Your full article (between 500 to 5000 words) - - Do check for grammatical errors or spelling mistakes

Synthetic Biology - A new reform in the biotechnological approaches

Authors: VINAY SHARMA AND PRAVEEN KUMAR
Assam Agricultural University Jorhat Assam-13
Email: *vinaysharma965@gmail.com*

Introduction

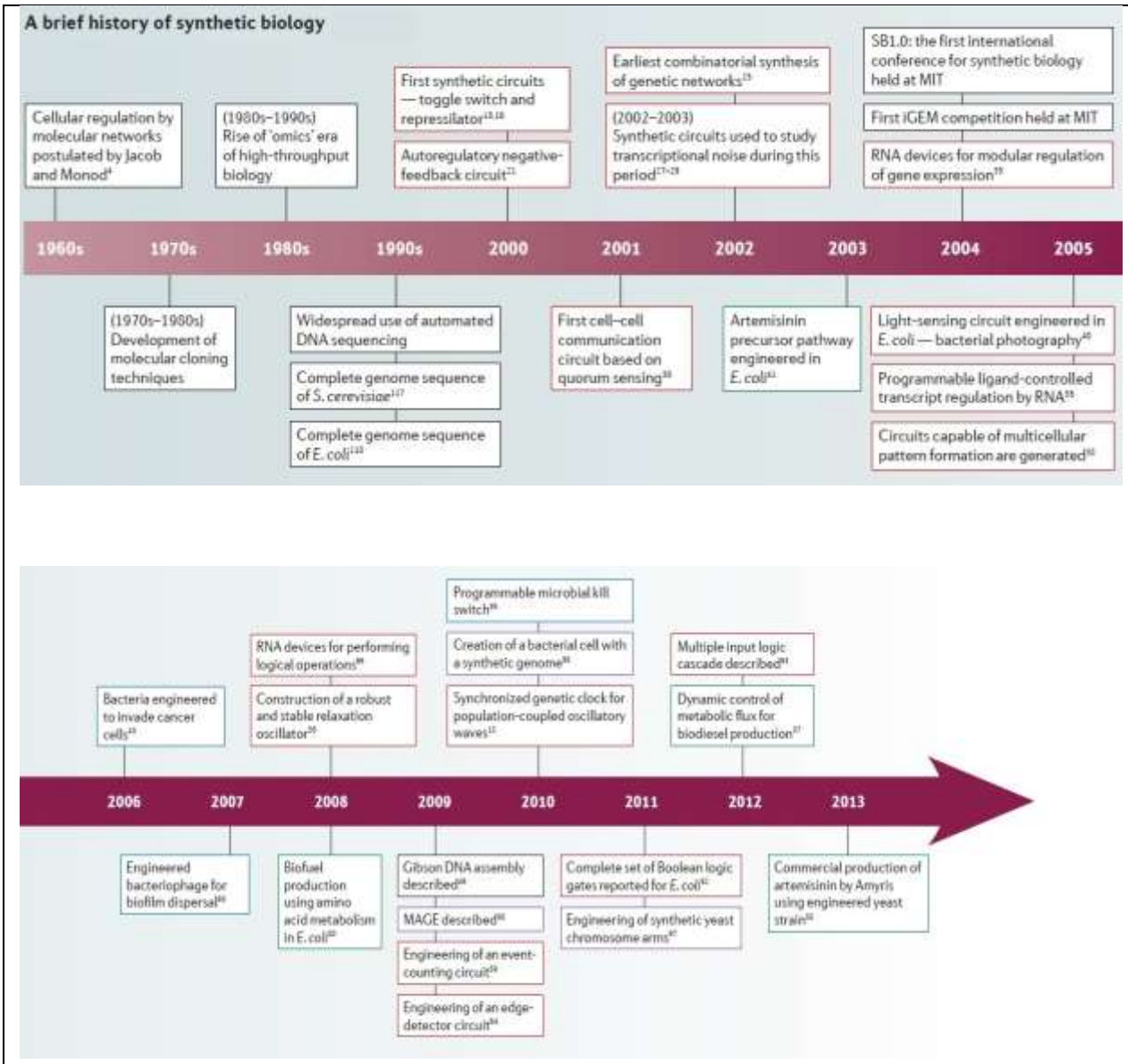
Genetic engineering with recombinant DNA is a powerful and widespread technology that enables biologists to redesign life forms by modifying or extending their DNA. Advances in this domain allow us to gain insight into the operating principles that govern living organisms, and can also be applied to a variety of fields including human therapeutics, synthesis of pharmaceutical products, molecular fabrication of biomaterials, crops and livestock engineering, and toxin detection with biological sentinels. While already providing great benefits, existing genetic engineering applications only hint at the possibilities for harnessing cells to our benefit.

Synthetic biology has been recently defined as the artificial design and engineering of biological systems and living organisms for purposes of improving applications for industry or biological research as it has expanded to many interdisciplinary fields such as biotechnology, evolutionary biology, molecular biology, systems biology, biophysics, computer engineering, and genetic engineering.

A brief history of synthetic biology

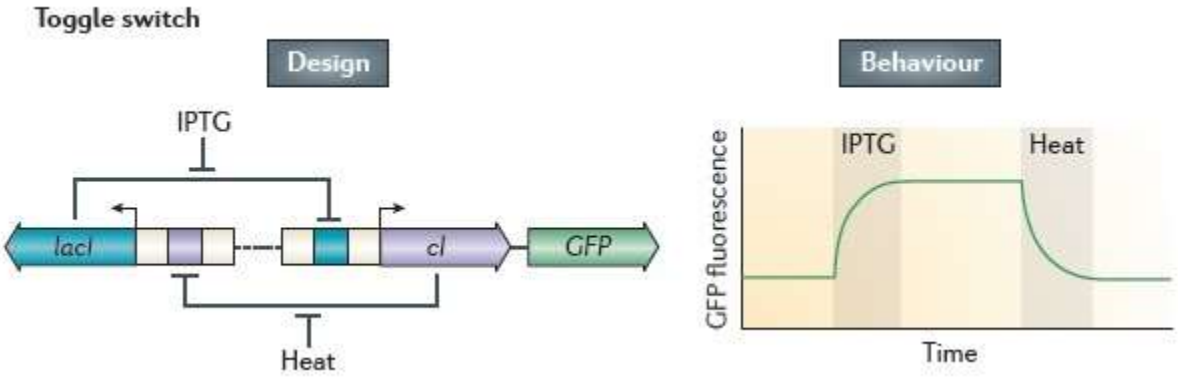
Chronologically the field is divided into three distinct periods and highlight scientific and cultural milestones for each period.

First, a foundational period, in which many of the characteristic experimental and cultural features of the field were established; second, an intermediate period, which was characterized by an expansion of the field but a lag in engineering advances; and third, a recent era of accelerated innovation and shifting practices, in which new technologies and engineering approaches have enabled us to advance towards practical applications in both biotechnology and medicine.



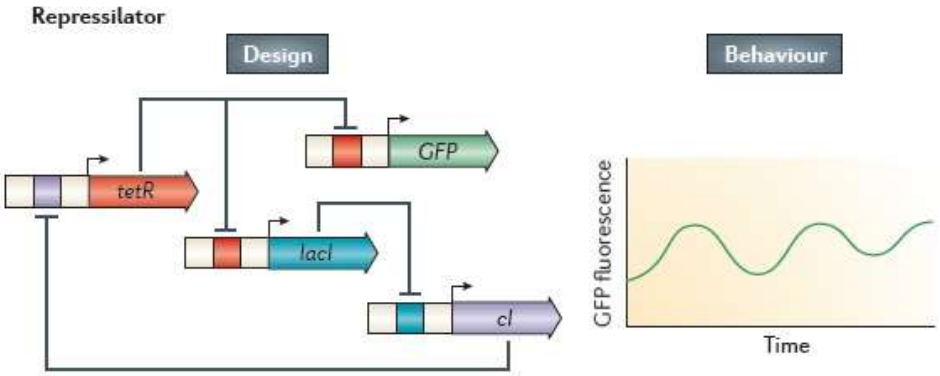
Landmark in the fiend of synthetic biology:

1. Toggle switch



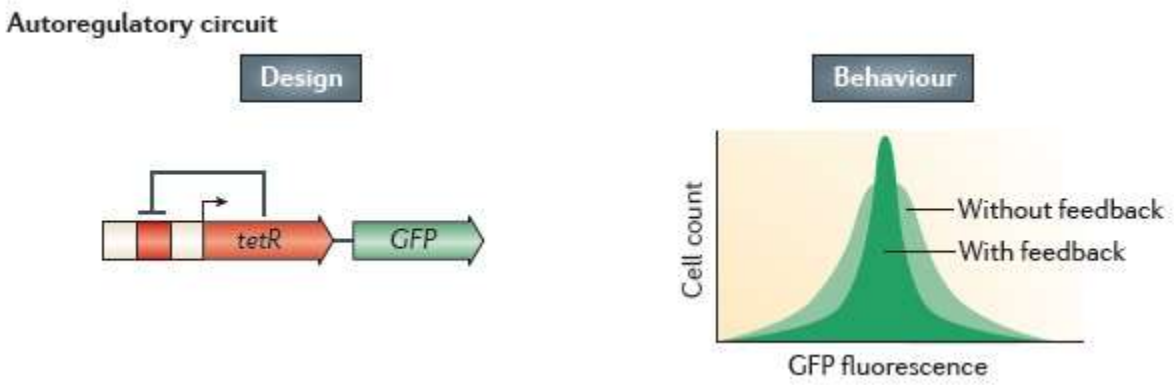
A pair of repressor genes (*lacI* and *cI*) are arranged to antagonistically repress transcription of each other, resulting in a bistable genetic circuit in which only one of the two genes is active at a given time. The toggle can be 'flipped' to the desired transcriptional state using environmental inputs to disengage one of the repressors from its operator (for example, IPTG (isopropyl-β-d-thiogalactoside) is used to disengage LacI and heat is used to disengage cI). Once the input is removed, the desired transcriptional state persists for multiple generations.

2. The repressilator



The circuit is constructed from three repressor–promoter interactions (between cI, LacI and TetR repressors and their associated promoters), which are linked together to form a ring-shaped network, in which TetR regulates a GFP-reporter node. When analysed at the single-cell level using time-lapse fluorescence microscopy, the circuit exhibits periodic oscillations in GFP expression, which persist for a number of generations; however, oscillations become dampened after a few periods and are generally noisy, with individual cells showing high variability in both the amplitude and period of their oscillations.

3. Autoregulatory circuit



In this circuit, TetR-mediated negative-feedback regulation of its own transcription results in a narrow population-wide expression distribution, as measured by the co-transcribed GFP reporter. The circuit demonstrates a principle that was long-appreciated in control-systems engineering and nonlinear dynamics — that noise in a system can be reduced by introducing negative feedback.

Building a genetic circuit *in vivo* requires tedious optimization of many often poorly understood parameters of protein–DNA interactions and mRNA and protein stabilities, among others.

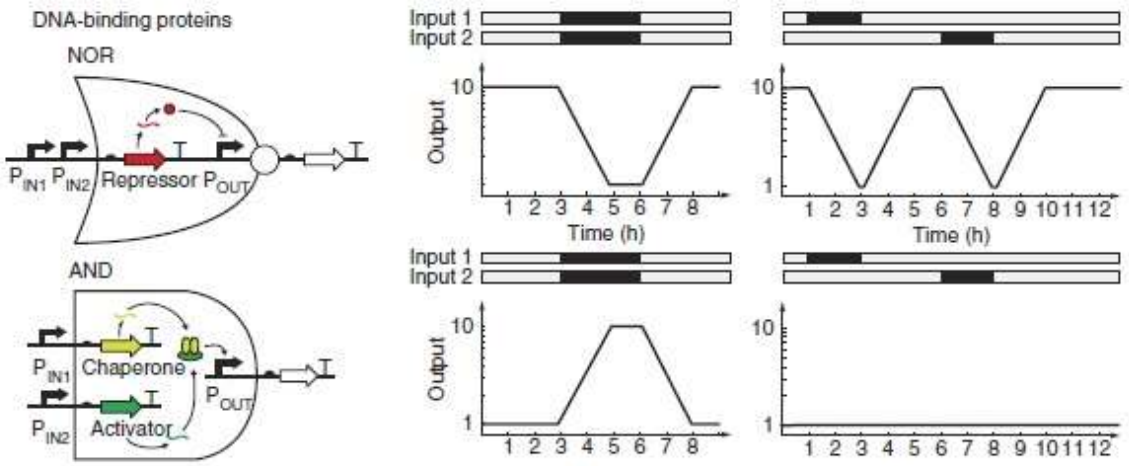
Genetic circuit design based on different regulator classes

Transcriptional circuits function by changing the flow of RNA polymerase (RNAP) on DNA. There are a number of regulators that influence this flux that have been used as the basis for building synthetic circuits.

DNA binding proteins:

Many families of proteins can bind to specific DNA sequences (operators). The simplest way to use these proteins as regulators is to design promoters with operators that block the binding or progression of RNAP. Such repressors have been built out of zinc-finger proteins, transcription activator–like effectors, TetR homologs, phage repressors and LacI homologs. A core set of three repressors were used to build many of the first synthetic circuits (CI, TetR, LacI) DNA-binding proteins can also function as activators that increase the flux of RNAP on DNA.

Many logic gates have been constructed with DNA-binding proteins. For example, NOT and NOR gates have been built by connecting input promoter(s) to a repressor that turns off an output promoter.



Other types of transcriptional logic gates have been built using pairs of proteins in which one either activates or inhibits the other. For example, AND gates have been built with artificially split proteins and activators that require chaperones. Similarly, NAND gates can be built with proteins that block the activity of an activator, such as anti-σ factors, which inhibit σ factors.

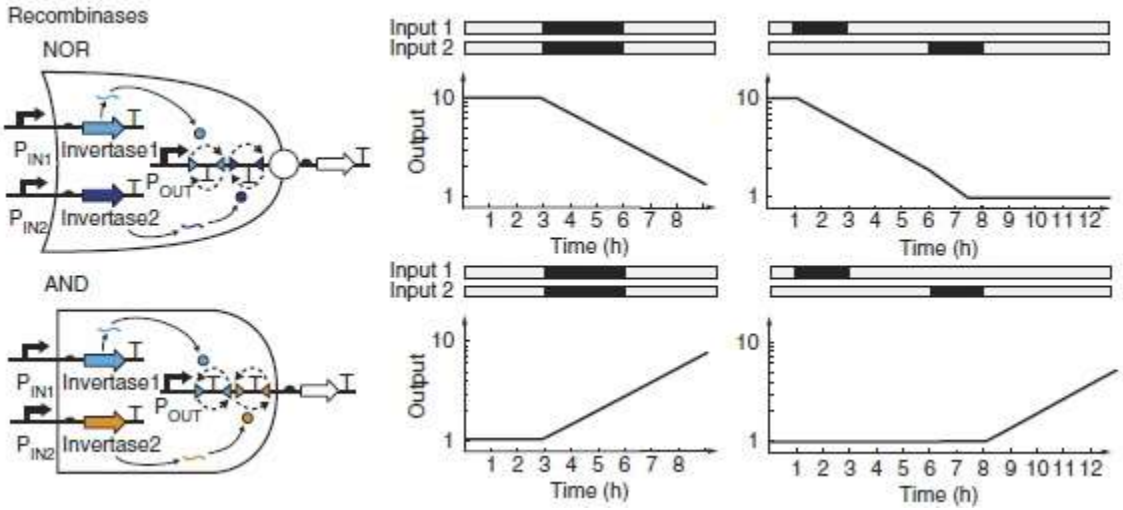
DNA-binding proteins have also been used to build circuits that incorporate positive and negative feedback loops, which form the basis for dynamic circuits, such as pulse generators, bistable switches, and oscillators. There are also several challenges in using DNA-binding proteins to build circuits. Individual transcription factors may appear nontoxic, but often a combination of multiple regulators can lead to acute toxicity.

Recombinase:

Recombinases are proteins that can facilitate the inversion of DNA segments between binding sites. Site specific recombinases often mediate 'cut-and-paste' recombination, during which DNA is looped, cleaved and religated. Two types of recombinases have been used to build genetic circuits. The first is tyrosine recombinases, such as Cre, Flp and FimBE, which require host-specific factor.

These recombinases can be reversible and flip the DNA in both directions, or irreversible and flip in only a single direction. The second class of recombinases is serine integrases, which catalyze unidirectional reactions that rely on double-strand breaks to invert DNA. Serine integrases typically do not require host factors and often have cognate excisionases that can be expressed independently to return the DNA to its original orientation.

Recombinases have been used to build switches, memory circuits, counters and logic gates. These proteins are ideal for memory storage because they flip DNA permanently, and once the DNA is flipped, its new orientation is maintained without the continuous input of materials or energy. In recombinase logic gates, these discrete physical states of the DNA can correspond to ON and OFF states (1 and 0).



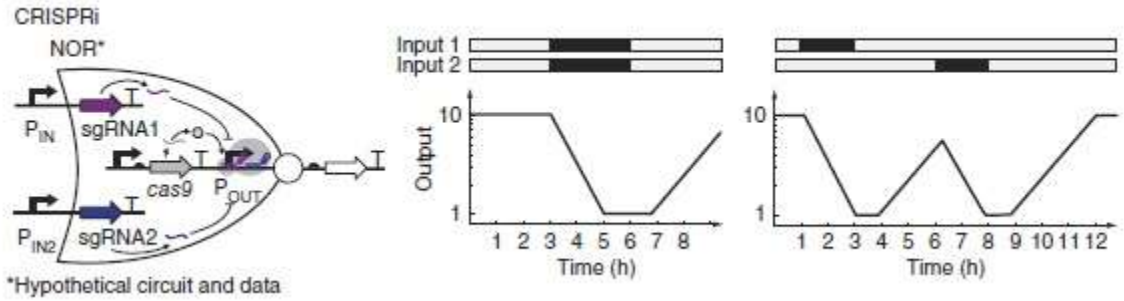
All two-input gates, including AND and NOR logic, have been constructed such that two input promoters express a pair of orthogonal recombinases, which change RNAP flux by inverting unidirectional terminators, promoters or entire genes.

CRISPRi:

Clustered, regularly interspaced, short palindromic repeat (CRISPR) arrays function as a bacterial 'immune system' that targets specific DNA sequence motifs for degradation.. CRISPR systems use a Cas (CRISPR-associated) nuclease and guide RNA to introduce double-strand breaks to specific DNA sequences. Mutant Cas proteins such as Cas9 that do not have nuclease activity have been developed and used as transcription factors that knock down gene expression by forming a DNA bubble that interferes with RNAP activity. CRISPR can also activate transcription by fusing an RNAP recruiting domain to catalytically inactive Cas9.

One advantage of CRISPR interference (CRISPRi) is the designability of the RNA-DNA complex. It is possible to imagine creating a very large set of orthogonal guide sequences that target different promoters. This set would enable the construction of large genetic circuits, but it would need to be experimentally screened because predicting guide RNA orthogonality is complicated.

In general, the properties of CRISPRi circuits will probably resemble DNA-binding protein circuits. Circuits based on CRISPRi are expected to operate on timescales similar to those of protein-based circuits because of the stability of the regulatory dCas9-sgRNA-DNAcomplex.



A current challenge in implementing CRISPRi circuits is toxicity, which is difficult to control. Toxicity could be the result of Cas9 binding to the host genome at protospacer-adjacent

motifs (such as NGG), forming bubbles that deleteriously affect host gene expression.

Selecting parts to tune the circuit response

Genetic circuits need to be tuned to meet the specifications required for a particular application. For example, a large dynamic range may be required to strongly activate a pathway. Similarly, low OFF states are desirable when expressing toxic proteins.

New libraries of well-characterized parts and computational tools have made it easier to design and tune genetic circuits. Moreover, new classes of insulators improve the reliability of these parts when they are placed in the local genetic context of a circuit. Additional biochemical tools, such as small RNA (sRNA), have been incorporated into circuits in order to provide more tuning knobs. In a prior review, we detailed advances in part design and tools that allow engineers to obtain reliable expression levels.

The response function of a digital logic gate can be shifted up or down by changing promoter strengths, RBS strengths or the proteins' degradation rates. Promoter strength can be altered with mutations in the promoter sequence or by selecting new promoters from a characterized library. Increased degradation can be achieved with N-terminal degrons.

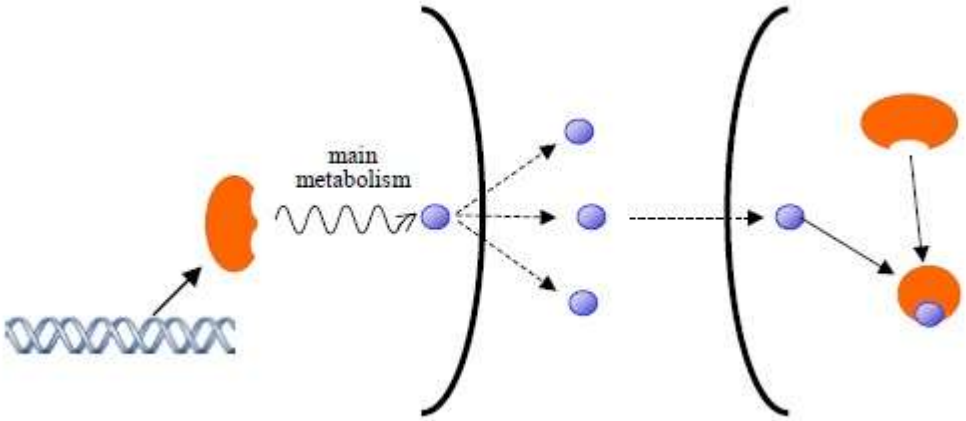
Circuit components are often distributed between multiple plasmids at different copy numbers in order to synthesize each component at the necessary level. However, when entire circuits are expressed on one plasmid, copy number can be shifted to simultaneously alter the circuit's dynamic range and threshold.

Interactions between synthetic circuits and the host organism

Genetic circuits are based on biochemical interactions within living cells. Most circuits use host resources to function, including transcription and translation machinery (e.g., ribosomes and RNAP), DNA-replication equipment and metabolites (e.g., amino acids). The availability of these resources and the details of the intracellular environment change significantly in different strain backgrounds, environmental conditions and media, and they also depend on cell density and growth rate.

Cell to cell communication

Cell-cell communication was discovered in bacteria about three decades ago (Hastings and Nealson, 1977). The ability to engineer both prokaryotic and eukaryotic communication systems with new cell- cell interaction capabilities will be central to the future engineering of multicellular structures.



The system allows us to control the extent of a chemical message that a sender cell transmits to a receiver cell, which subsequently activates a remote transcriptional response.

Quorum sensing is a bacterial communication system that allows cells to sense their own population density through the diffusion of a chemical signal encoded by their genes (Bassler, 1999). The quorum sensing system of certain marine prokaryotes (e.g. *Vibrio fischeri*) is responsible for light organ symbiosis with other animals.

Application:

Gene therapy

An important application area for synthetic biology is the development of innovative gene circuits for therapeutic purposes. Recent years have witnessed encouraging advancements in the field of cancer gene therapy in the creation of novel adenoviral vectors. For example, an oncolytic adenovirus dl1520, first coined by its creators, has shown potential use in specific targeting of tumor cells. Pioneered by McCormick and colleagues.

Drug Development

Many metabolites such as drugs are of natural origin but are difficult to extract from their native hosts. This rarity makes them expensive to produce in the quantities necessary to treat patients. In an effort to produce these rare drugs cheaply and in large quantities, microbes have been engineered by inserting the genes necessary to produce the drug or its precursor. Bacteria are suitable hosts because they grow rapidly to produce large quantities of the drug, and it is easier to extract the product from bacteria as compared with plants.

Biotechnology Applications

By bringing discovery and utilization of novel genetic components together with a rational design approach, synthetic biology opens up tremendous possibilities for environmental engineering and biomedical discovery. However, use of recombinant DNA techniques invites risks commonly associated with conventional genetic engineering such as horizontal gene

transfer to other species and introduction of new allergens and toxins to the environment.

Conclusion: The field of synthetic biology has grown considerably and has chartered many notable achievements. The pace of progress in synthetic biology will continue to accelerate as design and testing cycles rely less on the traditional molecular cloning tools that sustained the field in its design cycle. Early years and increasingly on DNA synthesis and high-throughput assembly methods. In the near future, workflow for a biological circuit engineer will no longer be limited by the pace of fabrication but instead by their ability to analyse circuit behavior and incorporate the data into the next design cycle.

As synthetic systems have become larger and more complex, their interactions with endogenous systems have become more pronounced. Biological circuit engineers will need to develop methods to account for the disparate and often heavy physiological burdens that synthetic systems place on their microbial hosts, perhaps borrowing lessons from metabolic engineering.

References (if any)

Brophy, J. A., & Voigt, C. A. (2014). Principles of genetic circuit design. *Nature methods*. *11*(**5**):

508- 520.
Cameron, D. E., Bashor, C. J., & Collins, J. J. (2014). A brief history of synthetic biology. *Nature Reviews Microbiology*, *12*(**5**), 381-390.
Weiss, R., Basu, S., Hooshangi, S., Kalmbach, A., Karig, D., Mehreja, R., & Netravali, I. (2003). Genetic circuit building blocks for cellular computation, communications, and signal processing. *Natural Computing*, *2*(**1**), 47-84.