CONTENTS

1)BACTERIAL GENETICS

Amino acids, Structure of DNA, nucleic acids, then we go on Central dogma

Protein

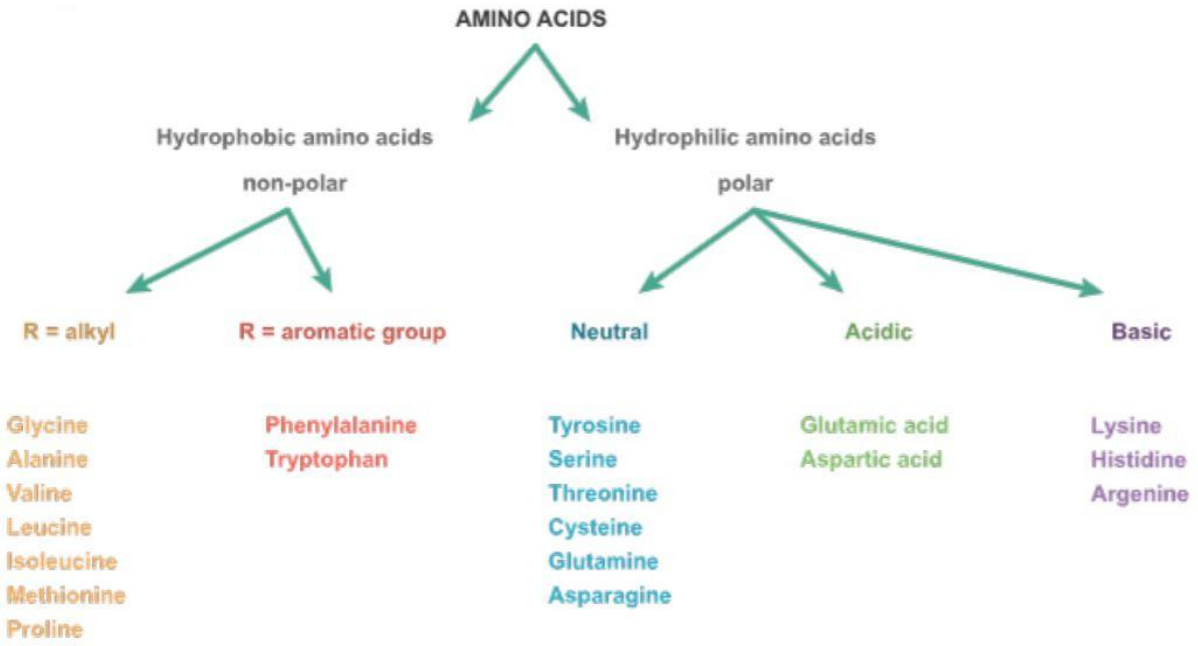
- 20% of the human body is made up of proteins. They are the large and complex molecules which have a critical role for normal functioning of cells.
- They are crucial for the maintenance of structure, function, and regulation of the tissues and organs.
- Amino acids are building blocks of proteins. Peptide bonds enables them to attach to one another and forming a long polypeptide chain.

Amino acid structure and its classification

• An amino acid molecule contains both a carboxyl group and an amino group. The amino group bonded directly to The alpha-carbons are called alpha-amino acids.
• The alpha carbon, Cα; bonded to a carboxylic acid, –COOH group; an amino, –NH2 group; a hydrogen atom; and an R group, and this is a unique feature for every amino acid

Classification of amino acids

- Based on the nature of their ‘R” group



Nucleic acids

Nucleic acids are targets of many important drugs, including several anticancer agents. There are two types of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA encodes the hereditary details and controls the growth and division of the cells. The genetic information stored in DNA is then transcribed into RNA, and the details in RNA are then translated for the synthesis of the proteins.

Nucleosides and Nucleotides

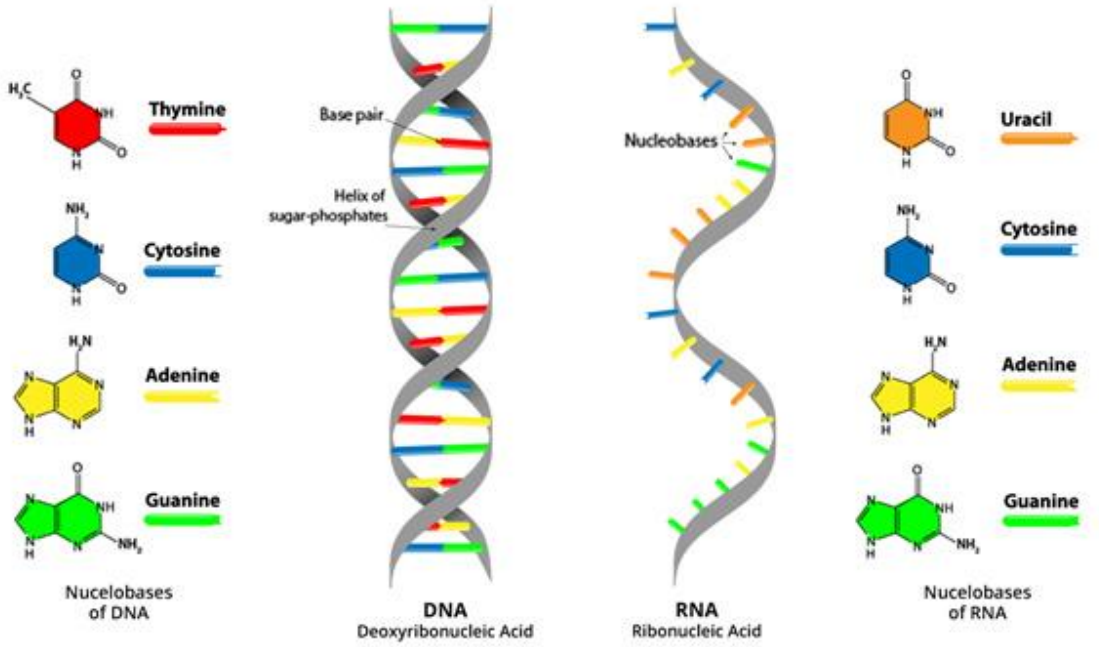
Nucleic acids are a five-membered ring of sugars linked by phosphate groups. The anomeric carbon of each sugar bonded to nitrogen of heterocyclic amine in a β-glycosidic linkage. In RNA, the five-membered sugar is D-ribose. In
DNA, the five-membered sugar, is 2'deoxy-D-ribose.
The difference in heredity among the species is determined by the sequence of the DNA. The 4 bases in DNA are : adenine, guanine, cytosine, and thymine. RNA also contains four bases. Three-adenine, guanine, and cytosine- are the same as in DNA. But the 4th base in RNA is uracil instead of thymine.
A compound with D-ribose or 2'-deoxy-D-ribose bonded with base is called nucleoside. Nitrogen bases guanine, cytosine, uracil, and thymine can bond to the sugar to give the corresponding nucleotides. Most commonly β linkage is the stereochemistry of the linkage between the base and the ribose sugar.
A nucleotide is a nucleoside with either the 5' or the 3'-OH group bonded in an ester linkage to phosphoric acid. The nucleotide where the sugar is D-ribose is called ribonucleotide, whereas the nucleotide with 2'-deoxy-D-ribose is called deoxyribonucleotide

Nucleic acids are polymers composed of nucleotide subunits linked by phosphodiester bonds. DNA and RNA are polynucleotides. Nucleotide triphosphate serves as a substrate precursor for the biosynthesis of nucleic acids. The nucleotides linked by the nucleophilic attack of 3'-OH of one nucleotide triphosphate on the - phosphorus of another nucleotide.

Genetics is the study of genes including the structure of genetic materials, what information is stored in the genes, how the genes are expressed and how the genetic information is transferred. Genetics is also the study of heredity and variation. The arrangement of genes within organisms is its genotype and the physical characteristics of an organism based on its genotype and the interaction with its environment, make up its phenotype. The order of DNA bases constitutes the bacterium's genotype. A particular organism may possess alternate forms of some genes. Such alternate forms of genes are referred to as alleles. The cell's genome is stored in chromosomes, which are chains of double stranded DNA. Genes are sequences of nucleotides within DNA that code for functional proteins. The genetic material of bacteria and plasmids is DNA. The two essential functions of genetic material are replication and expression

Structure of DNA

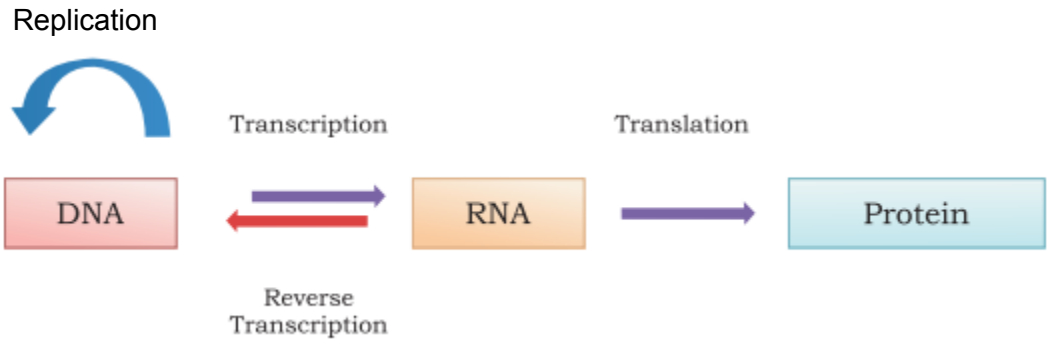
The DNA molecule is composed of two chains of nucleotides wound around each other in the form of "double helix".Double-stranded DNA is helical, and the two strands in the helix are antiparallel. The backbone of each strand comprises repeating units of deoxyribose and phosphate residue. Attached to the deoxyribose is purine (AG) or pyrimidine (CT) base. Nucleic acids are large polymers consisting of repeating nucleotide units. Each nucleotide contains one phosphate group, one deoxyribose sugar, and one purine or pyrimidine base. In DNA the sugar is deoxyribose; in RNA the sugar is ribose. The double helix is stabilized by hydrogen bonds between purine and pyrimidine bases on the opposite strands.



Ref: DNA vs. RNA – 5 Key Differences and Comparison December 18 2020 | by Ruairi J Mackenzie, Editor for Technology Networks

Central Dogma

The most important work of the cells is to synthesize proteins based on the codes present in the genes in DNA. Let's understand how these codes are read and how proteins are synthesized based on these codes.



Replication

DNA Replication: process of copying and synthesizing new DNA.. It Occurs in the nucleus before a cell divides so that each new cell produced has a complete set of chromosomes. It

takes place in the S phase of the replication. The replication begins at the Origins of Replication, the two strands open to form the Replication Forks (Y-shaped region). Now the new strands grow at the forks.There are several enzymes involved in this process such as helicase, DNA polymerase and ligase. HELICASE unwinds parental double helix .DNA POLYMERASE binds nucleotides to form new strands .LIGASE joins Okazaki fragments and seals other breaks in sugar phosphate backbone. Basically there are 4 steps involved in this process. The helicase unwind the DNA at ORI by breaking the hydrogen bonds.Before new DNA strands can form, there must be RNA primers present to start the addition of new nucleotides, now the DNA polymerase binds to each of the parental strands and starts to add nucleotides to the primer.The replication happens in the 5 ‘ to 3’ direction. In the 3’ to 5’ parental strand, the replication happens in fragments because replication moves in only one direction i.e. 5’ to 3’. So, small DNA segments called OKAZAKI FRAGMENTS are made on this strand.The final step is by DNA LIGASE to seal up all of the gaps in the DNA molecule, like the Okazaki fragments.

Complementarity
Adenine(A) always pairs with thymine(T) in case of DNA and in RNA to uracil(U) and guanine (G) with cytosine(C), in both DNA and RNA. Between adenine and thymine or uracil 2 hydrogen bonds and between guanine and cytosine 3 hydrogen bonds are formed. So during replication the primer attached to the parental strand for elongation of the strand, they follow this rule, now when it has to elongate it again follow the rule of bonding, in this way the system can conserve its genetic composition.
Say the parental strand is 5’ GAATTCCAAA 3’ ,then the complementary DNA strand during replication will be 3’ CTTAAGGTTT 5’. In case of transcription of the former sequence when DNA is transcribed into RNA , the complementary RNA strand would be 3’ CUUAAGGUUU5’.

Transcription

Transcription means the generation of RNA from DNA using RNA polymerase. The RNA polymerase unpairs and unwinds DNA as it reads. However, this process is less accurate than replication errors. This will not affect protein synthesis really because protein synthesis tolerates some errors. In this process, multiple RNAs can be sequenced from the same gene at the same time. It is determined by the cell type, environment, and other factors.
In bacteria:
• RNA polymerase binds to specific nucleotide sequences regions of the DNA called promoters,
• Promoters helps orient the polymerase in definite directions
•With the assistance of an accessory protein, called a sigma factor RNA polymerase binds to the promoter region of the DNA.
• RNA transcript is produced by ribonucleotide triphosphate additions
• Synthesis of RNA stops at the terminator sequence, usually of a poly A-T stretch of DNA
In eukaryotes, the situation is different in several ways:
1. There are different types of RNA polymerases, depending on the product is protein or RNA
2. RNA polymerase needs several helper proteins to bind to DNA to initiate RNA synthesis, such as transcription factors
3. Transcription factor binds to a specific DNA sequence upstream 25 nucleotides of the coding region for the protein TATA sequence or TATA box

4. Other proteins gather to form a large transcription complex
5. Chromatin-remodeling proteins are required in making DNA available from the wound histone structure
6. RNA chemically altered with methylated guanine at the 5' end and a poly-A sequence at the 3' end these serve the ribosome later to assure that the full recipe for a protein is present.
7. RNA is processed after synthesis by splicing to eliminate the non-coding regions termed introns
8. The nuclear pore complex selectively transports complete, spliced mRNA molecules to the cytosol
Splicing has evolutionary advantages, like increasing the diversity of proteins produced from a single gene. It also enables the formation of new proteins from combinations of different genes separated by long non-coding regions

Codon

A set of three base pairs constitutes a codon, which codes for a single amino acid. The "triplet code" is said to be degenerate or redundant because more than codon may exist for the same amino acid. For example, the codons AGA, AGG, CGU, CGC, CGA and CGG all code for arginine. There are 64 codons, of which 3 (UAA, UAG and UGA) are nonsense codons. They don't code for any amino acid, but act as stop codons. There are specific codons which code for start and stop sequences. The start codon (AUG) indicates the beginning of the sequence to be translated, and the stop codons (UAA, UGA, UAG) terminate the protein synthesis. With the exception of methionine, all amino acids are coded for by more than one codon. The DNA in a gene that are expressed into the protein product are called exons and the non-coding DNA segments are called introns. There are no introns in the bacterial chromosome. A segment of DNA carrying codons specifying a particular polypeptide is called a cistron or a gene.

Translation

The translation is the method of polymerization of amino acids to form a polypeptide. The order and sequence of amino acids are described by the sequence of bases in the mRNA. In a polypeptide, the amino acids are joined by a bond known as a peptide bond. The formation of a peptide bond needs energy. Hence, before the process, the amino acids are activated in ATP, which is then linked to their related tRNA, usually called the charging of tRNA or aminoacylation of tRNA. If two such charged tRNAs are brought close enough, the formation of a peptide bond between them would be promoted energetically. The presence of a catalyst would improve the rate of peptide bond formation. The ribosome is the cellular factory accountable for synthesizing proteins. It is composed of structural RNAs and about 80 distinct proteins. It exists as two subunits in its inactive state, a large and a small subunit. When the small subunit faces an mRNA, the process of translation of the mRNA to protein starts. There are two sites for the amino acids to bind to and thus, be close enough to form a peptide bond in the large subunit. The ribosome also functions as a catalyst (23S rRNA in bacteria is the enzyme- ribozyme) to

make the peptide bond.The RNA sequence, which is flanked by the start codon (AUG) and the stop codon and codes for a polypeptide, is the translational unit in the mRNA. An mRNA also has some extra sequences that are not translated and are called untranslated regions (UTR). The UTRs are present at both 5' -end (before start codon) and 3' -end (after stop codon). They are required for an adequate translation process. The ribosome binds at the start codon (AUG) od]f the mRNA for initiating the process, identified by the initiator tRNA. The ribosome continues to the elongation phase of protein synthesis. In this stage, complexes composed of an amino acid linked to tRNA sequentially bind to the proper codon in mRNA by forming complementary base pairs with the tRNA anticodon. The ribosome runs from codon to codon along the mRNA. Amino acids are joined one by one, translated into polypeptide sequences directed by DNA and represented by mRNA. In the end, a release factor binds to the stop codon, terminating translation and delivering the whole polypeptide from the ribosome.

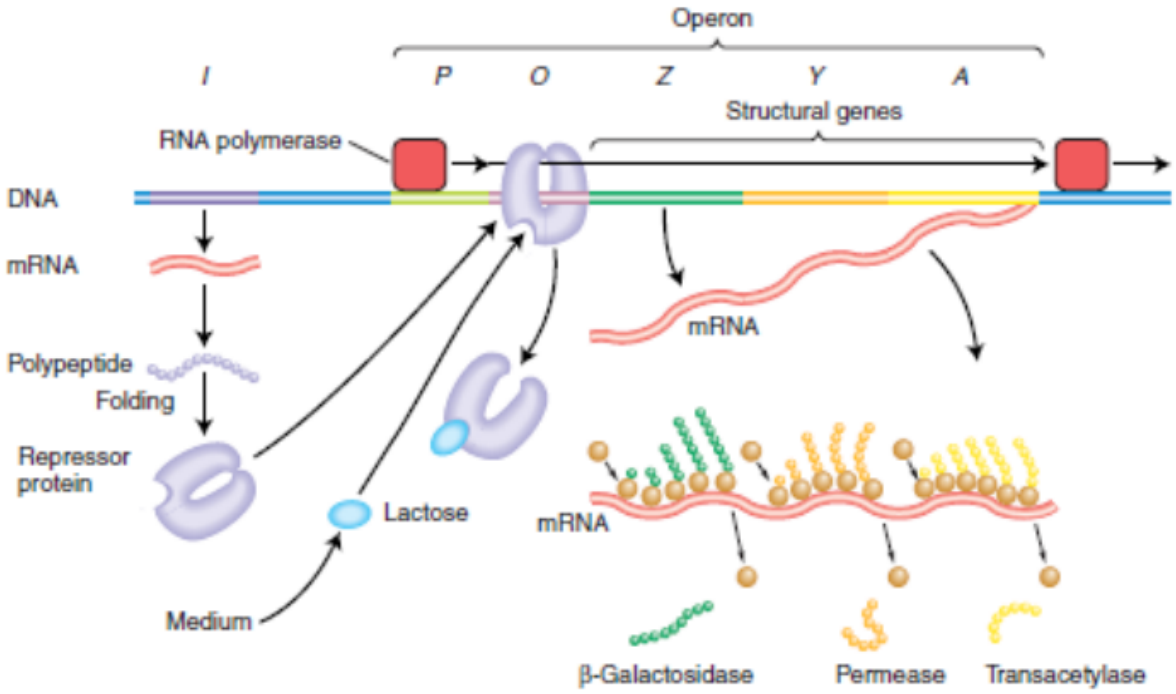
Structure of chromosome

In contrast to the linear chromosomes found in eukaryotic cells, most bacteria have single, covalently closed, circular chromosomes. Not all bacteria have a single circular chromosome: some bacteria have multiple circular chromosomes, and many bacteria have linear chromosomes and linear plasmids. Multiple chromosomes have also been found in many other bacteria, including Brucella, Leptospira interrogans, Burkholderia and Vibrio cholerae. Borrelia and Streptomyces have linear chromosomes and most strains contain both linear and circular plasmids. The chromosome of E coli has a length of approximately 1.35 mm, several hundred times longer than the bacterial cell, but the circular DNA is then looped and supercoiled to allow the chromosome to fit into the small space inside the cell.

2)NUCLEIC ACIDS, PROTEINS

Ques for the day : Do you think bacteria eat sugars? And if so, where do the get these sugars from?

Operon concept



The lac Operon (BIOT 4006: Genetics and Molecular Biology) : Dr. Saurabh Singh Rathore Department of Biotechnology MGCU

Some proteins (or enzymes) are always required by a bacterium, genes coding for such proteins are constitutively expressed. These genes are usually needed for the cell to survive.

Other genes that may not be needed at all times are regulated to conserve energy and cellular materials. Some proteins (or enzymes) are produced only when the need arises or when stimulated by certain environmental conditions. Such genes are normally repressed and are induced whenever required.

Repression is a method of inhibiting or decreasing the expression of specific genetic products. This inhibition is controlled by proteins called repressors, which usually block the binding of RNA polymerase to the template DNA. Induction is the opposite of repression, inducers act to “turn on” genes that are not constitutive.
The operon concept was first demonstrated by Jacob and Monad. Bacteria utilize a special energy saving system of genetic control called operons. The operon is a sequence of DNA that contains multiple genes used to produce multiple proteins for a single purpose.
An example of an operon is the lac operon in E. coli.

In order to break down lactose, E. coli must use a series of enzymes (beta-galactosidase, galactoside permease and transacetylase). The genes for these three enzymes are located in a row on the DNA and share a single promoter. Genes determining structure of a particular protein are called structural genes and the activity of structural genes are controlled by regulator genes, which lie adjacent to them. The genes lacZ, lacY and lacA which code for the three enzymes are the structural genes. lacI gene codes for the repressor protein, hence is the regulator gene. Between the lacI gene and the structural genes lie promoter and operator genes. For transcription of the structural genes, the enzyme RNA polymerase first has to bind to the promoter region. The operator region lies in between the promoter and structural genes and the RNA polymerase has to go through the operator region. Under normal circumstances, when the structural genes are not transcribed, the repressor protein is bound to the operator region thus preventing the passage of RNA polymerase from the operator region towards the operon. When lactose is available in the environment, the repressor protein leaves the operator region and binds to lactose because it has high affinity for lactose. This frees the operator region and the RNA polymerase enzyme moves towards the operon and transcribes the structural genes. The products of structural genes result in the metabolism of lactose. When lactose is no more available, the repressor protein goes back and binds to the operator region, thus stopping further transcription of structural genes. This way lactose acts both as inducer as well as a substrate for beta galactosidase

Secondary Structure:

DNA is a double-stranded nucleic acid, with the sugar-phosphate backbone outside and the base inside. The chains are held together by H- bonding between the base of one strand with the base of another strand. Adenine pairs with thymine, while guanine pairs with cytosine through two and three H-bonds, respectively. This means if we know the sequence bases in one strand, we will be able to sequence the bases in the other strand. If the two strands run in opposite directions, the strands are not linear. Instead, they are twisted into a helix around the common axis, which is known as a double helix.

3) PLASMID DESIGN AND ITS INTAKE BY HOST

Do you know about bioluminescent jellyfish?

They are jellyfishes which can produce light on their own. But have you ever wondered whether other organisms can also produce light on their own like these jelly fishes? Well this thought had struck the scientists too and they went on to develop genetically engineered mice which could glow. They took the gene found in certain bioluminescent jellyfish responsible for this trait into the DNA of the mice. And the skin, eyes and organs of the mice started glowing. This is an example of genetic engineering. But we have not yet narrated the most critical question: why? Why would one want to genetically manipulate life itself to do something it isn't supposed to? What is the advantage? What are the barriers we face in trying to tackle real-world issues using synthetic biology and bioengineering? Let us study each of the inquiries you may

have surrounding "why." The goal of synthetic biology research is to solve real-world obstructions using life itself. Nature presents us with a limitless list of structural proteins, enzymes, and genomic elements that allows us to direct almost any physical concern reasonably using biological molecules. Synthetic biologists will often find a protein from an organism that permits severe environments and will then introduce it into a harmless, non-pathogenic organism, such as E. coli K-12, so it can execute its function and have no further consequence.

All these are done by a method called molecular cloning. Molecular cloning is a set of methods, which are used to insert recombinant DNA into a vector. Vector is a carrier of DNA molecules that will replicate recombinant DNA fragments in host organisms. Now, to insert our gene of interest into our organism, we insert the target gene into a circular piece of DNA called a plasmid which we then insert into our target organism to express the required traits.

Q : Name some round objects, can there be a round DNA ?

Plasmid

Plasmids are small circular pieces of DNA that can replicate independently from the host's chromosomal DNA. They are mainly found in bacteria and exist naturally in archaea and eukaryotes such as yeast and plants. In nature, plasmids provide more functional benefits to the host, such as resistance to antibiotics, degradative functions,
and virulence. All-natural plasmids contain an origin of replication (which controls the host range and copy number of the plasmid). They typically include a gene that is advantageous for survival, such as an antibiotic resistance gene. In contrast, plasmids utilized in the lab are usually artificial and designed to introduce foreign DNA into another cell. Minimally, lab-created plasmids have an origin of replication, selection marker, and cloning site. The ease of modifying plasmids and the ability of plasmids to self replicate within a cell make them attractive tools for the life scientist or bio engineer.
Due to their artificial nature, lab plasmids are referred to as "vectors" or "constructs."To insert a gene of interest into a vector, scientists may utilize a variety of cloning methods (restriction enzyme, ligation independent, Gateway, Gibson, and more). The cloning method is ultimately chosen based on the plasmid that has to be cloned. Regardless, once the cloning steps are complete, the vector containing the newly inserted gene is transformed into bacterial cells and selectively grown on antibiotic plates.

Introduction to DNA Cloning

There are several steps of DNA cloning.

1. Extraction of DNA from cells
2. Plasmid Purification
3. Amplifying required gene segment using PCR and gel electrophoresis
4. Using restriction enzymes to cut gene segment and plasmids
5. Ligation to form recombinant plasmid
6. Transformation of recombinant plasmid into bacteria to form GMO
7. Blue-white screening

Extraction of DNA

When we perform the cloning experiments the primary raw material we require is our gene of interest. We know that the genome or the whole DNA sequence is so long that we have to cut them into small pieces to incorporate the DNA into the new host.
There are some molecular scissors to cut DNA at specific locations; they are called restriction enzymes. They can cut from the edges of a DNA or in the middle of the strand at a specific sequence called recognition sequence. Now we have obtained the small fragment of our gene of interest. When we cut the DNA with this enzyme it leaves a sticky end, ie some sequence flanks as single stranded
Now this DNA can't be transfected to the host cell directly for 2 main reasons, chance for intake will be less and secondly to check whether the gene of interest has been incorporated so that we have carriers or vectors. There are many types of vectors like Plasmid, cosmid, phagemid etc. For inserting our gene of interest which was cut with the restriction enzymes, we have to cut the vector also with the same restriction enzyme. Now we obtain a similar sticky end for the vector also. Due to complementarity of sequence both the vector and the DNA can bind together. Since this is happening in an external condition, there will be a nick between the vector and the DNA. In order to glue it together, we need molecular glue, i.e. ligase. The ligase will fix the nick and leave the vector- DNA complex.

For this step, traditionally, we can extract the DNA from the cells through an experiment and even using reagents available at home. We will explain this later in a video. But generally, in a molecular cloning lab, scientists, order gene sequences from companies who synthesize the gene sequences for them. So we can skip the DNA extraction step and directly move on to the next step!

Plasmid purification

We perform this step to extract the plasmids from the bacterial cells and to separate the genomic DNA from the plasmid DNA. We get plasmid purification kits which help us to do this step in the laboratory.

PCR & Gel electrophoresis

Watch 2 videos

Restriction enzymes

Restriction enzymes are also known as molecular scissors as their job is actually to cut DNA at specific sites. Each restriction enzyme recognizes a short, specific sequence of nucleotide

bases called a recognition/restriction site. These restriction sites are always palindromic in nature i.e. the base sequence reads the same backwards and forwards.
For eg,

The restriction enzyme EcoRI cuts after G from left and T from right in the sequence GAATTC. This cleavage leads to the formation of sticky ends which will help in pasting of the gene segment with the plasmid i.e. ligation in next step. The same restriction enzymes are used to cut both the gene segment and the plasmid to generate the same sticky ends. This will ensure the ligation of insert DNA into the plasmid.

Intake Of Plasmid by Host

Transformation

Exogenous DNA uptake by E Coli cells that changes the phenotype or genetic trait is called transformation. For cells to uptake exogenous DNA, they must first be made permeable so that the DNA can penetrate the cells. This state is known as competency. In nature, certain bacteria become competent due to environmental stresses. We can purposely cause cells to become competent by treating chloride salts of metal cations such as calcium, rubidium, or magnesium and cold treatment. These alterations affect the structure and permeability of the cell wall and membrane so that DNA can pass through. However, this makes the cells very fragile, and so they must be treated carefully while in this state. Competent cells differ in how well they take up DNA. We can express this. The number of cells transformed per 1 µg of DNA is called the transformation efficiency. Too little DNA can result in low transformation efficiencies, but too much DNA also hinders the transformation process.
E. coli from the source plate resuspended in an ice-cold $CaCl_2$ solution. Plasmid DNA is added to half of the cells before “heat shocked” in a 42°C water bath. The heat-shock step helps the entry of DNA into the bacterial cells. Recovery Broth added to the cell suspension, and the bacteria are permitted to recover for 30 minutes at 37°C. This recovery period enables the bacteria to repair their cell walls and to express the antibiotic resistance gene. Finally, the transformed E. coli are plated on LB plates and permitted to grow at 37°C overnight

Transduction

This process involves the introduction of genes into the host cell’s genome using viruses as vectors. The viruses used in gene transfer due to the following characteristics
• Efficiency of viruses to deliver their nucleic acid into cells
• High level of replication and gene expression. The foreign gene is packed into the virus particles to enter the host cell. The entering of virus particles containing the gene sequences of interest into the host cell and then to the nuclear genome is a receptor-mediated process. The vector genome undergoes complex processes ending up with ds-DNA depending on the vector that can persist as an episome or integrate into the host genome, accompanied by the expression of the candidate gene

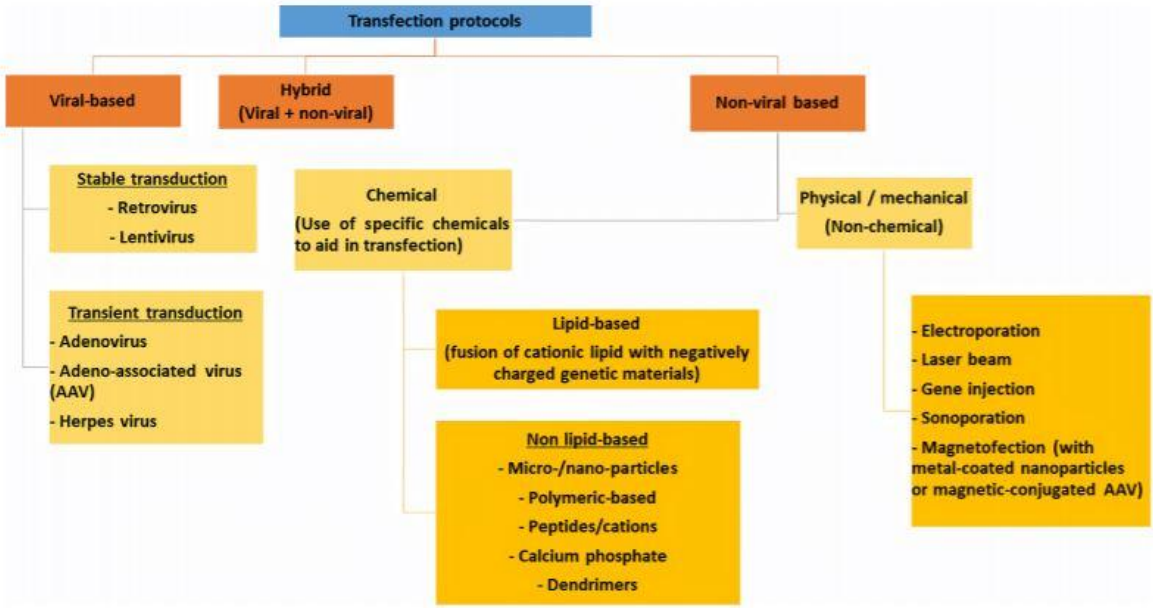
https://study.com/academy/lesson/bacterial-transformation-screening-and-interpreting-experimental-results.html

Transfection

Transfection is a process that forces nucleic acids into a cell.
Different methods can be used for transfection, such as chemical, physical or biological methods.
The transfected nucleic acids may stay transient or stable in the cell, dependent on the method of transfection

Selecting Bacteria with recombinant plasmids

The strain of E. coli bacteria used in gene cloning will be susceptible to all antibiotics. The only way these bacteria will be able to grow on media that contain the Ampicillin antibiotic is for them to acquire resistance when they are transformed with a plasmid. If an antibiotic-sensitive bacteria is transformed with the nonrecombinant plasmid, the bacteria could grow on Amp and produce a blue color. Cells that can grow will divide and divide and form colonies. If the bacteria lack a resistance gene, the antibiotic will either kill the cell or prevent it from dividing. When cloning genes with a plasmid that has the Amp resistance and LacZ genes, the insertion of new DNA occurs in the LacZ gene. Because new DNA will combine with the plasmid in the LacZ gene, recombinant plasmids will have an inactive LacZ gene. Bacteria colonies that have recombinant plasmids will be white, bacteria with the original, nonrecombinant plasmid will be blue. Therefore, the gene cloner will select white colonies from their plates



Ref: https://peerj.com/articles/11165.pdf

4) APPLICATIONS OF SYNTHETIC BIOLOGY & IGEM

APPLICATION OF SYNTHETIC BIOLOGY

So far, we have discussed how we can engineer a bacteria to express proteins of our interest. These engineered organisms can be used for various purposes, for example for food and drug production, pesticide replacement, enhanced nutrient composition, increased crop yields and many more. As for the boundaries of synthetic biology, engineered organisms continue to perform more reliably in their new environments, suggesting the sky's the limit for engineered organisms. The marked outperformance of organisms over conventional chemical methods also gives aid to the world of synthetic biology, with engineered organisms able to be more resilient, clean, and cost-effective.

To effectively express proteins in the organism, we clone the gene into a genetic circuit along with some components into the plasmid. The simplest of the genetic circuit is composed of a promoter, a ribosome binding site, a coding region and a terminator. A promoter is a sequence of DNA needed to turn a gene on or off. The process of transcription is initiated at the promoter. We can choose the promoter to be always on or metal sensitive or stress sensitive upon our wish in accordance with the need of the genetic circuit. A ribosome binding site, or ribosomal binding site (RBS), is a sequence of nucleotides upstream of the start codon of an mRNA transcript that is responsible for the recruitment of a ribosome during the initiation of translation.

The coding region essentially is the gene encoding the protein to be expressed. The terminator stops the translation process.

ETHICS

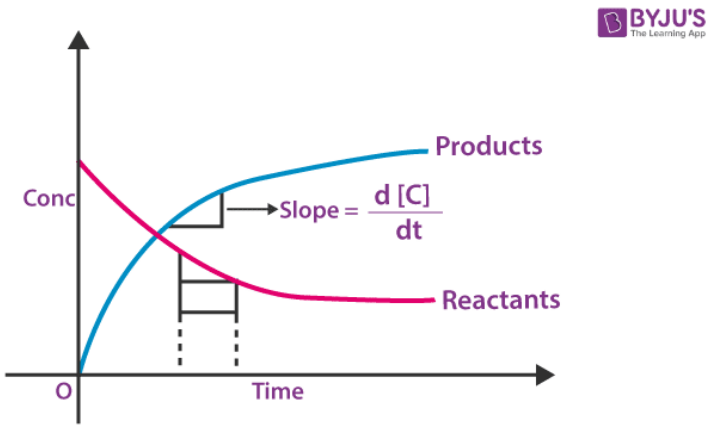
Synthetic biology holds out the possibility of significant benefits to humanity. However, it also raises some significant concerns. These include, for example, concerns regarding laboratory biosafety, the intensification of injustices and challenges that the discipline may pose to existing systems of intellectual property rights. Nevertheless, in our view, three concerns are anticipated to garner the most attention from bioethicists:
(1) concerns about 'playing God,' which have been notable in closely related areas of science;
(2) concerns about undermining the distinction between living things and machines, which attracted early attention from ethicists
(3) concerns about the intentional misuse of knowledge from synthetic biology, which has been among the most obvious concerns raised by non-ethicists
The unethical research study results should not be published

THE TASTE OF INTERDISCIPLINARY

Synthetic biology is solely not concerned with biology only.Quantification of the product, understanding the interaction (in silico), calculating the kinetic parameters, standardization of detection techniques are one of those key aspects that can not be done without mathematical modeling. Using the jugglery of differential equations, systems are defined and while solving them we obtain useful information. [Description-D.E and compare with normal equation for analogy].

Simple chemical Kinetics (for determining the constants in gene production)
Transcription factor,promoter,
Mathematical model is logical representation and quantification of biological processes requires help from other disciplines like chemistry, physics, math and computer science.
Let's look at the chemistry part:

1) Chemical Kinetics:
   Originally a part of physical chemistry, chemical kinetics can be used to understand various biological processes. Applying the knowledge of chemical reactions to biology? How do we do that? Before the application, let's look at some core concepts.
   Whenever a reaction takes place, the concentration of reactants reduced and that of product increases.



(reference: https://byjus.com/jee/chemical-kinetics/)

A + B -> C

Rate of appearance of C = $\Delta[C]/\Delta t$

rate of disappearance of A = $\Delta[A]/\Delta t$

Rate of disappearance of B = $\Delta[B]/\Delta t$

Rate of reaction = $(-1/a)*\Delta[A]/\Delta t = (-1/b)*\Delta[B]/\Delta t = (1/c)*\Delta[C]/\Delta t$

Average and instantaneous rate: The rate of reaction can be classified into average and instantaneous rate depending on the amount of time period.

If the time period taken is finite, then it's called average rate:r_{avg} = $\Delta[C]/\Delta t$

(Average rate doesn't give exact information in most cases about the completion of the reaction.)

So, for getting a broader insight into the time taken for completion and other purposes, "Instantaneous rate is used, which is represented as,

$$r_{inst} = \lim_{\Delta t \to 0} \frac{\Delta[C]}{\Delta t} = \frac{d[C]}{dt}$$

Now let's dive into the applications:

Growth of microorganisms

Let N = Number of cells

Reaction for cell division: N -> 2N

dN/dt = r*N

Reaction for death of a cell: N -> 0N

$dN/dt = -k_d*N$

Combining together we write,

$dN/dt = r*N - k_d*N$

2) Diffusion of proteins through the cell membrane.

Cell membrane is semipermeable and does not allow the transport of all the molecules.

Simple diffusion: The molecules that can pass through the membrane move from their high concentration to low concentration. (There are other modes of transport, but we

will stick to the simplest one). (if possible an animation for protein diffusion can be added)

The rate of **diffusion** is dependent on (1) temperature, (2) size of the particles, and (3) the size of the concentration gradient.

Diffusion coefficient is (derived using physics) that takes into account the effect of temperature and particle on the rate of diffusion. Higher is the temperature more is the diffusion coefficient and higher is the rate of diffusion. (this is for liquid medium) Diffusion coefficient along with concentration of the molecule can be used to form an equation. This can be used to determine the concentration of protein that has reached the other side and allow us to calculate how much protein should be produced for a fixed amount to come out or vice versa.

3) Use of softwares
    a) Use of python, Matlab for solving the equations and getting graphs. It is sometimes easier to get the code than solving it manually.
    b) Protein modelling

    The 3D structure of protein is necessary in case of understanding its biological activity and to modify it for resourceful purposes. With the advancement of computing and computer graphics, today several 3D structures of proteins are present in databases or data banks. These structures are then superimposed and studied to check the interaction with other proteins or small molecules which act as ligands.

    A very common example of using protein structures is in drug designing. By computational approach, several programs have been designed to predict the binding sites and study interactions between these molecules, this approach is called docking. The protein structures are placed in various orientations and then the best fit structures are ranked according to the score obtained from the algorithm present in the program. Thus, several drug molecules are designed by checking the interaction between different proteins or protein and ligand.

    All functions of proteins are dependent on their structure which gives us the idea about how small molecules build up to an entire organism.

Case study

The designer babies

A designer baby is a baby whose genetic makeup has been selected or altered, often to include a particular gene or to remove genes associated with a disease. This process usually involves analysing a wide range of human embryos to identify genes associated with particular diseases and characteristics, and selecting embryos that have the desired genetic makeup; this process is known as preimplantation genetic diagnosis. But this technique or artificially designing babies with favourable traits are highly unethical both in terms of rights as well as the health complications that can also result due to this procedure.

The other method, artificially designing babies, is to alter the genomic composition of a person by deleting inserting or mutating genes This process is not routinely performed and only one instance of this is known to have occurred as of 2019, where Chinese twins Lulu and Nana were edited as embryos, using CRISPR technology for HIV resistance.

In India there exist many strict laws and ministry arms like the Department of Biotechnology to prevent such actions. Across the world still in many countries can't access live human embryos/zygotes for experiments, in other countries where it is permitted, live embryos after use for experiments are strictly discarded.

IGEM

iGEM, or the International Genetically Engineered Machine Competition, intends to improve the field of applied synthetic biology on a global scale. Teams worldwide investigate and generate synthetic biology solutions to significant problems and present these solutions at a conference, known as the Giant Jamboree, every fall in Boston, Massachusetts. In the past, teams have inscribed every possible issue, from decomposing plastics and gum to purifying water from uranium pollution, developing an organism-based detection method for chlamydia, and purifying contaminated air. Many teams begin with the idea that it became a commercial product when the Giant Jamboree was held. There are over graduate, undergraduate, and even high school teams that participate in iGEM. Nevertheless, science is only half of the iGEM experience. The other half is known as Human Practices, which concentrates on how the projects covered in iGEM hit the world population and ethical concerns surrounding synthetic biology and education. This is all part of iGEM being an "open source" competition, in which the competition between teams is eliminated to allow for maximum scientific progress. Teams collaborate to help accomplish each other's goals and develop the field of synthetic biology to serve humankind. Previously, the 2019 IGEM IISER Tirupati team attempted to engineer an E coli that helps battle colon cancer in a cost-effective and targeted way. In 2020 the team engineered an *E. coli, which* gives it antibiotic degrading abilities, thus releasing the cell lysate of said *E. coli* on-farm excreta to degrade the excess antibiotic in the feces before releasing it to the environment. This year, we are using commensal bacteria in the fallopian tube of humans and engineers to produce an ovum-specific protein that acts on the ovum before fertilization of an ovum and thus causes hardening and acts as a potential female contraceptive. This method is a highly targeted, safe, reversible, and non-hormonal method with minor side effects. We will test our idea by checking the expression level of the protein and thus evaluate the efficiency of our plan before attending iGEM.

Reference

https://ncert.nic.in/textbook/pdf/kebt107.pdf
https://peerj.com/articles/11165.pdf
https://ncert.nic.in/ncerts/l/lebo106.pdf

Day 5

Interesting iGEM projects

1) Glowing tattoos -
   A 2019 iGEM team with their idea BTS made a tattoo that glows when an animal is in stress. It can do so by detecting the stress hormones called Cortisol released in the animal's body during the time of stress. -

   "BTS is an eukaryotic bioluminescent system that
   detects and visulaizes stress level in body.
   If the level of cortisol rises, BTS system will
   cause part of skin to light increasingly."

2) Pan-stain removal - A 2018 iGEM team from RUIA, Mumbai made an engineered bacteria to produce enzymes that can degrade the pan stains that we commonly see around our houses, buildings and footpaths. The bacteria produces certain enzymes that can degrade the colour of the pan stains, making an eco-friendly solution to pan stain problem and saving lakhs of rupees in maintenance charges .
   http://2018.igem.org/Team:Ruia-Mumbai/Description

In our project we aim to address this sociological problem by engineering bacteria to produce combination of enzymes (tannases, gallase and other bond specific enzymes-catechol 1,2 and 2,3 dioxygenases) extracellularly which target the polyphenolic rings in the catechu moiety and hence degrade the colour within few hours of application to the stains. Making it an environment friendly, time-saving, non-labour intensive, wide area application system without involvement of large quantities of water and corrosive chemicals.

3) Thera - PUFA : probiotic solution for COVID 19 :
https://2020.igem.org/Team:William_and_Mary/Description
TheraPUFA is a "smart" probiotic in that it can sense and suppress excess inflammation, which can occur during infection by SARS-CoV-2. TheraPUFA constitutively secretes arachidonic acid (AA), which has both pro- and anti-inflammatory metabolites, then switches to explicitly anti-inflammatory docosahexaenoic acid (DHA) in the case of excess inflammation, as indicated by high levels of pro-inflammatory cytokines. Both AA and DHA have demonstrated antiviral effects against enveloped and positive strand RNA viruses in vitro. Please visit our Design page to learn more about how TheraPUFA works to combat viral infection.

4) Fertility tracking chewing gum - A 2019 iGEM team from Denmark came up with Ovulaid; a hormone-based ovulation test in chewing gum. Using a yeast biosensor, we want to create a product that is comfortable and easy to use, and which gives a precise and comprehensive picture of the entire menstrual cycle.
Thier yeast biosensor will contain receptors detecting *both* LH and estrogen (and potentially in the future progesterone as well), as opposed to most tests currently on the market. When the biosensor detects an increase in LH or estrogen, it will produce a colored compound, changing the color of the chewing gum according to the hormone levels. For easy use, they also plan to create an app to use with the gum. Through a photo of the gum, the app will then precisely determine the intensity of the color and let the user know her fertility status. The results can be logged for future predictions of the fertile window for the individual user. :
https://2019.igem.org/Team:UCopenhagen/Description

5)
Microplastics - A 2016 iGEM team from Harvard University with their project plastiback Used the power of synthetic biology, to harness the natural ability of a microbe called *Ideonella Sakaiensis* to degrade PET and engineered it into a PET detection device. First, Plastiback breaks down PET plastic into nontoxic constituent parts. Then, one of the products of the breakdown, called terephthalic acid (TPA), is fed to a microbial fuel cell which only produces electricity when TPA is present.
http://2016.igem.org/Team:Harvard_BioDesign/Attributions

We want them to answer these 4 questions :-

1) Out of the projects shown, which project did you like the most ?
2) What problem do you want to solve ?
3) Think about how you can solve this problem with the help of synthetic biology?
4) Think about the ethics associated with your idea