NEWS AND VIEWS

# An overview of synthetic biology

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**Abstract**: Synthetic Biology is the combination of basic sciences with engineering. The aim of Synthetic Biology is to create, design, and redesign biological systems and devices to understand biological processes and to achieve useful and sophisticated functionalities to improve human welfare. When the engineering community took part in the discussion for the definition of Synthetic Biology, the idea of extraction and reassembly of "biological parts" along with the principles of abstraction, modularity, and standardization was introduced. Genetic Engineering is one of the many essential tools for synthetic biology, and even though they share the DNA manipulation basis and approach to intervene in the complexity of molecular biology, they differ in many aspects, and the two terms should not be used interchangeably. Some of the applications that have already been done by Synthetic Biology include the production of 1,4-butanediol (BDO), the antimalarial drug artemisinin, and the anticancer compound taxol. The potential of Synthetic Biology to design new genomes without immediate biological ancestry has raised ontological, political, economic, and ethical concerns based on the possibility that synthetic biology may be intrinsically unethical.



**KeyWords:** Synthetic Biology, Genetic Engineering.

## Introduction

### What is Synthetic Biology?

Synthetic Biology is an arising field of research that integrates basic sciences with engineering. The interdisciplinarity of Synthetic Biology is evident as it has evolved along with the progress made in Biology, Biotechnology, Molecular Biology, and Computer Science. The discovery of DNA as the molecule carrying the organisms' genetic information, the findings regarding the regulation of *E. coli's* lac operon, and the advent of recombinant DNA technology, all paved the way for Synthetic Biology. This field owes its further development to Computer Science, which made possible the construction of models that describe and predict the processes and interactions between and within biological systems. The goal of Synthetic Biology is to create, design, and redesign biological systems and devices to understand biological processes and to achieve useful and sophisticated functionalities to improve human welfare[1–6].

The term "synthetic biology" was not always associated with the design of biological systems. In the 80s, the term was first used in the literature to describe bacteria that were genetically engineered employing recombinant DNA technology. Later, in the early 2000s, synthetic biology was associated with the synthesis of non-natural organic molecules that could function in living systems. The current definition of Synthetic Biology began to crystallize when the engineering community took part in the discussion and introduced the idea of extraction and reassembly of "biological parts" along with the principles of abstraction, modularity, and standardization. Abstraction refers to dissecting the design procedure into several hierarchies as an effective way to handle complexity. The division of the engineering process into several more straightforward abstraction levels (DNA, parts, devices, and systems) allows designers to work at a specific level somewhat independently to build a part, device, or system. Modularity or decoupling is the degree to which a system can be separated into "functional blocks" or orthogonal components. Functional blocks can be combined to construct modules with different functionalities that do not interact with each other. Finally, standardization aims to provide tools and protocols to ensure predictability and reproducibility in biological experimentation. Nowadays, Synthetic Biology is characterized by two main lines of research. The first one is focused on the discovery, characterization, and creation of biological parts, whereas the other seeks to assemble said parts into systems of increasing complexity[1,4–12].

Biological parts are the building blocks in Synthetic Biology. These are segments of DNA that encode for specific and indivisible biological functions such as promoters, ribosome binding sites, protein-coding regions, and transcription terminators (Figure 1). According to the International Genetically Engineered Machine (iGEM) Foundation, biological parts are functional units that cannot be separated into smaller units, and that can be ligated to build sophisticated devices[13]. Two or more parts can be assembled to form construction intermediates that do not comprise a device. Devices are made up of two or more parts that when combined can perform a biological function. The Registry of Standard Biological Parts is a repository of biological parts ran by iGEM that is available for the public. It contains information about the sequence, design, and availability of thousands of parts. The biological parts found in the Registry meet the BioBrick standard. The standardization involves the addition of a BioBrick prefix and a suffix, which are standard cloning sites flanking the part's DNA sequence. The standardization of parts guarantees its compatibility and interchangeability because the restriction enzymes and ligation steps used to combine two BioBricks are independent of its sequences[10,14–18].

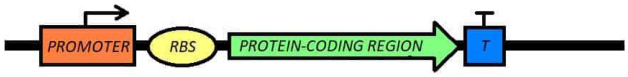


**Figure 1.** Basic devices with four biological parts: promoter, ribosome-binding site (RBS), protein-coding region, and terminator.

According to the Registry of Standard Biological Parts, there are five assembly standards and three assembly methods for BioBrick-compliant biological parts (Table 1). Assembly standards allow the assembly of parts using the prefix and suffix found on the plasmid backbones containing those parts.

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The BioBrick Standard (RFC 10) was introduced in 2007. This standard is the most used because the vast majority of parts are compatible with it. The RFC 10 uses restriction enzymes that recognize EcoRI, NotI, and XbaI in the prefix and SpeI, NotI, and PstI in the suffix. The main issue with BBF RFC 10 is that it produces an 8bp scar that results in a shift of the reading frame. Thus, BBF RFC 10 impedes the construction of fusion proteins. The standards developed in further years sought to solve this problem by creating scars that could be translated into amino acids. The BioBrick BB-2 Standard (RFC 12) was proposed in 2008. BioBricks compatible with RFC 12 are maintained in plasmid backbones that have EcoRI, NotI, and SpeI as the prefix and NheI, NotI, and PstI as the suffix. The scar that results from assembling parts using RFC 12 translates into the amino acids alanine and serine. The BglBricks Standard (RFC 21) was developed in 2009. BglBricks have restriction sites for EcoRI and BglII in the prefix and BamHI and XhoI in the suffix. The resulting scar corresponds to glycine and serine residues. The Silver Standard (RFC 23) is a modification of RFC 10 as it uses the same enzymes and restriction sites of BBF RFC 10; however, the scar that it produces has 6bp, which encodes for amino acids threonine and arginine. Finally, the Freigbur Standard (RFC 25) uses the same prefix and suffix of RFC 10 but adds NgoMIV and AgeI restriction sites in the prefix and suffix, respectively[13,18,19].

| Assembly Standards | Assembly Methods |
|---|---|
| BioBrick Standard (RFC 10) | 3A Assembly |
| BioBrick BB-2 Standard (RFC 12) | |
| BglBricks Standard (RFC 21) | Gibson Assembly |
| Silver Standard (RFC 23) | |
| Freigbur Standard (RFC 25) | Golden Gate Assembly |

**Table 1.** Assembly standards and assembly methods for the design of biological devices.

Assembly methods are compatible with most of the assembly standards. The Three Antibiotic (3A) Assembly uses the same restriction enzymes of RFC 10. However, the composite part resulting from the ligation of two parts is introduced in a plasmid with an antibiotic resistance that is different from the other two backbone vectors. This technique permits the selection of the vector with the composite part using antibiotic selection instead of using gel electrophoresis to purify the digested parts before ligation. Gibson Assembly is a scarless technique that allows the simultaneous assembly of multiple fragments. It uses a 5' exonuclease, a DNA polymerase, and a DNA ligase. Gibson Assembly does not require specific prefixes or suffixes as it uses PCR primers to produce overlapping BioBricks. Golden Gate Assembly facilitates the assembly of different fragments in one reaction. This technique is based on type IIs restriction endonucleases, usually BsaI, and a T4 DNA ligase. Type IIs endonucleases cut DNA sequences outside their recognition sites, leaving single-stranded overhangs of 4bp. The ligation product of Golden Gate Assembly lacks restriction sites, and the 4bp overlapping fragments can be designed in such a way that multiple parts can be ligated in a single direction[19–23].

### The Difference Between Synthetic Biology and Genetic Engineering

There tends to be confusion between Synthetic Biology and Genetic Engineering in which some might even use these terms interchangeably. However, the real problem is whether the difference between these terms is scientific or merely a matter of terminology. Although these two fields of biology share the DNA manipulation basis and approach to intervene in the complexity of molecular biology, they differ in many aspects. According to the Encyclopædia Britannica Synthetic Biology is a field of biology whose main objective is the creation of fully operational biological synthetic systems from the smallest constituents possible[24]. Whereas Encyclopædia Britannica defines Genetic Engineering as "the artificial manipulation, modification, and recombination of DNA or other nucleic acid molecules to modify an organism or population of organisms"[25]. Here, Genetic Engineering becomes one of the many essential tools for synthetic biology because while Synthetic Biology creates synthetic organisms with several biological parts, Genetic Engineering modifies already existing organisms.

One of the major differences between these fields of Biology is the use of engineering. Synthetic Biology relies intensively on the standardized concept of engineering involving the design of optimized genetic circuits with biological parts from many different species as well as industrial analysis and mathematical modeling to achieve this. Genetic Engineering, on the other hand, relies on the alteration of genetic material based on a set of methodologies and is often represented as a hit and miss activity. For this Genetic Engineering is considered a misnomer in which there is hardly any engineering involved. The engineering part in Genetic Engineering is considered a synonym for manipulation of genetics instead of optimization[26,27]. Another important difference between Synthetic Biology and Genetic Engineering is the potential risks. It is general consensus that the risks that Synthetic Biology poses are far more serious than Genetic Engineering due to scientists failing to recognize their limitations and overestimating their ability to control these organisms. Thus, GMOs are closer to patients with organ transplants rather than Frankenstein's monster[28].

### Applications of Synthetic Biology

The iGEM competition gathers teams of high schoolers, undergraduates, and graduates every year, from several countries, to present biological systems that have been developed using the biological parts available in the Registry. The goal of the competition is to promote the implementation of Synthetic Biology to design solutions for different problems. The projects presented in the competition are oriented to tackle issues in different areas, including therapeutics, manufacturing, food and nutrition, environment, energy, etcetera. In 2019, several projects were awarded in different categories. For instance, a project (Novosite) in the Therapeutics category had the objective of improving wound healing by creating an antimicrobial, cellulose-based bandage able to deliver peptides and enzymes with antimicrobial activity. The team engineered *Escherichia coli* and *Vibrio natriegens* to produce enzymes and peptides attached to a carbohydrate-binding domain (CBD). In the Manufacturing category, the project Paper Transformer was awarded first place. Paper Transformer was created to produce bacterial cellulose (BC) from short cellulose fibers found in wastepaper. To achieve this, the team engineered *E. coli* to hydrolyze cellulose and synthesize BC employing a dual plasmid system containing three devices: cellulose hydrolysis, BC synthesis, and a regulator. Chlamy Yummy was the award-winning project in the Environment category. The team developed a method for the degradation of polyethylene

terephthalate (PET), one of the most common plastics, to deal with the increasing contamination by plastics. They used *Chlamydomonas reinhardtii* as the chassis to produce PETase and MHETase enzymes, which degrade PET into its monomers.

Outside the context of the iGEM competition, Synthetic Biology has been successfully applied for the manufacture of biofuels and biopharmaceuticals. The most famous examples are the production of 1,4-butanediol (BDO), the antimalarial drug artemisinin, and the anticancer compound taxol. BDO is an important chemical intermediate used to make plastics, elastic fibers, and polyesters. No known organism is capable of synthesizing BDO, so its production relies on petroleum feedstocks. Researchers optimized two heterologous pathways for the synthesis of BDO in *E. coli.* The metabolic routes were divided into upstream and downstream pathways for the biosynthesis of 4-hydroxybutyrate (4HB) and the conversion of 4HB to BDO, respectively. To achieve BDO production in *E. coli* a combination of native enzymes from E.coli and heterologous enzymes from *Porphyromonas gingivalis*, *Mycobacterium bovis*, and *Clostridium acetobutylicum* was used. Additionally, the host metabolism was engineered to channel carbon and energy into the pathways by knocking out several genes involved in the formation of fermentation products and by modifying the host's TCA cycle[29–32].

Artemisinin is a natural compound produced by the plant *Artemisia annua*. The therapeutic properties of artemisinin against multidrug-resistant *Plasmodium spp*. were discovered in the 1970s. Even though artemisinin derivatives are considered as first-line antimalarial drugs, its availability is limited, and its price has fluctuated due to inconsistencies in *A. annua* yields. To ensure steady and higher production of artemisinin, researchers engineered *E. coli* to synthesize the artemisinin precursor, amorpha-4,11-diene, by introducing a heterologous isoprenoid pathway from *Saccharomyces cerevisiae*. The authors expressed the mevalonate pathway of yeast in *E. coli* together with a codon-optimized synthetic variant of the amorphadiene synthase found in *A. annua* (ADS). Two operons, top, and bottom, were assembled for mevalonate pathway expression in bacteria. The top operon transformed acetyl-CoA into mevalonate, whereas the bottom operon converted mevalonate to FPP. Then ADS turned FPP into amorphadiene. Subsequent projects have focused their efforts to produce artemisinic acid, the direct precursor of artemisinin, from the oxidation of amorphadiene[33,34].

Taxol (paclitaxel) is a terpenoid found in the Pacific yew tree (*Taxus spp.*). Taxol is a powerful anticancer drug that has been used to treat several types of cancers, including breast and lung cancer, leukemia, lymphoma, and sarcoma. Similar to artemisinin, the isolation of taxol from its vegetal source is expensive and time consuming due to low yields and the presence of other taxoids with similar chemical structures. To avoid the extraction of taxol from *T. brevifolia*, researchers engineered *S. cerevisiae* to produce paclitaxel by introducing heterologous genes involved in the taxol biosynthetic pathway and the isoprenoid pathway. The authors expressed in yeast heterologous geranylgeranyl diphosphate (GGPP) synthase from *Sulfolobus acidocaldarius* and a codon-optimized variant of taxadiene synthase from *T. chinensis*. GGPP is converted into taxadiene by the taxadiene synthase, which is further transformed into taxol following oxygenation. Also, to favor the production of GGPP, a truncated version of the yeast HMG-CoA reductase was expressed as well as a transcription factor mutant allele[35,36].

**The Issues of Synthetic Biology**

The fact that synthetic biology aims to fabricate biological interchangeable, standardized sequences of genes and even design new genomes without immediate biological ancestry has raised ontological, political, economic, and ethical concerns based on the possibility that synthetic biology may be intrinsically unethical. Some of the major concerns surrounding synthetic biology rely on questions about whether we have enough knowledge on structures and regulatory mechanisms and the ability to control DNA sequences and synthetic genomes and whether it is safe enough for its use in less-restricted settings. Some of these concerns also include, scientists overestimating their ability to control synthetic organisms and failing to recognize their own limitations, side effects of assuming the techniques work, public safety and social consequences, potential dangers of genetically modified organisms, the resilience of natural ecosystems and ultimate impacts on the habitats and species for which the targets were devised. Other considerations include antibiotic resistance, allergies, carcinogens, toxicity among human health, and horizontal gene transfer[27-32].

Also, as in many emerging technologies, there is a preoccupation for dual-use applications and the deliberate misuse of the technology for nefarious purposes. In this case, synthetic biology has given rise to the potential bioterrorism and biowarfare with the synthesis of lethal biological weapons if fallen into the wrong hands. For example, in 2013 the National Science Advisory Board for Biosecurity (NSABB) advised against the publication of papers including H5N1 influenza ¨gain of function¨ with the concern that this information could allow H5N1 influenza to become transmissible from mammal to mammal and act as a shortcut for the development of the deadly biological weapon. Another example of the potential misuse of synthetic biology could be the creation of pathogens more toxic than the preexisting, considering that this has happened before with traditional genetic engineering techniques with a vaccine-resistant strain of the mousepox virus. Some even believe that synthetic biology can pose a threat higher than nuclear technology. This is mainly because the information for synthetic biology and life sciences, in general, is mostly of public domain contrary to that of nuclear technology and because in the future synthetic biology may be cheap and portable contrary to nuclear technology, which is bulky and expensive. A potential way to reduce the risk of harmful misuse of synthetic biology is by applying regulations and policies that ensure enforcement of chemical and biological weapon conventions and rules for DNA sales benchtop DNA synthesizers[37-39,43-45].

Some emerging issues question how synthetic organisms will interact with already existing species and whether these will disrupt communities or be invasive and how will issues like these be regulated to avoid ¨garage biology¨. Also, synthetic biology has given rise to doubts on the impact that engineered organisms intended to generate services to benefit people will have on natural ecosystems that already deliver these services. Besides, there are also uncertainties in whether there will be interactions between synthetic and natural organisms, and if the public notion of what is natural will change and challenge the basis for conservation action. There are also concerns about humans ¨playing god¨ which could have a religious interpretation of humans taking the role of a higher being by avoiding the constraints of timescales and evolution. Therefore, ignoring the need for a natural template to create life from non-living inorganic matter ignoring human

limitations. Another ethical concern lies on whether synthetic biology may fall in between machines and living things because usually in synthetic biology organisms are referred to as ¨genetically engineered machines¨ or intracellular processes as ¨genetic circuits¨ thus allowing these metaphors to interpret synthetic biological organisms as machines. Also, this metaphor assumes that the behavior of a complex object or organism could be explained by reference to its parts[39,45,46].

In the political and economic side, synthetic biology raises concerns in Latin American countries such as Ecuador, Peru, Venezuela, Brazil, Colombia, and Mexico being these, countries with massive biodiversity of fauna, flora, bacteria, and microorganisms arguing that synthetic biology could strengthen the gap between developing and developed countries. This is because biotechnology companies can obtain patents for synthetic organisms, DNA synthesizer machines, and their digitalized genome maps on the argument that they did not exist in nature previously, for industrial purposes. The benefits of synthetic biology will reflect the economic interests of those able to invest, develop and patent them. Latin American countries have economies based on agriculture, with crops of potatoes, banana, corn, beans, and thousands of medicinal and culinary plants, which could jeopardize the raw material of new biotechnological productions. Therefore, there could be out-and-out biopiracy or bioprospecting to produce modifications in commonly used living organisms, to privatize them, leading developing countries to pay royalties for these. This could raise questions such as how will a balance be achieved between private risk and gain and public benefit and safety[37,39].

Bioethics currently have a higher priority for other ethical controversies that are nearer in the future, such as abortion, artificial intelligence, stem cells, human-non-human chimeras, and animal treatment; thus, synthetic bioethics haven't been evaluated in depth. The Presidential Commission for the Study of Bioethical Issues issued a report in December of 2010 stating the *New Directions: The Ethics of Synthetic Biology and Emerging Technologies* in which several issues are considered for precautionary and risk analysis. However, it is argued that insufficient work has been done to address the risks of this discipline, which requires attention, so strategies for mitigating the potential dangers be discussed accordingly. Also, it is considered that the preexisting traditional regulations related to laboratory management and pathogens are not enough for the emerging field of synthetic biology[42,45,47].

## Conclusions

To summarize, Synthetic Biology is the combination of basic sciences with engineering with the goal of creating, designing, and redesigning biological systems and devices to understand biological processes and to achieve useful and sophisticated functionalities to improve human welfare. Synthetic Biology is based on the idea of extraction and reassembly of "biological parts" along with the principles of abstraction, modularity, and standardization. It is important to remember that while Genetic Engineering is one of the many essential tools for synthetic biology and they share the DNA manipulation basis and approach to intervene in the complexity of molecular biology, they differ in many aspects, and the two terms should not be used interchangeably. The iGEM competition for the implementation of Synthetic Biology has attracted projects such as the creation of an antimicrobial, cellulose-based bandage able to deliver peptides and enzymes with antimicrobial activity, production of bacterial cellulose (BC) from short cellulose fibers found in wastepaper, and the degradation of polyethylene terephthalate (PET). Some of the applications that have already been done by Synthetic Biology include the production of 1,4-butanediol (BDO), the antimalarial drug artemisinin, and the anticancer compound taxol. The fact that Synthetic Biology has the potential to design new genomes without immediate biological ancestry has raised ontological, political, economic, and ethical concerns based on the possibility that synthetic biology may be intrinsically unethical.

## Bibliographic references




1. Del Vecchio D, Qian Y, Murray RM, Sontag ED. Future systems and control research in synthetic biology. Annu Rev Control. 2018;45:5–17.
2. Carbonell P, Radivojevic T, García Martín H. Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation. ACS Synth Biol. 2019 Jul 19;8(7):1474–7.
3. Linshiz G, Goldberg A, Konry T, Hillson NJ. The Fusion of Biology, Computer Science, and Engineering: Towards Efficient and Successful Synthetic Biology. Perspect Biol Med. 2012;55(4):503–20.
4. Benner SA, Sismour AM. Synthetic biology. Nat Rev Genet. 2005 Jul;6(7):533–43.
5. Yang J, Kim B, Kim GY, Jung GY, Seo SW. Synthetic biology for evolutionary engineering: from perturbation of genotype to acquisition of desired phenotype. Biotechnol Biofuels. 2019 May 9;12(1):113.
6. Singh B, Mal G, Gautam SK, Mukesh M. Synthetic Biology. Advances in Animal Biotechnology 2019:405–12. doi:10.1007/978-3-030-21309-1_36.
7. Agapakis CM. Designing Synthetic Biology. ACS Synth Biol. 2014 Mar 21;3(3):1218.
8. Xiang Y, Dalchau N, Wang B. Scaling up genetic circuit design for cellular computing: advances and prospects. Natural Computing 2018;17:833–53. doi:10.1007/s11047-018-9715-9.
9. Müller KM, Arndt KM. Standardization in Synthetic Biology. Methods in Molecular Biology Synthetic Gene Networks 2011:23–43. doi:10.1007/978-1-61779-412-4_2.
10. Chen YY, Galloway KE, Smolke CD. Synthetic biology: advancing biological frontiers by building synthetic systems. Genome Biol. 2012 Feb 20;13(2):240.
11. Porcar M, Latorre A, Moya A. What Symbionts Teach us about Modularity. Frontiers in Bioengineering and Biotechnology 2013;1. doi:10.3389/fbioe.2013.00014.
12. Decoene T, De Paepe B, Maertens J, Coussement P, Peters G, De Maeseneire SL, et al. Standardization in synthetic biology: an engineering discipline coming of age. Crit Rev Biotechnol. 2018 Jul 4;38(5):647–56.
13. igem.org [Internet]. [cited 2019 Dec 1]. Available from: https://igem.org
14. Wang Y-H, Wei KY, Smolke CD. Synthetic Biology: Advancing the Design of Diverse Genetic Systems. Annual Review of Chemical and Biomolecular Engineering 2013;4:69–102. doi:10.1146/annurev-chembioeng-061312-103351.
15. Galdzicki M, Rodriguez C, Chandran D, Sauro HM, Gennari JH. Standard Biological Parts Knowledgebase. PLoS ONE 2011;6. doi:10.1371/journal.pone.0017005.
16. Peccoud J, Blauvelt MF, Cai Y, Cooper KL, Crasta O, DeLalla EC, et al. Targeted Development of Registries of Biological Parts. PLOS ONE. 2008 Jul 16;3(7):e2671.
17. Ho-Shing O, Lau K, Vernon W, Eckdahl T, Campbell A. Assembly of Standardized DNA Parts Using BioBrick Ends in E. coli. In: Methods in molecular biology (Clifton, NJ). 2012. p. 61–76.
18. Li S-Y, Zhao G-P, Wang J. C-Brick: A New Standard for Assembly of Biological Parts Using Cpf1. ACS Synth Biol. 2016 Dec 16;5(12):1383–8.

19. Røkke G, Korvald E, Pahr J, Øyås O, Lale R. BioBrick Assembly Standards and Techniques and Associated Software Tools. DNA Cloning and Assembly Methods Methods in Molecular Biology 2013:1–24. doi:10.1007/978-1-62703-764-8_1.
20. Gibson DG, Young L, Chuang R-Y, Venter JC, Hutchison CA, Smith HO. Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods. 2009 May;6(5):343–5.
21. Engler C, Gruetzner R, Kandzia R, Marillonnet S. Golden Gate Shuffling: A One- Pot DNA Shuffling Method Based on Type IIs Restriction Enzymes. PLOS ONE. 2009 May 14;4(5):e5553.
22. Weber E, Engler C, Gruetzner R, Werner S, Marillonnet S. A Modular Cloning System for Standardized Assembly of Multigene Constructs. PLoS ONE 2011;6. doi:10.1371/journal.pone.0016765.
23. Andreou AI, Nakayama N. Mobius Assembly: A versatile Golden-Gate framework towards universal DNA assembly. PLOS ONE. 2018 Jan 2;13(1):e0189892.
24. Michael Rugnetta. Synthetic biology. Encycl Br Inc 2016. https://www.britannica.com/science/synthetic-biology (accessed December 1, 2019).
25. Adam Augustyn, Patricia Bauer, Brian Duignan, Alison Eldridge, Erik Gregersen, Amy McKenna, Melissa Petruzzello, John P. Rafferty, Michael Ray, Kara Rogers, Amy Tikkanen, Jeff Wallenfeldt, Adam Zeidan AZ. Genetic engineering. Encycl Br Inc 2019. https://www.britannica.com/science/genetic-engineering (accessed December 1, 2019).
26. Paras Chopraa AK. Engineering Life through Synthetic Biology 2006:401–10. doi:10.0000/CONTENT.IOSPRESS.COM.
27. A. O'Malley M, Powell A, Davies JF, Calvert J. Knowledge-making distinctions in synthetic biology. BioEssays 2008;30:57–65. doi:10.1002/bies.20664.
28. Paper O. Playing God in Frankenstein ' s Footsteps : Synthetic Biology and the Meaning of Life 2009:257–68. doi:10.1007/s11569-009-0079-6.
29. Singh SP, Bansal S, Pandey A. Basics and Roots of Synthetic Biology. Current Developments in Biotechnology and Bioengineering 2019:3–22. doi:10.1016/b978-0-444-64085-7.00001-0.
30. Yim H, Haselbeck R, Niu W, Pujol-Baxley C, Burgard A, Boldt J, et al. Metabolic engineering of Escherichia coli for direct production of 1,4-butanediol. Nat Chem Biol. 2011 Jul;7(7):445–52.
31. Liu H, Lu T. Autonomous production of 1,4-butanediol via a de novo biosynthesis pathway in engineered Escherichia coli. Metab Eng. 2015 May;29:135–41.
32. Katz L, Chen YY, Gonzalez R, Peterson TC, Zhao H, Baltz RH. Synthetic biology advances and applications in the biotechnology industry: a perspective. J Ind Microbiol Biotechnol. 2018 Jul;45(7):449–61.
33. Paddon CJ, Keasling JD. Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. Nat Rev Microbiol. 2014 May;12(5):355–67.
34. Martin VJJ, Pitera DJ, Withers ST, Newman JD, Keasling JD. Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. Nat Biotechnol. 2003 Jul;21(7):796–802.
35. Weaver BA. How Taxol/paclitaxel kills cancer cells. Bement W, editor. Mol Biol Cell. 2014 Sep 15;25(18):2677–81.
36. Engels B, Dahm P, Jennewein S. Metabolic engineering of taxadiene biosynthesis in yeast as a first step towards Taxol (Paclitaxel) production. Metab Eng. 2008 May;10(3–4):201–6.
37. Enrique J, Salgado L. The Promises of Synthetic Biology : New Bioartefacts and Their Ethical and Societal Consequences n.d.:179–94.
38. Keshava R, Mitra R, Gope ML, Gope R. Synthetic Biology : Overview and Applications. 2018. doi:10.1016/B978-0-12-804659-3.00004-X.
39. Redford KH, Adams W, Mace GM. Synthetic Biology and Conservation of Nature : Wicked Problems and Wicked Solutions 2013;11:2–5. doi:10.1371/journal.pbio.1001530.
40. Thompson PB. Bioethics Synthetic Biology Needs A Synthetic Bioethics 2012:37–41. doi:10.1080/21550085.2012.672676.
41. Parens E, Johnston J, Moses J. Do We Need " Synthetic Bioethics "? AAAS 2008;321. doi:10.1126/science.1163821.
42. Wang F, Zhang W. Synthetic biology : Recent progress , biosafety and biosecurity concerns , and possible solutions. J Biosaf Biosecurity 2019;1:22–30. doi:10.1016/j.jobb.2018.12.003.
43. Gronvall GK. Safety , security , and serving the public interest in synthetic biology. J Ind Microbiol Biotechnol 2018;45:463–6. doi:10.1007/s10295-018-2026-4.
44. Erickson B, Singh R, Winters P. Synthetic Biology : Regulating Industry Uses of New Biotechnologies 2011:1254–6.
45. Douglas T, Savulescu J. Synthetic biology and the ethics of knowledge 2010:687–94. doi:10.1136/jme.2010.038232.
46. Boldt J. Machine metaphors and ethics in synthetic biology 2018.
47. Gutmann A. The Ethics of Synthetic Biology: Guiding Principles for Emerging Technologies 2010:17–22.

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