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Review Article

Towards the first synthetic eukaryotic cell

Wangyue Xu [a], Yue Teng [b,1,*], Sijie Zhou [a,c,1,*]

[a] Frontiers Science Center for Synthetic Biology and Key Laboratory of Systems Bioengineering (Ministry of Education), School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
[b] State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China
[c] Frontiers Research Institute for Synthetic Biology, Tianjin University, Tianjin 300072, China

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ABSTRACT

With the rapid advance in synthetic biology and the expanding field of synthetic genomics, the realization of a redesigned yeast genome has become an achievable milestone. Multiple eukaryotic chromosomes, meticulously designed and synthesized, are now being systematically integrated to create an entirely synthetic eukaryotic cell. This comprehensive review examines the fundamental design principles and construction strategies, highlighting critical technological breakthroughs in pursuing the first synthetic eukaryotic cell. Additionally, it underscores the critical contributions of the Sc2.0 project, which has provided essential tools and engineered cellular platforms that have significantly accelerated research and industrial progress. The ethical and legal implications arising from synthetic eukaryotic life are also explored, offering insights into future research directions for synthetic eukaryotic genomes. The remarkable advances in deoxyribonucleic acid synthesis hold immense potential, promising to unlock new opportunities across medicine, industry, agriculture, and research.

1. Introduction

Deoxyribonucleic acid (DNA), the fundamental building block of life, contains the genetic information that defines an organism's characteristics. The study of genomes enables us to unravel the evolutionary patterns embedded within this data. Thanks to advancements in genome sequencing, editing and synthesis technologies, our understanding of the genome has reached unprecedented levels [1]. While we can now read and modify genomes artificially, fundamental questions arise: can we write genomes *de novo*? Is it possible to reconstruct an entirely new genome? For one thing, the process of creating a new life could potentially solve previously insurmountable problems for practical application and for another, there is great interest in the outcome of a created new life for pure science, the success of the outcome itself would have been proving that humans can make good use of the code of life. New life forms can be created by designing, synthesizing, and detecting synthetic genomes, leading to scientific breakthroughs.

The development of DNA synthesis and assembly technology, particularly the successful application of array-based DNA synthesis chip, has significantly reduced the cost of DNA synthesis and increased synthesis throughput [2], which makes it chemically feasible to write a genome from scratch. In recent years, scientists have used nonliving chemical substances to synthesize DNA and assemble it into chromosomes. By studying significant factors that cause cell death, inactivation, and growth defects, researchers aim to gain insights into the essence of life. Milestones include the 7.50 kb synthetic poliovirus in 2002 [3], the 5.38 kb synthetic φX174 phage in 2003 [4], the 0.58 Mb *Mycoplasma genitalium* genome in 2008 [5], the 1.08 Mb *Mycoplasma mycoides* genome in 2010 [6], and the 3.98 Mb *Escherichia coli* (*E. coli*) genome in 2019 [7]. The genome sizes of yeast and humans are approximately 12.1 Mb and 3,000 Mb, respectively, much larger than those of viruses and prokaryotes. Eukaryotic organisms, characterized by larger genomes, abundant transposons and unique structures such as telomeres and centromeres with highly repetitive sequences, pose significant challenges.

Saccharomyces cerevisiae (*S. cerevisiae*), commonly known as baker's yeast, is arguably the most extensively studied eukaryote. Its whole genome was sequenced as early as 1996, revealing 6,275 open reading frames (ORFs), of which 5,885 are protein-encoding genes [8]. As a eukaryotic organism, *S. cerevisiae* exhibits fundamental cellular processes like those in human cells, such as DNA replication, transcription, translation, and cell cycle regulation. As a model microorganism, *S. cerevisiae* is considered as a low-risk experimental

* Corresponding authors: State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China (Y. Teng); Frontiers Research Institute for Synthetic Biology, Tianjin University, Tianjin 300072, China (S. Zhou).

E-mail addresses: yueteng@sklpb.org (Y. Teng), sjzhou@tju.edu.cn (S. Zhou).

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subject, which helps mitigate ethical concerns associated with synthetic genome experiments. It benefits from a rich research background and well-established gene manipulation techniques, is easy to culture and multiplies in a laboratory. Consequently, *S. cerevisiae* is an appropriate model for studying complex eukaryotic genome engineering.

Synthetic genomics research is the landmark international collaborative effort in the Synthetic Yeast Genome Project, also known as the Sc2.0 project. This project, spearheaded by 21 leading research institutions from multiple countries, aims to design and build synthetic versions of all 16 chromosomes of *S. cerevisiae*. More importantly, the combining all 16 synthetic chromosomes into a single yeast cell generates a strain containing an entire synthetic genome [9]. The Sc2.0 project seeks to deepen our understanding of genome functions, basic biological processes, advanced disease treatments, and potential delay aging, ultimately benefiting all humanity. Nowadays, Scientists are inching closer to generating a synthetic eukaryotic cell.

This review will primarily focus on the progress made in synthetic eukaryotic chromosomes, highlighting design concepts and technical tools associated with their construction. Additionally, the review will discuss the applications of synthetic eukaryotic genomes and explore the ethical considerations surrounding their use.

2. Basic principles of synthetic eukaryotic genome synthesis

Genome synthesis encompasses a range of technologies rooted in synthetic biology, including genome sequencing, genome editing, DNA synthesis, and the hierarchical assembly of long DNA sequences. This approach surpasses small-scale gene editing, involving the comprehensive redesign of yeast chromosomes, large-scale *de novo* synthesis and debugging of DNA sequences, and their assembly into eukaryotic chromosomes to create living organisms. The Sc2.0 project aims to redesign and synthesize of the *S. cerevisiae* genometo achieve a highly adaptable and versatile synthetic yeast genome [10].

The target of the Sc2.0 project is to replace the natural sequence of *S. cerevisiae* with a designed sequence. It is not a mere replication of the *S. cerevisiae* genome on a frame-by-frame basis. On the contrary, its design is customized, intricate, and innovative. Repetitive regions and most introns are eliminated, and unstable regions, such as transposons, are removed. Transfer ribonucleic acid (tRNA) genes, hotspots of genomic instability, are relocated to a dedicated synthetic chromosome (recombine a tRNA neochromosome) [11]. The TAG termination codon is re-encoded as TAA to facilitate the insertion of non-standard amino acids. The most notable feature of the synthetic yeast genome's sequence design is the incorporation of numerous loxPsym specific recombination sites. Unlike the wild-type unidirectional recombination lox site, the 34 bp sequence of the loxPsym site possesses a reverse palindromic symmetric sequence structure, enabling it to recombine in either orientation [12]. Within the Sc2.0 project, researchers introduced a loxPsym site at the 3′ end of each non-essential gene. Cre recombinase can recognize these sites to initiate synthetic chromosomal recombination (synthetic chromosomal recombination and modification system mediated by loxP, SCRaMbLE) [13]. Genomic rearrangements include deletions, insertions, and inversions using the Cre recombinase and the bidirectional loxPsym site inserted into the 3′ untranslated region (UTR) of non-essential genes [14].

3. Methods and tools for synthetic eukaryotic genomes

3.1. Design of synthetic eukaryotic genomes

The global consortium research groups are distributed across many locations, Sc2.0 relies on the computer-aided sequence design tool BioStudio to enable a common assembly strategy and enforce a shared language to describe designed chromosomes [13]. They adhere to the three guiding principles of "robustness, stability, and variability" to systematically design the chromosomes of *S. cerevisiae*. To facilitate international collaboration and accessibility, W. Yu et al. [15] developed and launched a public online design platform, GenoDesigner, during the *de novo* synthesis of the *Physcomitrium patens* (earthmoss) genome. Another software named Geneious [16]has molecular biology and sequence analysis tools to enhance productivity. Some other genome design tools are shown in Table 1.

3.2. Building of Synthetic Eukaryotic Chromosomes

Most research teams adopted a hierarchical assembly strategy to synthesize chromosomes, which involves dividing the synthesized chromosome sequence into DNA fragments of various sizes. Initially, synthetic genes (Minichunks, -3 kb) are assembled into longer DNA fragments (Chunks, -10 kb) using Gibson assembly [17]. Subsequently, employing the SwAP-IN (switching auxotrophies progressively for integration) strategy [13], 3–5 long DNA fragments are simultaneously introduced into yeast cells, where they replace the corresponding wild-type regions (-30 kb) in the chromosomes through homologous recombination [12]. Using the meiotic recombination-mediated assembly (MRA) strategy [18], the intermediate strains were crossed, sporulated, and screened for spores in which the synthetic sequences were combined. Finally, complete synthetic chromosomes are constructed through homologous recombination to replace the native DNA sequence.

Synthetic chromosomes can be consolidated into a single strain by endoreduplication intercross [13]. Precisely, two strains with different synthetic chromosomes are mated, after destabilizing native chromosomes, and haploid strains with two or more synthetic chromosomes are obtained by sporulating and screening. A new strategy using chromosome elimination by Vika/vox, a site-specific recombination system orthogonal to Cre/loxP, was developed to accelerate consolidation [19]. The centromeres of native chromosomes were successively excised by Vika/vox recombination, eliminating of the native chromosomes. Another chromoduction phenomenon was developed, named chromosome substitution [20], which can directly transfer individual chromosomes to a recipient haploid strain that already carries multiple synthetic chromosomes.

3.3. Editing of synthetic eukaryotic chromosomes

During the chemical synthesis of the genome, abnormalities and mismatches in base sequences can significantly affect the growth and functionality of synthetic cells, indicating flaws in the synthesized genome. Consequently, identifying and rectifying these defects constitute the two significant challenges encountered in gene assembly.

Interestingly, note that genotype and growth phenotype defects occurred during the design and construction process of synV. To solve these problems, and more importantly, to expedite the process of whole chromosome replacement and detect potential issues in synthetic sequences at an early stage, Y. Wu et al. [21] introduced a high-throughput localization strategy known as pooled PCR tag mapping (PoPM), which proved to be an efficient method for identifying growth defective targets and locating errors in synthetic chromosome construction. This approach can be extended to any synthetic chromosome containing PCRTags, enabling the efficient repair of errors. This technology was used to identify design bugs in the synV sequence of this version, enabling the redesign of the synV chromosome. By iterating the "design-synthesis-test" cycle, the perfect synV [22] chromosome was successfully synthesized.

Y. Zhao et al. [20] developed a systematic and efficient bug-mapping strategy exploiting loss of heterozygosity (LOH) in diploids called clustered regularly interspaced short palindromic repeats (CRISPR) Directed Biallelic URA3-assisted Genome Scan, or CRISPR D-BUGS. By checking the fitness of the synthetic strains, they can find

Table 1
Genome design tools for synthetic eukaryotic genomes.

Design tools	Description	Uniform resource locators (URLs)	Reference
BioStudio	This tool supports the design and editing of synthetic genomes, specially for yeast originally.	https://app-studio.bioturing.com/	[13]
GenoDesigner	A public online design platform for the synthesis of the *Physcomitrium patens* genome.	https://github.com/WenfeiY/P.patens_geno_design	[15]
Benchling	A cloud-based platform that supports sequence design, clustered regularly interspaced short palindromic repeats (CRISPR) guide design, and molecular biology workflows.	https://www.benchling.com/platform	For commercial purpose
Geneious	A comprehensive tool for deoxyribonucleic acid (DNA) sequence analysis, cloning, and gene design, offering features for sequence assembly and annotation.	https://www.geneious.com/	[16]
SnapGene	A tool for visualizing and documenting molecular biology procedures, particularly useful for designing plasmids and cloning experiments.	https://www.snapgene.com/	For commercial purpose

the "fitness boundary" at which derivative strains shift phenotypically from unhealthy to healthy under specific conditions.

The difficulties of precisely customizing and synthesizing long chromosomes have been dealt with, greatly enhancing the efficiency and accuracy of constructing such DNA. As a result, the scientific community has established precise chemical synthesis techniques for synthetic genomes.

4. The development status and applications

In 2011, Sc2.0 achieved a significant milestone by synthesizing the first synthetic chromosome arm, synIXR, which marked the commencement of the international project [12]. In 2014, Sc2.0 reported the synthesis of the first yeast chromosome, synIII [23]. Subsequently, in 2017, five additional chromosomes, namely synII [24], synV [22], synVI [25], synX [21], and synXII [18], were successfully synthesized. By 2023, the synthesis of nine more chromosomes, namely synI [26], synIV [27], synVII [28], synVIII [29], synIX [30], synXI [31], synXIV [32], synXV [33] and synXIII [34], along with a tRNA neochromosome [11] had been reported. The synthesis of the remaining one chromosome is expected to be complete by the end of 2024, with one piece and completion of the jigsaw puzzle. The design and synthesis of these synthetic chromosomes represent a significant milestone in synthetic genomics. Researchers have successfully synthesized all 16 chromosomes and a tRNA neochromosome of *S. cerevisiae*, creating 17 partially synthetic yeast strains. Each haploid strain contains only one synthetic chromosome, leaving most of the genome native.

The next challenge lies in merging these chromosomes into one cell and creating a yeast organism possessing entirely artificially synthesized chromosomes within its cells. synI is designed to be attached to synIII so that the karyotype is not altered by introducting the tRNA chromosome [26]. S. Zhou et al. [19] accomplished the consolidation of 5.5 synthetic chromosomes into a single cell, utilizing the Vika/vox site-specific recombination system, which operates independently of the Cre/loxP system, to achieve chromosome deletion. By gradually eliminating the wild-type chromosomes corresponding to the synthetic ones, they integrated 5.5 synthetic chromosomes (synII, synIII, synV, synVI, synIXR, and synX, approximately 2.61 Mb) into haploid strains. In parallel, Y. Zhao et al. [20] successfully merged 6.5 full-length synthetic chromosomes into the same cell using the "endoreduplication intercross" strategy, and proposed the next-generation method, chromosome substitution, to produce the syn7.5 strain. Compared with the "endoreduplication intercross" strategy, the latter can directly transfer individual chromosomes to a recipient haploid strain that already carries multiple synthetic chromosomes by chromoduction without the process of meiosis. Combined with CRISPR D-BUGS, it can significantly expedite the consolidation of the remaining synthetic chromosomes into a single cell.

In the future, the 16 artificially synthesized chromosomes will serve as a robust foundation, analogous to constructing piers for a high-speed railway bridge spanning the sea (Fig. 1). This significant development will position yeast as the first eukaryotic organism with a fully synthetic genome, enabling it to function seamlessly akin to a high-speed bullet train. This breakthrough will bridge the gap between the known and the unknown, guiding us from the familiar shores to unexplored territories.

SCRaMbLE enables the altering the 3′ Region [35] of these genes, providing a valuable resource for studying their functions. This method accelerates phenotypic evolution and facilitates the analysis of the relationship between genome structure and function [10]. SCRaMbLE induces profound and comprehensive changes in pathways, chromosomes, or even the entire genome, which other methods may not achieved through other methods [36]. It mainly develops from technological progress, practical applications, and biological mechanism contributions.

First, SCRaMbLE has undergone technological evolution and technological innovation. To address the concerns regarding uncontrolled rearrangement caused by leaky expression, B. Jia et al. [37]designed a genetic AND gate to achieve precise control of genome rearrangements in synthetic yeast. L. Hochrein et al. [38] developed a light-controlled Cre (L-SCRaMbLE) system with rapid and fully reversible performance advantages. The scope of rearrangement expands with the increase in the number and diversity of loxP sites utilized. A yeast strain comprising 894 loxPsym sites distributed across 5.5 chromosomes was constructed [19], and 263,520 rearrangement events were detected in total. L. Cheng et al. [39] constructed a yeast strain containing 83 loxPsym sites distributed across all 16 chromosomes. SCRaMbLE of SparLox83R produces versatile genome-wide genomic rearrangements, obtain strains with increased tolerance to nocodazole and stains with rapid adaptation of tolerance to acetic acid. C. Cautereels et al. [40] established a set of 16 orthogonal LoxPsym variants. They demonstrated their use for multiplexed genome engineering in both prokaryotes (*E. coli*) and eukaryotes (*S. cerevisiae* and *Z. mays*), significantly expanding the Cre-LoxP toolbox for applications in genome editing, metabolic engineering, and other controlled recombination events.

Second, its application holds the potential to enhance growth adaptability and increase chemical production in cell factories. B.A. Blount et al. [41] conducted rapid genome recombination in yeast con-

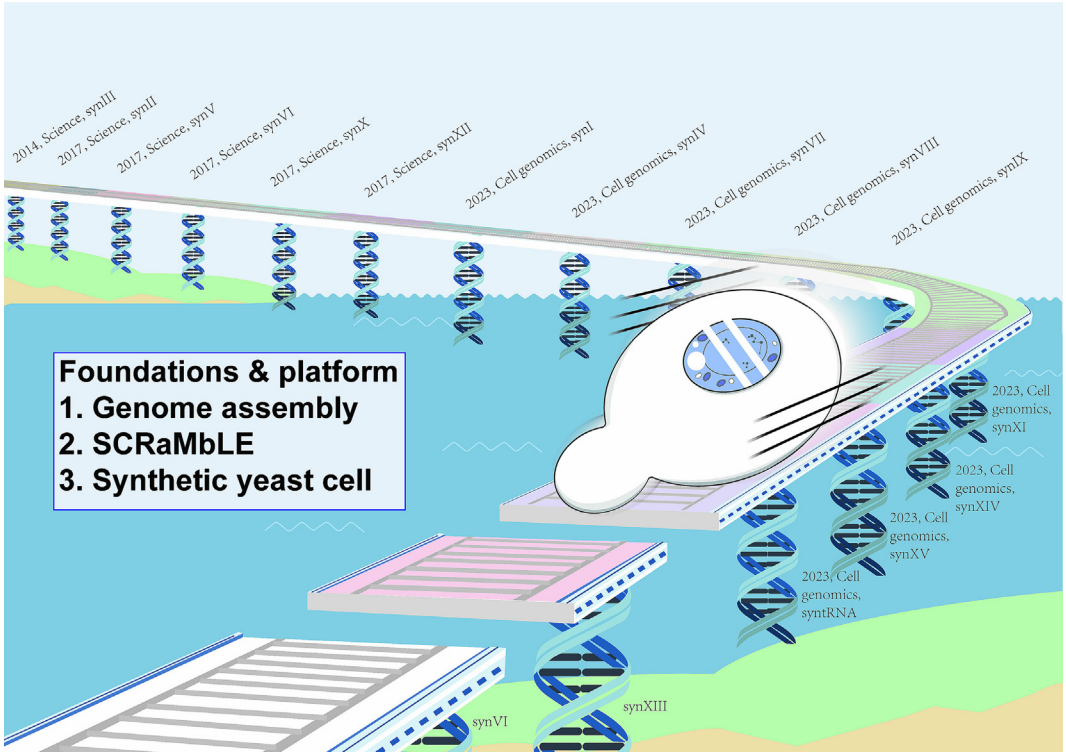

Fig. 1. Towards the creation of the first synthetic eukaryotic cell.

taining synV chromosomes, resulting in increased production of violacein or penicillin. Leveraging the strength of the ability to manipulate the Sc2.0 genome for large-scale recombination, non-synthetic yeasts can also play an essential role by SCRaMbLE. B. Jia et al. [37] and Y. Shen et al. [42] demonstrated that by mating haploid Sc2.0 strains with haploid non-conventional yeast strains, the synthesized Sc2.0 chromosomes not only functioned in heterozygous diploid yeast but also yielded diploid strains with improved phenotypes, such as enhanced growth at high temperatures, through SCRaMbLEing. B. Jia et al. [37] also employed MuSIC (multiplexed SCRaMbLE iterative cycling) in heterozygous diploid yeast, further diversifying genotypes, increasing the diversity of rearranged libraries, and continuously increasing carotenoid production.

SCRaMbLE can be applied not only *in vivo* but also *in vitro*. Y. Wu et al. [43] and W. Liu et al. [44] applied SCRaMbLE to a pathway or a group of genes *in vitro*, targeting smaller segments than the entire chromosome. They employed SCRaMbLE like enzymes to rearrange gene parts and reintroduce them into cells to observe the resulting phenotypes, which can increase production effectively.

Third, SCRaMbLE also advances research on biological mechanisms. S. Zhou et al. [19] generated a genome rearrangement library using strains containing 5.5 synthetic chromosomes. They discovered a specific rearrangement pattern of the synthetic chromosomes by analyzing these rearrangement events. The preference for rearrangement was found to be a synergistic effect of chromatin accessibility and spatial contact probability, with rearrangement tendencies occurring in spatially close with open chromatin. C. Zhou et al. [34] constructed the synXIII and conducted a study to reveal the impact of synthetic chromosomes and aging. The study suggests that Sc2.0 yeast has the potential for unveiling new aging-related genes and gene-gene interactions underlying replicative lifespan.

Progress in eukaryotic synthetic genomics has been impressive, but the costs of DNA synthesis remain a barrier for genome-scale projects. Accelerating progress could require breakthroughs in the efficiency of DNA synthesis, similar to previous breakthroughs in DNA sequencing [1]. Projects involving synthetic genomics are arduous and resource-intensive endeavors, the synthesis of other eukaryotic organisms may not materialize until the cost of genome synthesis is significantly reduced. Building upon the Sc2.0 project, Boeke and Church introduced the Human Genome Writing Project (HGP Write) in 2016 [45], thrusting synthetic eukaryotic genomes into the public spotlight. One of its key objectives is to achieve a 1,000-fold reduction in the cost of higher eukaryotic genome synthesis by 2029, thereby enabling large-scale genome projects.

The human genome is much larger than the yeast genome. Leopold Parts and George Church developed a toolbox [46] to create scale rearrangements by highly multiplexed recombinase recognition site insertion into repetitive sequences with CRISPR prime editing. Using the conception of SCRaMbLE, they obtained many human cells with megabase-sized rearrangements per cell.

5. The ethical issues

In synthetic biology, the main concerns related to biosafety are the potential damage of dangerous biological agents to the environment and public health. A report signed by three European Scientific Committees states that “new challenges in predicting risks are expected due to emergent properties of SynBio products and extensive genetically engineered systems, including, 1) the integration of protocells into / with living organisms, 2) future developments of autonomous protocells, 3) the use of nonstandard biochemical systems in living cells, 4) the increased speed of modifications by the new technologies for DNA synthesis and genome editing, and 5) the rapidly evolving Do It Yourself (DIY)-bio citizen science community, which may increase the probability of unintentional harm” [47]. The challenges of adapting and applying artificial intelligence (AI) principles to synthetic biology not be denied specialized testing needed for medical AI such as safety, food and drug administration (FDA), and privacy [48].

The debate on biosafety in synthetic biology is open; measures for biocontainment, guidelines, and regulations in this area have already

Table 2
Guidelines and laws for the code of conduct on biosafety.

File name	Leading country or region	Publish year
Biosafety in Microbiological and Biomedical Laboratories (BMBL)	America	1984
Convention on Biological Diversity (CBD)	the United Nations Environment Programme (UNEP)	1993
The Cartagena Protocol on Biosafety	the United Nations Environment Programme (UNEP)	2000
Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (Text with EEA relevance)	European Union	2003
Biosecurity Law of the People's Republic of China	China	2020
The Tianjin Guidelines for the Code of Conduct on Biosafety for Scientists	China	2021
Global guidance framework for the responsible use of the life sciences: mitigating biorisks and governing dual-use research	The World Health Organization (WHO)	2022
The Measures of the Ethical Reviews of Life Science and Medical Research Involving Humans	China	2023

been proposed. Biocontainment measures can be divided into physical measures and biological mechanisms. Physical measures include the optimization of equipment and processes. Biological mechanisms include means to make sure that the engineered trait will not be expressed when the organism escapes from the lab [49] and make the synthetic genes more easily traceable by "genetic barcodes" [6].

Here are some guidelines and laws around the world regarding biosafety; see Table 2.

In 2021, *the Tianjin Guidelines of the Code of Conduct in Biosafety* for Scientists was established. It urges governments and research institutions worldwide to reinforce oversight and self-discipline to ensure that biological science serves humanity's best interests and prevents misuse. They advocate for enhancing researchers' biosafety awareness across multiple domains, including research responsibility, dissemination of achievement, technology popularization, and international collaboration.

An overarching concern is regenerating of any virus and many bacterial pathogens, including the smallpox virus through DNA synthesis. Consequently, restricting DNA synthesis to legitimate researchers becomes crucial. Reputable DNA synthesis companies already employ screening measures, to mitigate this risk by comparing all orders against a list of Class A pathogens [50].

6. Summary and outlook

As physicist Richard Feynman wrote, "What I cannot create, I do not understand." The synthesis of yeast genomes by scientists serves the true purpose of studying and comprehending genes, demonstrating our ability to decipher and harness the genetic codes crafted by nature. The endeavor is driven by the aspiration to create something significantly distinct from natural design, with the primary objective of developing yeast strains that facilitate the acquisition of new biological knowledge.

The exploration of artificial eukaryotic genome synthesis holds the potential to deepen our understanding of gene function and regulatory mechanisms in organisms. It offers novel insights and methods for unraveling the intricate relationship between genes and diseases. Microorganisms can be designed and engineered with specific metabolic pathways and functions, finding applications in biological manufacturing and environmental remediation. This technological advancement opens new possibilities for industrial production and environmental protection. Furthermore, synthetic eukaryotic genomes enable the creation of model organisms with tailored genomic structures, facilitating research into disease mechanisms, drug development, and other areas of biomedical inquiry by providing innovative tools and resources. Synthetic biology can provide new practical measures to fulfill biosafety requirements, such as synthetic auxotrophies, genetic barcodes or ingenious genetic circuits. However, these may be insufficient, and research in this area must continue.

S. cerevisiae has been extensively utilized in various applications, such as food fermentation, chemical bioproduction in microbial cellular factories, and as a tool for medical research. It has played a pivotal role in numerous fundamental discoveries in cell biology, such as telomeres [51], cell cycle [52], and autophagy [53]. Sc2.0 demonstrated the feasibility of designing and synthesizing a fully functional eukaryotic genome, establishing a proof-of-concept for synthetic genomics. This success provides a validated model for applying similar approaches to other eukaryotic organisms, encouraging further research in more complex species. The Sc2.0 project has led to the development and refining various technologies and methodologies for genome synthesis, editing, assembly, and debugging. These tools can be adapted and applied to other organisms, facilitating the design and construction of synthetic genomes in different species. A case in point is BioStudio. Although initially designed for the yeast genome, its editing capabilities and underlying design principles apply equally to many other eukaryotic organisms. Customization may be necessary to address the target organism's unique genomic features and extend BioStudio's functionality to other eukaryotic species, including managing genome size, the complexity of repetitive sequences, specific regulatory elements, and other species-specific genomic attributes. Undoubtedly, the yeast can enable the progress of similar work in other species from multiple dimensions, including conceptual and technical advancements, and propel us forward like an accelerated high-speed train from a familiar shore into uncharted territories.

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Conflcit of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Wangyue Xu: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yue Teng:** Writing – review & editing, Supervision, Project administration. **Sijie Zhou:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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