These bioproducts include one of the most widely used flavoring agents, vanillin (a secondary metabolite and main constituent of natural vanilla originally obtained from cured seed pods of the vanilla orchid, *Vanilla planifolia*); the citrus-flavored sesquiterpene, valencene (a natural aroma component of citrus fruits such as *Citrus sinensis* oranges and commercially used as a flavor ingredient in food and drink, personal care, and household products); the citrus-flavored sesquiterpenoid nootkatone (naturally found in *Citrus paradisi* grapefruit with food-fragrance and insect-repellent properties); and the stilbenoid, resveratrol (a polyphenolic phytoalexin found in red *Vitis vinifera* grapes and believed to have several health benefits). Evolva expects to commercialize another two products within the next year: saffron (the world’s most expensive spice naturally derived from the flower *Saffron crocus*) and stevia (an increasingly sought-after zero-calorie sweetener usually extracted from the plant *Stevia rebaudiana*), and their work on sandalwood, agarwood, and ergot alkaloids is well advanced. At least two of these compounds, vanillin (3-methoxy-4-hydroxybenzaldehyde) and resveratrol (trans-3,5,4′-trihydroxystilbene), are also of interest to the wine industry.

*Vanillin:* Oak-aged red wines are known to contain traces of vanillin. *De novo* biosynthesis of vanillin in *S. cerevisiae* was achieved by incorporating an engineered pathway, which included 3-dehydroshikimate dehydratase from the dung mold *Podospora pauciseta*; an aromatic acid reductase from a bacterium belonging to the *Nocardia* genus; phosphopantetheinyl transferases from *E. coli* and/or *Corynebacterium glutamicum*; and overexpression of an O-methyltransferase gene from *Homo sapiens* (Figure 15).[33–38] To prevent the reduction of vanillin to vanillyl alcohol, this heterologous pathway was engineered into a mutant *S. cerevisiae* strain containing a deletion in the *ADH6* alcohol dehydrogenase gene. To decrease product toxicity, the *Arabidopsis thaliana* UGT72E2 glycosyltransferase gene was added. The yield of free vanillin was further improved in this vanillin-glucoside producing strain, by using an *in silico* metabolic engineering strategy involving deletions of the native yeast *PDC1* and *GDH1* genes. Significantly, increased levels of free vanillin were achieved by employing a “global metabolic strategy” and “model-guided network engineering” prior to local pathway manipulation.

*Resveratrol:* Red wines fermented with grape skins contain varying levels of resveratrol depending on the cultivar and growth conditions of the grapes and the length of skin contact during vinification.[39] Resveratrol is a natural plant stress metabolite produced at increased levels when grapevines are exposed to fungal infection, wounding, or ultra-violet radiation in
vineyards. In addition to its defense properties in grapevines, resveratrol in red wine is widely associated with human health benefits relating to anti-aging, anti-diabetic, anti-inflammatory, anti-thrombotic, and anti-tumor properties in pre-clinical trials. However, data from these inconclusive pre-clinical tests remain to be supported with clinical trials with large cohorts. Nevertheless, earlier work on the development of resveratrol-producing S. cerevisiae strains fed with expensive p-coumaric acid or aromatic amino acids [39] has recently been superseded by the construction of a metabolically engineered strain capable of de novo production of resveratrol from glucose or ethanol via tyrosine as an intermediate (Figure 16).[40] The first step was to introduce the resveratrol biosynthetic pathway, comprising tyrosine ammonia-lyase from the bacterium Herpetosiphon aurantiacus, 4-coumaryl-CoA ligase from A. thaliana and resveratrol synthase from V. vinifera. The yield of resveratrol was significantly improved by the overexpression of feedback-insensitive alleles of the ARO4-encoded 3-deoxy-D-arabino-heptulosonate-7-phosphate and ARO7-encoded chorismate mutase, and the ACC1-encoded acetyl-CoA carboxylase boosting the supply of the precursor malonyl-CoA. Commercially relevant production levels in fed-batch fermentation were achieved by multiple integration of the resveratrol pathway genes into the genome of a robust S. cerevisiae strain.

However, the synthetically engineered yeast cell factory with the highest commercial profile is the
high-yielding \textit{S. cerevisiae} strain capable of producing artemisinic acid, a precursor of the potent anti-malarial compound, artemisinin (Figure 17). Artemisinin is a sesquiterpene endoperoxide naturally produced by the sweet wormwood plant \textit{Artemisia annua}. The unstable supply of plant-derived artemisinin caused regular shortages and price fluctuations for the manufacturers of “artemisinin-based combination therapies” (ACTs) for “uncomplicated” malaria caused by the parasite \textit{Plasmodium falciparum}. Commercialization of a more reliable source of semi-synthetic artemisinin was achieved by engineering the complete biosynthetic pathway to artemisinic acid into \textit{S. cerevisiae}.\cite{41}

In this bioengineered strain, acetyl-CoA was converted to mevalonate through GAL-regulated overexpression of \textit{ERG10}, \textit{ERG13}, and \textit{tHMG1} (encoding a truncated HMG-CoA reductase). By placing \textit{ERG8}, \textit{ERG12}, and \textit{ERG19} under the GAL induction system, mevalonate was converted into isopentenyl diphosphate at increased levels.\cite{41} \textit{IDI1} overexpression was also controlled by the GAL system to efficiently convert isopentenyl diphosphate to dimethylallyl diphosphate, which, in turn, was converted to farnesyl diphosphate by GAL-induced overexpression of \textit{ERG20}. To down-regulate a competing reaction of joining two farnesyl diphosphate moieties to form unwanted squalene, the \textit{ERG9} gene was linked to the copper-regulated \textit{CTR3} promoter. By adding copper sulfate to the medium, farnesyl diphosphate was converted via the amorpha-4,11-diene synthase (ADS) route to amorphadiene instead of squalene. Amorphadiene was oxidized to artemisinic alcohol by GAL-regulated overexpression of \textit{CYP7AV1}, \textit{CPR1}, and \textit{CYB5}. GAL-induced overexpression of \textit{ADH1} led to the oxidation of artemisinic alcohol to artemisinic aldehyde, which was then oxidized to artemisinic acid by the overexpression of \textit{ALDH1} under the control of the GAL system. A practical, efficient, and scalable chemical process was developed to convert artemisinic acid to artemisinin by using a chemical source of singlet oxygen.\cite{41} These bioengineered yeast strains and chemical processes developed by renewable products company Amyris formed the basis for the commercial
production of semi-synthetic artemisinin launched by pharmaceutical company Sanofi in 2014. It is hoped that this will help stabilize the supply of artemisinin for derivatization into active pharmaceutical ingredients (e.g. artesunate) for incorporation into anti-malarial ACTs.

Inspired by these early successes in using yeast as cell factories, several other research breakthroughs are in the making. For example, by imitating the modularity of diterpene biosynthesis in plants, more than 40 “new-to-nature” combinations of Class I and II diterpene synthases have recently been constructed. Four industrially relevant “targets” were achieved in scalable biotechnological production of therapeutically important diterpenoids involving the use of bioengineered strains of *S. cerevisiae* as cell factories.[42]

**Bioengineered wine yeast uncorks a future with synthetic DNA**

History tells us that wine yeast strain development is well positioned to, once again, benefit from technological advances made with the genetic and genome engineering of non-wine strains of *S. cerevisiae*. To date, using genetic engineering [20,43,44] and non-GM techniques,[45] such as classical breeding (hybridization), adaptive laboratory evolution, and mutagenesis, several non-GM and GM wine strains have been developed with increased robustness, fermentation performance, health-related properties, and/or sensory attributes. Examples include strains that produce wines with lower alcohol levels,[46–55] mutants that limit the production of unwanted hydrogen-sulfide off-flavors and volatile acidity,[56–58] and strains that produce desirable esters,[59,60] terpenes,[61] and thiols (Figure 18).[62–70] Efforts are also underway to express the α-guaiene-2-oxidase from grapevine in *S. cerevisiae*, thereby equipping wine yeast to transform grape-derived α-guaiene to the sought-after spicy aroma compound of Shiraz wine, rotundone.[71–73] Several of these improved wine strains were patented but, so far, only two GM strains have been cleared by regulators in the USA and Canada for commercialization.
Figure 17. A synthetically engineered strain of *Saccharomyces cerevisiae* designed and developed for the production of artemisinic acid, a precursor of the potent anti-malarial compound, artemisinin.[41]

Figure 18. A range of genetically engineered wine strains from *Saccharomyces cerevisiae* (i) to decrease the concentration of alcohol, thereby reducing “hotness” in wine; (ii) to increase the safety of wine products by restricting the production of urea, thereby limiting the production of ethyl carbamate; (iii) to reduce the formation of hydrogen sulfide, thereby eliminating H$_2$S-associated off-flavors; (iv) to increase the release and conversion of nonvolatile grape-derived thiols into volatile aromas, thereby increasing fruitiness in wine; (v) to enable yeast to assimilate malate and convert malic acid into lactic acid without the involvement of malolactic bacteria, thereby simplifying the winemaking process; and (vi) to optimize the ester content, thereby producing more-complex and well-balanced wines.
In 2005, a transgenic malolactic strain, ML01, became the first GM wine yeast to be released to market. This strain contains the mae1 malate transporter gene from the fission yeast Schizosaccharomyces pombe and the mleA malolactic gene from the wine bacterium Oenococcus oeni.[74,75] Placed under the control of the S. cerevisiae PGK1 promoter and terminator signals, both these genes were chromosomally integrated. A second commercially available strain, ECMo01, contains an extra copy of the S. cerevisiae DUR1,2 urea-amidolysis gene, which is expressed under the PGK1 regulatory sequences. Both GM strains offer potential benefits to winemakers and wine consumers alike. In the case of ML01, no malolactic fermentation driven by bacteria is required, thereby simplifying the winemaking process and avoiding the potential health risks associated with biogenic amine production by some bacteria. The constitutive overexpression of the DUR1,2 urea-amidolysis gene in ECMo01 drives the conversion of urea into ammonia and carbon dioxide, thereby removing the substrate for ethyl carbamate production, a suspected carcinogen.[76] Despite these potential benefits, the ongoing anti-GMO campaigns prevented widespread uptake of these two GM wine yeasts in commercial wine production.[27,77,78]

The 10-year delay caused by the anti-GM sentiments in the marketplace, however, did not deter scientific progress in yeast laboratories around the world. On the contrary, the pace of unraveling the genetic make-up of wine yeasts to inform further strain development accelerated over the past few years. For example, since the publication of the first complete genome sequence of a wine yeast strain (AWRI1631) in 2008,[79] the genomes of at least six other widely used commercial wine yeast strains (AWRI1796, EC1118, QA23, VIN7, VIN13, and VL3) were sequenced and compared with the genomes of laboratory S. cerevisiae strains (S288c and Sigma1278b) as well as genomes of commercial Saccharomyces strains used in the baking, brewing, biofuel, raiji, and saké (Figure 19).[28,29,80–86] Valuable insights were gained from these studies— we are now much closer to fully understand what makes a wine yeast tick.[87]

As compared to the S. cerevisiae S288c reference strain, an additional stretch of ~200 kb spread across distinct genomic loci (encoding single and multigene clusters) was found to be present in other S. cerevisiae strains, including wine strains.[79] Strain-specific loci seem to reside in the subtelomeric regions – S. cerevisiae’s epicenter of genetic diversity. To date, at least three main loci were identified as loci that distinguish specific classes of industrial strains.[87] First, the RTM1 locus (a member of a subtelomeric three-gene cluster) is mainly present in ale and distilling strains, and, to some extent, in strains that carry a set of genes specific to wine yeasts. This wine-specific set of genes constitutes a second industry-defining locus. This wine-specific locus comprises a cluster of five genes. Interestingly, this gene cluster displays strain differences in copy number, genomic location, and gene order most likely due to mobilization into, and throughout, wine-strain genomes as a circular intermediate via a yet to be understood process. The third industry-specific locus entails the evolutionary differences in biotin prototrophy amongst certain S. cerevisiae strains. More specifically, while the majority of strains, including those used in winemaking and brewing, are biotin auxotrophs, strains used for the production of saké acquired the capacity to synthesize biotin de novo over time, presumably because of evolutionary pressures in the low-biotin fermentations of saké mash.[87]

In addition to these three industry-specific loci, several strain-specific genes were identified.[87] Three wine-relevant examples of strain-specific genes are the FSY1 gene encoding a H+/fructose symporter, two paralogues, MPR1 and MPR2, conferring resistance to L-azetidine-2-carboxylic acid, and the β-lyase encoding gene, IRC7. An effective H+/fructose symporter is thought to provide a selective advantage to strains expressing FSY1 in highly concentrated mixtures of glucose and fructose during the fermentation of grape must. Wine strains that are able to consume fructose effectively are less likely to produce sluggish or stuck fermentations during vintages in which heat waves distort the usual 1:1 ratio of glucose and fructose in grape juice. Strains that carry the MPR-family paralogues are generally more stress tolerant by decreasing the toxic effects of reactive oxygen. It is believed that the fermentation performance of MPR-carrying strains is more robust. IRC7-expressing strains release more volatile thiols during fermentation, thereby producing wine with more fruity characters.[64]

Valuable genomic insights were also gained into the Saccharomyces sensu stricto clade, comprising the S. cerevisiae complex (consisting of clearly defined “pure” lineages based strictly around geographic or industrial limits, and mosaic strains that appear to be the result of outcrossing between multiple pure lineages), and seven distinct Saccharomyces species (S. arboricolus, S. cariocanus, S. eubayanus, S. kudriavzevii, S. mikatae, S. paradoxus, and S. uvarum).[87] As an example, genomic comparisons revealed that the thiol-releasing wine yeast, VIN7, has an allotriploid hybrid genome with S. cerevisiae and S. kudriavzevii origins.[81] The understanding of the genetic basis of this “natural” hybrid’s unique capacity to produce wines with a guava-like aroma informed strain hybridization programs aimed at
the improvement of fruitiness in specific styles of white wine. Once such strain-specific “flavor” genes are uncovered, they will undoubtedly also become the target for “synthetic software engineers”, constructing complex aroma-enhancing metabolic pathways in specific yeast strains.

In this regard, the first “synthetically engineered” wine yeast strain has just popped a cork revealing a whiff of raspberries in an experimental Chardonnay wine. In a world-first, a wine strain of *S. cerevisiae* was equipped with a biosynthetic pathway – comprising four separate enzymatic activities – to produce the highly desirable raspberry ketone, 4-[4-hydroxyphenyl]butan-2-one (Figure 20).[4] This phenylpropanoid is the primary aroma compound found in raspberries and it is also present in other fruits, vegetables and berries, such as blackberries, grapes, and rhubarb.

Plant-derived raspberry ketone is uneconomical for commercialization due to its low yield and chemically manufactured derivatives of this valuable flavoring agent fetch much lower prices than the naturally derived form.[88] In earlier work, it was demonstrated that it is possible to produce raspberry ketone from *p*-coumaric acid in heterologous *E. coli* and *S. cerevisiae*.
strains. However, the high cost of \( p \)-coumaric acid as a substrate and the trace amounts of this phenylpropanoid obtained in the previous iteration of a bioengineered yeast strain, did not offer a profitable option for commercial production of raspberry ketone. The recent de novo production of raspberry ketone in a haploid wine strain (AWRI1631) of \textit{S. cerevisiae} was achieved via metabolic pathway engineering and synthetic enzyme fusion.

The phenylpropanoid pathway starts with the conversion of phenylalanine to \( p \)-coumaric acid via cinnamate or directly from tyrosine to \( p \)-coumaric acid. Conversion of \( p \)-coumaric acid to raspberry ketone requires three additional enzymatic steps including a condensation reaction between coumaroyl-CoA and malonyl-CoA. To construct a biosynthetic pathway for the de novo production of raspberry ketone in a wine strain of \textit{S. cerevisiae}, the following codon-optimized genes were synthesized and integrated into the yeast’s HO locus: the phenylalanine ammonia lyase from an oleaginous yeast, \textit{Rhodosporidium toruloides}; the cinnamate-4-hydroxylase from the well-characterized model plant, \textit{A. thaliana}; and the coumarate CoA ligase 2 gene from parsley, \textit{Petroselinum crispum}, fused by a rigid linker to the benzalacetone synthase from rhubarb, \textit{Rheum palmatum}.

This “synthetic” wine yeast is capable of synthesizing raspberry ketone at concentrations almost two orders of magnitude above its predicted sensory threshold in Chardonnay grape juice under standard wine fermentation conditions, while retaining the ability to ferment the must to dryness. This first small step marks a giant leap forward for wine yeast into an unwritten future with synthetic DNA.

**Treading the “synthetic” tightrope with care**

Science is a constant balancing act, especially when it comes to walking the thin tightrope that spans the chasm between scientific freedom and social responsibility in the unknown territory of synthetic genomics. Even the best-balancing acrobats in research would find it challenging, at times, to make treading the high-tensioned wire of synthetic bioengineering or low-hanging slackline of “bio-error”/“bio-terror” look effortless – they
have to carefully consider the complex gravitational forces of perception and control every step of the way across the gorge caused by this emerging science. The pioneering “extreme tightrope-daredevils” in Synthetic Biology cannot afford to lose their footing even once.

It is for this reason that the Sc2.0 partners (consisting of scientists from different backgrounds, diverse settings, and many nations) have developed and formally adopted a common set of principles to guide this massive, collaborative Synthetic Biology project (Figure 21). To participate in the Sc2.0 project, every partner organization has signed a legally binding agreement that each individual researcher working across the “nodes” will strictly abide by the rules outlined in a published statement on ethics and governance. This Sc2.0 ethics and governance statement addresses issues that go to the heart of societal benefits, intellectual property, safety, and governance of this international Synthetic Yeast Genome Project.[3]

All members of the Sc2.0 consortium undertook to conduct and promote their work for the benefit of humankind and not to bring harm. The Sc2.0 team members agreed to engage with the public and to be transparent and open about their work. Intellectual property rights will not be taken on the ultimate strain containing the 16 synthetic chromosomes, nor on the intermediary clones and strains generated as part of the Sc2.0 project. Data and materials generated by this project will be made available to other researchers.

Figure 21. Balancing scientific freedom and social responsibility with a project-level agreement and ethics and governance statement to guide the international Synthetic Yeast Genome (Sc2.0) Project (adapted from Silva et al. [3]).
with regard to safety or compliance with the Sc2.0 agreement, and to revisit the Sc2.0 agreement as the project progresses and the technologies it uses develop to ensure that any risk by this work is appropriately managed.

This large international Synthetic Yeast Genome Project is, therefore, regulated by existing biosafety legislation and regulations pertaining to recombinant DNA research, and self-governed by the above Sc2.0 agreement and governance structure. To maintain a healthy balance between freedom and responsibility in synthetic genomics research, the Sc2.0 team is of the view that such project-level agreements are an important, valuable, and flexible model of self-regulation for similar global, large-scale Synthetic Biology projects to maximize the benefits and minimize potential harms.[3]

Beyond the horizon

The world that we live in today is vastly different from the world of a century ago. We have become used to living in an ever-changing world of digital democracy and ubiquitous surveillance in smart megacities; a dangerously divided and income-polarized world of cyber and drone warfare; an overpopulated and polluted world with finite natural resources; and an interconnected world that is increasingly dependent on satellite-guided precision agriculture, “on-demand” big data analyses, cloud-computing, bionics, artificial intelligence, and automated robotics where “virtual reality” has indeed become reality. One wonders what the world will look like in 2050 or even 2100? When one pauses and imagines a future world, surreal visions – colored by science fiction movies – of brain-to-machine interfaces in post-human societies, space tourism, moon-mining, and even alien intelligence might slide through one’s mind. Perhaps more realistically from today’s perspective and in the context of this article’s topic, one could envisage a secure and “green” world powered by renewable biofuels; an adequate supply of affordable, nutritious, safe foods and clean water; and effective accessible medicines and treatments for all. Will synthetic chloroplasts and artificial leaves enable us to eventually grow green, self-powered buildings; will synthetic yeast cell factories provide the next generation biofuels, food enzymes, antibiotics, vaccines, and personalized medicines; and will synthetic genomics accelerate the discovery of beneficial gene therapies and cures for cancers and dementia?

Nobody can answer these questions with certainty; however, it is certain that Synthetic Biology will impact a future world in a very significant way. The unimaginable is about to be made possible by synthetic genome engineering. We are, yet again, standing on the brink of a new scientific frontier of unknown opportunities and unimaginable perils, a frontier of unfulfilled hopes and unfulfilled fears. Close collaboration among researchers across the boundaries of multiple disciplines, regulators and thought-leaders in society will have to carefully survey the wonders and terrors that await us and the legacy we will be leaving for future generations.

Past generations of pioneers, discoverers, inventors, and innovators demonstrated that the best way to navigate the uncharted and stormy oceans of a new science is not to take an overly pessimistic view complaining about the wind direction nor to be overly optimistic expecting the wind to change; the best way forward is to be realistic and adjust the sails. As realists, we need to embrace the winds of change and adjust our sails so that we can positively influence our destiny over the next fifty years and beyond.

As synthetic genomics is breathing new life into wine yeast research and stretching the realms of possibility, the winds of change originating from Synthetic Biology will undoubtedly also sweep through the vineyards, wineries, boardrooms, and consumer markets of the global wine industry. However, this will not be the first time that winemaking will have to weather the stormy winds of change generated by new discoveries, technologies, and inventions. It is well understood that the art and science of winemaking are natural companions and that the opposing forces of tradition and innovation have often tested this 7000-year-old companionship to its core. History shows us that when art and science combine harmoniously, great wines emerge; however, when the “natural” tension between tradition and scientific innovation is ill-informed and mismanaged, opinions diverge into centrifugal forces that prevent a constructive dialog and, ultimately, progress.

This article charges all stakeholders and thought-leaders in the global wine sector, to inform themselves about the opportunities and challenges afforded by Synthetic Biology. The time has come to engage in a constructive dialogue about the potential impact of synthetic genomics and the arrival of wine yeast 2.0 strains on their practices and products.

Acknowledgements

The author acknowledges the ongoing support from all members of the international Synthetic Yeast Genome Project (Sc2.0) consortium, and the research contributions of his team at Macquarie University: Thomas Williams, Heinrich Kroukamp, Hugh Goold, Natalie Curach, and Ian Paulsen and collaborators at The Australian Wine Research Institute, Anthony Borneman, Dariusz Kutyna, Danna Lee, and Daniel
Johnson. I am grateful to Rae Blair, Tori Hocking, and Erin Semon for helping to proofread the manuscript, and to Bill Hope, Frantz Kantor and The Drawing Book Studios for creating the artwork used in the diagrams.

Disclosure statement
The author declares that he does not have any competing interests. The Synthetic Biology initiative at Macquarie University is financially supported by an internal grant from the University, and external grants from Bioplatforms Australia, the New South Wales (NSW) Chief Scientist and Engineer, and the NSW Government’s Department of Primary Industries.

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