Synthetic Biology of Genetic Circuit

[1]Mallar, K. N. and [2]Prasenjit, D.

[1] M.Sc. (Agri.), Dept. of Biotechnology, AAU, Jorhat, Assam

[2] M.Sc. (Agri.), Dept. of Biotechnology, UAS, Dharwad, Karnataka

Correspondence mail id: mallar2015@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.

Well, Synthetic biology is the future of today's Genetic engineering or Biotechnology. 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. The similarity of silicon diode based electronic engineering and gene circuit based synthetic biology has been shown (Fig. 1).

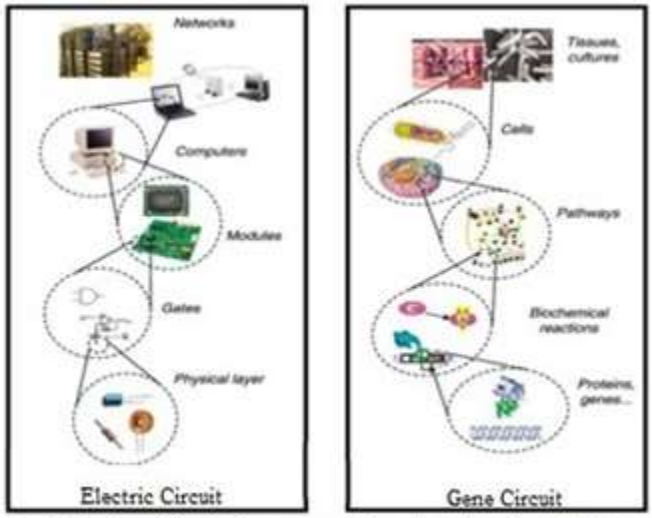


Fig. 1. Relation ship in between Electric circuit and Gene circuit

How Synthetic biology is different?

Genetic engineering (last 30 years):

Recombinant DNA
Polymerase Chain Reaction (PCR)
Automated sequencing

Synthetic biology adds:

Automated construction - separate design from construction.
Standards - create repositories of parts that can be easily composed.
Abstraction - high-level models to facilitate design.

A brief history of synthetic biology

The first ever studied gene circuit was "*lac Operon*" which is a naturally occurring phenomenon and after that man started to develop new gene circuit based protein-nucleic acid interactions. Chronological development has been shown in Fig. 2.

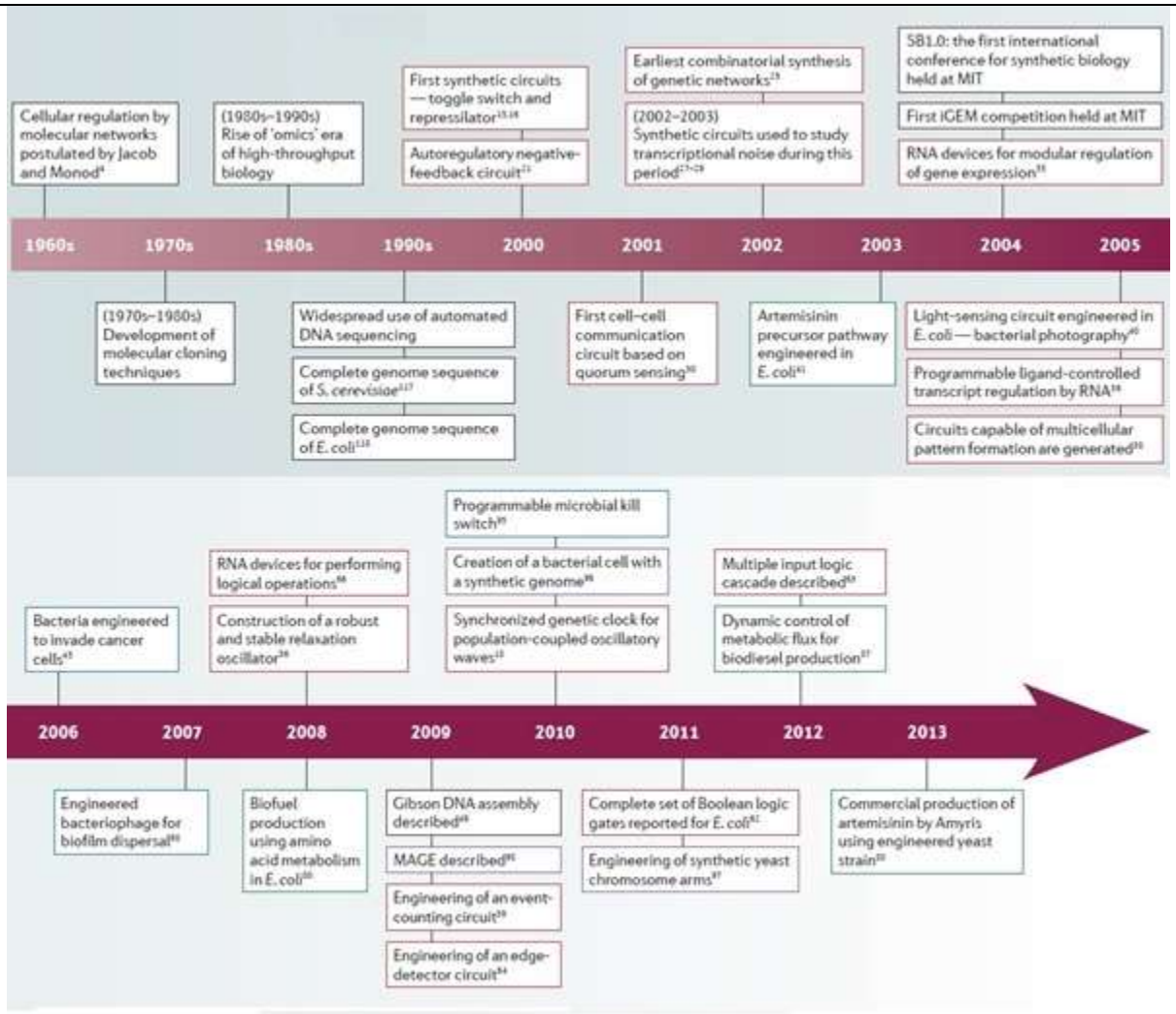


Fig.2. Brief History of Synthetic Biology

Landmark in the fiend of synthetic biology:

- **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. (Fig. 3)

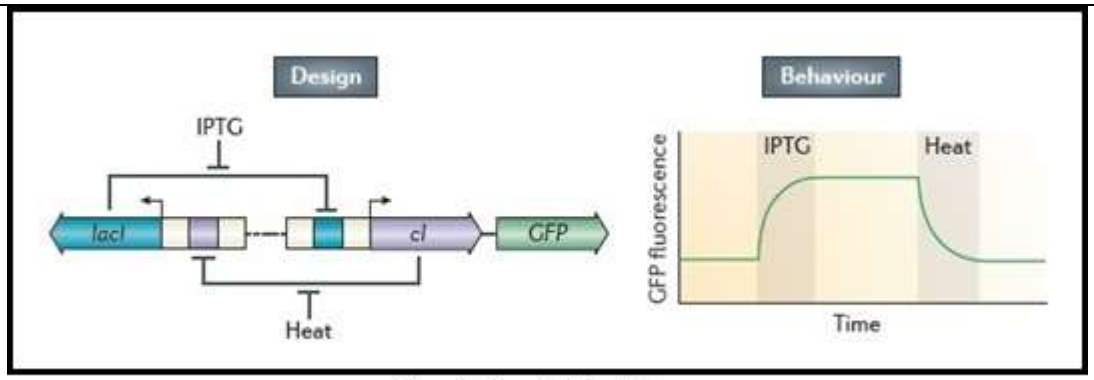


Fig. 3. Toggle Switch

Auto regulatory 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. (Fig. 4)

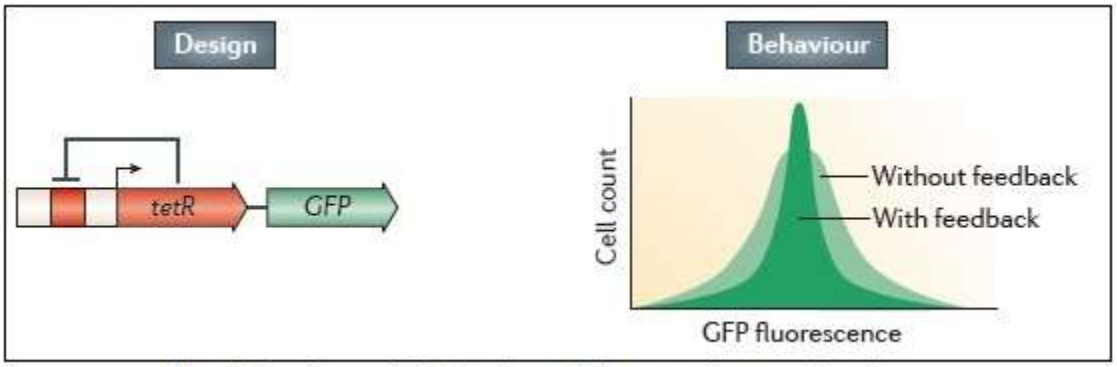


Fig. 4. Design and Behaviour of Autoregulatory circuit

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. (Fig. 5)

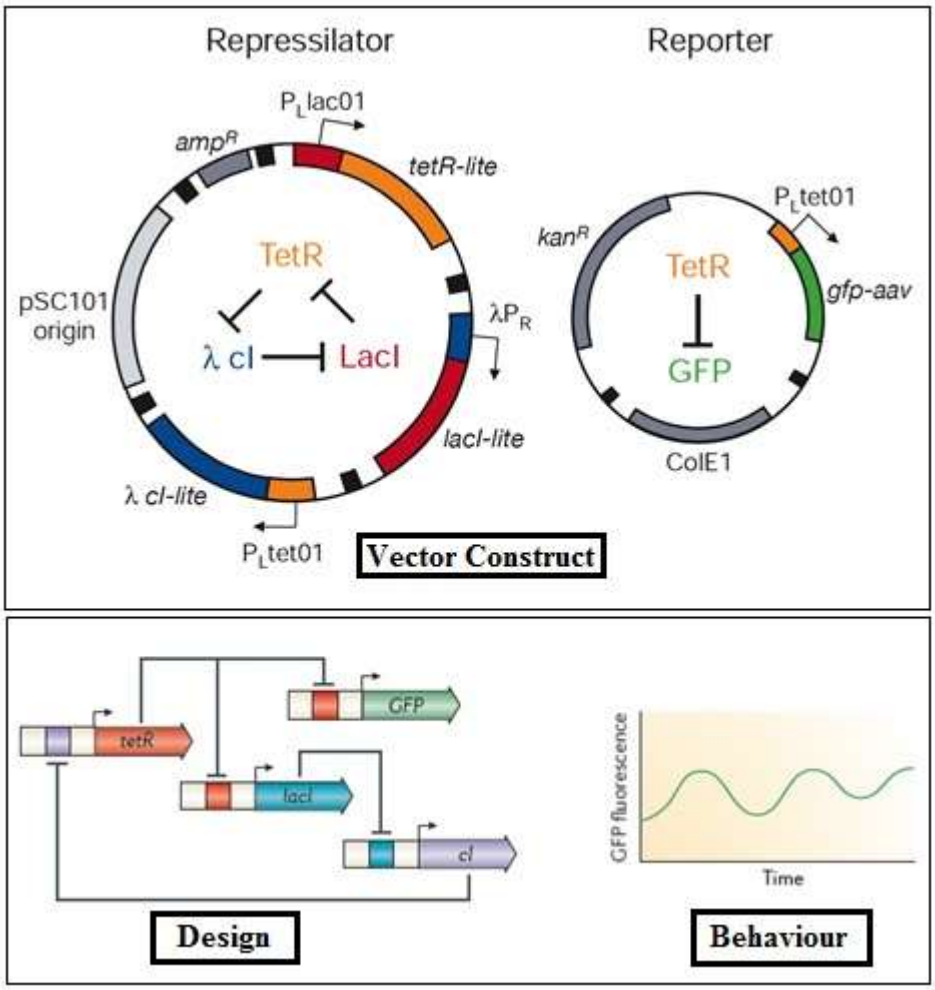


Fig. 5. Vector construct, Design and Behaviour of Repressilator

Genetic circuit design based on different regulator classes

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. 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. (Fig. 6)

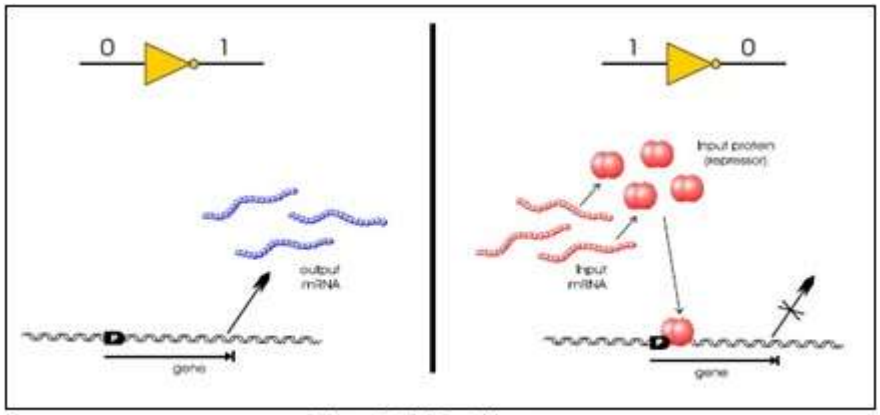
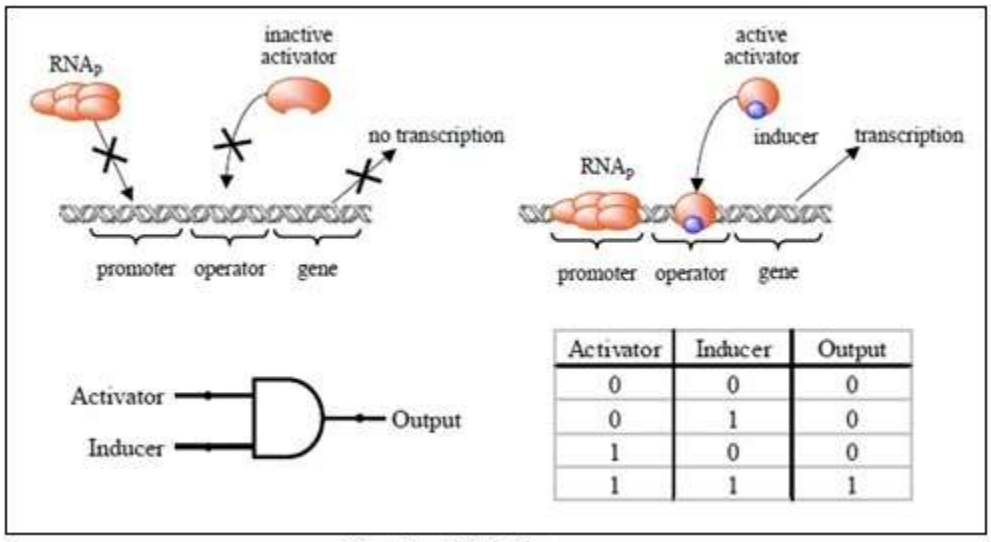


Fig. 6. NOT Gate

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.



Activator	Inducer	Output
0	0	0
0	1	0
1	0	0
1	1	1

Fig. 7. AND Gate

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. (Fig. 8)

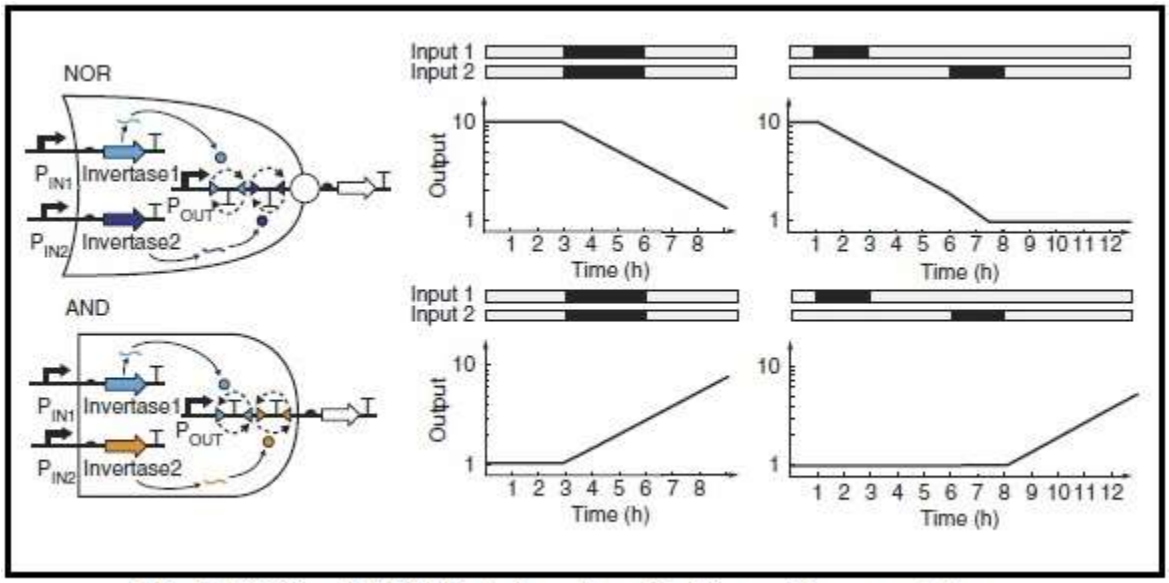


Fig. 8. NOR and AND Gate based on the Recombinase protein

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. (Fig. 9)

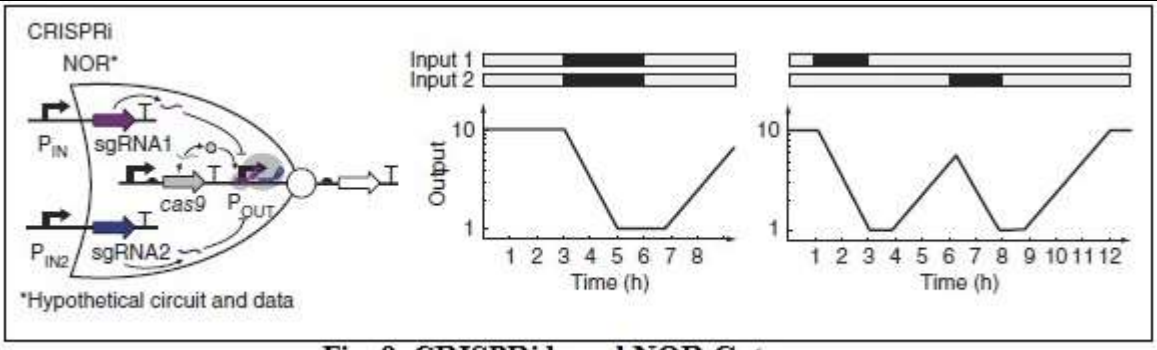


Fig. 9. CRISPRi based NOR Gate

A current challenge in implementing CRISPRi circuits is toxicity, which is difficult to control.

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-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. (Fig. 10)

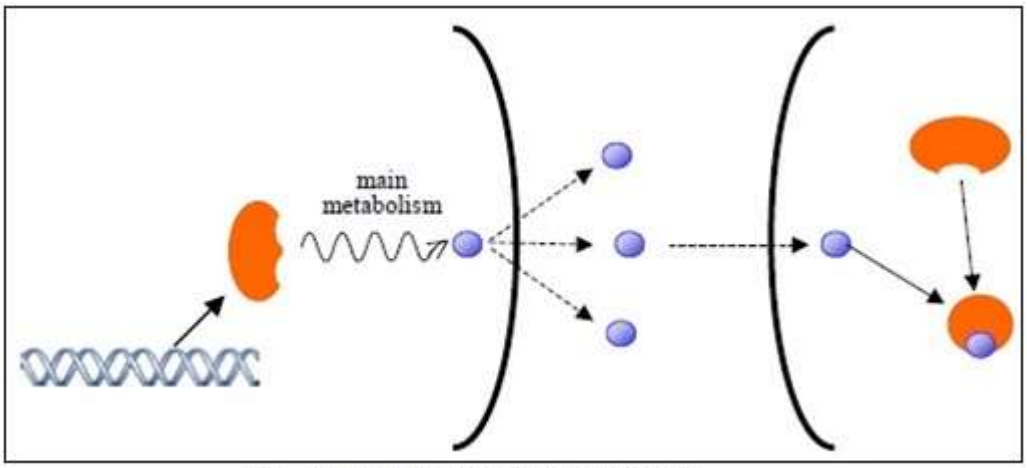


Fig. 10. Cell to cell communication

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 of Synthetic Biology:

- ✓ Gene therapy
- ✓ Drug Development
- ✓ Biotechnology Applications

Genetic circuits in 'smart' plants that sense environmental stimuli and implement a response. The circuit, built into chloroplasts, integrates sensors for drought (pSpark), temperature (pCBF) and plant maturity (pSAG12) to control pesticide (Bt) production and drought tolerance (IPT). (Fig. 11)

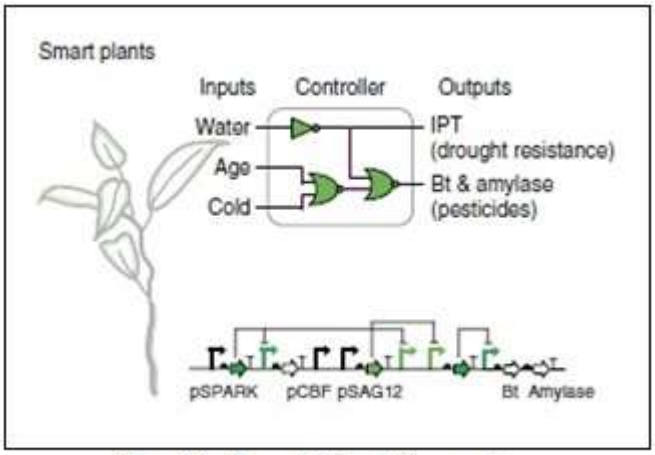


Fig. 11. Smart Plant Concept

International Genetically Engineered Machine (iGEM) is an independent, non-profit organization dedicated to education and competition, the advancement of synthetic biology, and the development of an open community and collaboration. http://parts.igem.org/Main_Page provides a library of gene circuit parts, devices and proteins which can be ordered for developing new gene-circuits also.

REFERENCES

1. Cameron, D. E., Bashor, C. J., & Collins, J. J. (2014). A brief history of synthetic biology. *Nature Reviews Microbiology*, *12*(5), 381-390.

2. Tu, D., Lee, J., Ozdere, T., Lee, T. J., & You, L. (2007). Engineering gene circuits: foundations and applications.

3. 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.

4. Yokobayashi, Y., Weiss, R., & Arnold, F. H. (2002). Directed evolution of a genetic circuit. *Proceedings of the National Academy of Sciences*, *99*(26), 16587-16591.

5. Brophy, J. A., & Voigt, C. A. (2014). Principles of genetic circuit design. *Nature methods*, *11*(5), 508-520.

Terms - Do not remove or change this section (It should be emailed back to us as is)

- This form is for genuine submissions related to biotechnology topics only.
- You should be the legal owner and author of this article and all its contents.
- If we find that your article is already present online or even containing sections of copied content then we treat as duplicate content - such submissions are quietly rejected.
- If your article is not published within 3-4 days of emailing, then we have not accepted your submission. Our decision is final therefore do not email us enquiring why your article was not published. We will not reply. We reserve all rights on this website.
- Your article will be published under our "Online Authors" account, but you will be clearly indicated as the original author inside the article. Your name and email address will be published. If we feel it is not feasible for us to publish your article in HTML format then we may publish it in PDF format.
- Do not violate copyright of others, you will be solely responsible if anyone raises a dispute regarding it.
- Similar to paper based magazines, we do not allow editing of articles once they are published. Therefore please revise and re-revise your article before sending it to us.
- Too short and too long articles are not accepted. Your article must be between 500 and 5000 words.
- We do not charge or pay for any submissions. We do not publish marketing only articles or inappropriate submissions.
- Full submission guidelines are located here: http://www.biotecharticles.com/submitguide.php
- Full Website terms of service are located here: http://www.biotecharticles.com/privacy.php

As I send my article to be published on BiotechArticles.com, I fully agree to all these terms and conditions.