

Genomic Synthetic Biology: A Brief Introduction, Its Applications, and Ethical Laws

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Abstract:

Synthetic biology is now a prominent field that correlates different fields of science like engineering, computer studies, molecular analysis, the biological study of chemicals, and others into novel biological experiments of designing, which involve a series of activities rather than a distinct work. It is a collection of technological and scientifically based experiments that involve the principles of engineering applications to produce and generate robust biological systems like viruses, make different types of protein, and other biological materials. A specific protocol is required in synthetic biology for converting the product from one step to another. For example, there are different strategies for converting the simple target PCR sequence into a sequence-verified clone. For genomic, proteomic, and metabolomic analysis, different and complex protocols and tools are required, and most know certain experiments in synthetic biology. Their task includes organizing the experimental plan, a suitable protocol, maintaining the quality control assessments and performance, and storing the part of the encoded sequence in a controlled environment. Synthetic biology tries to create organisms from the ground up that may be used to serve people as an environmental mediator, microbial factory, or disease fighter. Not only this, synthetic biology also creates non-natural nucleic acid, non-natural amino acid, gene circuits, and biochemical production. Synthetic biology has gained more interest involving life sciences like healthcare and medicine, gene synthesis, and pathogen modification for transformation, especially in primary and secondary health care systems. During the use of synthetic biology, you have to take care of different rules and regulations of NIH and FDA, and different government policies around the globe. As it is a very important field, it is also very important to maintain the laws and ethics of different countries and use them as wisely as possible.

Keywords: Synthetic Biology, Genomics, Ethics, pathogens, DNA

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1. Introduction:

Synthetic biology is a collection of technological and scientific experiments that involve the principles of designing applications to produce and reconstruct robust biological systems. It involves software systems to design the complementary DNA sequence of individuals along with promoters or coding domains. Their task includes organizing the experimental plan, a suitable protocol, maintaining the quality control assessments,

performance, and storing the part of the encoded sequence in a controlled environment. The size of the DNA sequence ranges from 100 nucleotide bases to megabase chromosomes (Kim et al., 2024). The main work in synthetic biology is the DNA synthesis methodology, which depends on whether the part of interest is constructed or obtained by the target amplification by PCR, through a restriction enzyme, or by direct synthesis. The quality and storage of the part of DNA depends on the route from which the part of the coded sequence is obtained (Rourke, 2022).

Synthetic biology is now a prominent field that correlates molecular biology, computer science, biochemistry, and engineering into novel experiments of genetic design. It is a series of activities rather than a distinct work (Robaey et al., 2018). In recent years, there has been a trend of thinking about biological components performing multiple tasks rather than one at a time. Gene regulatory networks and protein signaling pathways are influenced at the molecular level, while at the cellular and tissue level, the molecular components are involved in their feedback and inhibition. Finally, at the population level, various genetic features may influence phenotypic status. Synthetic biology also has its applications in cyanobacteria and algae. It involves the development of "BioBricks," endogenous enhancers, plasmid vectors, and so on [4(Krishnamurthy et al., 2016).

Synthetic biology has diverse applications in many scientific fields, the most important among them. Synthetic biology is a series of activities, or in other words, a series of operations. These steps further require quality control for each step (Yang et al., 2013). The sample is taken and then subjected to barcoding for further study and easy understanding. After that gene of interest is identified and fluorescently or radioactively labeled for easy detection, it is then subjected to product making (Fig. 1). A specific protocol is required for converting the product from one step to another step. There are different strategies for converting the simple PCR target sequence into a sequence-verified clone (Grant et al., 2002). Synthetic biology techniques are being used to improve product manufacturing (Naseri & Koffas, 2020).

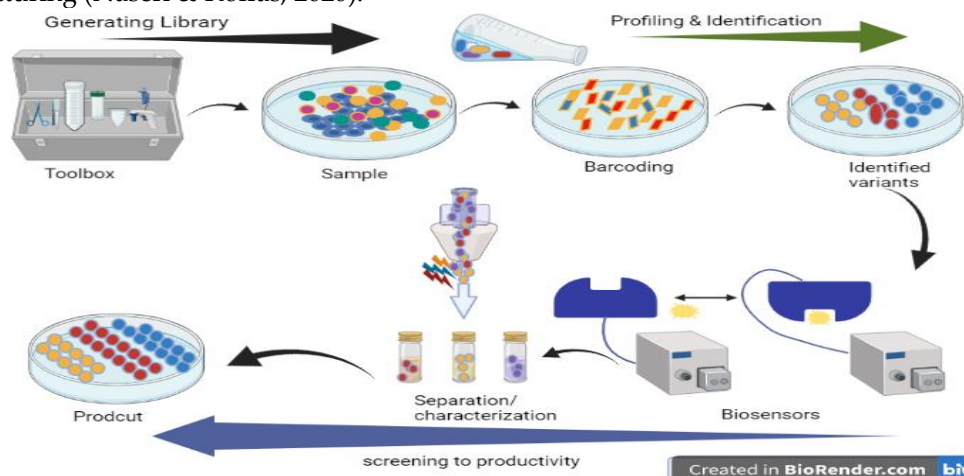


Figure 1. Combinatorial optimization is accelerated by synthetic biology. The synthetic biology toolbox includes DNA modification tools that give combinatorial optimization approaches using a variety of tools, such as regulators and genome editing tools (black arrow). Members of the combinatorial library can be tracked via barcoding throughout the screening process (green arrow). Biosensors combined with high throughput monitoring methods like flow cytometry increase library member selection for isolation (blue arrow).

1.1. Target DNA synthesis

DNA is traditionally synthesized through recombinant procedures, chemical synthesis, and other methods, which provide scientists the opportunity to conceptually design novel DNA sequences (Fig. 2). Commercially, services capable of synthesizing DNA in the tens

of kilobases range are now accessible (June & Levine, 2015). By transferring a DNA sequence to a firm, a new DNA sequence can be created. Blue Heron, Generate, DNA 2.0, and Gene Script are among the companies that have produced a DNA template. These companies minimize the time and provide convenience for the rapid production of genes, eliminating the undesirable structures and increasing gene expression (Shi et al., 2022). It can be seen that using different methods, different results can be achieved (Fig.2). Now, there is a need for producing larger DNA fragments by using new DNA synthesizing technologies. It has been reported that the size of synthesized DNA has increased from 75 kb to 582,970 kb in length between 1970 and 2008. Recently, J. Craig Venter assembled a DNA of 583 kb by the assembly of 101 pieces of DNA fragments, 5-6 kb in length. The whole process is done in two steps; the first in vitro enzymatic process is used to produce six large fragments of DNA; the genome is created using in vivo yeast recombination in the second step. Finally, the synthetic product was made by overlapping fragments directly in yeast (Barnes et al., 2011).

1.2. Protein synthesis

To turn naturally occurring proteins into variations or desired proteins, a variety of technologies are employed. The important ones are directed evolution and rational design methods. Since rational design needs inadequate information about protein structure, functions, dynamics, and other factors, directed evolution is an extra efficient method for humanizing premature protein functionalities (R. Liu et al., 2022) Although directed evolution is the most effective method for researching protein attributes like activity, stability, compatibility, and selectivity, this technology has drawbacks, such as the employment of microorganisms linked to protein function with a smaller size of nearly 300 nucleotides. To develop novel proteins with desirable functionality, the computational tool was combined with the direct evolution method. The development of a Kemp elimination enzyme is a good example of this (van Doren et al., 2013).

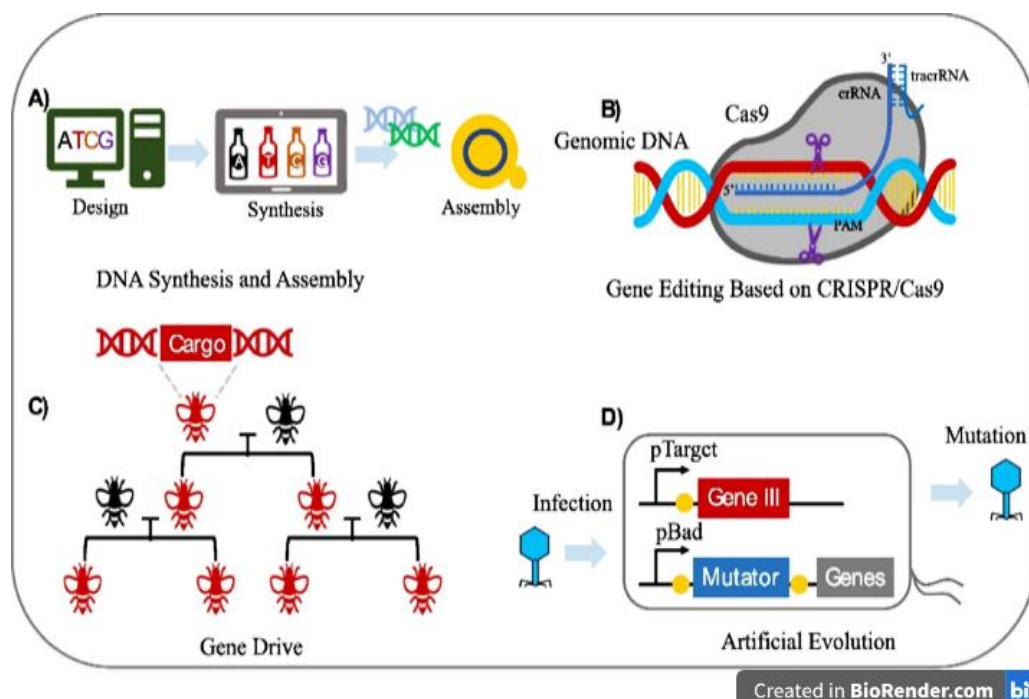


Figure 2. Schematic of cutting-edge technologies in synthetic biology. A) DNA synthesis and assembly; B) CRISPR/Cas9-based gene editing; C) Gene drive; and D) phage-assisted continuous evolution.

2. Genomics, Proteomics, and Metabolomics as Analysis tools in synthetic biology

The ability to correlate genotypes and phenotypes has been greatly improved by advancements in genomics tools such as DNA microarrays and high-throughput sequencing (Lim et al., 2025). Microarray is a familiar process to analyze the level of mRNA in contrast with many genes. Through this, scientists can analyze the change in phenotype due to changes in the environment genetics [14(H. Gao et al., 2010). Chromatin Immunoprecipitation (CHIP) is a technique that is most widely used to determine genomics, in which the gene of interest is separated from the chromatin sample to determine its sequence. Recently, the CHIP technique has been associated with sequences and is known as CHIP-IP seq. It gives higher resolution, lower noise, etc., and is followed by a sequence (P. H. B. Chen et al., 2025).

Proteomics is used to quantify and determine all expressed genes and their products in different locations. Proteomics uses mass spectrometry as one of the most efficient techniques, where a stable label isotope tag is recognized by a mass spectrometer (Kelle, 2013). Another suitable technique is activity-based proteomics for the global function of enzymes, in which chemical probes are used to specifically target the enzyme's active site. Metabolomics is complementary to proteomics as it discusses the different activities and synthesis of small molecules on a large scale, within cells, biofluids, tissues, or organisms. Proteomics gives information about how many proteins are present, while metabolomics provides the proteins doing work on the biological molecule. Recently, metabolomics has been reported to be used in determining the biochemical pathway of engineered proteins (Geiger & Spatz, 2016).

3. Synthetic virology as controllable nanodevices from the perspective of synthetic biology

Synthetic virology employs engineering principles and technologies to program viruses with novel functionalities. The three primary methodologies are (a) directed evolution of virus capsids, (b) rational capsid design, and (c) bioinformatics-driven design. Although the methodologies are sometimes employed separately, there are natural synergies at the intersections of the three that lead to hybrid designs, such as employing bioinformatics studies to influence how libraries of the viruses are created for directed evolution patterns. One more key viewpoint in artificial virology is to think of viruses as nanoscale devices that sense exogenous/endogenous inputs and respond with chosen outputs (Brun et al., 2017). This segment provides a beginning and modernization to a prior survey of the synthetic virology area (Guenther et al., 2014). We will focus on the three ways of viral vector or tools of synthetic biology techniques in particular (Powell et al., 2016).

3.1. Directed evolution of virus capsids

Directed evolution is a process that uses circles of alteration and choices to discover mutants that fit users distinct objectives, much like natural evolution (DeFrancesco, 2021). William Stemmer issued one of the widely used directed evolution procedures (White, 2021) and was a pioneer in many of its applications in bacteria (M. Y. Chen et al., 2019) retroviruses (Guenther et al., 2014), enzymes (M. Y. Chen et al., 2019), and enzymes (Railard et al., 2001). Various mutagenesis procedures, such as accidental point mutation (e.g., error-prone polymerase chain reaction), DNA recombination (e.g., DNA shuffling), and casual peptide presence, can be used to achieve directed evolution. The readers are recommended to an in-depth examination of the guided progression of viral vectors for further information (Tenoever, 2020). They are further advised to consult the literature (O'Neil & Hoess, 1995) for further information on peptide-exhibit-based conducted evolution (Tenoever, 2020). Adenovirus (Ad) (Kuhn et al., 2008), adeno-associated viruses (AAV) (Tenoever, 2020) simplex virus (HSV) (Christians et al., 1999), Murine leukemia virus (MLV) (Soong et al., 2000), and tobacco etch virus (TEV) [25](Tenoever, 2020) are just a few of the viruses whose capsids are altered. AAV alternatives famous for dissuading the CNS were swapped in a recent study to generate a virus capable of effective in vivo oligodendrocyte gene transfer (Powell et al., 2016).

Directed evolution has also been used to generate cancer-specific viral treatments. OvAd1, an ovarian tumor-targeting adenoviral vector, was produced using directed evolution, e.g., Ad-binding knob was randomly mutated to form an adenovirus library which was chosen for a viral variation with increased contagion in a 3D culture of ovarian tumor cells (Kuhn et al., 2016).

3.2. Rational capsid design

To confer new skills onto a viral particle, rational design methodologies employ past awareness of the virus and efficient "components" that may be introduced into the capsid of the virus. Proteins and peptides that have been heritably incorporated into viral capsids will be discussed in this section (Peng et al., 1999). Rational design methodologies are used to describe how to make the virus bio-activatable in response to an intracellular or extracellular stimulus.

MMPs (matrix metalloproteinases) are increased in a variety of illnesses, including cancer (Mühlebach et al., 2010). MMP-receptive viruses have been developed to receive protease activity from outside the cell, resulting in targeted transduction to disease areas (Evans et al., 2016). The Moloney murine leukemia virus (MMLV) was used to create individual earliest protease-receptive viruses (Schneider et al., 2003). An EGFR-binding antibody section was linked to the envelope of the virus via an MMP bifurcation (changing in MMP qualitative nature of a system as a parameter) region on the virus. These altered MMLV vectors were unable to integrate into cells until enough MMP activity was present to slice the antibody segment from the envelope of the virus, permitting the virus to incorporate and take over the host cell. As a result, the MMLV would only be infectious in areas with high MMP activity, such as sick areas. Intratumorally inserted MMP-alerted MMLV vectors have been proven to react in subcutaneous tumor models (Peng et al., 1999).

3.3. Bioinformatics-driven capsid design

Protein structure-based and sequence-based tools are two types of computational approaches for designing viral vectors. AAVs, for instance, are divided into serotypes and clades based on phylogenetic, genomic, and functional similarities (Greber & Gomez-Gonzalez, 2021). The alignment of the capsid genes of different AAV serotypes reveals an elevated level of similarity among them, suggesting that viral phenotypic changes in features are probably mediated by modest dissimilarity in genotypes. Over the last few decades, significant work has revealed that phenotypic diversities in (wt) AAV serotypes are linked to inconsistent loop areas in the capsid (Zinn et al., 2015). Novel viral features have been discovered because of modifying these highly variable areas (Adachi et al., 2014)]. For example, an AAV capsid collection was created by changing solely AAV2's changeable surface loops, and a mutant capable of transducing glial cells was discovered after selection (Belkin & Wang, 2022).

Phylogenetic analysis may be used with virus capsid sequence alignments to construct novel ancestral variations from disreputable relations. These techniques were utilized to create and contrast a library of ancestral AAV variants of great thermal stability, but few of them were more infectious than others (Smith et al., 2016). Ancestral 80, a potential AAV ancestor, was discovered using an ancestral library technique (Zinn et al., 2015). Anc80 is a hypothetical ancestor between AAV4 and AAV5, as well as the other regularly utilized serotypes. Anc80 has been shown to efficiently dissuade hair cells in the cochlea, making Anc80-based vectors attractive for the management of hearing loss (Suzuki et al., 2017). Next-generation sequencing (NGS) technologies using barcoded AAV collections can be utilized to offer further knowledge about viral phenotype, genotype, and their connections, in addition to utilizing currently known viral variant sequence data. For instance, over 200 AAV capsid varieties with various genetic barcodes were created and combined into a single vector library, where the AAV barcoded collection was administered in vivo (Cheema et al., 2022). This method, known as AAV Barcode-Seq,

enabled a high-output study of each AAV alternative's biodistribution. An additional study employing a method like AAV Barcode-Seq might find novel sequence–utility correlations in the capsids of viruses in a shorter time.

Instead of protein sequence, bioinformatic methods may be used to analyze protein structure. SCHEMA, a method for predicting ideal crossover locations for the production of DNA-shuffled libraries, is a structure-based computational implementation. SCHEMA determines the number of remnant-to-remnant connections that are disrupted during recombination using 3D structural knowledge. During recombination, reducing the number of broken connections should improve the chances of producing structurally intact viral offspring. This method has been utilized to AAV capsids to create new recombinant capsids and mutants with novel properties, i.e., they can dissuade brain stem cells (Ojala et al., 2018). Structure-based design methodologies are anticipated to grow more, which can disclose more fundamental knowledge about capsid structures.

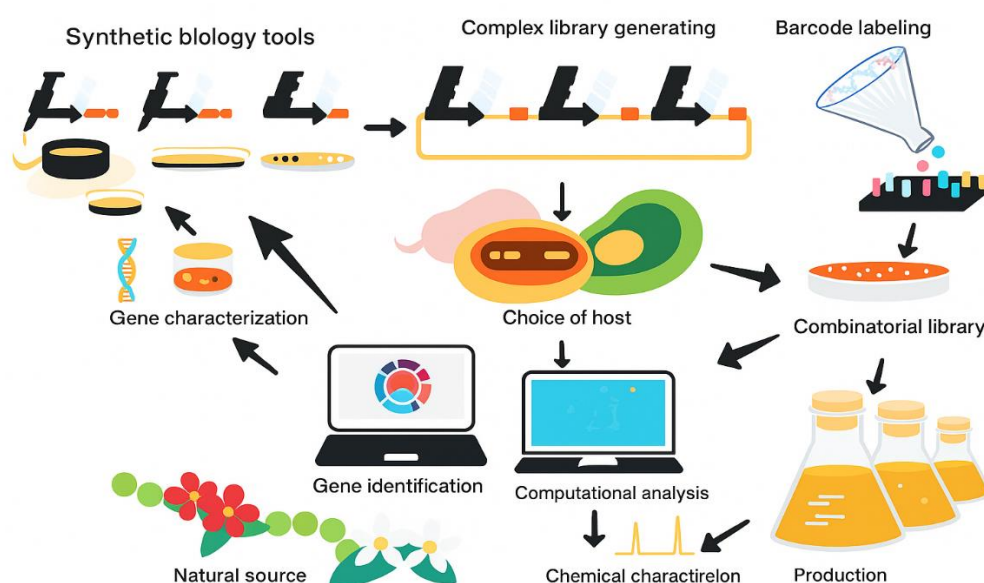


Figure 3. An overview of bioinformatics design and evaluation to obtain optimal performance is shown in this diagram

Combinatorial optimization approaches are established using synthetic biology technologies, while biosensors and barcoding technologies are used to characterize the created library, as shown in Fig. 3. The blue area of the figure demonstrates that synthetic biology techniques are used in metabolic engineering to create chemicals from a defined biosynthesis in a chosen host. Combinatorial optimization procedures can be used to improve the manufacturing of particular chemicals. On the basis of genome-scale metabolic modeling, the data gathered from the combinatorial library and its pre-characterized elements are computationally integrated to construct mathematical models to assist the early design processes for the chosen host. Based on an understanding of how different biological subsystems interact, the computational results reveal which synthetic pathways are the most viable in a given target species and which host pathway genes must be boosted or repressed. The greatest combinatorial library producers can supply extensive information for models aimed at uncovering fundamentals of how synthetic devices function in host systems [7(Naseri & Koffas, 2020).

4. Applications of synthetic biology

Synthetic biology finds diverse applications in various fields related to the life-functioning molecules and the pathways involved in their synthesis, as well as functions (Bhatia et al., 2024)It performs its function in polymers like nucleic acid and proteins and also helps in

studying the involved pathways. At the second stage, it also involves molecular functioning in which biochemical networks give rise to self-replicating life entities. In the end, synthetic biology can be branched into the multicellular level so that it may be called synthetic ecology (Cox et al., 2010). Synthetic biology has its application in every field, as mentioned in Fig. 4.

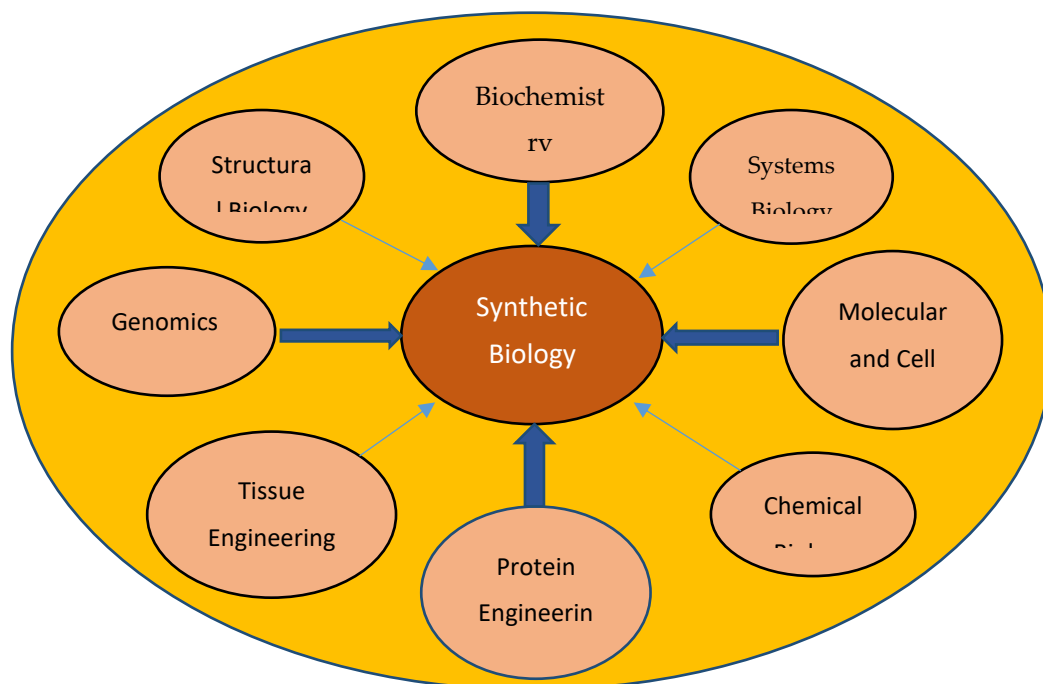


Figure 4. General overview of the application of synthetic biology in different fields of science.

4.1. Synthetic biology works on changes at the Molecular level

At a molecular level, synthetic biology is shown to change the basic properties of macromolecules such as proteins and nucleic acid and alter or manipulate properties that are not present in them by nature. At the molecular level, the role of synthetic biology can be discussed both in terms of non-natural nucleic acids and non-natural amino acids.

Non-Natural Nucleic Acid: DNA is the fundamental molecule that aids in the storage and operation of the central dogma and the triplet codes A, T, C, and G, which are involved in the complexity of life. Synthetic biology's essential design units in non-natural nucleic acids are a) nucleotide choice, b) number of nucleotides, and c) the triplet code (Haseltine & Patarca, 2024). To perform the synthesis by non-natural means, the most important thing is its compatibility with natural DNA and RNA enzymes as a polymerase. The main purpose of non-natural synthesis is to enhance the stability of polymerase as well as to increase the base pairs (Stracquadanio et al., 2016).

Non-Natural Amino Acid: Synthetic biology performs its function in non-natural amino acid synthesis to alter the natural sequence of proteins to achieve novel protein functions and properties. One of the valuable synthesis tools in non-natural amino acid research is protein engineering (Laserson et al., 2005). In vivo, site-specific alterations are made in non-natural amino acid synthesis that require some of the components. One is amino-acyl transferase, which replaces tRNA with an amino acid that is not found in the host system normally (Beites & Mendes, 2015). The amino acid analogues contain many properties regarding fluorescence and photo-regulation. Some of the strategies must be used to enhance the synthesis in non-natural ways. By introducing non-natural base pairs,

one is to expand the genetic code. Another also used quadruplet code for non-natural amino acids (Di Camillo et al., 2012).

4.2. Network Level/Pathway

There are two main focuses using synthetic biology at the network level or pathway: one is to explore the gene regulatory design, and the other is to perform a more complex transformation. Several tools are used at this level for synthesis (Kanigowska et al., 2016).

Gene Circuits: Gene circuits are similar to electronic circuits. Biological gene circuits, on the other hand, are not likely to contest the computational capacity of their silicon complement. They also lack a channel that can predict genetic behavior (Bi et al., 2013). It has been revealed that before translation, transcription, and after translation, the output of gene circuits can be controlled [51](Tsai et al., 2010).

Biochemical Production: Metabolic synthesis of biochemical metabolites is another prominent study field at the pathway and system level where enzymes are involved in the biochemical production rather than any equipment. It is not to be confused with the creation of therapeutic proteins. The ultimate product is protein, and the machinery to make it is already present in the cell's DNA template [52](Z. Liu et al., 2022).

4.3. Whole-Cell/Organism Level

Cells have been uniting to develop an organization that is durable and well-organized in its atmosphere and endurance since the moment of revolution. At this level, synthetic biology tries to create organisms from the ground up that may be used to serve people as an environmental mediator, microbial factory, or disease fighter (Lorenzo et al., 2018). It has been revealed that self-replicating organisms are undergoing chemical synthesis. Because of their tiny genomes and basic functions, DNA and RNA are easier to synthesize chemically (Toni & Tidor, 2013). Vaccine production is one of the immediate uses of chemically synthesized viruses. Now it is possible to make a change in the genome of the virus to attenuate its virulence. The non-enzymatic assembly and replication can also be done by these techniques (Pauwels, 2009).

4.4. Multi-Cell Level

In multicellular organisms, cells are highly distinguished and categorized into organs to perform specific and composite functions of the body. Synthetic biology at the multicellular stage is based on some thoughts. It may be communication and division of labor. Communication can be achieved by the exchange of signal molecules or by the exchange of metabolites. Different domains of life can also demonstrate communication, as mammalian and bacterial cells do (Bacchus et al., 2013).

4.5. Application in Healthcare and Medicinal Use

Synthetic biology is propelling substantial advancements in biomedicine, which will result in considerable healthcare breakthroughs. Patients are so far making the most from CAR (chimeric antigen receptor) expertise, which modifies the patient's immune cells (T-cells) to detect and destroy tumor cells (Khan et al., 2022).

In individuals with hereditary disorders like epidermolysis bullosa or severe combined immune deficiency (SCID), these genetically modified viruses are being utilized to fix faulty genes (Dunbar et al., 2018).

The capacity to develop patients' body cells into induced multipotent stem cells is advancing our knowledge of their condition, lowering the usage of animals in the study, and opening the path for tailored medications and cell treatments.

4.6. DNA assembly technology

DNA fragments were initially cleaved and ligated by restriction endonuclease and ligase in the 1970s, signaling the commencement of the biotechnology revolution in genetic engineering (Nishi et al., 2022). Synthetic biology preserves recombinant DNA-based technology while introducing additional requirements and concepts. In general, the major aims of DNA assembly technology are to build a simpler, more efficient approach that can be used to assemble bigger and more complicated fragments (Nishi et al., 2022) and to

establish the assembly standard to achieve operational standards (Ellis et al., 2011; Nishi et al., 2022). Tom Knight et al. proposed the BioBrick standard in 2003, and it was the first DNA in vitro splicing standard. The approach makes use of a collection of standardized restriction endonuclease restriction sites, including prefixes (such as EcoR I and Xba I sites) and (SpeI and PstI) sequences at both ends of each biological element. The cleavage of these sites and subsequent ligation provides a standardized DNA splicing mechanism (Dou & Bennett, 2018)

4.7. De novo synthesis of DNA

Although native DNA sequences may be modified in vitro and in vivo to overcome numerous issues. De novo DNA synthesis offers several benefits (Kosuri & Church, 2014). To begin with, designing a DNA sequence with new functionality sometimes necessitates the mutation of a large number of or even whole sequences, which is easily accomplished using de novo synthesis technology. Second, in the study of genetic processes, artificially produced sequences are frequently preferable to natural sequences because they may be developed and used to test theories (S. Li et al., 2017). Third, many sequences, such as those spliced with metagenomic data, find it challenging to get natural templates for further amplification and alteration. The current study covered DNA de novo synthesis methods, such as huge-scale single-stranded DNA synthesis, further assembly of it into larger double-stranded DNA sequences, and some of the issues encountered throughout the process (Súnico et al., 2021).

4.8. Column oligonucleotide synthesis

Todd et al. were the first to effectively synthesize oligonucleotides in the 1950s (Olesiak et al., 2009). Most oligonucleotides are now produced using automated equipment and the solid-phase phosphoramidite technique (the standard method for synthesizing DNA and RNA oligonucleotides (short strands of nucleic acids) (Abe et al., 2025). The mechanism is a four-step cycle that yields a new nucleotide throughout each cycle: 1) deprotection, 2) coupling, 3) capping, and 4) oxidation (Tang et al., 2020). This automated process can synthesize 96–384 nucleotides in total, with each nucleotide containing roughly 10 to 100 nmol. Chemical synthesis processes have constraints in terms of synthesized length and error rate for the following reasons (S. Li et al., 2017). To begin, each cycle's yield must be extremely high, especially when producing long-chain oligonucleotides. For 200 μ L oligonucleotides, for example, even if each cycle yields 99 percent, only 13 percent of the full-length product may potentially be achieved. Furthermore, the depurination step, particularly with adenine, causes difficulties in the synthesis of lengthy oligonucleotides (LeProust et al., 2010). Finally, even correctly produced oligonucleotides may be subject to some degree of inaccuracy (Binkowski et al., 2005). As a result, more research is needed to create new chemical technology to boost the length and quality of the synthesis (S. Li et al., 2017).

4.9. Synthesis of Gene

The process of synthesizing a sequence of oligonucleotides (typically 5–50) into bigger pieces (usually 200–3,000 bp) is known as gene synthesis, and the gene here refers to the length of the gene. Initially, the Khorana research team used T4 DNA ligase to bind the oligonucleotides to a sequence of 80–200 bp (Shi et al., 2022). The ligase reaction linked the complementary overlapping chains into larger segments in this linking-based technique. Later, the common ligase was replaced with a heat-resistant ligase to perform ligation at high temperatures (50–65°C), limiting the creation of secondary structure in the oligonucleotide chain (Cheema et al., 2022). The most prevalent splicing approach is polymerase cycling assembly (PCA) (S. Li et al., 2017). The polymerase is used in this procedure to stretch the overlapping segments into double-stranded DNA fragments (Meydan et al., 2018). PCR amplification, as well as final cloning and sequencing, is required for both the linking-based technique and PCA. Both systems have their benefits and drawbacks. Because the probability of heterozygosity and ligation of erroneous sequences is

low, linking-based synthesis lowers the error rate. However, oligonucleotide chains must completely cover both the top and lower chains, and the 5' end must be phosphorylated; this is a more costly procedure (S. Li et al., 2017).

5. Tools for Genome Editing

In the past, genome editing technologies advanced quickly. Genome editing was first done using the homologous recombination approach (J. Gao et al., 2022). However, due to the different efficiencies of different systems and the need to introduce screening markers such as resistance, site-specific recombinase systems, such as Cre protein (cyclization recombinase) and the corresponding loxP sequences, the Flp-FRT system, and C31 integrase-ATT sites (Lertwattanasakul et al., 2022), were used, which significantly improved the efficiency. The loxP, FRT, and ATT sequences must first be inserted into specified places in the genome, followed by the introduction of location recombinant proteins, which allow genomic DNA to be rearranged. Site-specific protein cleavage has progressed fast in recent years, from meganuclease (Muth et al., 2021) to zinc-finger nuclease (Urnov et al., 2010), transcription activator-like effector nucleases (TALENs) [75], and CRISPR (clustered regularly interspaced short palindromic repeats) (Ran et al., 2013), among others. All of these methods cause double-stranded DNA breaks in cells, which are subsequently used to alter recombinant repair via non-homologous end-joining repair or an extra homologous recombination template. However, lengthy sequences are generally inserted into initial sequence-specific sites, causing discomfort. Later, it was discovered that the zinc finger protein, which binds to sequence-specific DNA, may be designed into the nuclease ZFN with particular recognition capabilities by fusing the tandem repetitions of the zinc finger protein with the Fok I nuclease domain. TALEN is another designed nuclease (Vojvoda et al., 2022).

6. Pathogen Modification and Artificial Synthesis

The reform and creation of pathogens based on an available pathogen's gene sequence is an interesting area of synthetic biology. Cello et al. (2002) employed chemical techniques to create a whole poliovirus cDNA, which was then transcribed by RNA polymerase into RNA (Cello et al., 2002). By incubating cytoplasmic extracted HeLa cells with transcribed RNA from poliovirus cDNA, they were able to generate the infectious virus. The Spanish influenza virus was successfully synthesized by Tumpey et al. (2005) using the following technique (Tumpey et al., 2005). The scientists inserted all eight coding gene sections of the 1918 Spanish influenza virus into the genomic DNA of a common influenza virus based on a release of the virus's genome sequence. Viral elements were then isolated from human kidney cells that had been infected with an influenza virus. Although pathogen synthesis can aid scientific study on viral pathogenesis and therapeutic expansion, pathogen alteration or production is a risky effort (Thiel, 2018). During pathogen synthesis, biosafety and biosecurity, as well as cybersecurity, are major concerns that must be addressed.

7. Pathogen Detection and Vaccine Research Using Synthetic Biology

Pathogen detection is an interesting area of synthetic biology. Considering COVID-19 as some nations consider it to be influenza, substantial disease characterization information and studies have revealed that COVID-19 is not equivalent to epidemic influenza in terms of fitness risks or possible societal impact (Murch & DiEuliis, 2019). Synthetic biology approaches appear to play a vital role in the development of delicate and precise analytical kits, vaccinations, and medications in the battle in opposition to COVID-19, according to evidence (Súnico et al., 2021).

SARS-CoV-2 detection might be aided by synthetic biology. The use of CRISPR-Cas technology in the detection of pathogens has drastically decreased the expenditure of detecting SARS-CoV-2, with the overall price tag being much less as compared to a traditional RT-PCR test (Palaz et al., 2021). SARS-CoV-2 and its mutations may also be distinguished using the CRISPR-Cas13-based detection approach (Rahimi et al., 2021; Tonsager

& Stargell, 2024). By inclusion of customizable nuclease PfARNGO with the RT-PCR technique, Wang et al. (2020) created a more receptive, considerably more precise, and perfect PfAgo-based identification of SARS-CoV-2 that is able to also discriminate mutations of coronavirus (Y. Wang et al., 2021).

The development of a SARS-CoV-2 vaccine has made tremendous progress thanks to synthetic biology. Many vaccines that have been created or are currently being industrialized, such as viral vector-based vaccines, subunit vaccines, inactivated vaccines, and so on, are now based on synthetic biology (F. Wang et al., 2021). However, major occurrences in the creation of vaccines have occurred in the past (Haynes et al., 2020) It is critical to produce efficient vaccines under strict bio-risk evaluation and control (Strizova et al., 2021). Serious unfavorable measures have earlier stimulated the suspension of tests in the recent progress of the COVID-19 vaccine, while an independent review committee completed an inclusive evaluation of fatalities connected with vaccination, as was completed in the chimpanzee adenovirus vector vaccine learning (Belete, 2021). Vaccine biosafety is ingrained in the whole development and usage process. It is vital to make sure that security hazards (e.g., vaccine-associated enhanced disease (VAED)) are discovered, weighed, and measured in opposition to possible benefits to introducing an efficient COVID-19 vaccination as broadly, quickly, and securely as feasible.

8. Risks and shortcomings of synthetic biology

Synthetic biology is an illustration of dual-use expertise: it has many potential benefits, but it also has the potential to impose damage. This has raised concerns that it might injure individuals or harm the environment, either purposefully or accidentally. For example, our capacity to create viruses to be a more efficient vehicle for gene treatments of catastrophic genetic illnesses is extremely valuable; nevertheless, manipulating viruses might lead to the production of even more lethal infections by those seeking to damage (El Karoui et al., 2019).

The unknown and remote likelihood of the associated hazards to the treatment methods might inhibit the advancement of helpful technologies. Researchers, their host organizations, and subsidy organizations ought to (and do) think about whether the intended study may be abused. Measures should be established and explicitly stated to decrease the risk of misuse and its repercussions by participating in perspective-examining activities as well as uniting conversation with controlling authorities and the media; the synthetic biology community can become aware of and respond to these problems (El Karoui et al., 2019).

9. Synthetic Biology's Opportunities and Challenges

Synthetic biology has evolved dramatically over the last two centuries because of its importance in digitalization and automation, providing strong tools to create and even manufacture living forms. There have been numerous initiatives to elevate the consciousness of the resulting risks (biosecurity, biosafety, and cyberbiosecurity) in this area. We must take necessary actions to address the potential and difficulties posed by synthetic biology and give the remarkable beneficial contributions of artificial biology to people's lives, well-being, surroundings, and environment, as well as the problems created by its use (J. Li et al., 2021).

10. Ethical laws and Regulation of synthetic biology

Synthetic Biology for Human Well-being—The Legal Issues and Ethical (SYBHEL) was a three-year working European project. It was used to wonder about the legal and ethical issues on SynBio to govern the signs of human health (Hill et al., 2012). SynBio describes engineering in biochemistry, computer science, and other fields to design new applications to work. It can distinguish the new technologies to collect multipurpose engineered production. All the visions of synthetic biology can enlighten its social promises. It can involve bio-based processes in energy, environment, computer sciences, fuel, and medicine (Mukherjee et al., 2009).

Different biological products are governed by policies, rules, and laws endorsed by the US government. Pathogens are categorized according to their pathogenicity. In terms of laboratory management, the National Institutes of Health (NIH) and the US Centers for Disease Control and Prevention (CDC) have produced "Biosecurity in Biomedical and Microbiology Research Centers," a guidebook on suggestions for pathogen physical containment (Berns, 2014). The federal Food, Drug, and Cosmetic Act governs all pharmaceuticals in the United States (FDCA).

The US government developed DURC policy, which governs the oversight of scientific life research. Life science is a study that can reasonably be expected, based on current knowledge and information, to supply facts, foodstuffs, or techniques that could be openly misapplied to cause a significant risk to communal health and security, animals' crops and plants, materials, surroundings, or general safety (J. Li et al., 2021).

In European countries, legislation on genetic modification of organisms (GMOs) and most research in the subject of synthetic biology include genetic engineering, which governs the usage as well as the marketing of GMOs and related products (Buhk, 2014). The EU has established a definite working cluster to evaluate operations of modern biotechnology in plant propagation and other genetic changes to limit the extent of the law's implementation. Labeling, adequate control, transshipment, and protected use in study atmospheres are all covered by a succession of directives issued by the European Union for GMOs and developing biotechnology (Keiper & Atanassova, 2020).

In China, synthetic biology is rapidly evolving, particularly in the domains of higher biomanufacturing, developed enzyme engineering, microbial genetic propagation, and biomedicine. In response to bio-threats, China has enacted rules and conventions on synthetic biology to ensure biosecurity and biosafety (J. Li et al., 2021). The Biosafety Law of the People's Republic of China was disseminated in 2020 by the Standing Committee of the National People's Congress, which represents China's ultimate legislature. This biosafety law cannot be violated by any local system, governmental system, or departmental system (J. Li et al., 2021).

Conclusion

The purpose of the present review is to have a clear understanding and updates via literature references about the different aspects of synthetic biology, including its core concept, comparative overview of the methods/procedures involved in processes that come under the scope of synthetic biology, application of synthetic biology in different fields, and the global laws regarding ethical issues associated with synthetic biology practices. Nowadays, synthetic biology has become the most striking and booming area of research. Groundwork is now being laid to bring it to reality due to its vibrant technology. As mentioned in this review, meaningful applications exist at every scale. However, even more enhancing features can be obtained by combining the results occurring at each level. Enzymes can be improved by better solubility, stability, and function. These can be integrated and produce desirable results. Genome engineering can also be optimized to increase titer and purity using synthetic biology.

Synthetic biology is a new area based on standardization, modularization, and characterization engineering principles, as well as systematic planning (it is not a direct extension of genetic engineering). The development of increasingly low-cost and dependable gene sequencing and synthesis methods that are becoming widely commercially available has been a key driver of synthetic biology, if not the key to its genesis, making viruses new vectors for production and using it for medical uses and health care.

Further research

The present review was to recognize the patents of synthetic biology on the methodological approach. The construction of new and minimal genomes in the prospect that does not violate moral boundaries in technology further advances. However, efforts are made

on public attempts and community to recognize the purpose of science and its religious ethical concerns to debate. The only step we have to take is the line of research independently. There is some ethical concern that the technique of synthetic biology works to create life. It contributes to the therapeutic system based on a synthetic genome and expanded genetic code and is designed for specific personalized drugs. One of the most promising future directions is the expansion of therapeutic systems. Synthetic genomes may be tailored to create next-generation biologics, including smart vaccines, enzyme therapies, and precision-targeted drugs. The future also points toward the routine use of expanded genetic code, allowing the incorporation of non-standard amino acids to create novel proteins with unique functions. These innovations are paving the way for personalized medicine, where drugs are not only custom-designed for individual patients but also delivered via programmable living cells. As synthetic biology evolves, global conversation is around its ethical, religious, and social implications. Future frameworks will require stronger collaboration between scientists, ethicists, policymakers, and the public. This interdisciplinary dialogue will be essential to navigate concerns over “creating life,” genetic manipulation, and the potential misuse of technology. Importantly, future research must be carried out with transparency and independence. Publicly funded projects and open-access knowledge sharing will ensure that synthetic biology serves the common good, rather than exclusive commercial interests. Community engagement initiatives will also be key to fostering public understanding and trust.

Conflict of Interest

The authors report no conflicts of interest.

Availability of data and material

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Books, research articles, and the internet.

List of Abbreviations

AAV: (Adeno-associated viruses)

CAR: (chimeric antigen receptor)

CHIP: (Chromatin Immunoprecipitation)

CRISPR: (Clustered regularly interspaced short palindromic repeats)

HSV: (Herpes simplex virus)

MMPs (matrix metalloproteinases)

MLV (Murine leukemia virus)

MMLV: (Moloney murine leukemia virus)

SCID: (Severe Combined Immune Deficiency)

TEV: (Tobacco Etch virus)

TALENs (Transcription activator-like effector nucleases)

CRISPR (Clustered regularly interspaced short palindromic repeats)

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