SYNTHETIC BIOLOGY 101

iGEM

Presented to you by:

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What is iGEM?

Muhammad Shehryar Hamid

iGEM stands for "International Genetically Engineered Machine", and it is a competition in synthetic biology where teams of high school, undergraduate, and postgraduate students are welcomed to participate. Inaugurated in 2003, iGEM provides a platform where biologists, engineers, computer scientists, and students from various other fields of study gather together in teams to design and engineer biological systems. iGEM started as a summer course at the Massachusetts Institute of Technology in 2003, and the competition itself was first held in 2004, when 5 teams participated.[1] It is now held annually, with over 300 teams consisting of 5600 members from all over the world participating in 2016.[2] The event of the competition where all the teams present their projects and research is called the Giant Jamboree, and it takes place in Boston, USA.

The aim of iGEM is to encourage the use of synthetic biology to construct artificial biological systems, or to redesign and engineer natural biological systems. There are various components that are a part of the iGEM competition. One of the components is the laboratory work. All teams are provided with a standard kit of biological parts which consist of DNA sequences with different functionalities. Using this kit, the teams design a biological system that ideally

[1] 2016 "iGEM About" http://igem.org/About

[2] 2016 "iGEM 2016 Giant Jamboree" http://2016.igem.org/Giant_Jamboree

solves an ongoing problem in the world. This system can be in the form of a device, or just a conceptual proof of application. The teams use their time until the Giant Jamboree to conduct research and to design and construct prototypes of the biological systems of interest. This work is also accompanied by the documentation of a standard BioBrick part or device that is important to the project. These BioBrick parts are DNA sequences imperative to the biological systems, and several BioBrick parts form a BioBrick device.

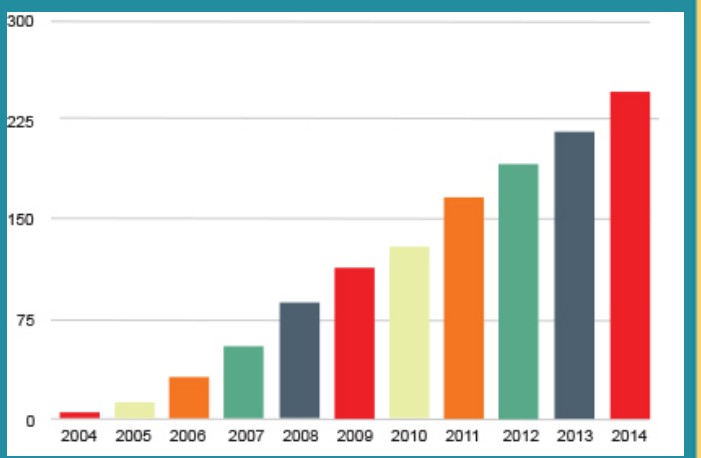


Figure 1: The number of teams that participated in each edition of iGEM from 2004-2014 [3]

[3] Figure 1 accessed from: http://igem.org/About

Apart from the scientific components of iGEM, another important part is 'human practices', which calls for public engagement to the projects. This engagement can be in any form, as not only does it aim to promote awareness about synthetic biology, but it also considers the public opinion on various issues that are central to the projects. The teams establish dialogues where the community can express their opinions. The teams are required to respond to the suggestions gathered upon their investigation by making appropriate amendments and configurations to their projects.

Other important components of iGEM include the Team Wiki, which is an online platform where the teams can upload all their project details and descriptions. The Team Wiki provides the teams with an opportunity to present their project in an interesting manner, by breaking it down into the various steps which help to explain their process of ideation and their results. Along the timeline, teams are also required to choose a standard or special track which their project is geared

towards. These tracks include ‘Manufacturing’, ‘Environment’, ‘Hardware’, etc. The teams are also required to prepare posters and presentations which describe their projects.[4]

An integral part of iGEM is the Giant Jamboree, where every team has to present their project, and this is usually done by various poster and presentation sessions. This is the moment where a team can showcase the end product that came out of the months of research that was conducted. It is also an opportunity to meet with other students who have spent their time along the same lines working on their projects. The endless possibilities in the world of synthetic biology, and the extraordinarily wide range of projects, are manifested at the Giant Jamboree. The teams are awarded medals and other special prizes for their work over the months, and there are also various workshops and social events that take place at the Giant Jamboree. The Giant Jamboree marks the end of an edition of iGEM.

[4] 2016 “iGEM Medal Requirements”

Giant Jamboree 2016 [5]

Over the years, iGEM teams have identified various issues of pressing concern, and have addressed them in creative and efficient ways. One such example is that of the Heidelberg 2014 team, that won the Grand Prize in the undergraduate section, at the Giant Jamboree of that year. The Heidelberg 2014 team decided to work on circularization of proteins, which no other iGEM team had previously worked on before. Proteins are linear molecules that fold in intricate ways. The Heidelberg 2014 team used synthetic biology to increase the heat and pH stability of proteins, whilst also making them resistant to exopeptidases (enzymes that digest proteins), by linking

[5] Accessed from: http://2017.igem.org/Community/Mentorship

together the extrema of a linear protein molecule to generate circular proteins. The team discovered that rigid linkers provide greater stability in circularizing proteins, and so they created a software tool called “CRAUT” that allowed users to design appropriate rigid linkers, which reciprocatively helped their project.[6] The Heidelberg 2014 project is just an example of the achievements that iGEM teams accomplish. iGEM projects range from dealing with widespread diseases such as malaria and tuberculosis, to the creation of biosensors based on pigment production. There is still plenty of room for discovery and exploration for future iGEM teams.

[6] 2016 “iGEM Judging Handbook”

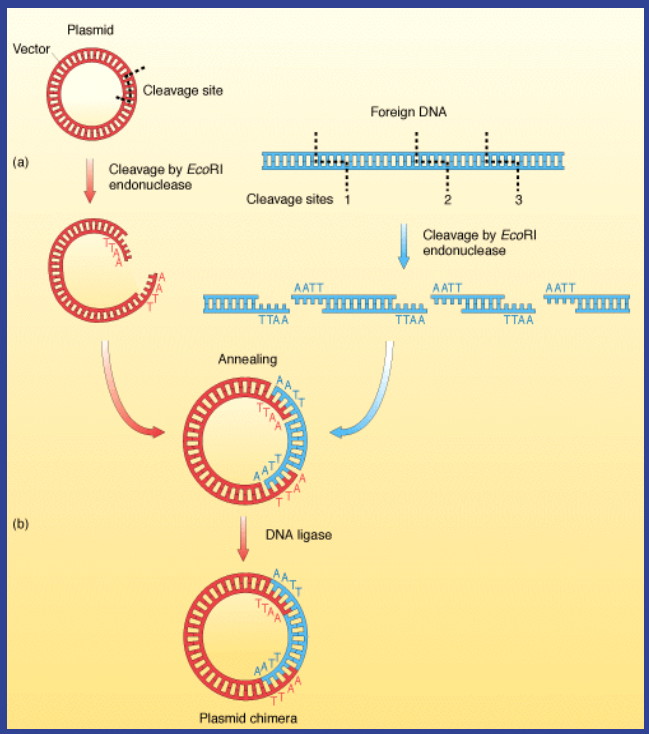
A Brief History of Synthetic Biology

Diego Kleiman

The word “synthetic” can be used to describe artificial products generated by the combination of elemental parts. In this way, a commonly accepted definition of synthetic biology is “the design and construction of biological devices or systems for useful purposes”(1). This definition indicates that the goal of synthetic biology is to artificially manufacture living systems in order to accomplish certain objectives, such as solving food crises or producing medicines at faster rates.

Although the interest in designing biological systems to solve defined problems is recent, the idea of synthesizing or recreating living systems in a laboratory is not.

1. Porcar M & Peretó J (2014) *Synthetic Biology: From iGEM to the Artificial Cell*, (Springer Netherlands, Dordrecht).



What is a restriction enzyme? Source: University of Miami, Biology Dept.

In Mary Shelley’s novel *Frankenstein; or, The Modern Prometheus* (1818), Dr. Victor Frankenstein creates a creature and brings it to life, showing that the possibility of creating artificial life existed in the imagination of the people at the time.

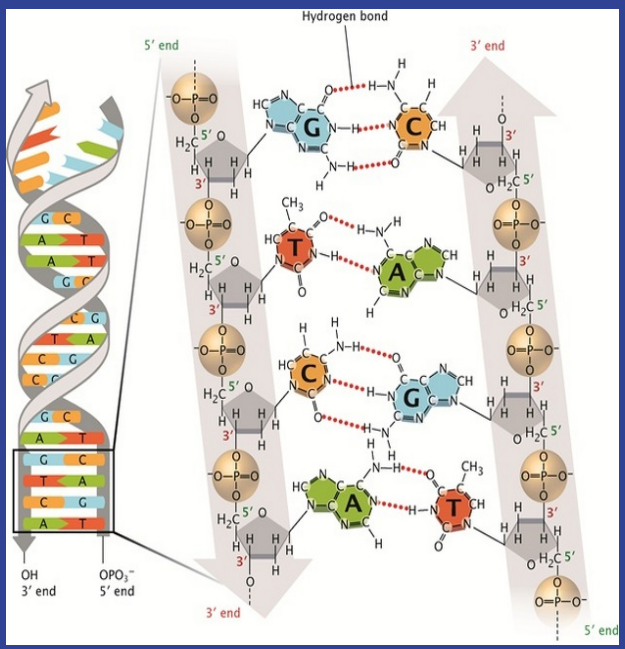
The publication of this novel was preceded by the work of physicians such as William Harvey (discovered blood circulation) and Luigi Galvani(the father of electrophysiology), who studied the behavior of living systems from a physical perspective in the seventeenth and eighteenth centuries, respectively.

However, it was not until 1912 that Jacques Loeb articulated the desire to study life from a strictly physicochemical view and to manufacture biological systems in *The Mechanistic Conception of Life*.

In reality, Loeb did not attempt to achieve the goal of synthesizing living systems despite having contributed many discoveries to the life sciences. That same year, the term "synthetic biology" was used for the first time by Stéphane Leduc as the title of the second volume of his *Études de Biophysique* (1912). Nonetheless, Leduc was only able to mimic biological structures through chemical reactions (1).

2. Cameron DE, Bashor CJ, & Collins JJ (2014) A brief history of synthetic biology. *Nat Rev Micro* 12(5): 381-390.

Throughout the twentieth century, new discoveries and technological inventions allowed the development of what would be termed "genetic engineering" in the 1970's (2).



Structure of a DNA (Adapted from: ***Pray, L. (2008) Discovery of DNA structure and function: Watson and Crick. Nature Education 1(1):1000)***

Understanding how living cells encode the information necessary to carry out their vital functions and mastering modification of said information was a prerequisite for the development of synthetic biology. In this way, genetic engineering was the precursor of synthetic biology.

The discovery of the DNA structure by James Watson, Francis Crick, and Rosalind Franklin (not attributed in the original paper) in 1953 was crucial in understanding how DNA encodes information. The knowledge of the chemical structure of DNA, in addition to subsequent discoveries, allowed Crick to enunciate the central dogma of molecular biology in 1958 (3). He stated that the information insegments of DNA denominated genes is transcribed into similar RNA molecules, which then guide the synthesis of proteins that accomplish numerous cellular functions (4). But how do cells regulate this transfer of information? In 1961, the *lac* operon from the bacteria *Escherichia coli* was characterized by François Jacob and Jacques Monod (5).

The *lac* operon is an example of a genetic regulatory mechanism, which limits the transfer of the information encoded in the DNA to particular situations; in this case, the *lac* operon triggers the transcription of a given set of genes from DNA to RNA when the bacteria has access to the sugar lactose, but not glucose, as a food source. This example served as a guide to understand how genes are regulated in bacteria.

Despite understanding how the information flow worked in a living cell, scientists had no tools to manipulate this information.

During the 1960's and 1970's, Werner Arber, Hamilton Smith, and Dan Nathans purified and characterized restriction enzymes, proteins that behave like "molecular scissors" which cut DNA at specific sites and permit researchers to create novel combinations of DNA sequences by combining the

3. Pray LA, Clancy S, Smith A, Shaw K, & Phillips T (2010) The Elaboration of the Central Dogma. (Nature Publishing Group Education, Cambridge, MA).

4. Crick FH (1958) On protein synthesis. *Symp Soc Exp Biol*, p 8.

5. Monod J & Jacob F (1961) General conclusions: teleonomic mechanisms in cellular metabolism, growth, and differentiation. *Cold Spring Harbor symposia on quantitative biology*, (Cold Spring Harbor Laboratory Press), pp 389-401.

resulting pieces (6). This discovery was, arguably, the birth of genetic engineering. Nonetheless, this tool and the sequencing technologies available were not enough for researchers to design a biological system.

During the 1990's, tools to sequence entire genomes and to draw metabolic networks were developed. The main idea was to apply engineering design concepts (e.g., the modularity or independence of the functional units in a system) to the design of biological systems (2).

Technology to synthesize long DNA sequences artificially provided further flexibility to the work of synthetic biologists. This approach has produced innovations including the engineering of a metabolic pathway in *E. coli* to produce the precursor of the antimalarial drug, artemisinin. This means that researchers were able to equip bacteria with all the DNA parts required to produce a medicine that naturally found in the plant Artemisia *annua*, from which artemisinin can only be extracted at slow rates (1).

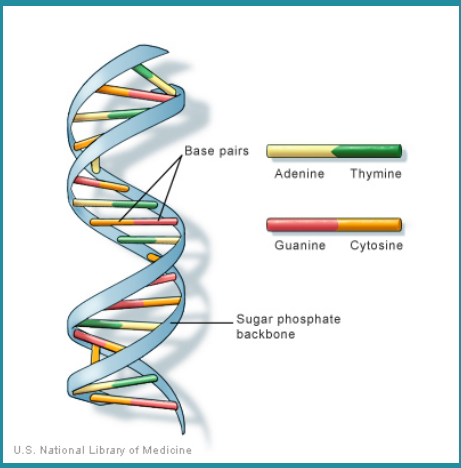
It is remarkable that throughout the years, the desire to recreate life in the laboratory shifted in favor of modifying already existing organisms to accomplishing feats such as the production of the antimalarial drug. In a machine, each part executes a well-known and characterized function of a process. However, in cells, different components can interact in unpredictable ways, so a part that has been proven useful in one system may fail to work the same way in a different one (2). These drawbacks pose fundamental questions regarding the possibility of applying engineering principles in a biological context. Only time will tell if synthetic biologists will overcome these barriers to produce safe and reliable biological systems to solve some of the most pressing issues the world faces.

6. Chial H (2014) Restriction Enzymes. (Nature Publishing Group Education, Cambridge, MA).

Rewriting Life As We Know It

Khairunnisa Semesta

The genome of an organism comprises a myriad of genes. The gene itself is a sequence of nucleotides that form DNA, a molecule composed of a deoxyribose sugar, a phosphate group, and one of the four bases - adenine (A), tyrosine (T), guanine (G), or cytosine (C).



DNA and Types of Bases[1]

[1] U.S. National Library of Medicine

The genetic information stored In DNA holds a paramount role, as it dictates many cellular functions in an organism.

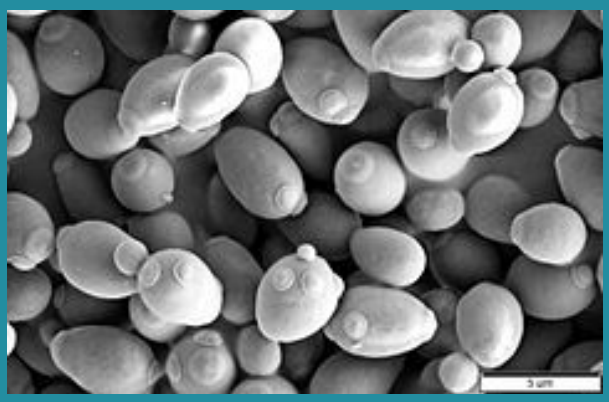
The field of synthetic genomics has been marked with advances in making “designer genomes”, which comprise of a wide range of approaches from genome size reduction to complete genome synthesis. Synthetic genomics is, in a way, rewriting life. Why would scientists be interested in creating these designer genomes? Synthesizing genomes provides researchers with an innovative approach to study gene functions as well as understand how organisms function at the systems level, which includes considering the complexity of interactions in an organism. For many researchers, investigating the smallest genome size to create viable life

also becomes a pertinent question to this pursuit. Many gene products often perform similar essential functions in an organism, making neither gene essential. Creating a streamlined organism by removing non-essential genes is useful to establish conserved functions as well as study the effect of reintroducing each gene individually.

What would happen if the genome were reduced? In 2006, a group of researchers reduced the genome size of the bacteria *Escherichia coli* by 15%. The researchers eliminated nonessential genes and sequences. Reduction of the *E. coli* genome presents an attractive opportunity to increase its metabolic efficiency while preserving good growth profiles and protein production[2]. In 2016, scientists were able to replace 7 of 64 genetic codons in *E. coli* — sequences that code for amino acids — with others that produce the same components[3].

[2] Posfai, G. "Emergent Properties of Reduced-Genome Escherichia Coli." *Science* 312.5776 (2006): 1044-046

The pursuit to synthesize designer chromosomes is also active in eukaryotic species, particularly in the yeast *S. cerevisiae*.



S. cerevisiae[4]

The synthesis of eukaryotic genomes is more challenging than prokaryotic genomes due to its complexity. However, the concept of synthesizing eukaryotic chromosome is based upon similar principles: after designing the chromosome with the desired changes, 10-kbp pieces of this chromosome are

[3] Ostrov, N., et al. "Design, synthesis, and testing toward a 57-codon genome." Science 353.6301 (2016): 819-822

[4] Feldmann, Horst *Yeast. Molecular and Cell bio*. Wiley-Blackwell (2010)

assembled from chemically synthesized oligonucleotides. These pieces contain unique restriction sites - sequences where the DNA can be "cut" using special enzymes - to allow ligation of these pieces into larger 30-kbp or 50-kbp pieces.

Diagram of Restriction Sites[5]

Lastly, by exploiting the highly recombinogenic nature of yeast, 30–50-kbp pieces of the wild-type sequence can be replaced by the corresponding synthetic ones. In 2014, researchers reported the first eukaryotic synthetically designed chromosome based on chromosome III of *S. cerevisiae*[6]. Currently, an international consortium of scientists has accepted the challenge to synthesize the remaining 15

[5] Thermo Fisher Scientific

[6] Annaluru, N., et al. "Total Synthesis of a Functional Designer Eukaryotic Chromosome." Science. 344.6179 (2014): 55-58

chromosomes of the Sc2.0 yeast genome in the Synthetic Yeast Genome Project. Like the Mycoplasma genome, the next step after building Sc.20 is to determine the minimal genome would be for its function and reproduction[7].

The major advances in synthetic biology, particularly synthetic genomics, have laid new avenues for scientific research. Synthesizing whole genomes, which previously seemed unthinkable, is now within reach through the collaborative efforts of scientists around the world. Now, the Human Genome Project—write (HGP-write), an ambitious proposal to synthesize the human genome, is also on the table. Jef Boeke of New York University, one of the proponents of the HGP-write, is also a leading researcher in yeast genome synthesis. This proposal has been met with

[7] Annaluru, Narayana, Sivaprakash Ramalingam, and Srinivasan Chandrasegaran. "Rewriting the Blueprint of Life by Synthetic Genomics and Genome Engineering." Genome Biology 16.1 (2015)

praises and criticisms alike. Although the current goal of HGP-write is to enable the technology that can synthesize long strands of genetic material at a reasonable price, misinformation often contributed to a flurry of concerns. For example, critics cite the lack of justification and the ethical problems that it might raise, such as the rise of designer babies as well as gene patenting. However, the proponents of HGP-write argue that this project has the potential to lower the cost of gene editing, increase the possibility of making living cell lines for medical research, and pave the way for major technological advancements in synthetic biology. Since the project will also require large amounts of funding to overcome the cost of synthesizing DNA, the project is currently trying to win broader support from the public.

Genetically Modified Organisms (GMOs)

Laura Karpauskaite

What is GMO?

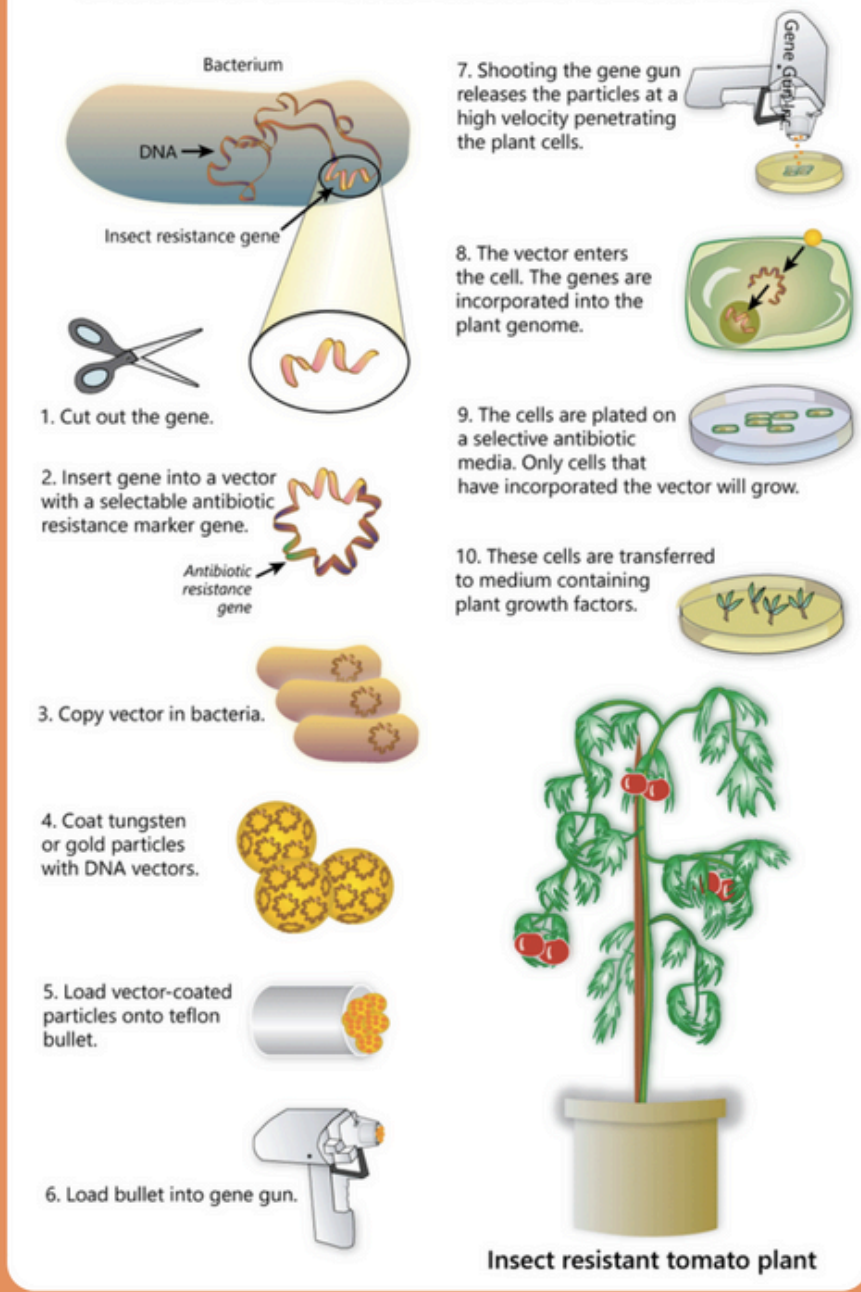
Genetically modified organisms (GMOs) are created through biotechnology and genetic engineering processes. Genetic modifications are driven by the need to find new treatments for diseases, to limit and eradicate starvation. Although GMOs have been extensively discussed in the media only recently, the first GMO (bacteria producing human insulin) has been developed in 1982 and has been available in the market for people suffering from diabetes. The development of GMOs truly started in 1970s after Stanford University researchers created the first gene sequence and recombinant DNA.[2]

How does it work?

GMO production is based on reproducing the natural selection processes with genetic manipulations. Through subtraction or substitution genetic material of different species is combined to create a new species with desired traits. Molecules of DNA from different sources are combined and placed into a cell and then a new host. After the organism reproduces the modified genome is passed to the offspring, which has the genetic modifications with changes in certain biological functions within the organism.[2]

1. Qaim M & Kouser S (2013) Genetically Modified Crops and Food Security. *PLOS ONE* 8(6):e64879.

2. Watts CPDBASBS (2016) Genetically Modified Organisms. (Salem Press).



3. Genetic Science Learning Center. "Genetically Modified Foods." Learn.Genetics. July 15, 2013. http://learn.genetics.utah.edu/content/science/gmfoods/.

Applications

Scientist predict that soon the demand for food will exceed the food produced by traditional agriculture methods. With genetic modifications the crops could be resistant to diseases, weeds and pests, which would increase the yield of the crops. The scientists have already created plant species that are able to survive high temperatures and rainfall, thus allowing the cultivation of previously uncultivable areas.[3]

Scientist are also using the same technology to try and solve problems in medical and environmental fields. Genetic engineers can produce ecologically friendly batteries, bacteria, which could produce biodegradable plastics or could potentially protect the endangered species. [5]

GMOs and UAE

GM crops are not grown in the GCC, however products such as corn or soybean are imported from countries that have more lenient regulations on GM products (e.g. US).

5. Watts CPDBASBS (2016) Genetically Modified Organisms. (Salem Press).

The laws concerning GM crops and related products depend on each country as there is no set of international law for acceptable amount of GM material in food. Therefore, although some countries as Saudi Arabia, Russia, Brazil, Australia and EU require labelling if the food GM content is higher than 0.9-1%, other countries such as US, Canada, Philippines, Thailand and Taiwan, do not label food with less than 5% of GM content as genetically modified.[6] Due to this discrepancy, in 2008 the GSO created a subcommittee for laws concerning biotechnology, especially GM products. Although there is not much published information about GM foods in UAE, a study published in 2011 has confirmed that GM foods are present in UAE market. The study has also shown that even some supposedly non-GM crops have genetically modified material present, which indicates that the lack of proper international regulations create confusion about what exactly are GM products.[6]

6. Premanandh J, Maruthamuthu M, Sabbagh A, & Al Muhairi S (2012) Short communication: Prevalence of genetically modified foods (GM foods) in the United Arab Emirates. *Food Control* 25:10-12.

E. coLAMP

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The most common type of bacterial infection stems from contact with *Escherichia coli*, which when ingested can cause a variety of symptoms ranging from nausea to diarrhea. Shiga toxin-producing *E. coli* (STECs) are responsible for the majority of foodborne *E. coli* infections because it produced inhibits protein synthesis in all cells. Although most countries now have stringent food safety regulations in place to prevent the sale of contaminated foods, small scale manufacturers, particularly street food vendors, often do not have access, time or pressure to consult laboratories about the safety of their food.

Our project aims to produce a portable device that allows detection of STEC through the use of loop-mediated isothermal amplification (LAMP), a technique that is similar to, but more sensitive than, polymerase chain reaction (PCR). We are targeting the genes that have been identified in shiga-toxin producing *E. coli*, namely *stx1B*, *stx2B*, *rfbE*, and *eae*. For each gene, we designed a set of 4-6 primers, which includes the forward and backward outer primers, the forward and backward inner primers, and the forward and backward loop primers if applicable.

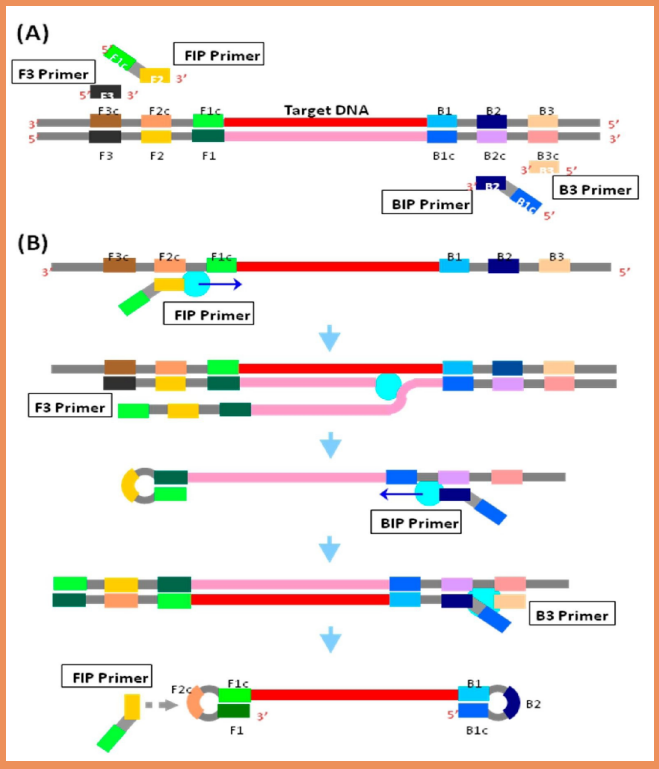


Figure 1. Illustration of LAMP Reaction[1]

The forward inner primer initiates the amplification, followed by the strand-displacing DNA polymerase which separates the target DNA duplex. Synthesis initiated by the forward outer primer at an upstream target region subsequently displaces the first product, causing a stem-loop structure to form at the end of the first product due to the inner primer sequence complementarity. The annealing and strand-displacing processes continue from the opposite direction, yielding a dumbbell-shaped structure that contains more annealing sites for further amplification.

The device consists of two main components: a heater and a cartridge. The cartridge will contain three type of chambers connected by microfluidic channels and valves. The first chamber is a cooking chamber where the samples will be heated to 95℃, triggering the lysis process. The LAMP amplification will occur in the second chamber featuring temperature of 65℃. The result of is visualized by a colorimetric assay using a color-changing dye in the third chamber. The reaction tubes will be prepared in powder form and premixed, ensuring that the user only needs to insert their food sample for testing. The device is also designed such that the cartridge is easily disposable and a new cartridge can be inserted every time for a new food sample. This ensures the whole setup to be easy to use and clear from contamination.

The end goal is to provide food vendors an opportunity to easily and quickly detect the presence of STEC in their food to ensure that they are complying with government standards. The results of each test will be uploaded into a database that provides consumers with the date, location and result of each STEC test. This will ensure that both vendor and consumer are safe, leading to a decrease in the incidence of foodborne *E.coli* infections.

[1]http://what-when-how.com."Novel Molecular Diagnost Platform for Tropical Infectious Diseases"

The Road to Food Safety

Muhammad Shehryar Hamid

The aim of NYUAD iGEM team, E.CoLAMP, is to build a device that can test for the presence of Shiga toxin-producing *Escherichia coli* (STEC), to ensure that food safety is regulated and not compromised by food vendors. For improved prototyping and designing of this device, various food vendors were surveyed.

During the summer 2017, NYUAD team for iGEM created the initial prototypes and prepared the biological reactions for a device that would test for the presence of Shiga toxin-producing *Escherichia coli* (STEC) in food. The prototypes were yet to go through simplifications in terms of designs and device features. To help with the process, we conducted some surveys regarding food safety, and how it relates to our iGEM project. These surveys were conducted in Indonesia and Pakistan thus engaging international communities of food vendors to obtain a diverse set of results.

Surveys were conducted in places ranging from coffee shops and restaurants to street food stalls. Having conversations with food vendors allowed us to get their perspective about the device which enabled our team to tailor the prototypes according to the need of the consumers.

In these conversations that took place, the vendors were asked about any precautions they took to ensure food safety. The responses were rather surprising. While a few vendors claimed that some efforts were made to ensure that the food they sold was healthy and safe to eat, few clearly stated that they never did anything to make sure that the food was safe.

The responses were rather surprising. While a few vendors claimed that some efforts were made to ensure that the food they sold was healthy and safe to eat, few clearly stated that they never did anything to make sure that the food was safe.

For such vendors, earning a living by making a profit was the priority.

Some ways of checking for food safety included purchasing raw meat and spices from trustworthy sources, storing ingredients in clean places and only selling freshly made food. These checks showed that many of the food vendors took responsibility in some way to ensure the safety of their food. However, none of them said “yes” when they were asked if they had heard about STEC before.

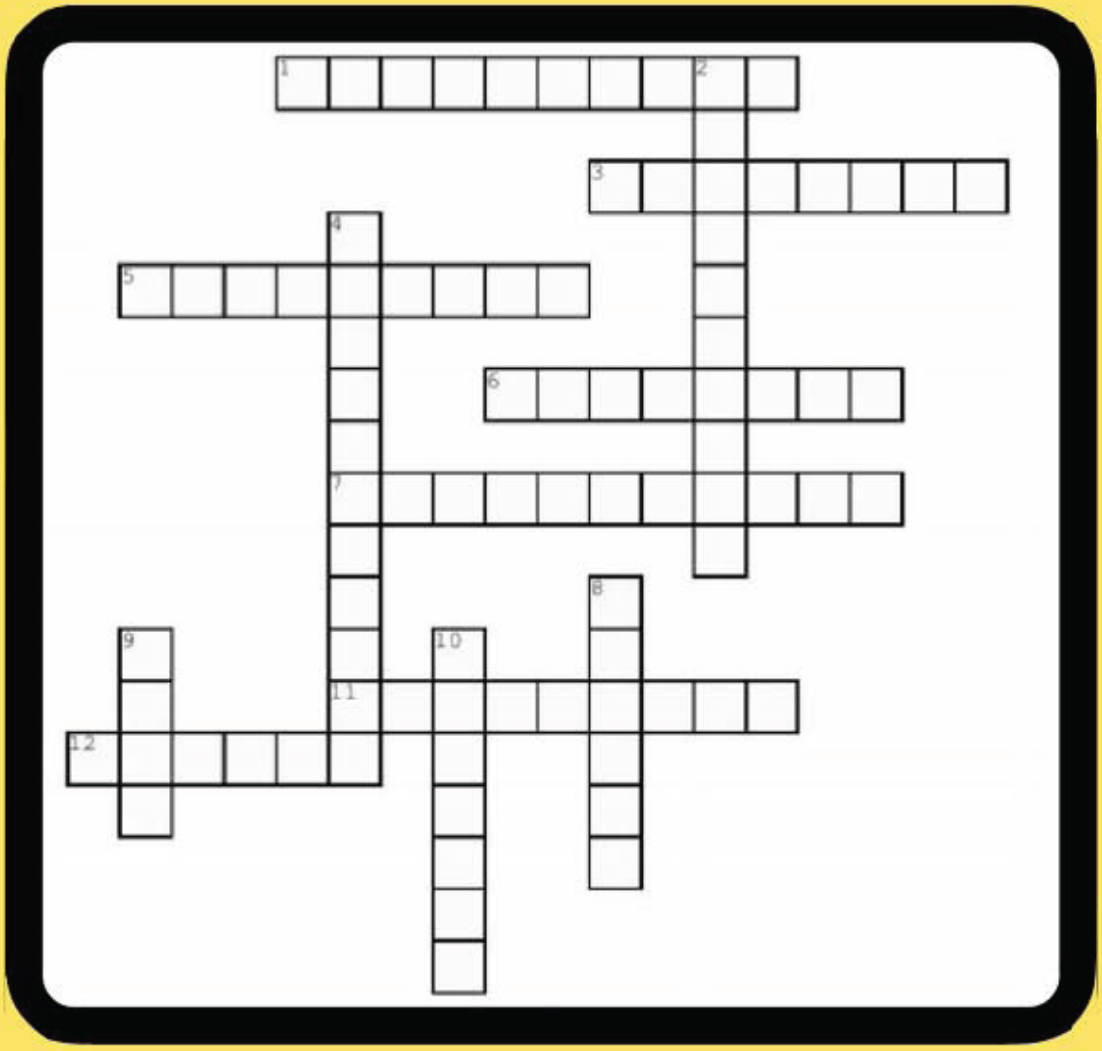
Following this, they were told that STEC refers to the strains of bacteria which produces Shiga toxin that can cause foodborne illnesses. The vendors were then asked if they would be interested in acquiring equipment to detect STEC in food samples, and 90% of the vendors replied in the affirmative after which they were asked of the features they would want to see in the detection device. The suggestion received included the results should be easy to visualize; device should be portable, easy to use and should provide results within 2 hours. This information was really important, because the detection device was initially targeted to food vendors so that they could test for the safety of their food.

The vendors were then asked about the cost they would be willing to pay to purchase this equipment. Some vendors claimed that they would only pay up to $10 USD for the device, whereas others were willing to pay up to $100 USD. On average, those surveyed were willing to pay $60 USD for a STEC detection device. Most of the vendors were hesitant

when they were asked if they would periodically acquire a kit of reagents in order to use this equipment, but on average, they were willing to pay $10 USD for such a kit if it was required. The NYUAD team, thus, took note to make the detection device as cost effective and affordable as possible.

Finally, the food vendors were asked for any suggestions that they might have and the responses recorded said that such a device should not be dangerous, and should be lightweight for easy use with easily comprehensible results.They also mentioned that the device could be targeted to the initial manufacturers that are at the top of the food chain, and serve as the initial source of all food products. The vendors mentioned that the government should be involved in regulating food safety at every part of the food chain, and the provision of this STEC detection device should be subsidized by their governments. All surveyed agreed that safe food is the key to productive business and the creation of a healthy environment.

Crossword



Across

1. The genus of the bacteria with the smallest genetic material created at the JCVI
3. Genetically engineered specimen which genome is altered by adding or removing genetic material
(Genetically Organism)
5. A member of a large group of unicellular microorganisms that have cell walls but lack organelles and an organized nucleus
6. The abbreviation of the ambitious proposal to synthesize the human genome
7. "Molecular scissors" that cut DNA at specific sites L Enzymes)
11. The field that focuses on the design and construction of biological devices or systems for useful purposes L Biology)
12. The complete set of genes in an organism

Down

2. Engineering design concept related to the independence of the units in a system
4. TIle name of the sugar in DNA
8. A unit made up of linked genes that is thought to
regulate other genes responsible for protein synthesis
9. International Genetically Engineered Machine,
the world's largest synthetic biology competition
10. TIle substance that was produced by the first
genetically engineered organism in 1982