A beginners guide to SynBio

and all about iGEM

iGEM
IISER Bhopal
2023

Welcome, young minds, to the extraordinary world of synthetic biology! I am your friend Bacti-Bob, and I am so eager to introduce you to the treasures of Synthetic Biology, which I lovingly call SynBio. SynBio is a world where biology entwines with the principles of engineering to create biological systems that perform desired tasks! Brace Yourselves, for a wondrous adventure awaits you!

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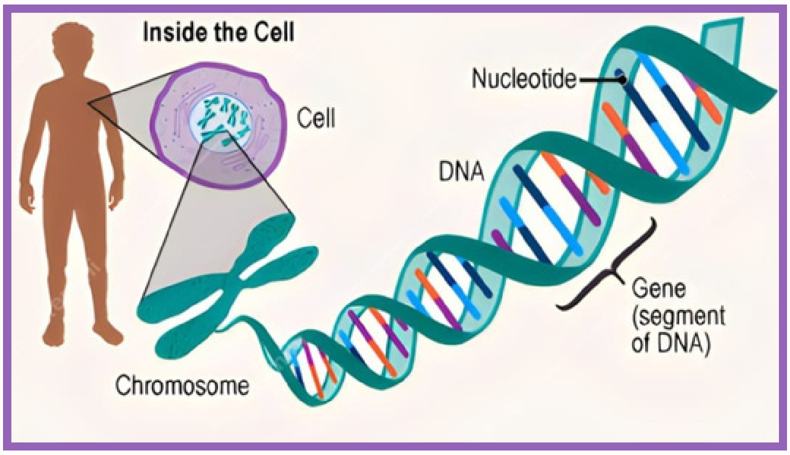
What's SynBio?

We all know how buildings are made, right? Bricks, when placed on top of one another, can make a building. You can have bricks made up of different materials, and arrange them in a specific manner to make different kinds of buildings, employing various techniques to do so.

Synthetic Biology is *that*, but for life. Only instead of bricks, the building blocks here are various components like genes, cells, enzymes, and a lot more! And what you end up making, is a system that performs a function that *you* designed it to do!

To understand all this, we need to go back to the basics and first look at what a **GENE** is: Simply put, a gene is a set of instructions that help perform a function.
What kind of function?
It can be anything, but almost all of it involves a protein,
and that's exactly what a gene makes!

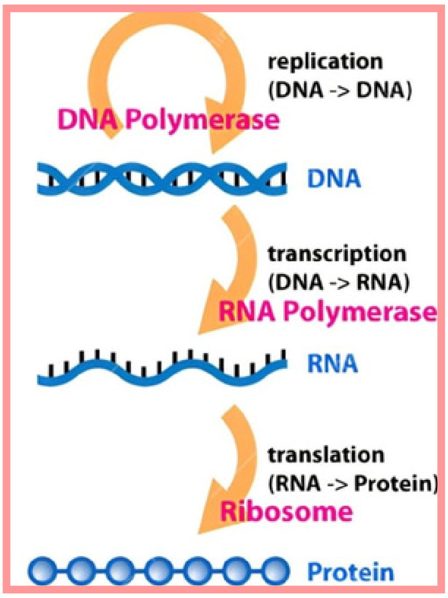


Now, what is this gene made up of? It's the good old **DNA**! You must have heard of it, right? It's a sequence of nucleotides, and the different ways that sequence is arranged, make up the different genes. You know the interesting part? All the innumerous sequences that we find in DNA are all made up of a combination of just 4 nucleotides -adenine (A), thymine (T), guanine (G),and cytosine (C)!

How exactly does this work? To answer that we need to understand the famous **Central Dogma of Life:**

It defines the flow of information from DNA to proteins. Here's the simple version, which has 2 steps-

- **Transcription**: DNA → RNA
- **Translation**: RNA → Proteins

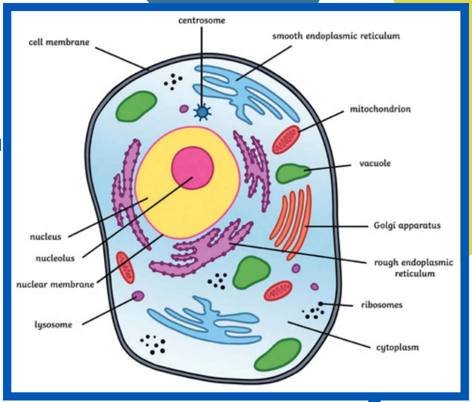


DNA is a double-stranded sequence of nucleotides. One of its strands is copied,forming a single-stranded RNA called messenger RNA (mRNA). This process is called **Transcription**. RNA Polymerase facilitates this process. One big difference in mRNA and the DNA it is copied from,is that every thymine nucleotide in the sequence is replaced by one other nucleotide, Uracil (U).

Now let's look at how this mRNA is then "translated" to proteins. What exactly is being "translated" here? You see, every triplet of nucleotides 'codes' for a particular amino acid (Amino acids are the building blocks of proteins!) These triplets of nucleotides are called codons. In total, there are 64 codons possible, combining the 4 nucleotides in different orders. But remember! Not all these codons code different unique amino acids. Together, they code for 20 different amino acids, and all the proteins we see in nature are a combination of these. That's what happens in the process of **translation**! The codons on an mRNA are "translated" to their corresponding amino acids, forming a chain of amino acids, which makes up a protein.

So you see, a particular gene, after going through transcription and translation, will give rise to its corresponding protein, which in turn will perform some relevant function.

CELL

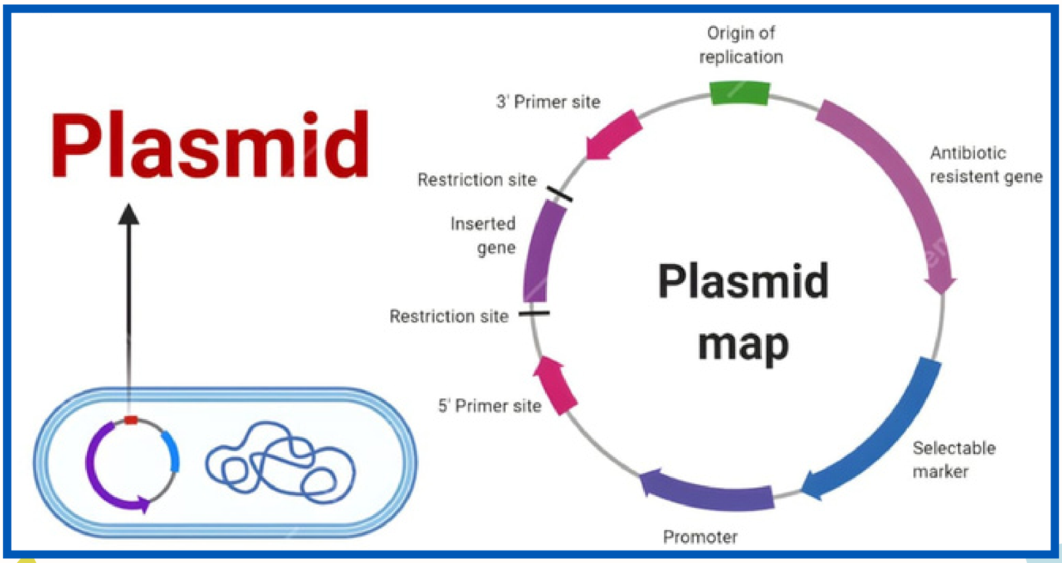


We all know what a cell is by now - the smallest unit that can live on its own and that makes up all living organisms and the body's tissues.

Now, we need something that can “express” a gene of interest, which means to produce the protein that the gene codes for. That’s where cells come in. New genes can be introduced into these cells, which are then transcripted and translated by them to get the desired product. Single-celled organisms are one of the most prevalent in Synthetic Biology. Even among them, various strains of the bacteria E. coli are particularly popular. In some cases, eukaryotic cells such as yeasts, and certain insect and mammalian cells are also used, depending on the requirements.

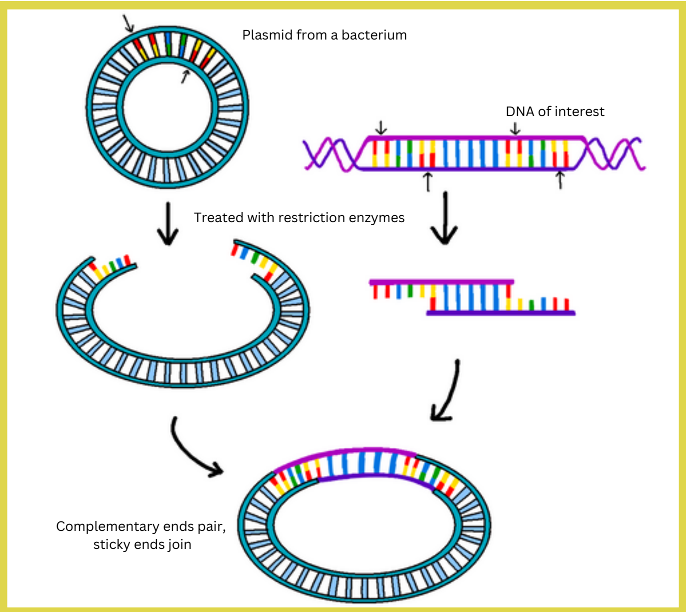
How do we transport the gene of interest into this cell? That’s where VECTORS come in.

A vector is a DNA molecule that can be thought of as a vehicle, with the gene of interest as the passenger. We first insert the gene into the vector, and then insert this vector into the cell. Plasmids, which are circular DNA molecules, are the most commonly used vectors. Some other vectors in practice are cosmids, viral vectors, and artificial chromosomes.



How is the gene inserted in a vector?

Enzymes, namely, RESTRICTION ENDONUCLEASES and DNA LIGASE play an essential role here, analogous to scissors and glue. Sounds weird? It's really not. Plasmids have particular sites (called restriction sites) in its sequence which can be recognised and cut by these restriction enzymes. Different enzymes have unique restriction sites. These cuts produce something called "sticky ends" at the cut ends of the plasmid. The same enzyme is used to isolate the gene of interest, which will have the same "sticky ends", making it compatible to join with the plasmid. But that's not enough, and we use another enzyme, called DNA LIGASE, which acts as a glue and joins the gene with the plasmid.



Now that we have an idea of the "building blocks", let's get into how we go about "arranging" these blocks. I'm talking about the tools and techniques that we use to bring the concepts and theoretical methods mentioned above into reality. We'll look into some of them here.

Genome Sequencing

We need proper information of what a DNA sequence looks like and how it is analysed before we can begin making any changes to it. Genome Sequencing is a method that helps us find out the genomic sequence of DNA. It's possible to deduce the entire genome of an organism now. We can analyse the sequence with great resolution, with every single nucleotide base laid out for us to view.

Most of synthetic biology boils down to editing genes. One of the most popular methods of doing so is using **CRISPR-Cas9**. It stands for Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-ASsociated protein 9. Quite a mouthful, huh? Let's stick to CRISPR-Cas9. This method is adapted from a neat little trick, that bacteria use to protect themselves from viral invasions.

When a virus infects bacteria, the bacteria incorporate small sections of the viral DNA into their own DNA in a regular pattern (called the CRISPR array). This helps the bacteria "remember" the virus in case of future infections, much like how our bodies' immune system works against certain antigens. And when the virus does infect again, the bacteria now releases RNA segments from the CRISPR array, which bind to the corresponding points in the viral DNA. This tags the DNA, which is then cut by enzymes like Cas9, thus degrading the viral DNA.

Now, how does this help us with editing DNA? Similar to how the bacteria produces RNA which binds to specific points in DNA, it's possible to create small pieces of RNA which can bind to a desired point in a DNA. This RNA is also attached to Cas9, and so, when the RNA binds at the location, it then guides Cas9 towards it, which then does its job and makes a cut there. After that, the cell's own repair mechanism is used to then edit the DNA, which can include, adding, deleting, or altering a certain segment, or adding a customised piece of DNA or gene.

Polymerase Chain Reaction (PCR)

PCR helps in the gene assembly aspect of synthetic biology. It helps to synthesise specific DNA fragments. This includes the gene of interest. We can amplify the number of gene fragments exponentially by synthesising them in repeating cycles.

How does it work? There are 3 main steps:

1. **Denaturation** - the double-stranded DNA is heated, and the strands separate.
2. **Annealing** - short DNA molecules called primers attach (anneal) to the ends of the target DNA sequence.
3. **Elongation** - An enzyme DNA Polymerase helps fill the gap between the primers, essentially making the single strand of the gene fragment into double-stranded DNA.

These 3 steps make up 1 cycle. This cycle is repeated several times, and each time the DNA fragment is duplicated twice more than the previous cycle, resulting in exponential growth by the time the last cycle is complete.

To build anything, proper equipment is essential. The same goes for all the techniques mentioned above. Here are some of the science-y gears extensively used in a lab-

- Thermal Cyclers: Used for PCR
- SDS-PAGE Apparatus and Gels: Used for separating and analysing proteins
- Chromatography Columns: Used for separation and purification of proteins
- Spectrophotometer: Used for measuring the concentration of DNA, RNA or proteins

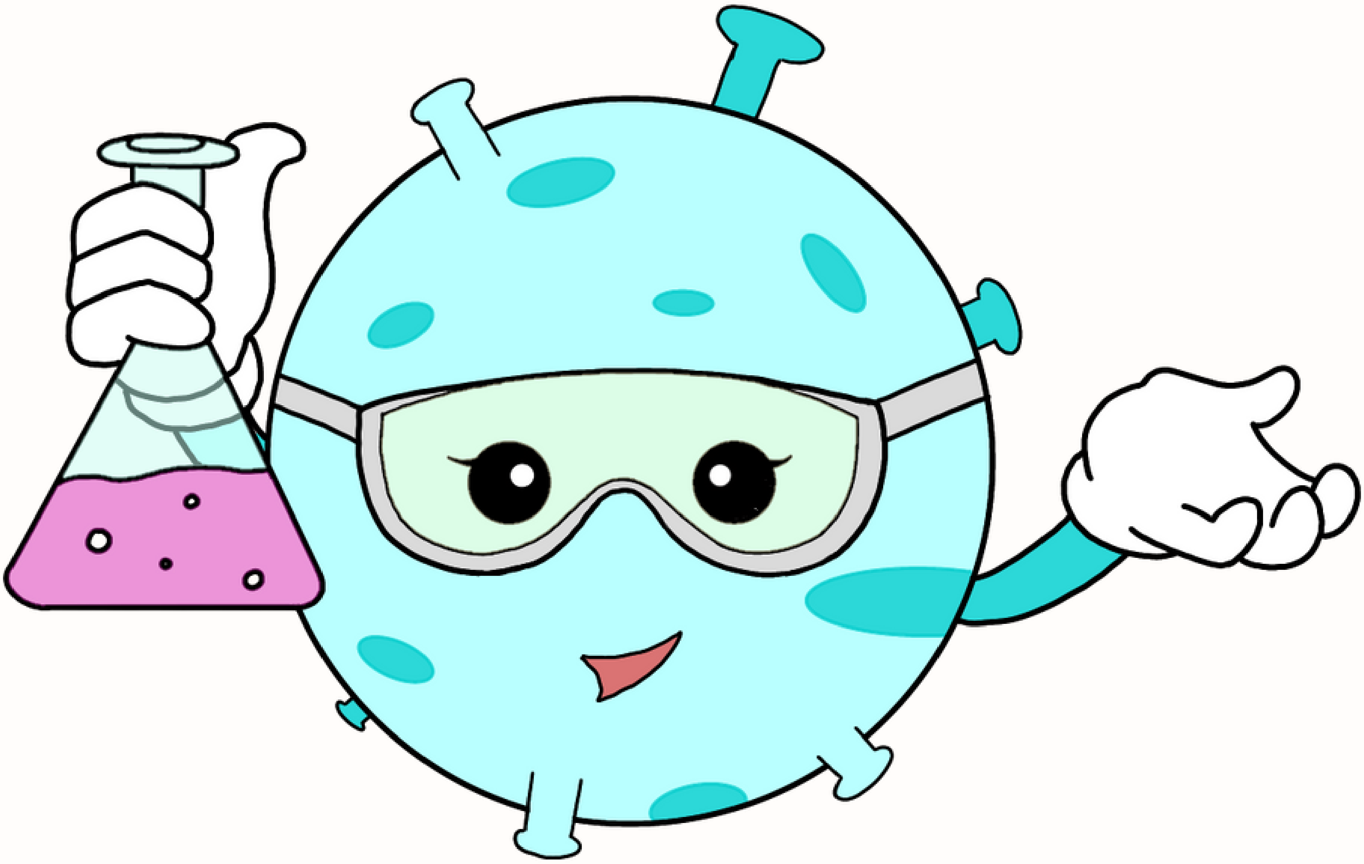
And a lot more, depending on the requirements of the experiment. Of course, apart from these, we have the standard lab equipments like Laminar Flow Hoods, Micropipettes, Centrifuges, Petri Dishes, MCTs, pH Meters, Vortex, Autoclaves, Water Baths, Hot Plates, Ovens (and more), which are absolutely necessary for most lab activities.

Anagrams

1. **GENERANTS**
2. **CHEBOIT**
3. **AERONOIC PLAYERS MACHINE**
4. **PREHEAT YEN**
5. **CHARMERS TILE**
6. **CORVET**
7. **CIGNESQUE**

1. TRANSGENE
2. BIOTECH
3. POLYMERASE CHAIN REACTION
4. GENE THERAPY
5. THERMAL CYCLERS
6. VECTOR
7. SEQUENCING

But you know what? There are some things you can do without any of these fancy equipment, in the comfort of your own home! Yes, you read that right!



Extract DNA at home

Everything we have discussed so far has revolved around this elusive DNA, which apparently you can't even see! Does it even exist?! Let's put an end to all doubts, and have a nice look at it on our own. You can do it easily, with things readily available at your home!

Procedure

1. First, we need a DNA source. You can select anything like bananas, spinach, peas, and strawberries. The list is non-exhaustive, and you can use other things too, depending on availability. Crush your source of choice to a pulp. You can use a blender, or go old school with a mortar and pestle!
2. In a separate bowl, take water and add salt. The aim is to make a highly concentrated solution.
3. Add the dishwashing liquid to this solution and mix. Avoid making a lot of bubbles.
4. Add this mixture to the pulp you made in Step 1, mix thoroughly and let it stand for 15 minutes.
5. Filter this mixture using a strainer, and collect the filtrate in a clear glass. It would be excellent if you happen to have a test tube at your disposal, but a narrow glass works too.
6. Now comes the crucial step. Take some *ice-cold* sanitiser the same amount as your filtrate, and *carefully* add it to the filtrate, by pouring gently along the walls of the container. *Do not mix!* You should get two separate layers, the filtrate at the bottom and the alcohol above it.
7. Allow this to rest for at least 5 minutes. You should start seeing some white cloudy substance forming and rising in the alcohol layer. *That's DNA!* If you don't see it clearly, let it rest for some more time.
8. If you want, you can use a paper clip or something similar to pull out the DNA gently.

Materials required

- Water (~100 mL)
- Banana/Spinach/Peas/Strawberries
- Dishwashing liquid (~2 tbsp.)
- Salt (~5 g)
- Ice-cold Alcohol (Don't fret! Just use liquid sanitisers with 70%+ alcohol)
- Strainer/Sieve
- Bowls
- A glass


So there you go! You just extracted some tangible proof that DNA does exist!

Riddle me this

I slice DNA with precision and might,
What am I,
With my precise incise?

Restriction Endonuclease

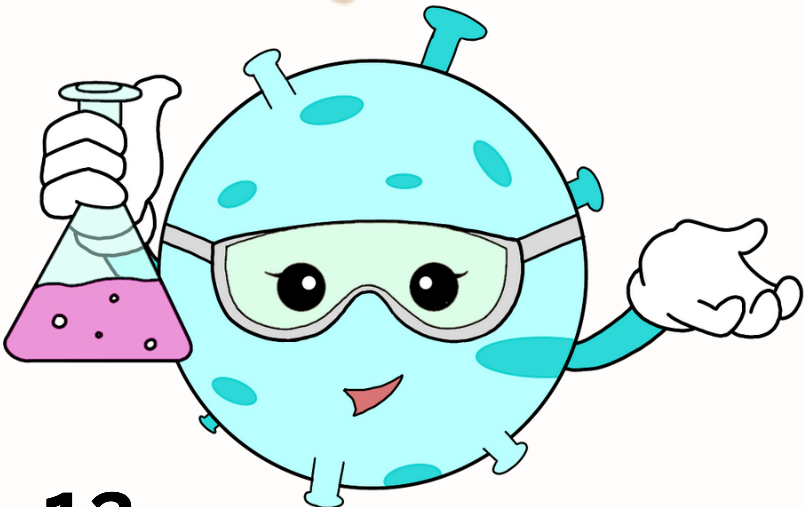
SynBio in Pop Culture

So, my curious pals, I am certain you all enjoy watching sci-fi movies and reading fictional literature! Many of these books, TV shows, and films are inspired by real-life events, where the creators take inspiration from our geneticists and scientists to build awe-inspiring worlds with genetically modified organisms or mythical animals with extraordinary abilities.

How did these amazing beings come to be? and how do these fantastic universes function in the movies?

The solution is easy: they employed Genetic Engineering and other Synthetic Biology approaches to bring these organisms and worlds to the screen for our enjoyment!

Let me present some examples of movies and books you must have watched and read that used unique SynBio ideas to create media that has ended up amassing a huge fan following over the years.



1. **Jurassic Park**

On top of my list is Jurassic Park! I am sure all of you are familiar with Jurassic Park. Who doesn't love watching these gigantic prehistoric beasts recreated on screen? We've all come to adore the Jurassic Park franchise for its incredible depiction of Dinosaurs and the adventures that the protagonists have with them. These Dinosaurs were created using the ancient extracted DNA of the extinct Dinosaurs, integrating the DNA of other modern organisms like frogs, cuttlefish, and snakes to fill the gene sequence gaps and then cloning them using artificial eggs.

2. **Amazing Spider-Man**

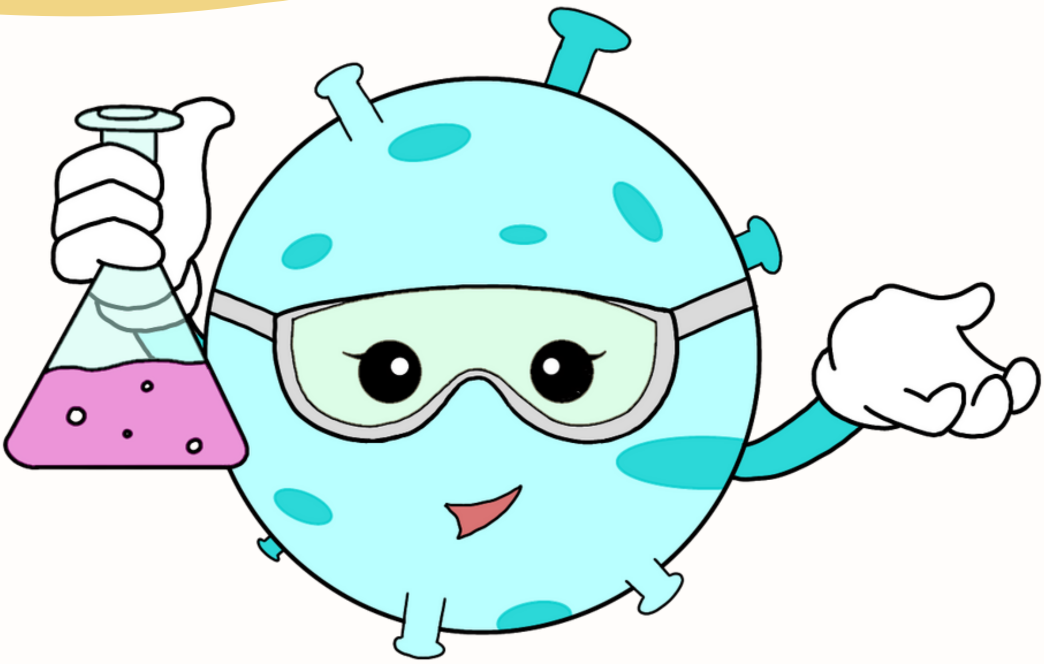
Our favourite web-slinging superhero got his superpowers after being bitten by a genetically modified spider. Although this is a far-fetched and implausible idea of genetic engineering, the base concept of altering the spider's DNA to make it such that it becomes fluid enough to combine with the genetic material of any organism derives its inspiration from Genome Editing techniques that are used in real life by scientists to edit an organism's DNA.

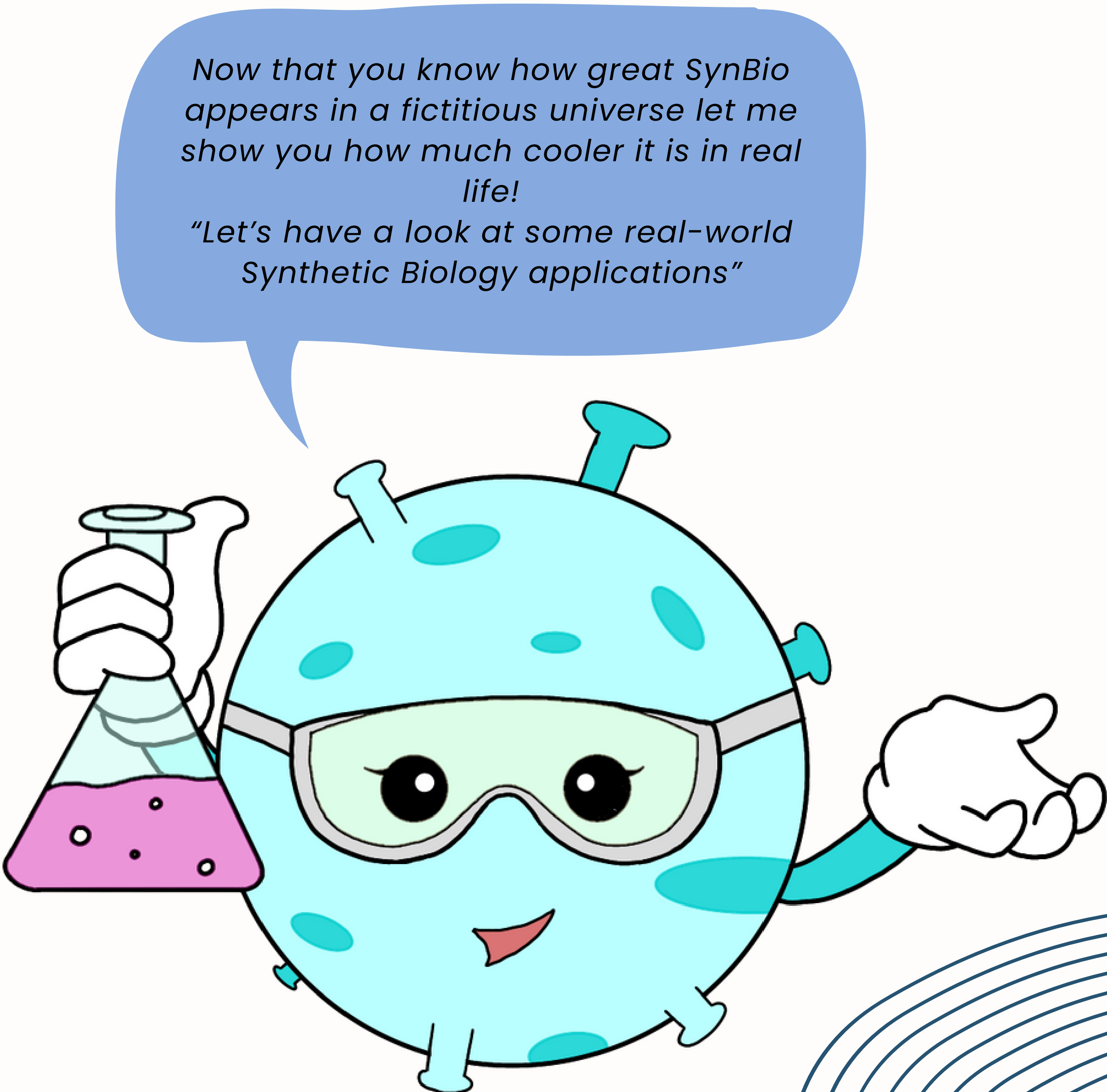
3. **Frankenstein**

This classic science fiction novel, also known as the first science fiction story, tells the story of a scientist on a mad quest to create life; this is accomplished through intense genetic modifications and combining the genetic material of various organisms; however, it results in the creation of the monstrous organism. It shows how genetic experiments frequently go wrong if not carried out professionally and using accurate scientific methodologies.

Dearest Friends, it's important to remember that these examples aren't scientifically accurate depictions of synthetic biology in the real world. They take creative liberties and often invent scientific elements for storytelling. So, while they're great for entertainment, please don't take them as factual representations of genetic engineering as it exists today. Believe me; real-world Synthetic Biology is much more awesome!

Can you think of other fascinating examples of Synthetic Biology in the media?




Now that you know how great SynBio appears in a fictitious universe let me show you how much cooler it is in real life!
“Let’s have a look at some real-world Synthetic Biology applications”

SynBio in Actuality

1. **GMOs:** Genetically Modified Organisms- These are plants, animals, or microbes whose genetic material has been altered to perform functions they would not naturally accomplish. Most of these GMOs have been modified for human consumption and utility, while some are limited to use as 'Model' organisms to study the functions of specific genes.

- *GM Salmon*- This fish has been genetically modified to continuously produce growth hormone, allowing it to be sold as a large adult fish despite being in its juvenile stage. Isn't this awesome?

- *GM Crops*- Various crops have also had genetic alterations made to them. This allows them to grow faster, even in harsh conditions with minimal resources. It also gives them resistance to pests so that the use of chemical pesticides can be reduced.

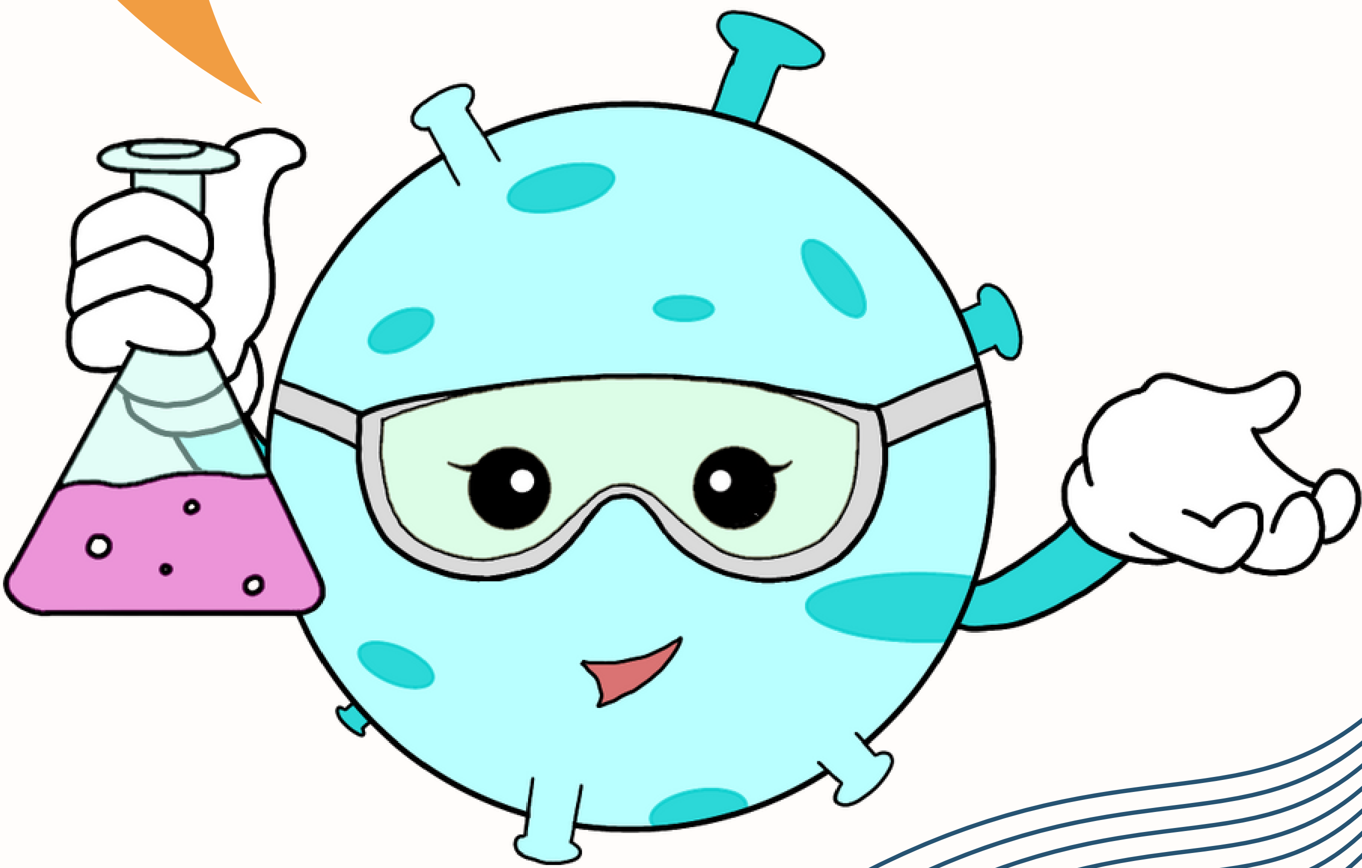
- *GM Mosquitoes*- In laboratory, trials on genetically manipulating mosquito species to reproduce weak progeny are underway; however, if these GM mosquitoes mate with natural species, a hybrid species may emerge. This is an excellent example of genetic engineering having harmful consequences if not done responsibly.

2. **Gene Therapy**- Gene therapy attempts to cure sickness or increase your body's capacity to fight disease by replacing a broken gene or adding a new gene. Gene therapy can potentially cure various ailments, including cancer, cystic fibrosis, heart disease, diabetes, haemophilia, and AIDS. An example of Gene Therapy is when Adenosine Deaminase Deficiency(ADA) is treated by inserting the gene responsible for producing adenosine deaminase into the deficient lymphocytes extracted from the affected individual. These modified lymphocytes are then injected back into the patient.

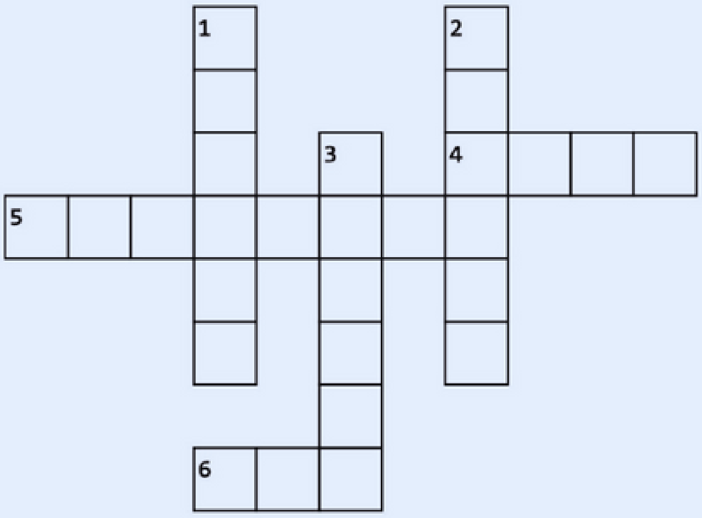
3. **Transgenic Animals**- These animals have successfully incorporated and accepted foreign-engineered DNA from another organism. Such an organism can pass on the 'transgene' to all of its offspring. These animals are used to research gene functions and their impact on live bodies, most notably humans. They are also used extensively in developing medicines as newly developed drugs can first be tested on transgenic animals before they are administered to humans. The most common transgenic animals are mice and rats, followed by other animals like pigs, sheep, fish, and dogs.

4. **Genetically Engineered Insulin**- This is a well-known example of SynBio in Medicine; the genes that generate the insulin chains were inserted into E.coli plasmids, and the E.coli bacteria subsequently made insulin, which was extracted and used to treat Type 2 Diabetes in humans.

We've explored the marvels of genetic engineering and the endless possibilities it offers, but don't you wonder if all of these trials are successful? Are they safe? With these insane mind-boggling processes around them, how can scientists keep track of the regulations? What happens if something goes wrong? After all, we are tinkering with Life's Blueprint! To answer your concerns, scientists must adhere to stringent rules and Biosafety Protocols to avoid something terrible from occurring. They must also address the Bioethics of Synbio practices



Biogrid



Across

4. set of instructions that help perform a function.

5. the superstars of Synthetic Biology

6. the tool that helps in gene assembly

Down

1. vehicle of DNA

2. glue of DNA

3. the infinity gauntlet of gene editing

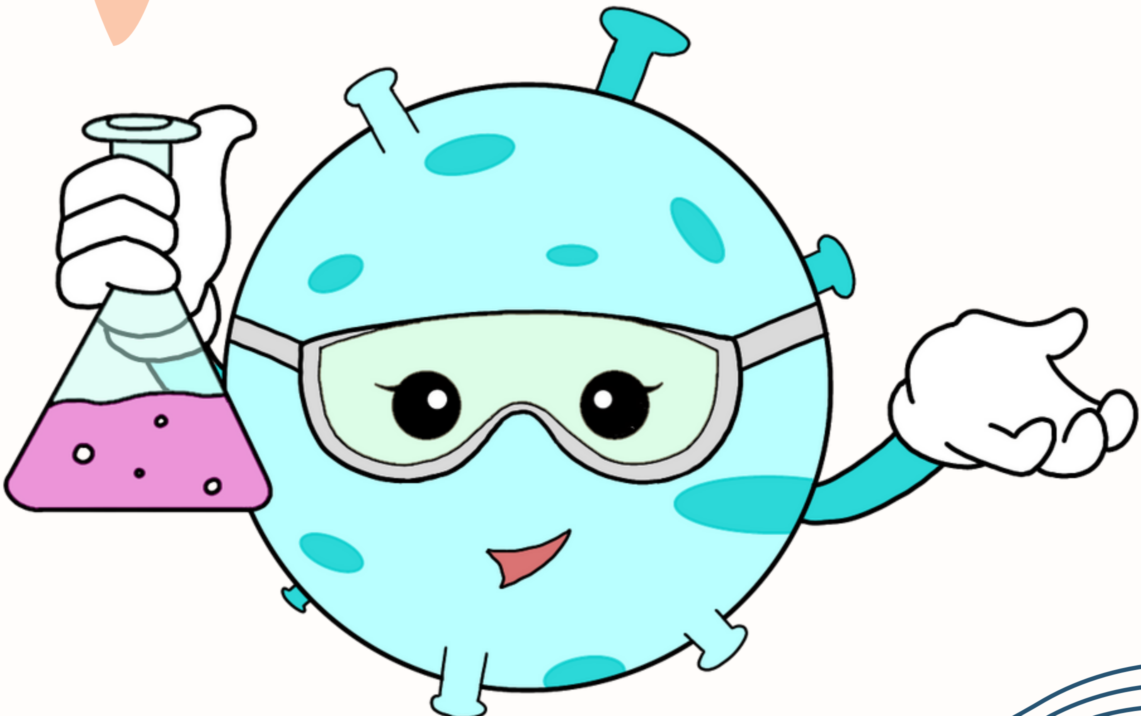
BioSafety and Bioethics

Human manipulation of living genetic components is a complicated process that needs strict laws to guarantee that when these technologies and practices are released, they do not disrupt the natural environment or ecosystems.

- Biosafety refers to the measures and precautions scientists must take to ensure the safe handling and containment of these genetically modified organisms. Like superheroes who protect the innocent, Biosafety Protocols shield us from potential risks and ensure that synthetic biology remains a force for good, preventing abuse and heedless experiments. When we manipulate genes and engineer life forms, there is always a chance that something unexpected could happen. Researchers follow strict guidelines and regulations to prevent accidental release or unintended consequences that could harm our environment, ecosystems, or human health.
- Bioethics is concerned with the moral and ethical issues that arise when we seek to change the fabric of life and customise natural rules. When experiments include live biological systems, it is critical to follow a code of conduct, and researchers must think about the many outcomes that may occur from the information and innovation they bring to synthetic biology.

To ensure that the BioSafety and Bioethics Regulations are followed, various governing organisations are established at both the global and national levels.
One such organisation in India is the GEAC- Genetic Engineering Appraisal Committee, a regulatory body that approves the GMOs before they are released or used for commercial purposes.
On the global level, The International Bioethics Committee oversees the progress and activities related to life sciences and Synbio. It was set up by UNESCO to evaluate the methods, processes, and techniques carried out on biological systems so that no unethical and destructive procedures are performed.

Dearest Friends, it makes me immensely happy that we took this journey of exploring the captivating world of SynBio, and now that we are nearing its end, you all must remember to be thoughtful in your actions when dabbling with Synthetic Biology techniques because, as Uncle Ben told Spidey- With great power comes great Responsibility! Continue reading to discover about iGEM, an organisation dedicated to make the world a better place by encouraging young people like you to love and learn about SynBio.



iGEM stands for International Genetically Engineered Machine.
It is an annual synthetic biology competition that invites students worldwide to design, create, and test innovative biological systems utilising genetic engineering techniques. The teams attempt to solve issues ranging from pollution and climate change to serious medical problems like coronary artery disease.

iGEM aims to bring together diverse communities of students across the globe to promote synthetic biology as a tool to solve global problems. iGEM isn't just a competition, it is an international organisation that has started many initiatives to foster an aptitude in Synbio and spread awareness about its many facets, especially amongst the youth. It also provides a platform for participants to collaborate and exchange ideas with fellow students, researchers, and industrialists.

The iGEM Grand Jamboree is the competition's grand finale. All short-listed teams assemble to display their projects and compete for thrilling awards in categories like Best Presentation, Best Wiki, and many more.

iGEM's High School Division:
iGEM has a special division for high school students enabling them to engage in synthetic biology. This is an excellent opportunity for students who are interested in Synbio to dabble with various techniques and develop a project they are passionate about.
Students get a head-start on developing their scientific skills and gain practical laboratory experience designing and assembling functional biological systems.

iGEM and IISERB

Since 2018, IISERB has been participating in iGEM, creating ingenious biological systems as solutions to tackle a variety of problems.

- The iGEM 2022 Project, called Stubburn, endeavoured to overcome the problem of burning crop residue to clear land for the next cropping season. The bacteria Bacillus subtilis was genetically modified to break down the stubble of diverse crops such as rice, wheat, soybean, and millet. This alternate strategy aimed to significantly cut carbon emissions, which have grown due to smoke clouds produced by agricultural residue burning. The engineered bacillus strain also assisted in extracting vanillin from partially decomposed wheat and paddy. This vanillin may subsequently be utilised to produce bioplastic. Stubburn is an innovative technique to solve two problems simultaneously with a cheaper economic value.

- IISERB 2021 Project, named BLAST, intended to lessen cancer burden by considerably shrinking tumor size using an efficient anticancer therapy that induces tumor-cell-specific apoptosis while causing no harm to normal cells. The approach was to take advantage of Bifidobacterium longum. This obligate anaerobe has been proven in vivo to cluster in hypoxic tumor areas and release tumor-specific apoptosis-inducing peptides in the tumor microenvironment.It proved to be a successful attempt at cancer therapy by using synthetic biology tools.

Check out the Wikis of STUBBURN & BLAST to know more about the dynamic efforts team IISERB put into creating these ambitious projects -

https://2022.igem.wiki/iiser-bhopal/
https://2021.igem.org/Team:IISER_Bhopal

iGEM's Entrepreneurial Activities

- iGEM supports the entrepreneurial ambitions of its participants through the iGEM StartUp Division. It funds and boosts projects into high-impact enterprises that address the most significant global challenges. To encourage BioEconomic growth, iGEM provides a platform to aspiring BioFounders by organizing conferences, StartUp Showcases, Summer School, BioHackathon, and BioInnovation Fair. These events act as a platform for participants to exchange ideas and create robust networks.

- From the initial spark of an idea to the final presentation at the Jamboree, iGEM encourages students to explore biotechnology and the frontiers of responsible scientific advancement. Its mission is to encapsulate the transformative power of Synthetic Biology and make it accessible to students worldwide.

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