



Design and Characterization of the Synthetic Microbial Genome

10

Nomthandazo Twala, Mutsa M. Takundwa, Earl Prinsloo,
and Deepak B. Thimiri Govinda Raj

Contents

Introduction	278
Concept of Synthetic Biology	281
Strategies to Develop Synthetic Genomes	283
The Workflow for Designing and Engineering Synthetic Genomes	283
Evolution of Synthetic Genome	286
Future Perspective: Lactic Acid Bacteria Synthetic Genomes	292
References	295

Abstract

Synthetic genomics is a field of engineering biology that involves modifying or constructing viruses, bacteria, and eukaryotic cells with synthetic genomes. Synthetic genomes are used to alter or create new functions in cells, such as the production of high protein expression. This chapter examines the latest discoveries in synthetic genomics, focusing on the study design and modern strategies applied to create synthetic genomes. Several techniques are used to engineer and modify both eukaryotic and prokaryotic cells. The study conducted by Fredens and colleagues discovered the first living organism with a fully synthetic DNA code, called *Escherichia coli* Syn61, by using a prokaryotic cell. The Yeast 2.0 project is a well-known example of synthetic genomics where eukaryotic fungi, *Saccharomyces cerevisiae*, were genetically modified with artificial genomes to

N. Twala · M. M. Takundwa · D. B. Thimiri Govinda Raj (✉)
Synthetic Nanobiotechnology and Biomachines, Synthetic Biology and Precision Medicine Centre,
CSIR, Pretoria, South Africa

Biotechnology Innovation Centre, Rhodes University, Makhanda, South Africa
e-mail: Ntwala1@csir.co.za; mtakundwa@csir.co.za; dgovindaraj@csir.co.za

E. Prinsloo
Biotechnology Innovation Centre, Rhodes University, Makhanda, South Africa
e-mail: e.prinsloo@ru.ac.za

create a fully synthetic yeast strain. These artificial genomes are used by researchers to better understand how genomes function, the organism's metabolism, and the production of chemical molecules for medicines or biofuels.

Keywords

Synthetic genome · *Escherichia coli* · *Saccharomyces cerevisiae* · Lactic acid bacteria

Introduction

Synthetic biology (SynBio) is a new interdisciplinary field of study which combines biology and various branches of engineering to design and create new biological components as well as the redesign of existing organismal systems (Brooks and Alper 2021; Xu et al. 2023). The advances in knowledge and techniques of SynBio technology gave rise to synthetic genomics which introduces new functional modules or artificially modified genomes from pre-existing life forms into an organism (in most cases, microorganisms) which results in the reprogramming of the cells' DNA transforming the organism to serve as a chassis (Hamese et al. 2023; Wang et al. 2023; Ma et al. 2024a). In this field, a synthetic genome is an artificially constructed DNA built from scratch by scientists, and a microbial chassis is defined as a platform that acts as a framework containing genetic components along with biological resources that will allow the components to function.

The origins of SynBio can be traced back to the 1960s, where Jacob and Monod discovered the first gene regulatory mechanism, known as the lactose operon (lac operon, followed by the development of molecular cloning and PCR in the 1970s and 1980s) (Rheinberger 2023). By May 1996, the first complete synthetic yeast genome was sequenced using automated DNA sequencing techniques and advanced computational tools (Chen et al. 2022). The early 2000s marked the emergence of this growing biotechnology field, where scientists designed the first synthetic gene circuits, namely, the genetic toggle switch and the repressilator, both important for controlling gene expression (Lezia et al. 2021). Finally, the 2010s and 2020s contained significant scientific advancements, combining computer tools and molecular biology to design and produce microbial cell factories or chassis for vaccine, therapeutic, and industrial applications, among others (Crocker et al. 2021; Hamese et al. 2023; Mugwanda et al. 2023). In 2010, scientists working at the J. Craig Venter Institute (JCVI) designed and engineered the first self-replicating synthetic bacterial cell (Pelletier et al. 2021), *Mycoplasma mycoides* (*M. mycoides*) JCVI-syn1.0, which was later modified into *M. mycoides* JCVI-Syn3.0 by the same research group (Gibson et al. 2010). As of 2020, scientists recently used advanced technologies to design and engineer personalized medicine for various diseases including type 2 diabetes (Venkatachalapathy et al. 2021), gene therapy for cancer treatment and vaccine development, namely, mRNA vaccine with enhanced efficacy and stability for infectious diseases (Zhang et al. 2023). Table 10.1 illustrates the brief history of synthetic biology.

Table 10.1 Timeline showing the history of synthetic genomics. This timeline starts from the 1980 and expands to the 2020s

Year	Milestone reached	Source
1980	In 1983, Kary Mullis discovered the molecular technique, known as Polymerase Chain Reaction (PCR).	Mullis (1990)
	The first recombinant Vaccine for Hepatitis B was developed by Maurice Hilleman and his team in 1986.	Pattyn et al. (2021)
1990s	Francisco Mojica was the first to discover the principles of CRISPR technology in 1993.	Shmakova et al. (2022)
2000s	In 2002, Eckard Wimmer's group at Stony Brook University successfully engineered the first synthetic virus, the poliovirus, from its chemical code.	Moles et al. (2024)
	In 2003, the Chinese SFDA approved the first gene-targeted therapy product, called Gendicine, indicated for head and neck cancer.	Arabi et al. (2022)
	The first synthetic bacterial genome assembled, <i>M. genitalium</i> JCVI-1.0.	Gibson et al. (2008)
2010s	The construction of the world's first minimal synthetic bacterial cell, <i>M. mycoides</i> JCVI-syn1.0.	Gibson et al. (2010)
	In 2012, Emmanuelle Charpentier and Jennifer Doudna developed the CRISPR-CAS9 technology, revolutionizing genome editing.	Strzyz (2020)
	In August 2017, the FDA approved the first CAR T cell therapy, tisagenlecleucel (Kymriah) for the treatment of Acute Lymphoblastic Leukemia.	Mitra et al. (2023)
2020s	The Pfizer's COVID-19 vaccine was the first mRNA vaccine to receive full FDA approval in the United States.	Vitiello and Ferrara (2021)

Scientists use synthetic genomes to study biological systems of natural organisms which help in understanding how genomes function and the complex biological systems of the organisms (Fu and Shen 2024). These genomes are also used for engineering organisms with desired traits important for vaccine production of emerging viruses and pandemics, like the mRNA vaccines for COVID-19 that were created in less than one year (Venter et al. 2022; Demongeot and Fougère 2023). Additionally, they help to develop new technologies which assist with medicine production by creating new therapeutic proteins for pharmaceutical or industrial use, and food production by creating synthetic crops that are tolerant to stress conditions, have higher yield and use nutrients efficiently (Ye et al. 2024). They are also used for waste management using the process of bioremediation for the purpose of removing pollutants from water, air, and soil to keep our environment clean (Kuppan et al. 2024). Furthermore, these artificial genetic materials can be used for advanced cell therapy by expressing desired proteins or providing new biological features, such as targeting cancer cells with high specificity and developing pathogen resistance treatments for infectious diseases.

The main challenges in designing and engineering synthetic genomes include: i) difficulties in accurately assembling large DNA fragments (Ma et al. 2024b); ii) unintended consequences which could have unknown or unintended effects on

cellular function causing health problems; iii) and the potential release of engineered organisms (synthetic viruses or bacteria or cells) to the environment which may cause health or safety issues (Bohua et al. 2023). These challenges can be countered by using recently advanced technologies, such as computational genome modeling, before running experiments to identify the presence of essential genes and minimizing the genome by removing non-essential genes (Xu et al. 2023). The minimal synthetic genomes have improved stability characteristics, are easy to model, and grow rapidly (LeBlanc and Charles 2022). In addition, scientists are also using next-generation sequencing (NGS) technology for the analysis of the whole genome and CRISPR-CAS9 editing tools to precisely manipulate DNA sequences within a genome. The use of SynBio and bioinformatics tools to design and modify bacteria resulting in the production of oral vectors or the secretion of biomolecules is an emerging area of research with great clinical potential (Guo et al. 2023). The secreted biomolecules (peptides, cytokines, enzymes, or bacteriocins) function as probiotics which can strengthen the immune system and protect against infectious diseases (Silva et al. 2020). The key areas of future development in synthetic genomes include: i) enhanced organ engineering used to produce functional synthetic organs for transplantation; ii) personalized medicine to target specific disease mutations resulting in efficient therapies (Stefanov and Fussenegger 2022; Yan et al. 2023; Nazir et al. 2024); iii) using sustainable biofuel production to precisely convert renewable resources to biofuels (Carrillo-Martínez et al. 2023); iv) enhanced vaccine development by creating synthetic viruses with optimized antigen presentation which could lead to faster response time to emerging diseases (Viana Invenção et al. 2021; Khan et al. 2022).

Over the past three decades, the world has experienced various disease outbreaks, some of which led to disastrous outcomes, such as high death rates, as we were not properly prepared to develop effective medical countermeasure responses in time. The major pandemic outbreaks experienced in the twenty-first century include respiratory corona viruses, namely, the SARS-CoV1 which had 8422 reported cases with 916 deaths (Moghaddasi et al. 2021), and the most recent SARS-CoV2 (COVID-19 virus) which had 79,232,555 confirmed cases and 1,754,493 deaths by December 2020 (Bhadoria et al. 2021; Deb et al. 2025). Followed by mutated influenza pandemic viruses of the twentieth century, the influenza A H1N1 with reported cases close to 700 million to 1.4 billion (Bhadoria et al. 2021) with 18,500 reported deaths (Eslami et al. 2025), the influenza A H2N2 which caused the 1957 Asian Flu pandemic resulted in over 1 million deaths, and the influenza subtype H3N2 which resulted in a mortality rate between 1 to 4 million (Honigsbaum 2020). Evidence shows that the frequency of reported epidemic and pandemic infectious disease outbreaks has increased, with predictions that this rise will continue due to environmental changes and global travel, among others. So, WHO designed an R&D blueprint list of priority diseases and included Disease X. The goal of this list is to accelerate the production of medical countermeasures for future health crisis to prevent social, economic, and political disruptions. Therefore, in the current study, the authors aim to design and engineer *Lactobacillus casei* (*L. casei*) and/or *Lactococcus lactis* (*L. lactis*) microbial cell platforms that will function as prototypes

for the rapid production of vaccines against an unknown disease (Disease X) using computational methods, bioinformatics tools, and experimental molecular techniques. The development of the *L. lactis* and *L. casei* microbial cell factories is critical for protein production, gene therapy, and vaccine production which will lessen the impact of the next pandemic. In this chapter, the author discusses the concept of synthetic biology, the evolution of synthetic genomes, and strategies used by research scientists to design artificial genomes to understand the characterization of the synthetic genome better.

Concept of Synthetic Biology

Synthetic biology involves manipulating or reprogramming DNA sequences of organisms to give them new or altered functions. Synthetic biology has a wide range of applications, which include healthcare, pharmaceutical, industrial, biotechnological, biomedical, and environmental fields (Garner 2021). This field of study has generated basic tools, namely, DNA synthesis, DNA sequencing, chassis, transcriptional engineering, genome modification, and computer-aided tools to address many societal issues and needs. For example, in previous studies, SynBio researchers have modified and engineered bacteria that can produce biofuels (Zhang et al. 2021) and detect heavy metals using microbial biosensors (Somayaji et al. 2022). In other studies, researchers engineered microorganisms that were used during fermentation to produce many daily food products such as bread, chocolate, yogurt, and beverages. There are two different but complementary approaches for SynBio used to design artificial microbial chassis: the top-down approach which derives microbial chassis from the biological cells of living organisms, and the bottom-up approach which produces artificial microbial chassis de novo, for novel applications. These research strategies can be used to engineer and modify both eukaryotic and prokaryotic cells as seen in previous studies. The synthetic biological systems designed using SynBio tools are intended to maintain the central dogma for efficient production of biomolecules used to solve societal challenges, such as vaccine development, replacing fossil fuels, food production, and improving drug targeting systems (Sun et al. 2022; Bhatia et al. 2025).

The top-down approach produces synthetic chassis from existing living organisms with the aim of reducing their genome by repeated deletions of non-essential genetic components resulting in “minimal” cells (Kim et al. 2024). The synthetic chassis designed using the top-down approach is usually living and still closely related to the biological cell that created it but performs new or enhanced functions. The minimal cell’s genome only encodes a minimum number of essential genes needed for the cell to survive. In 2016, Xu and coworkers mentioned that these minimal cells are expected to assist researchers to better understand and regulate essential life processes. The key steps in top-down approach workflow include: i) genome annotation and analysis to identify functional regions of the genome such as essential genes; ii) target gene deletion by using genetic engineering editing tools like CRISPR-CAS9 to precisely remove or modify specific genes of known genomes to form a synthetic cell with desired

characteristics; iii) validation and characterization to assess viability and functionality after each experimental manipulation; iv) and interactive optimization by repeating rounds of gene deletion and manipulation, based on the results of previous characterization, to reduce the genome, see Fig. 10.1.

The advantages of this approach include access to the host cell's machinery (Hamilton et al. 2021; Karbalaeei-Heidari and Budisa 2024), faster development compared to the bottom-down strategy and improved experimental control since it uses known biological pathways (Denies et al. 2020). A well-known example for this approach is the minimal genome projects, which allow the identification of essential genes of a specific organism. The *Mycoplasma genitalium* (*M. genitalium*) genome sequenced in 1995 is the smallest known genome of all living organisms, used in a study by Fraser and colleagues, who discovered that the parasitic bacterium has 517 genes making this microorganism ideal for “minimal” cell development (Feng et al. 2024). Subsequently, in 2004, Gil and his colleagues performed computational analysis and experimental applications to further remove non-essential DNA segments from the genome of *M. genitalium* bacterium, reducing the number of genes to 206 for the bacterial cell to maintain its ability to live, grow, and evolve (Kumar et al. 2023b; Kim et al. 2024).

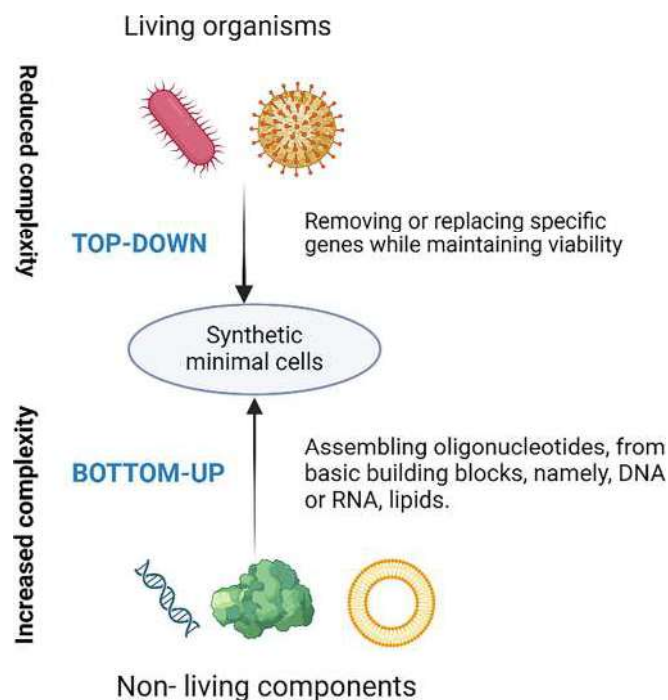


Fig. 10.1 The top-down and bottom-up synthetic biology approaches for the design and synthesis of synthetic minimal cells. (Created with [BioRender.com](https://www.biorender.com))

Compared to the top-down approach, the bottom-up approach starts from scratch using three essential elements to synthesize an entire genome (Hamese et al. 2023). It aims to construct a minimal synthetic cell capable of replication and evolution by assembling a stack of biological and or non-biological components (Xu et al. 2016). The first component of this approach is the molecules carrying information (DNA or RNA) which define the cell's nature and function; followed by the plasma membrane which serves as the barrier of the cell, they are semi-permeable meaning they allow some molecules into the cell, block some nutrients, and remove the waste products. Finally, the third component is metabolism process which generates energy for the cells to grow, maintain structures, reproduce, survive, evolve, synthesize, and respond to the environment. The bottom-up approach has many advantages which include helping scientists understand the mechanisms and origins of life; constructing new applications such as biosensors (Gaba et al. 2024); and creating advanced drug-delivery platforms or microbial cell factories that can be used in a wide range of applications to improve the standard of life (Emir Dilemiz et al. 2021). A well-known example of a bottom-up approach is the design and creation of *M. mycoides* JCVI-Syn3.0 bacterium, where scientists built the synthetic genome piece by piece from short DNA fragments (oligonucleotides) (Hutchison et al. 2016; Singh 2020). Figure 10.1 demonstrates the flow of the top-down and bottom-up approaches in synthetic biology.

Strategies to Develop Synthetic Genomes

The Workflow for Designing and Engineering Synthetic Genomes

As previously described, synthetic genomes are artificial genetic materials produced from manipulating existing life forms or by producing new DNA molecules. The design and production of artificial cells start with minimal genome synthesis using computational and laboratory genetic engineering tools to determine the following: i) essential genes which can be added into the synthetic genome using the bottom-up approach; ii) or non-essential genes which can be removed from existing genomes using the top-down approach. The resulting minimal synthetic cells contain only essential genes, which are important for cell survival, replication, and metabolism. Figure 10.2 illustrates the general workflow of designing and constructing a synthetic genome following the design-build-test-learn and repeat cycle of synthetic genomics.

Step 1: Designing the Sequence of the Genome

Scientists design synthetic genomes using computational models to differentiate between essential and non-essential genes located on the genome. This method involves stripping existing genomic data to contain only essential genes or replacing existing genes with chemically synthesized DNA to form artificial cells with enhanced functions (Xu et al. 2023). The first step of designing a genome is data collection from open databases like National Center for Biotechnology Information

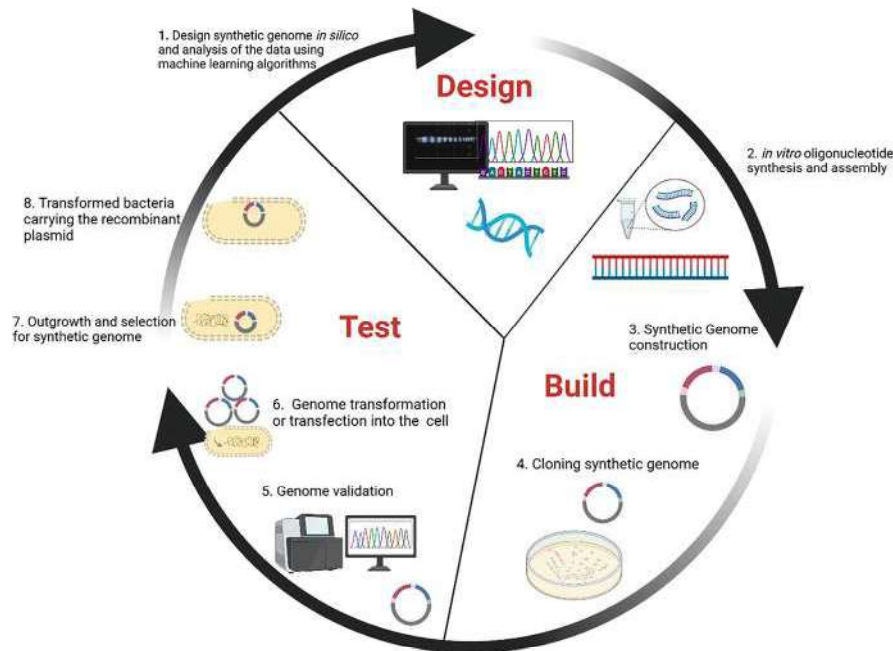


Fig. 10.2 An overview of the general workflow for synthetic genome design and construction. (Illustration created with [BioRender.com](https://www.biorender.com))

(NCBI), Kyoto Encyclopedia of Genes and Genomes (KEGG), Cluster of Essential Genes (CEG), and Database of Essential Genes (DEG) (Guo et al. 2021). Followed by the analysis of the data using machine learning algorithms, such as generative adversarial networks (GANs), recurrent neural networks (RNNs), and convolutional neural networks (CNNs) to predict essential genes which researchers use to create computational models (Shen et al. 2022). The methods used to test the performance of the models with essential genes include cross-validation, holdout validation, bootstrapping, and permutation tests, among others (Guo et al. 2021; Wilimitis and Walsh 2023). After testing the models, computational software is developed to implement the analysis and predict algorithms (Guo et al. 2021). The final stage involves the use of the software to design minimal synthetic genomes by deleting non-essential genes using specific computational tools, namely, Minesweeper and GAMA (Rees-Garbutt et al. 2020).

Step 2: Oligonucleotide Synthesis

Oligonucleotide synthesis is a highly automated process of chemically constructing short precise sequences of single-stranded DNA or RNA molecules known as oligonucleotides (Currin et al. 2021). The oligonucleotides are used in various applications including DNA sequencing, genetic testing, and molecular cloning (Kumar et al. 2023a). The desired oligonucleotide chains are built using two

methods, either the liquid-phase method which uses soluble polymer supports (usually polystyrene and polyethylene glycol) to immobilize reagents and catalysts; or the solid-phase method which utilizes phosphoramidite molecules as building blocks for the synthesis (Creusen et al. 2020). The solid-phase approach is the most widely used oligonucleotide synthesis method, which sequentially binds acid-activated phosphoramidite nucleotides to a growing DNA sequence attached to a solid support. The phosphoramidite nucleotides are added in the 3' to 5' direction and each cycle goes through four critical reactions, namely, detritylation, coupling, capping, and oxidation (Creusen et al. 2020). The cycle is initiated by the removal of the 5'-DMT (4,4'-dimethoxytrityl) protecting groups from the last base, making it reactive to the new nucleotide. Once the DMT has been removed, the next nucleotide is added to the growing chain of oligonucleotide by reacting to the free 5'-hydroxyl groups (5'-OH) of the solid-support-linked nucleotide; followed by the capping step where an acetyl group for DNA or a tert-butylphenoxyacetic anhydride reagent for RNA is added to any unreacted 5'-OH groups of the growing oligonucleotide chain to prevent deletion mutations, degradation or formation of unwanted side products. The final step adds oxygen to stabilize the bonds of the coupled nucleotides by converting unstable phosphite triester bonds formed during the coupling reaction step to the stable phosphate triester (Kiesman et al. 2021). These cycles repeat a series of these four steps until the desired oligonucleotide sequence is complete. At the end of synthesis, oligonucleotides are cleaved from the solid-phase support using substances such as ammonium hydroxide, which also removes the remaining protecting groups from heterocyclic bases and phosphodiester backbones (Yamamoto et al. 2023; Ma et al. 2024b). Oligonucleotide chain is ready for purification after the removal of any remaining protective groups. The purification step is crucial for the selection of only full-length oligonucleotides from capped, truncated fragments and other impurities using different methods, such as polyacrylamide gel electrophoresis (PAGE), "Trityl ON" purification, or the most used high-performance liquid chromatography (HPLC).

Step 3: The Assembly of the Oligonucleotides

Oligonucleotide assembly is a process that joins purified oligonucleotides together to create larger double-stranded DNA sequences. The oligonucleotides assembly process can be performed using polymerase chain reaction (PCR) technique to assemble the oligonucleotides, or the ligase chain reaction (LCR) which joins the overlapping synthetic oligonucleotides together to form complete synthetic genes. The PCR assembly method for creating long DNA molecules includes two steps: the first step of PCR involves a pool of oligodeoxynucleotides annealing and elongating to produce a full-length synthetic genome. In the second PCR step, the specific length of the desired artificial genome is amplified using primers specific to the desired length. In contrast, the LCR method uses DNA ligase enzymes which link complementary ends of DNA strands to build a complete synthetic DNA genome through repeated cycles of denaturation-annealing-ligation, guided by oligonucleotide "bridges" (short DNA sequences).

Step 4: Delivery of Synthetic Genome into the Cell

The resulting synthetic genome produced in step three must be analyzed using technologies like NGS to verify accuracy by checking the structure of the whole genome before cloning into an organism (Satam et al. 2023). The cloning process starts with isolation of the desired DNA sequence from the synthetic genome and choosing a vector like plasmids to carry the foreign DNA inserts; followed by cutting of the plasmid DNA at specific sequence sites using restriction nuclease enzymes. The final step involves inserting the gene of interest into the cut site of plasmids using ligase enzymes to seal the sticky ends resulting in a recombinant plasmid containing antibiotic resistance gene. The antibiotic resistance gene helps plasmids to survive in the presence of antibiotics during the selection step. The recombinant DNA plasmid is introduced into bacterial cells, such as *Escherichia coli* (*E. coli*), *L. lactis*, or *L. casei* through heat shock transformation process which compels them to take up the synthetic DNA. The bacterial colonies containing plasmids are antibiotic resistant, so they will survive nutrients containing antibiotics on the agar plates, while the ones without plasmids die. The transformed bacteria carrying the recombinant plasmid are amplified and validated using technologies such as PCR for further use in downstream applications like medicine and biotechnology, including biofuel production, crop improvement, vaccine, and antibody production (Mugwanda et al. 2023; Ye et al. 2024).

Evolution of Synthetic Genome

The field of synthetic genomics has evolved over time with new discoveries of technology and the development of new tools allowing for more ambitious experiments. Scientists use a combination of modern biotechnologies such as DNA synthesis, NGS, and CRISPR-CAS9 editing tools to produce viral or bacterial cells containing synthetic genomes (Venter et al. 2022). The evolution of synthetic genomes began in the early 2000s with the successful synthesis of viral genomes, like poliovirus cDNA (~7.9 kb), which paved the way for designing and creating more complex organisms such as bacteria (Fu and Shen 2024). The key milestones in synthetic genomics evolution include the first synthetic bacterial genome (*M. mycoides* JCVI-Syn1.0), followed by the discovery of the minimal bacterial cell (*M. mycoides* JCVI-Syn3.0), the *E. coli* and yeast synthetic genomes, and synthetic viruses (Fig. 10.3). The main goal is to use these artificially constructed cells for various applications including medicine, agriculture, research, and industry.

Minimal Synthetic Bacterial Cell

Mycoplasma genitalium is the smallest known bacteria with minimal metabolic requirements, lack of genomic redundancy, and capable of self-replication. The key characteristics of *M. genitalium* microbes include a lack of cell wall, very small circular DNA size (around 580 kb), 482 protein-coding genes, and a low Guanine + Cytosine (G + C) content. These characteristics make this bacterium an ideal candidate for constructing synthetic genomes for future applications. In a study

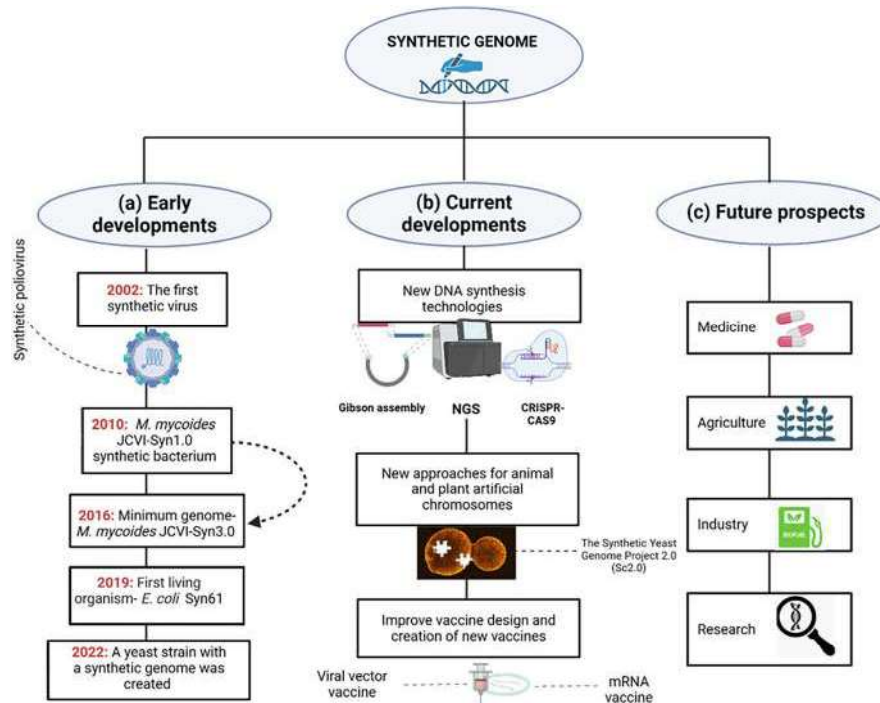


Fig. 10.3 Schematic diagram showing the evolution of synthetic genomes since the early 2000s to present. (a) Overview of synthetic genomes. (b) The current developments of synthetic genomes. (c) Synthetic genomes along with emerging technologies are expected to lead to new opportunities for revolutionizing methods of medicine production, crop production, and biofuel production. (Illustration created with [BioRender.com](https://www.biorender.com))

conducted by Gibson and colleagues, a bottom-up synthetic approach was used to create the largest man-made DNA structure by assembling and chemically synthesizing 582,970 base pairs (bp) DNA genome of *M. genitalium* bacterium, resulting in synthetic genome named *M. genitalium* JCVI-1.0 (Gibson et al. 2008). The researchers assembled overlapping “cassettes” of 5–7 kilobase pairs (kbp) from chemically synthesized oligonucleotides, joined by in vitro recombination to make intermediate assemblies, which were all cloned to form bacterial synthetic chromosomes in *E. coli*, and later transferred into yeast to fully assemble the length of the genome. Following the 2008 studies, Gibson and collaborators used digitized genome sequence information of parent *Mycoplasma mycoides* to produce the first complete synthetic genome containing 901 genes, known as *Mycoplasma mycoides* (*M. mycoides*) JCVI-Syn1.0 (1.08-mega-bp) which was chemically synthesized, and transformed into a recipient host cell, *Mycoplasma capricolum* (*M. capricolum*), to create new artificial *M. mycoides* cells which function like a living bacteria by self-replicating, resulting in cell growth (Gibson et al. 2010).

In a recent study, researchers used the bottom-up construction approach to design and modify *M. mycoides* JCVI-Syn1.0 genome to form *M. mycoides* JCVI-Syn3.0

bacterium (Singh 2020). The synthesis process started from short synthetic DNA or RNA molecules, then assembled gradually into a genome via methods such as Assembly PCR and transformation association recombination cloning in yeast, followed by the removal of the genome from yeast and installed into *M. capricolum* cell to test its robust growth (Xu et al. 2023). The *M. mycoides* JCVI-Syn3.0 bacterium is the first real minimal cell produced in a lab and has a minimal genome of 531 kbp containing 473 genes (438 protein-coding and 35 RNA-coding) needed for the survival of a living bacterium. The research concluded that *M. mycoides* JCVI-Syn3.0 is a versatile synthetic chassis for studying major gene functions, gene manipulation, metabolic pathways, and exploring whole-genome design to better understand life and improve applications. Similarly, in other studies, using a different kind of microorganism, lactic acid bacteria (LAB), scientists reported that LAB are versatile microbial chassis important for food fermentation and as probiotics promoting health (Wu et al. 2021; Mugwanda et al. 2023). Figure 10.4 shows the overview of the development of the first synthetic bacterial cell (JCVI-Syn3.0).

***Escherichia coli* and Yeast Synthetic Genomes**

Escherichia coli is a gram-negative, rod-shaped, non-spore forming, coliform bacteria belonging to the genus *Escherichia* and are mostly found in the lower intestines of humans and animals but can also occupy other habitats such as the environment, food, and water (Basavaraju and Gunashree 2023). The *E. coli* strains are the most studied bacterial cell models in labs for synthetic biology because they are well-characterized, versatile, and easy to manipulate (Kurokawa and Ying 2020). Additionally, this bacterium is used in many applications including genetic engineering for constructing and testing genetic circuits; genetic research for understanding how

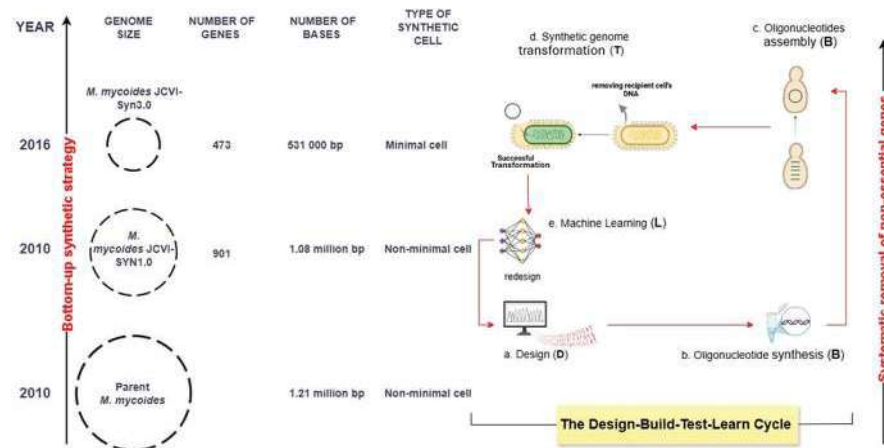


Fig. 10.4 The evolution of the first minimal synthetic cell developed by the J. Craig Venter Institute (JCVI) Synthetic Biology research group using the Design-Build-Test-Learn (DBTL) method. (The diagram was created using diagrams.net (formerly draw.io))

genes influence health and disease; the production of biopharmaceuticals like insulin; and metabolic engineering used to produce chemicals for food and agriculture (Basavaraju and Gunashree 2023). The top-down reduction of *E. coli* microbe is another example of bacterium used as a host for engineering chassis that can be used for medical and industrial applications (Singh 2020). The wild type *E. coli* strain K-12 MG1655 was the first fully sequenced genome studied in 1997, followed by the genome sequencing study of another wild type *E. coli* strain W311 (Kurokawa and Ying 2020), aiming at deleting non-essential genes from the genome giving rise to new strains. Choe and colleagues reported that most of the *E. coli* strains had grown equivalent to their parental strain but showed a higher productivity level compared to the ancestral strain (Choe et al. 2016).

Recently, researchers at the Medical Research Council Laboratory of Molecular Biology in Cambridge used the most common model organism (*E. coli* bacterium) to create the first living organism with a fully synthetic DNA code. This living synthetic bacterial strain with redesigned genetic code is called *Escherichia coli* Syn61 (contains four million-base genome). The research team developed a robust method using an iterative recombination-based approach, REXER (Replicon Excision Enhanced Recombination) and GENESIS (Genome Stepwise Interchange Synthesis), to substitute more than 100 kilobases (kb) genomic DNA of *E. coli* with synthetic DNA fragments (Robertson et al. 2021; Gao et al. 2024). The synthetic genome of strain Syn61 was computationally designed to contain 61 codons for protein synthesis by replacing and removing specific codons (Xu et al. 2023). The team replaced all occurrences of the serine amino acid codons TCG and TCA with AGC and AGT, respectively, and the TAG stop codon was replaced with TAA in all open reading frames (ORF). Furthermore, they deleted genes for the tRNAs that decode TCG and TCA, and the gene for RF-1 which terminates protein synthesis at the TAG stop codon (Spinck et al. 2023). Finally, the redesigned genetic code was chemically synthesized and added to *E. coli* bacterium where it replaced the organism's natural genome. The success of the Syn61 genome project shows for the first time that living organisms can be created with compressed genetic codes and still be functional for use in creating industrial bioprocesses, producing proteins with new functions and other applications in research (Xu et al. 2023).

The yeast microbe, specifically the species *Saccharomyces cerevisiae* (*S. cerevisiae*), is considered a model organism, like *E. coli* bacteria, but while *E. coli* is a model prokaryote, *S. cerevisiae* is a model eukaryote, making it valuable for studying eukaryotic cell biology (Parapouli et al. 2020). They are both widely used in research because of their simple genomes, fast growth rates, and well-studied characteristics. The *S. cerevisiae* microbe is a single-celled fungus, usually 5–10 μm in diameter, facultative anaerobic, contains 12,068 kb nuclear genomic DNA, and has a thick polysaccharide cell wall (Parapouli et al. 2020). They are usually spherical, cylindrical, or oval cells and are found in different habitats like soil, on plants, and in sugary liquids (fruit and flowers). The genome of *S. cerevisiae* was the first completely sequenced eukaryotic organism by Goffeau in collaboration with other research scientists from around the world. In 1996, it was reported that *S. cerevisiae* contains 6275 genes compactly organized in 16 chromosomes, of

which 5885 were predicted to be protein-encoding genes; which was later reduced to 5570 protein-encoding genes due to the deletion of hypothetical genes and pseudo-genes. Since then, the yeast genome continues to be studied by scientists working in different fields like genetics, biotechnology, and synthetic biology to improve yeast strains for industrial applications.

In 2010, scientists used a single yeast colony (S288C) along with modern sequencing techniques to construct an updated reference genome sequence for the *S. cerevisiae* yeast strain, known as S288C 2010. The significance of S288C 2010 yeast strain is to enhance our understanding of eukaryotic genomes and it can be used as an anchor for further research. In another study by Murakami and coworkers, the top-down synthetic strategy was used to produce novel eukaryotic yeast strains, where the genome of *S. cerevisiae* was sequenced completely using a computer program that was created to predict non-essential regions on the yeast genome. Followed by the process of PCR-mediated chromosome splitting (PCS) which enabled the splitting of the yeast chromosome at any desired site, resulting in the deletion of mutants (MFY1158, MFY1160, and MFY162), the remaining essential genes in the yeast genome provided increased levels of ethanol and glycerol production. Synthetic Yeast Genome Project Sc2.0 (Yeast 2.0 project) is another well-known example of synthetic genomics where organisms are genetically modified with artificial genomes, with the key difference being, yeast is a eukaryotic fungus while *E. coli* Syn61 is a prokaryotic bacterium (Venter et al. 2022). The Yeast 2.0 project was first launched in 2014, and it is led by Professor Jef Boeke from New York University in collaboration with other international researchers. This project aims to construct the first fully synthetic eukaryotic organism by using the open-source tool BioStudio to redesign and replace genes within the yeast genome. The BioStudio computer tool tracks all changes made to the native yeast chromosome and facilitates common assembly strategy and shared language for describing designed chromosomes. To date, research scientists have successfully synthesized and characterized all 16 native chromosomes of *S. cerevisiae* microorganism including the 17th de novo synthetic tRNA neo-chromosome (Zhao et al. 2023). Furthermore, over seven synthetic chromosomes were successfully combined into a single functional cell resulting in a yeast strain containing more than 50% Synthetic DNA. The completion of a fully synthetic eukaryotic cell is an ongoing study with the aim of consolidating the remaining synthetic chromosomes starting with the most recent modified version of *S. cerevisiae*, which will result in a genome with 100% synthetic DNA. The Yeast 2.0 project could lead to advancements in the field of synthetic biology, medicine, food, and biofuel production, including understanding metabolic pathways in eukaryotes. Figure 10.5 demonstrates the construction of the first prokaryotic and eukaryotic cells using *E. coli* and budding yeast *S. cerevisiae* strains, respectively.

Synthetic Viruses

In a globalized world, the interactions between humans and various animal populations have increased due to several reasons such as deforestation and agricultural expansion, thereby resulting in increased risk of “zoonotic disease spillover.” The term “zoonotic disease spillover” refers to transmission of infectious disease

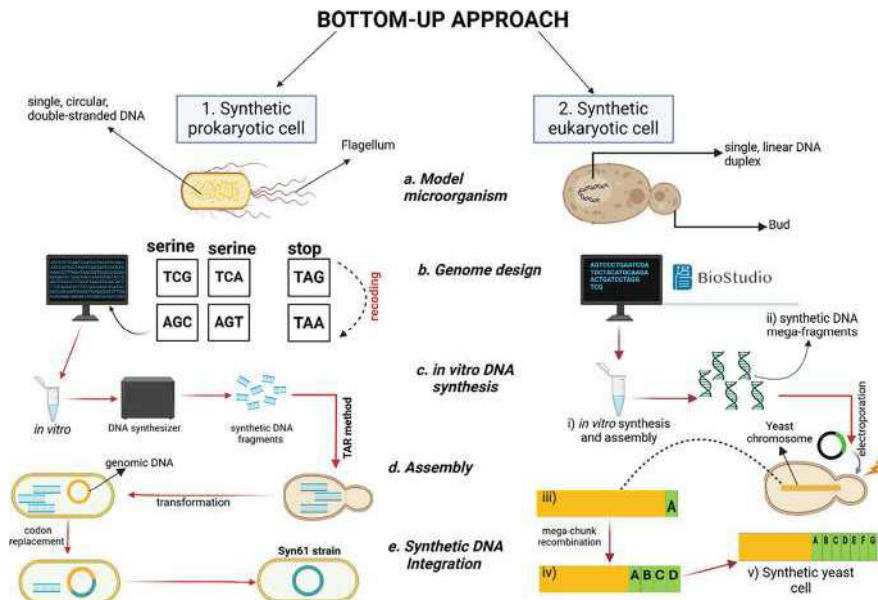


Fig. 10.5 Illustration of the generation of the first prokaryotic and eukaryotic living microorganisms controlled by synthetic DNA material. (Diagram created with [BioRender.com](https://www.biorender.com))

pathogens (bacteria, fungi, viruses, or parasites) from vertebrate animals to people (Ellwanger and Chies 2021). Therefore, there is a high chance of another pandemic outbreak. Synthetic vaccines are considered the best countermeasure response because they are safer and have improved therapeutic efficacy compared to traditional vaccines. Synthetic viruses are constructed in the lab (in vitro) using viral DNA through the process of synthetic virology. Synthetic virology is a multidisciplinary field that uses a combination of computational biology, molecular technology, and bioinformatic tools to build viral vectors that trigger adaptive immune responses which can prevent and protect against diseases (Brisse et al. 2020; White 2021). The production of synthetic viruses includes the following steps: i) sequencing a virus's whole genome and using it to create a synthetic version; ii) then oligonucleotides are synthesized and assembled to create a synthetic virus; iii) alterations of the structure and function of a virus's genetic information can be made; iv) synthetic virus morphological evaluation using transmission electron microscopy (TEM) to ensure a detailed image of the virus's internal structure and surface features before validation of the host (White 2021).

The first synthetic virus engineered was the poliovirus, which was chemically assembled in 2002 from synthetic oligonucleotides (Moles et al. 2024). Researchers used the basic synthetic genome construction workflow as described previously. They used commercially available information of poliovirus chemical code, then engineered the DNA version of the virus, known as synthetic cDNA, before RNA as it is unstable, and they used RNA polymerase enzyme to transcribe the DNA into viral RNA. After transcription, the virus was assembled from synthetic

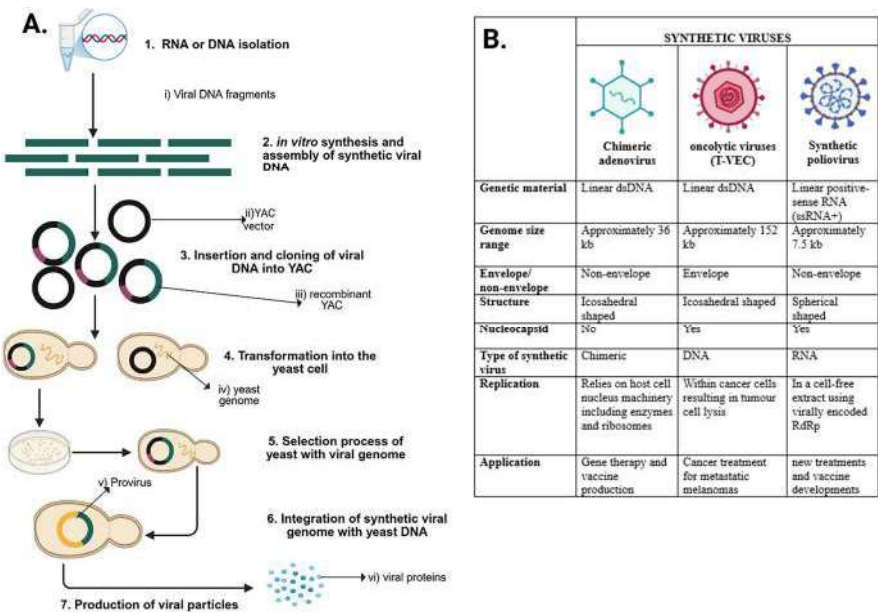


Fig. 10.6 The general steps of designing and engineering synthetic viruses to use against various diseases (a). (b) The table showing examples of various synthetic viruses produced from viral particles like proteins and their characterizations. Talimogene laherparepvec (T-VEC); double stranded deoxyribonucleic acid (dsDNA); positive sense ribose nucleic acid (ssRNA+). (Illustration created with [BioRender.com](#))

oligonucleotides resulting in artificial poliovirus which killed mice and was nearly identical to the original virus. The synthetic poliovirus study was used as proof-of-concept in the synthesis and assembly of other viral genomes including phiX174 bacteriophage, the 1918 Spanish influenza virus, Herpes simplex virus type 1 (HSV-1), horsepox virus, and the recent SARS-CoV-2 (COVID-19) virus, among others (Moles et al. 2024; White 2021). Synthetic viruses have many potential uses in medicine such as vaccine production, treating drug-resistant infections, and cancer treatments (bin Umair et al. 2022). Additionally, they can be used in research applications to study viral replications, host-virus interactions, and viral evolution. Figure 10.6 demonstrates the general construction of synthetic viruses (a) along with the characteristics of various types of synthetic viruses (b).

Future Perspective: Lactic Acid Bacteria Synthetic Genomes

Lactic acid bacteria are a functional heterogeneous group of gram-positive, non-spore forming, immotile, lack cytochrome, rod or cocci-shaped microorganisms that convert soluble carbohydrates into lactic acid and their DNA has low G + C content (Cho et al. 2020; Wu et al. 2021). The LAB microorganisms are natural inhabitants of human reproductive and gastrointestinal tract (GIT) and promote health benefits

(Wu et al. 2021). This group of bacteria has high tolerance to low pH, heat treatments, increased levels of salt concentrations, and can survive in the presence of additives (Onyeaka and Nwabor 2022). They are divided into two physiological groups, homo-fermentative or hetero-fermentative, based on the type of sugars available for fermentation. Homo-fermentative LAB metabolizes carbohydrates into lactic acid as the main end-product, while by contrast, hetero-fermentative species produce lactic acid in addition to other products, namely, ethanol, carbon dioxide, and acetic acid (Sharma et al. 2020). Apart from being widely used in the food fermentation industry, most LAB strains are considered ideal candidates used to produce microbial chassis that can be modified and engineered for vaccine, therapeutic, and industrial applications (Mugwanda et al. 2023). This is due to their ability of adapting to various environments, their metabolic functions, their small genome size approximately 2–3 mega-base pair (bp), and the simplicity in sugar and energy metabolism (Sharma et al. 2020; de Jesus et al. 2022).

They are also regarded as the live vehicles for the delivery of therapeutic molecules, including antigens, cytokines, antibiotics, peptides, and enzymes to the mucosa of the human GIT (Wu et al. 2021; Mugwanda et al. 2023). Additionally, due to their probiotic properties, LABs are regarded as GRAS (generally recognized as safe) by the United States Food and Drug Administration (FDA), playing an important role in maintaining health, and the prevention, and treatment of different human diseases (Cho et al. 2020; de Jesus et al. 2022). The most widely studied LAB species are the *L. casei* and *L. lactis* bacteria belonging to genus *Lactobacillus* and *Lactococcus*, respectively, with *Lactobacillus* being the largest genus which currently contains about 261 validly described species (Onyeaka and Nwabor 2022). The *L. casei* microbes are facultatively hetero-fermentative, meaning they can either be homo- or heterofermentative bacteria. Previous studies have successfully reported that engineered bacterial strains (chassis) belonging to the *L. casei* group, may decrease lactose intolerance, food allergies, diarrhea, and even constipation (Jarocki et al. 2020). Similarly, other studies also reported on the positive use of this bacteria, proposing that it can be used as new anti-cancer therapeutic agents, treatment for colon diseases, and treatments for inflammatory bowel diseases by stimulating the immune system, and restoring microbial balance (Chen et al. 2023). Other researchers used a close relative of *L. casei* species, *L. lactis* bacteria, to create a synthetic microbial chassis. The *L. lactis* microbes are used to manufacture lactic acid, cheese, and other dairy foods through the homo-fermentation process (Saleena et al. 2023). Like *L. casei*, they are regarded as efficient microbial chassis used to produce biomolecules, such as heterologous proteins that play a role in different applications including biopharmaceutical industry. The results of several studies have demonstrated the use of engineered or modified *L. lactis* bacteria as vehicles delivering antigens or cytokines or therapeutic proteins to the immune system. Preisser and team reported that orally administered *L. lactis* carrying cytokines, namely, interleukin-4 (*IL-4*) and interleukin-10 (*IL-10*), were used to trigger an immune response against Type 1 Diabetes (Preisser et al. 2021). In another recent study, orally modified *L. lactis* bacteria were used as vaccine against Porcine deltacoronavirus to test the mucosal immune responses (Zhai et al. 2023). The

LAB species are used as live vectors because of their non-invasive nature thus reducing the risk of side effects, and they are non-pathogenic due to their safety profile. Looking at past and current studies, LAB microbes have been proven to be beneficial in vaccine development, food industry, and pharmaceutical applications. Therefore, in the current study, the authors aim to design and engineer *Lactobacillus casei* (*L. casei*) and/or *Lactococcus lactis* (*L. lactis*) microbial cell platforms that will function as prototypes for the rapid production of vaccines against an unknown disease (Disease X) using computational methods, bioinformatics tools, and experimental molecular techniques (Fig. 10.7). The development of the *L. lactis* and *L. casei* microbial cell factories is critical for gene therapy and vaccine production which will lessen the impact of the next pandemic. So, in this current study, the authors will use LAB species, specifically *L. casei* and *L. lactis* strains with minimal genomes to design and construct efficient microbial cell factories for Disease X vaccine production. The author will use modern technologies, such as NGS for data analysis; digital PCR for quantification of DNA libraries and validation of NGS results; CRISPR-associated protein 9 (CRISPR-Cas9) system for editing or modifying genes (knock-in and/or knock-outs); and finally validate the success of gene engineering through CRISPR-CAS9 by NGS technology. This will be one of the first Lactochassis platforms using LAB microbes for vaccine development. The newly designed microbial cell factories can be used for antigen and vaccine production reducing the response time to less than 6 months. Additionally, these synthetic

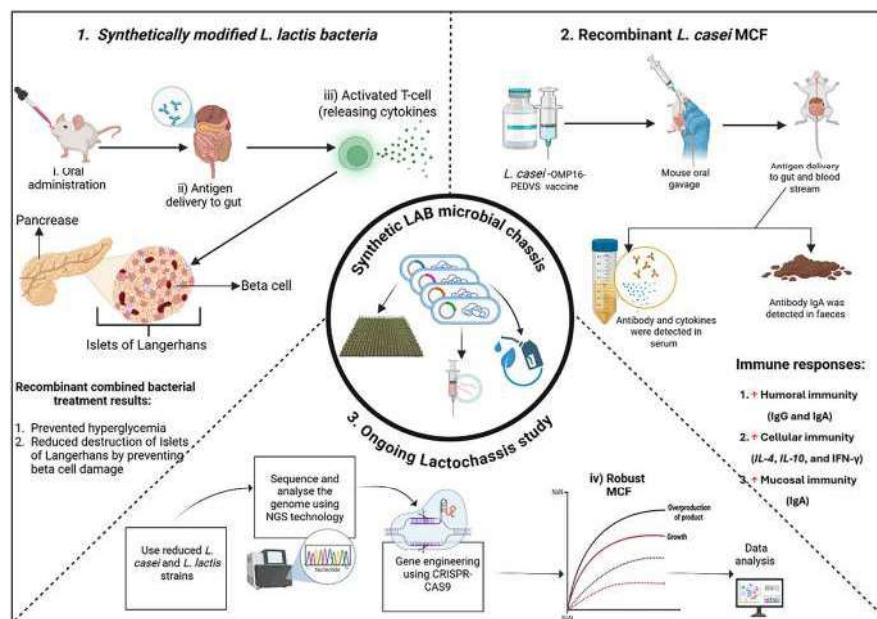


Fig. 10.7 Diagram showing the use of lactic acid bacteria (LAB) microbial cell factories (MCFs) in diverse applications, including, food production, vaccine development, and biofuel production amongst others. (Created with [BioRender.com](https://www.biorender.com))

microbial platforms can also be used for communicable diseases, cancer studies, and neurogenerative diseases, among others. Furthermore, the project will foster collaboration between laboratories with different scientific backgrounds and establish many international and industrial partnerships.

Thus, previous research has demonstrated the significance of using the newly emerging multidisciplinary field of study, synthetic biology, in creating artificial genes with new or enhanced functions. The engineered artificial genes are introduced into the host cell using an integration of computational tools and molecular techniques to produce synthetic organisms or chassis which are used in many applications including healthcare for vaccine development, agriculture for crop production, pharmaceutical for manufacturing drugs, and industrial for biofuel production. As human populations grow, so does deforestation and agricultural expansion into wildlife habitats which results in increased interactions between humans and wild animals leading to high risks of “zoonotic disease spillover.” So, since there is a high possibility of a future pandemic health crisis, synthetic vaccines engineered using the field of synthetic genomics are considered the best counter-measure response to infectious diseases. The synthetic genomic tools are used to construct personalized medicine for type 2 diabetes, gene therapy, and for cancer treatments, amongst others. The synthetic genomes are designed on the computer, then built in the lab via oligonucleotide synthesis and assembly, followed by gene manipulation by insertion of synthetic material into the host cell to study biological systems for further use in industrial and biopharmaceutical applications. Although there are challenges like error correction during synthesis, the authors are confident that synthetic biology is the future of engineering and manufacturing medicines, drug-targeted therapies, and many more.

Acknowledgments The work reported herein was made possible through funding by the South African Medical Research Council (SAMRC) through its Division of Research Capacity Development (RCD) under the Chan Soon-Shiong Family Foundation (CSSFF). The content hereof is the sole responsibility of the authors and does not necessarily represent the official views of the SAMRC.

References

- Arabi F, Mansouri V, Ahmadbeigi N (2022) Gene therapy clinical trials, where do we go? An overview. In: Biomed Pharmacother 153. <https://doi.org/10.1016/j.biopha.2022.113324>
- Basavaraju M, Gunashree BS (2023) *Escherichia coli*: an overview of main characteristics. In: *Escherichia coli* – old and new insights. IntechOpen. <https://doi.org/10.5772/intechopen.105508>
- Bhadoria P, Gupta G, Agarwal A (2021) Viral pandemics in the past two decades. J Family Med Prim Care 10(8). https://doi.org/10.4103/jfmpc.jfmpc_2071_20
- Bhatia A, Sobti RC, Sharma S (2025) Synthetic biology: engineering life for sustainable applications. In: Role of science and technology for sustainable future: volume 2-Applied sciences and technologies. Springer Nature Singapore, Singapore, pp 61–73. https://doi.org/10.1007/978-981-97-5177-8_4
- bin Umair M, Akusa FN, Kashif H, Seerat-e-Fatima, Butt F, Azhar M, Munir I, Ahmed M, Khalil W, Sharyar H, Rafique S, Shahid M, Afzal S (2022) Viruses as tools in gene therapy,

- vaccine development, and cancer treatment. *Arch Virol* 167(6). <https://doi.org/10.1007/s00705-022-05432-8>
- Bohua L, Yuexin W, Yakun O, Kunlan Z, Huan L, Ruipeng L (2023) Ethical framework on risk governance of synthetic biology. *J Biosaf Biosecur* 5(2). <https://doi.org/10.1016/j.jobbb.2023.03.002>
- Brisse M, Vrba SM, Kirk N, Liang Y, Ly H (2020) Emerging concepts and technologies in vaccine development. *Front Immunol* 11. <https://doi.org/10.3389/fimmu.2020.583077>
- Brooks SM, Alper HS (2021) Applications, challenges, and needs for employing synthetic biology beyond the lab. *Nat Commun* 12(1):1390. <https://doi.org/10.1038/s41467-021-21740-0>
- Carrillo-Martínez JC, Díaz-Zaragoza M, Ocegüera-Contreras E, Ortiz-Torres G (2023) Synthetic biology: on the development of genetic circuits and their potential applications in biosciences and biofuels. *TIP Rev especializada ciencias químico-biol* 26. <https://doi.org/10.22201/fesz.23958723e.2023.538>
- Chen Y, Li F, Nielsen J (2022) Genome-scale modeling of yeast metabolism: retrospectives and perspectives. *FEMS Yeast Res* 22(1):foac003. <https://doi.org/10.1093/femsyr/foac003>
- Chen Y, Gao H, Zhao J, Ross RP, Stanton C, Zhang H, Chen W, Yang B (2023) Exploiting lactic acid bacteria for inflammatory bowel disease: a recent update. *Trends Food Sci Technol* 138. <https://doi.org/10.1016/j.tifs.2023.06.007>
- Cho SW, Yim J, Seo SW (2020) Engineering tools for the development of recombinant lactic acid bacteria. *Biotechnol J* 15(6). <https://doi.org/10.1002/biot.201900344>
- Choe D, Cho S, Kim SC, Cho BK (2016) Minimal genome: worthwhile or worthless efforts toward being smaller? *Biotechnol J* 11(2). <https://doi.org/10.1002/biot.201400838>
- Creusen G, Akintayo CO, Schumann K, Walther A (2020) Scalable one-pot-liquid-phase oligonucleotide synthesis for model network hydrogels. *J Am Chem Soc* 142(39). <https://doi.org/10.1021/jacs.0c05488>
- Crocker H, Gorda B, Pelosse M, Thimiri Govinda Raj DB, Berger I (2021) SynBac: enhanced baculovirus genomes by iterative recombineering. *Methods Mol Biol* 2305. https://doi.org/10.1007/978-1-0716-1406-8_7
- Currin A, Parker S, Robinson CJ, Takano E, Scrutton NS, Breitling R (2021) The evolving art of creating genetic diversity: from directed evolution to synthetic biology. *Biotechnol Adv* 50. <https://doi.org/10.1016/j.biotechadv.2021.107762>
- de Jesus LCL, da Silva TF, Glória R d A, Freitas A d S, Américo MF, Fernandes LJ d S, Campos GM, Gomes GC, Santos RCV, Carvalho RD d O, Barh D, Azevedo V (2022) Lactic acid bacteria-based beverages in the promotion of gastrointestinal tract health. In: *Microbiome, immunity, digestive health and nutrition: epidemiology, pathophysiology, prevention and treatment*. Academic. <https://doi.org/10.1016/B978-0-12-822238-6.00008-X>
- Deb BR, Dev A, Begum M, Chowdhury SM (2025) Clinical profile of suspected and confirmed COVID-19 pneumonia patients admitted in a Tertiary Care Hospital, Sylhet, Bangladesh. *Jalalabad Med J* 19(1):11–20. <https://doi.org/10.3329/jmj.v19i1.79048>
- Demongeot J, Fougère C (2023) mRNA COVID-19 vaccines—facts and hypotheses on fragmentation and encapsulation. *Vaccine* 11(1). <https://doi.org/10.3390/vaccines11010040>
- Denies MS, Liu AP, Schnell S (2020) Are the biomedical sciences ready for synthetic biology? *Biomol Concepts* 11(1). <https://doi.org/10.1515/bmc-2020-0003>
- Ellwanger JH, Chies JAB (2021) Zoonotic spillover: understanding basic aspects for better prevention. *Genet Mol Biol* 44(1). <https://doi.org/10.1590/1678-4685-GMB-2020-0355>
- Emir Diltemiz S, Tavafoghi M, De Barros NR, Kanada M, Heinämäki J, Contag C, Seidlits SK, Ashammakhi N (2021) Use of artificial cells as drug carriers. *Mater Chem Front* 5(18). <https://doi.org/10.1039/d1qm00717c>
- Eslami M, Fadaee Dowlat B, Yaghmayee S, Habibian A, Keshavarzi S, Oksenysh V, Naderian R (2025) Next-generation vaccine platforms: integrating synthetic biology, nanotechnology, and systems immunology for improved immunogenicity. *Vaccine* 13(6):588. <https://doi.org/10.3390/vaccines13060588>
- Feng Y, Su C, Mao G, Sun B, Cai Y, Dai J, Ma Y (2024) When synthetic biology meets medicine. *Life Med* 3(1):lnae010. <https://doi.org/10.1093/lifemedi/lnae010>

- Fu X, Shen Y (2024) Synthetic genomics: repurposing biological systems for applications in engineering biology. *ACS Synth Biol* 13(5):1394–1399. <https://doi.org/10.1021/acssynbio.4c00006>
- Gaba S, Chauhan N, Chandra R, Jain U (2024) Future advances of artificial biosensor technology in biomedical applications. *Talanta Open* 9. <https://doi.org/10.1016/j.talo.2024.100301>
- Gao H, Qiu Z, Wang X, Zhang X, Zhang Y, Dai J, Liang Z (2024) Recent advances in genome-scale engineering in *Escherichia coli* and their applications. *Eng Microbiol* 4(1). <https://doi.org/10.1016/j.engmic.2023.100115>
- Garner KL (2021) Principles of synthetic biology. *Essays Biochem* 65(5). <https://doi.org/10.1042/EBC20200059>
- Gibson DG, Benders GA, Andrews-Pfannkoch C, Denisova EA, Baden-Tillson H, Zaveri J, Stockwell TB, Brownley A, Thomas DW, Algire MA, Merryman C, Young L, Noskov VN, Glass JI, Venter JC, Hutchison CA, Smith HO (2008) Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. *Science* 319(5867). <https://doi.org/10.1126/science.1151721>
- Gibson DG, Glass JI, Lartigue C, Noskov VN, Chuang RY, Algire MA, Benders GA, Montague MG, Ma L, Moodie MM, Merryman C, Vashee S, Krishnakumar R, Assad-Garcia N, Andrews-Pfannkoch C, Denisova EA, Young L, Qi ZN, Segall-Shapiro TH, ... Venter JC (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329(5987). <https://doi.org/10.1126/science.1190719>
- Guo Y, Ju Y, Chen D, Wang L (2021) Research on the computational prediction of essential genes. *Front Cell Dev Biol* 9. <https://doi.org/10.3389/fcell.2021.803608>
- Guo P, Wang S, Yue H, Zhang X, Ma G, Li X, Wei W (2023) Advancement of engineered bacteria for orally delivered therapeutics. *Small* 19(48):2302702. <https://doi.org/10.1002/sml.202302702>
- Hamese S, Mugwanda K, Takundwa M, Prinsloo E, Thimiri Govinda Raj DB (2023) Recent advances in genome annotation and synthetic biology for the development of microbial chassis. *J Genet Eng Biotechnol* 21(1). <https://doi.org/10.1186/s43141-023-00598-3>
- Hamilton RA, Mampuy R, Galaisi SE, Collins A, Istomin I, Ahteensuu M, Bakanidze L (2021) Opportunities, challenges, and future considerations for top-down governance for biosecurity and synthetic biology. In: *Emerging threats of synthetic biology and biotechnology: addressing security and resilience issues*. Springer, pp 37–58. <https://doi.org/10.1007/978-94-024-2086-9>
- Honigsbaum M (2020) Revisiting the 1957 and 1968 influenza pandemics. *Lancet* 395(10240). [https://doi.org/10.1016/S0140-6736\(20\)31201-0](https://doi.org/10.1016/S0140-6736(20)31201-0)
- Hutchison CA, Chuang RY, Noskov VN, Assad-Garcia N, Deerinck TJ, Ellisman MH, Gill J, Kannan K, Karas BJ, Ma L, Pelletier JF, Qi ZQ, Richter RA, Strychalski EA, Sun L, Suzuki Y, Tsvetanova B, Wise KS, Smith HO, ... Venter JC (2016) Design and synthesis of a minimal bacterial genome. *Science* 351(6280). <https://doi.org/10.1126/science.aad6253>
- Jarocki P, Komon-Janczara E, Glibowska A, Dworniczak M, Pytko M, Korzeniowska-Kowal A, Wzorek A, Kordowska-Wiater M (2020) Molecular routes to specific identification of the *Lactobacillus casei* group at the species, subspecies and strain level. *Int J Mol Sci* 21(8). <https://doi.org/10.3390/ijms21082694>
- Karbalaie-Heidari HR, Budisa N (2024) Advanced and safe synthetic microbial chassis with orthogonal translation system integration. *ACS Synth Biol* 13(9):2992–3002. <https://doi.org/10.1021/acssynbio.4c00437>
- Khan MA, Amin A, Farid A, Ullah A, Waris A, Shinwari K, Hussain Y, Alsharif KF, Alzahrani KJ, Khan H (2022) Recent advances in genomics-based approaches for the development of intracellular bacterial pathogen vaccines. *Pharmaceutics* 15(1):152. <https://doi.org/10.3390/pharmaceutics15010152>
- Kiesman WF, McPherson AK, Diorazio LJ, Van Den Bergh L, Smith PD, Northall JM, Fettes A, Wang T, Mehlmann M, Raza S, Held G (2021) Perspectives on the designation of oligonucleotide starting materials. *Nucleic Acid Ther* 31(2). <https://doi.org/10.1089/nat.2020.0909>
- Kim K, Choe D, Cho S, Palsson B, Cho BK (2024) Reduction-to-synthesis: the dominant approach to genome-scale synthetic biology. *Trends Biotechnol*. <https://doi.org/10.1016/j.tibtech.2024.02.008>

- Kumar S, Arora A, Kumar R, Senapati NN, Singh BK (2023a) Recent advances in synthesis of sugar and nucleoside coumarin conjugates and their biological impact. *Carbohydr Res* 530. <https://doi.org/10.1016/j.carres.2023.108857>
- Kumar N, Samant S, Singh K, Reshamwala SM (2023b) Minimal cells and genome minimization: top-down and bottom-up approaches to construct synthetic cells. In: *Biomanufacturing for sustainable production of biomolecules*. Springer Nature Singapore, Singapore, pp 17–44. https://doi.org/10.1007/978-981-19-7911-8_2
- Kuppan N, Padman M, Mahadeva M, Srinivasan S, Devarajan R (2024) A comprehensive review of sustainable bioremediation techniques: eco friendly solutions for waste and pollution management. *Waste Manag Bull* 2(3):154–171. <https://doi.org/10.1016/J.WMB.2024.07.005>
- Kurokawa M, Ying BW (2020) Experimental challenges for reduced genomes: the cell model *Escherichia coli*. *Microorganisms* 8(1). <https://doi.org/10.3390/microorganisms8010003>
- LeBlanc N, Charles TC (2022) Bacterial genome reductions: tools, applications, and challenges. *Front Genome Ed* 4. <https://doi.org/10.3389/fgeed.2022.957289>
- Lezia A, Miano A, Hasty J (2021) Synthetic gene circuits: design, implement, and apply. *Proc IEEE* 110(5):613–630. <https://doi.org/10.1109/JPROC.2021.3134169>
- Ma S, Su T, Lu X, Qi Q (2024a) Bacterial genome reduction for optimal chassis of synthetic biology: a review. *Crit Rev Biotechnol* 44(4):660–673. <https://doi.org/10.1080/07388551.2023.2208285>
- Ma Y, Zhang Z, Jia B, Yuan Y (2024b) Automated high-throughput DNA synthesis and assembly. *Heliyon* 10(6). <https://doi.org/10.1016/j.heliyon.2024.e26967>
- Mitra A, Barua A, Huang L, Ganguly S, Feng Q, He B (2023) From bench to bedside: the history and progress of CAR T cell therapy. *Front Immunol* 14. <https://doi.org/10.3389/fimmu.2023.1188049>
- Moghaddasi H, Rezaei-Tavirani M, Samadi L (2021) The role of systems biology and synthetic biology in appearing and managing COVID-19. *J Cell Mol Anesth* 6(6). <https://doi.org/10.22037/jcma.v6i1>
- Moles CM, Basu R, Weijmarshausen P, Ho B, Farhat M, Flaar T, Smith BF (2024) Leveraging synthetic virology for the rapid engineering of vesicular stomatitis virus (VSV). *Viruses* 16(10):1641. <https://doi.org/10.3390/v16101641>
- Mugwanda, K., Hamese, S., Van Zyl, W. F., Prinsloo, E., Plessis, M. Du, Dicks, L. M. T., & Thimiri Govinda Raj, D. B. (2023). Recent advances in genetic tools for engineering probiotic lactic acid bacteria. In *Biosci Rep* (Vol. 43, Issue 1). <https://doi.org/10.1042/BSR20211299>
- Mullis KB (1990) The unusual origin of the polymerase chain reaction. *Sci Am* 262(4). <https://doi.org/10.1038/scientificamerican0490-56>
- Nazir A, Hussain FHN, Raza A (2024) Advancing microbiota therapeutics: the role of synthetic biology in engineering microbial communities for precision medicine. *Front Bioeng Biotechnol* 12:1511149. <https://doi.org/10.3389/fbioe.2024.1511149>
- Onyeaka HN, Nwabor OF (2022) Lactic acid bacteria and bacteriocins as biopreservatives. In: *Food preservation and safety of natural products*. <https://doi.org/10.1016/b978-0-323-85700-0.00012-5>
- Parapouli M, Vasileiadis A, Afendra AS, Hatziloukas E (2020) *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiol* 6(1). <https://doi.org/10.3934/microbiol.2020001>
- Pattyn J, Hendrickx G, Vorsters A, Van Damme P (2021) Hepatitis B vaccines. *J Infect Dis* 224. <https://doi.org/10.1093/infdis/jiaa668>
- Pelletier JF, Sun L, Wise KS, Assad-Garcia N, Karas BJ, Deerinck TJ, Ellisman MH, Mershin A, Gershenfeld N, Chuang RY, Glass JI (2021) Genetic requirements for cell division in a genomically minimal cell. *Cell* 184(9):2430–2440. <https://doi.org/10.1016/j.cell.2021.03.008>
- Preisser TM, Da Cunha VP, Santana MP, Pereira VB, Cara DC, Souza BM, Miyoshi A (2021) Recombinant *Lactococcus lactis* carrying IL-4 and IL-10 coding vectors protects against type 1 diabetes in NOD mice and attenuates insulinitis in the STZ-induced model. *J Diabetes Res* 2021. <https://doi.org/10.1155/2021/6697319>

- Rees-Garbutt J, Chalkley O, Landon S, Purcell O, Marucci L, Grierson C (2020) Designing minimal genomes using whole-cell models. *Nat Commun* 11(1):836
- Rheinberger HJ (2023) Beyond nature and culture: modes of reasoning in the age of molecular biology and medicine. In: *Medical anthropology*. Routledge, pp 491–502
- Robertson WE, Funke LFH, de la Torre D, Fredens J, Wang K, Chin JW (2021) Creating custom synthetic genomes in *Escherichia coli* with REXER and GENESIS. *Nat Protoc* 16(5). <https://doi.org/10.1038/s41596-020-00464-3>
- Saleena LAK, Teo MYM, How YH, In LLA, Pui LP (2023) Immunomodulatory action of *Lactococcus lactis*. *J Biosci Bioeng* 135(1). <https://doi.org/10.1016/j.jbiosc.2022.10.010>
- Satam H, Joshi K, Mangrolia U, Waghoo S, Zaidi G, Rawool S, Thakare RP, Banday S, Mishra AK, Das G, Malonia SK (2023) Next-generation sequencing technology: current trends and advancements. *Biology* 12(7). <https://doi.org/10.3390/biology12070997>
- Sharma A, Gupta G, Ahmad T, Kaur B, Hakeem KR (2020) Tailoring cellular metabolism in lactic acid bacteria through metabolic engineering. *J Microbiol Methods* 170. <https://doi.org/10.1016/j.mimet.2020.105862>
- Shen X, Jiang C, Wen Y, Li C, Lu Q (2022) A brief review on deep learning applications in genomic studies. *Front Syst Biol* 2. <https://doi.org/10.3389/fsysb.2022.877717>
- Shmakova AA, Shmakova OP, Karpukhina AA, Vassetzky YS (2022) CRISPR/Cas: history and perspectives. *Russ J Dev Biol* 53(4). <https://doi.org/10.1134/s1062360422040075>
- Silva DR, Sardi J d CO, Pitangui N d S, Roque SM, da Silva ACB, Rosalen PL (2020) Probiotics as an alternative antimicrobial therapy: current reality and future directions. *J Funct Foods* 73. <https://doi.org/10.1016/j.jff.2020.104080>
- Singh V (2020) Advances in synthetic biology. In: Springer eBooks. <https://doi.org/10.1007/978-981-15-0081-7>
- Somayaji A, Sarkar S, Balasubramaniam S, Raval R (2022) Synthetic biology techniques to tackle heavy metal pollution and poisoning. *Synth Syst Biol* 7(3):841–846. <https://doi.org/10.1016/j.synbio.2022.04.007>
- Spinck M, Piedrafita C, Robertson WE, Elliott TS, Cervettini D, de la Torre D, Chin JW (2023) Genetically programmed cell-based synthesis of non-natural peptide and depsipeptide macrocycles. *Nat Chem* 15(1). <https://doi.org/10.1038/s41557-022-01082-0>
- Stefanov BA, Fussenegger M (2022) Biomarker-driven feedback control of synthetic biology systems for next-generation personalized medicine. *Front Bioeng Biotechnol* 10:986210. <https://doi.org/10.3389/fbioe.2022.986210>
- Strzyz P (2020) CRISPR-Cas9 wins Nobel. *Nat Rev Mol Cell Biol* 21(12). <https://doi.org/10.1038/s41580-020-00307-9>
- Sun L, Ma X, Zhang B, Qin Y, Ma J, Du Y, Chen T (2022) From polymerase engineering to semi-synthetic life: artificial expansion of the central dogma. *RSC Chem Biol* 3(10):1173–1197. <https://doi.org/10.1039/D2CB00116K>
- Venkatachalapathy P, Padhilahouse S, Sellappan M, Subramanian T, Kurian SJ, Miraj SS, Rao M, Raut AA, Kanwar RK, Singh J, Khadanga S, Mondithoka S, Munisamy M (2021) Pharmacogenomics and personalized medicine in type 2 diabetes mellitus: potential implications for clinical practice. *Pharmacogenomics Person Med* 14. <https://doi.org/10.2147/PGPM.S329787>
- Venter JC, Glass JI, Hutchison CA, Vashee S (2022) Synthetic chromosomes, genomes, viruses, and cells. *Cell* 185(15). <https://doi.org/10.1016/j.cell.2022.06.046>
- Viana Invenção M d C, Melo AR d S, de Macêdo LS, da Costa Neves TSP, de Melo CML, Cordeiro MN, de Aragão Batista MV, de Freitas AC (2021) Development of synthetic antigen vaccines for COVID-19. *Hum Vaccin Immunother* 17(11). <https://doi.org/10.1080/21645515.2021.1974288>
- Vitiello A, Ferrara F (2021) Brief review of the mRNA vaccines COVID-19. *Inflammopharmacology* 29(3). <https://doi.org/10.1007/s10787-021-00811-0>
- Wang C, Zeng HS, Liu KX, Lin YN, Yang H, Xie XY, Wei DX, Ye JW (2023) Biosensor-based therapy powered by synthetic biology. *Smart Mater Med* 4. <https://doi.org/10.1016/j.smaim.2022.10.003>

- White RA (2021) The future of virology is synthetic. *mSystems* 6(4). <https://doi.org/10.1128/msystems.00770-21>
- Wilimitis D, Walsh CG (2023) Practical considerations and applied examples of cross-validation for model development and evaluation in health care: tutorial. *JMIR AI* 2. <https://doi.org/10.2196/49023>
- Wu J, Xin Y, Kong J, Guo T (2021) Genetic tools for the development of recombinant lactic acid bacteria. *Microb Cell Fact* 20(1). <https://doi.org/10.1186/s12934-021-01607-1>
- Xu C, Hu S, Chen X (2016) Artificial cells: from basic science to applications. *Mater Today* 19(9). <https://doi.org/10.1016/j.mattod.2016.02.020>
- Xu X, Meier F, Blount BA, Pretorius IS, Ellis T, Paulsen IT, Williams TC (2023) Trimming the genomic fat: minimising and re-functionalising genomes using synthetic biology. *Nat Commun* 14(1). <https://doi.org/10.1038/s41467-023-37748-7>
- Yamamoto K, Fuchi Y, Ito Y, Hari Y (2023) Expansion of phosphoramidite chemistry in solid-phase oligonucleotide synthesis: rapid 3'-Dephosphorylation and strand cleavage. *J Org Chem* 88(5). <https://doi.org/10.1021/acs.joc.2c02195>
- Yan X, Liu X, Zhao C, Chen GQ (2023) Applications of synthetic biology in medical and pharmaceutical fields. *Signal Transduct Target Ther* 8(1):199. <https://doi.org/10.1038/s41392-023-01440-5>
- Ye X, Qin K, Fernie AR, Zhang Y (2024) Prospects for synthetic biology in 21st century agriculture. *J Genet Genomics*. <https://doi.org/10.1016/J.JGG.2024.12.016>
- Zhai K, Zhang Z, Liu X, Lv J, Zhang L, Li J, Ma Z, Wang Y, Guo H, Zhang Y, Pan L (2023) Mucosal immune responses induced by oral administration of recombinant *Lactococcus lactis* expressing the S1 protein of PDCoV. *Virology* 578. <https://doi.org/10.1016/j.virol.2022.12.010>
- Zhang J, Chen Y, Fu L, Guo E, Wang B, Dai L, Si T (2021) Accelerating strain engineering in biofuel research via build and test automation of synthetic biology. *Curr Opin Biotechnol* 67: 88–98. <https://doi.org/10.1016/j.copbio.2021.01.010>
- Zhang G, Tang T, Chen Y, Huang X, Liang T (2023) mRNA vaccines in disease prevention and treatment. *Signal Transduct Target Ther* 8(1). <https://doi.org/10.1038/s41392-023-01579-1>
- Zhao Y, Coelho C, Hughes AL, Lazar-Stefanita L, Yang S, Brooks AN, Walker RSK, Zhang W, Lauer S, Hernandez C, Cai J, Mitchell LA, Agmon N, Shen Y, Sall J, Fanfani V, Jalan A, Rivera J, Liang FX, ... Boeke JD (2023) Debugging and consolidating multiple synthetic chromosomes reveals combinatorial genetic interactions. *Cell* 186(24):5220–5236.e16