# GENETICS

## Methods of Plant Breeding for Crop Improvement:

### Introduction of New Varieties of Plants:

It is a process of introducing high yielding varieties of plants from one place to another. Such plants are called as exotic species. These imported plant materials may carry pathogens and pests, hence they are thoroughly tested in a plant quarantine before being introduced to the fields. e.g Phaseolus mungo was introduced from China.

### Selection:

Selection is one of the oldest methods of plant breeding in which individual plants or groups of plants are sorted out from a mixed population based on the morphological characters.

### Methods of selection:

#### i. Mass selection

Seeds of best plants showing desired characters are collected from a mixed population. The collected seeds are allowed to raise the second generation. This process is carried out for seven or eight generations. At the end, they will be multiplied and distributed to the farmers for cultivation.

Some common examples for mass selection are groundnut varieties like TMV–2 and AK–10. Its schematic representation is given below.

#### ii. Pureline selection

Pureline is "the progeny of a single individual obtained by self breeding". This is also called as individual plant selection. In pureline selection large numbers of plants are selected from a self-pollinated crop and harvested individually.

Individual plant progenies from them are evaluated separately. The best one is released as a pureline variety. Progeny is similar both genotypically and phenotypically.

#### iii. Clonal selection

A group of plants produced from a single plant through vegetative or asexual reproduction are called clones. All the plants of a clone are similar both in

genotype and phenotype. Selection of desirable clones from the mixed population of vegetatively propagated crop is called clonal selection.

**Polyploidy Breeding:**

Sexually reproducing organisms have two complete set of chromosomes in their somatic cells. This is called diploid (2n). The gametic cells have only one set of chromosome. This is called haploid (n). An organism having more than two sets of chromosomes is called polyploid (Greek: Polys = many + aploos = one fold + eidos = form). Such condition is called Polyploidy. It can be induced by physical agents such as heat or cold treatment, X-rays and chemical agents like colchicine.

**Achievements of polyploidy breeding:**

- Seedless watermelons (3n) and bananas (3n).
- TV-29 (triploid variety of tea) with larger shoots and drought tolerance.
- Triticale (6n) is a hybrid of wheat and rye. To make this plant fertile polyploidy is induced. It has higher dietary fibre and protein.
- Raphano brassica is an allotetraploid by colchicine treatment.

**Mutation Breeding:**

Mutation is defined as the sudden heritable change in the nucleotide sequence of DNA in an organism. It is a process by which genetic variations are created which in turn brings about changes in the organism. The organism which undergoes mutation is called a mutant.

The factors which induce mutations are known as mutagens or mutagenic agents. Mutagens are of two types namely physical mutagens and chemical mutagens.

**i. Physical mutagens**

Radiations like X-rays, α, β and γ-rays, UV rays, temperature etc. which induce mutations are called physical mutagens

**ii. Chemical mutagens**

Chemical substances that induce mutations are called chemical mutagens. e.g. Mustard gas and nitrous acid. The utilisation of induced mutation in crop improvement is called mutation breeding.

**Some achievements of mutation breeding are**

- Sharbati Sonora wheat produced from Sonora-64 by using gamma rays.
- Atomita 2 rice with saline tolerance and pest resistance
- Groundnuts with thick shells

**Hybridization:**

Hybridization may be defined as the process of crossing two or more types of plants for bringing their desired characters together into one progeny called hybrid. Hybrid is superior in one or more characters to both parents. Hybridization is the common method of creating genetic variation to get improved varieties.

**Genetic Engineering:**

Genetic engineering is the manipulation and transfer of genes from one organism to another organisms to create a new DNA called as recombinant DNA(rDNA). The term recombinant is used because DNA from two different sources can be joined together. Hence, genetic engineering is also called as recombinant DNA technology.

**Techniques of Genetic Engineering – Basic Requirements:**

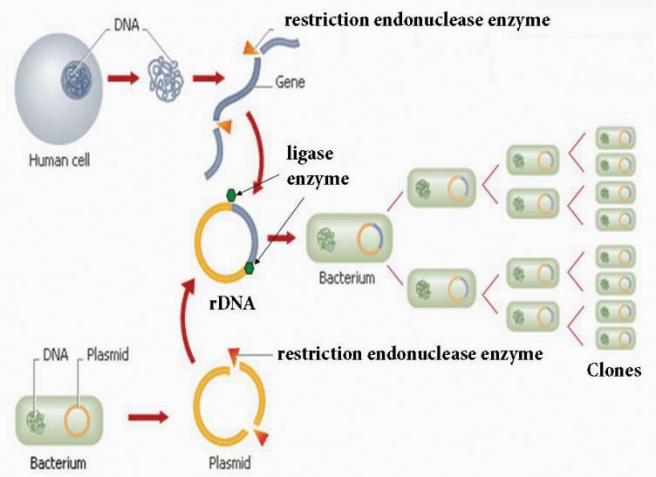
Important discoveries that led to the stepping stone of rDNA technology were

1. Presence of plasmid in bacteria that can undergo replication independently along with chromosomal DNA.
2. Restriction enzymes cuts or break DNA at specific sites and are also called as molecular scissors.
3. DNA ligases are the enzymes which help in ligating (joining) the broken DNA fragments.

**Gene Cloning:**

What reminds to your mind when you hear the word clone? Of course, 'DOLLY' the cloned sheep. The carbon copy of an individual is often called a

clone. However, more appropriately, a clone means to make a genetically exact copy of an organism. In gene cloning, a gene or a piece of DNA fragment is inserted into a bacterial cell where DNA will be multiplied (copied) as the cell divides.




**A brief outline of the basic steps involved in gene cloning are:**

1. Isolation of desired DNA fragment by using restriction enzymes
2. Insertion of the DNA frag2 ment into a suitable vector (Plasmid) to make rDNA
3. Transfer of rDNA into bacterial host cell (Transformation)
4. Selection and multiplication of recombinant host cell to get a clone
5. Expression of cloned gene in host cell. Using this strategy several enzymes, hormones and vaccines can be produced.

**Genetic Disorders:**

A genetic disorder is a disease or syndrome that is caused by an abnormality in an individual DNA. Abnormalities can range from a small mutation in a single gene to the addition or subtraction of an entire chromosome or even a set of chromosomes. Genetic disorders are of two types namely, Mendelian disorders and chromosomal disorders.

**Mendelian disorders:**

Alteration or mutation in a single gene causes Mendelian disorders. These disorders are transmitted to the offsprings on the same line as the Mendelian pattern of inheritance. Some examples for Mendelian disorders are Thalassemia,

albinism, phenylketonuria, sickle cell anaemia, Huntington's chorea, etc., These disorders may be dominant or recessive and autosomal or sex linked.

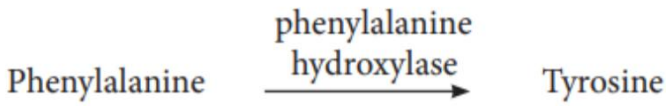
**Thalassemia:**

Thalassemia is an autosomal recessive disorder. It is caused by gene mutation resulting in excessive destruction of RBC's due to the formation of abnormal haemoglobin molecules. Normally haemoglobin is composed of four polypeptide chains, two alpha and two beta globin chains. Thalassemia patients have defects in either the alpha or beta globin chain causing the production of abnormal haemoglobin molecules resulting in anaemia.

Thalassemia is classified into alpha and beta based on which chain of haemoglobin molecule is affected. It is controlled by two closely linked genes HBA1 and HBA2 on chromosome 16. Mutation or deletion of one or more of the four alpha gene alleles causes Alpha Thalassemia. In Beta Thalassemia, production of beta globin chain is affected. It is controlled by a single gene (HBB) on chromosome 11. It is the most common type of Thalassemia and is also known as Cooley's anaemia. In this disorder the alpha chain production is increased and damages the membranes of RBC.

**Phenylketonuria:**

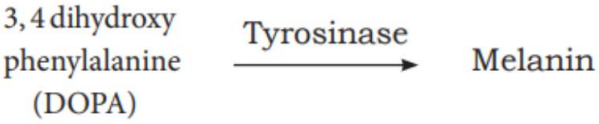
It is an inborn error of Phenylalanine metabolism caused due to a pair of autosomal recessive genes. It is caused due to mutation in the gene PAH (phenylalanine hydroxylase gene) located on chromosome 12 for the hepatic enzyme "phenylalanine hydroxylase" This enzyme is essential for the conversion of phenylalanine to tyrosine. Affected individual lacks this enzyme, so phenylalanine accumulates and gets converted to phenylpyruvic acid and other derivatives. It is characterized by severe mental retardation, light pigmentation of skin and hair. Phenylpyruvic acid is excreted in the urine.




**Albinism:**

Albinism is an inborn error of metabolism, caused due to an autosomal recessive gene. Melanin pigment is responsible for skin colour. Absence of

melanin results in a condition called albinism. A person with the recessive allele lacks the tyrosinase enzyme system, which is required for the conversion of dihydroxyphenyl alanine (DOPA) into melanin pigment inside the melanocytes. In an albino, melanocytes are present in normal numbers in their skin, hair, iris, etc., but lack melanin pigment.




**Huntington's chorea:**

It is inherited as an autosomal dominant lethal gene in man. It is characterized by involuntary jerking of the body and progressive degeneration of the nervous system, accompanied by gradual mental and physical deterioration. The patients with this disease usually die between the age of 35 and 40.

**Chromosomal Abnormalities:**

Each human diploid (2n) body cell has 46 chromosomes (23 pairs). Chromosomal disorders are caused by errors in the number or structure of chromosomes. Chromosomal anomalies usually occur when there is an error in cell division. Failure of chromatids to segregate during cell division resulting in the gain or loss of one or more chromosomes is called aneuploidy. It is caused by non-disjunction of chromosomes. Group of signs and symptoms that occur together and characterize a particular abnormality is called a syndrome. In humans, Down's syndrome, Turner's syndrome, Klinefelter's syndrome, Patau's syndrome are some of the examples of chromosomal disorders.

**Autosomal aneuploidy in human beings:**

Several autosomal aneuploidies have been reported in human being e.g. Down's syndrome (21-Trisomy), Patau's syndrome (13-Trisomy).

**1. Down's Syndrome/Trisomy – 21**

Trisomic condition of chromosome - 21 results in Down's syndrome. It is characterized by severe mental retardation, defective development of the central nervous system, increased separation between the eyes, flattened nose, ears are malformed, mouth is constantly open and the tongue protrudes.

**2. Patau's Syndrome/Trisomy-13**

Trisomic condition of chromosome 13 results in Patau's syndrome. Meiotic non disjunction is thought to be the cause for this chromosomal abnormality. It is characterized by multiple and severe body malformations as well as profound mental deficiency. Small head with small eyes, cleft palate, malformation of the brain and internal organs are some of the symptoms of this syndrome.

**Allosomal abnormalities in human beings:**

Mitotic or meiotic non-disjunction of sex chromosomes causes allosomal abnormalities. Several sex chromosomal abnormalities have been detected. Eg. Klinefelter's syndrome and Turner's syndrome.

**1. Klinefelter's Syndrome (XXY Males)**

This genetic disorder is due to the presence of an additional copy of the X chromosome resulting in a karyotype of 47,XXY. Persons with this syndrome have 47 chromosomes (44AA+XXY). They are usually sterile males, tall, obese, with long limbs, high pitched voice, under developed genitalia and have feeble breast (gynaecomastia) development.

**2. Turner's Syndrome (XO Females)**

This genetic disorder is due to the loss of a X chromosome resulting in a karyotype of 45,X. Persons with this syndrome have 45 chromosomes (44 autosomes and one X chromosome) (44AA+XO) and are sterile females. Low stature, webbed neck, under developed breast, rudimentary gonads lack of menstrual cycle during puberty, are the main symptoms of this syndrome.

**Packaging of DNA helix:**

The distance between two consecutive base pairs is 0.34nm ($0.34\times10^{-9}$m) of the DNA double helix in a typical mammalian cell. When the total number of base pairs is multiplied with the distance between two consecutive base pairs ($6.6 \times 10^{-9} \times 0.34 \times10^{-9}$ m/bp), the length of DNA double helix is approximately 2.2 m. (The total length of the double helical DNA = total number of base pairs × distance between two consecutive base pairs). If the length of E. coli DNA is 1.36 mm, the number of base pairs in E. coli is $4 \times10^{6}$ m ($1.36 \times 10^{3}$ m/$0.34 \times10^{-9}$). The length of the DNA double helix is far greater than the dimension of a

typical mammalian nucleus (approximately $10^{-6}$ m). How is such a long DNA polymer packaged in a cell?

Chromosomes are carriers of genes which are responsible for various characters from generation to generation. Du Praw (1965) proposed a single stranded model (unineme), as a long coiled molecule which is associated with histone proteins in eukaryotes. Plants and animals have more DNA than bacteria and must fold this DNA to fit into the cell nucleus. In prokaryotes such as E. coli though they do not have defined nucleus, the DNA is not scattered throughout the cell. DNA (being negatively charged) is held with some proteins (that have positive charges) in a region called the nucleoid. The DNA as a nucleoid is organized into large loops held by protein. DNA of prokaryotes is almost circular and lacks chromatin organization, hence termed genophore.

In eukaryotes, this organization is much more complex. Chromatin is formed by a series of repeating units called nucleosomes. Kornberg proposed a model for the nucleosome, in which 2 molecules of the four histone proteins H2A, H2B, H3 and H4 are organized to form a unit of eight molecules called histone octamere. The negatively charged DNA is wrapped around the positively charged histone octamere to form a structure called nucleosome. A typical nucleosome contains 200 bp of DNA helix. The histone octameres are in close contact and DNA is coiled on the outside of nucleosome. Neighbouring nucleosomes are connected by linker DNA (H1) that is exposed to enzymes. The DNA makes two complete turns around the histone octameres and the two turns are sealed off by an H1 molecule. Chromatin lacking H1 has a beads-on-a-string appearance in which DNA enters and leaves the nucleosomes at random places. H1 of one nucleosome can interact with H1 of the neighbouring nucleosomes resulting in the further folding of the fibre. The chromatin fiber in interphase nuclei and mitotic chromosomes have a diameter that vary between 200-300 nm and represents inactive chromatin. 30 nm fibre arises from the folding of nucleosome, chains into a solenoid structure having six nucleosomes per turn. This structure is stabilized by interaction between different H1 molecules. DNA is a solenoid and packed about 40 folds. The hierarchical

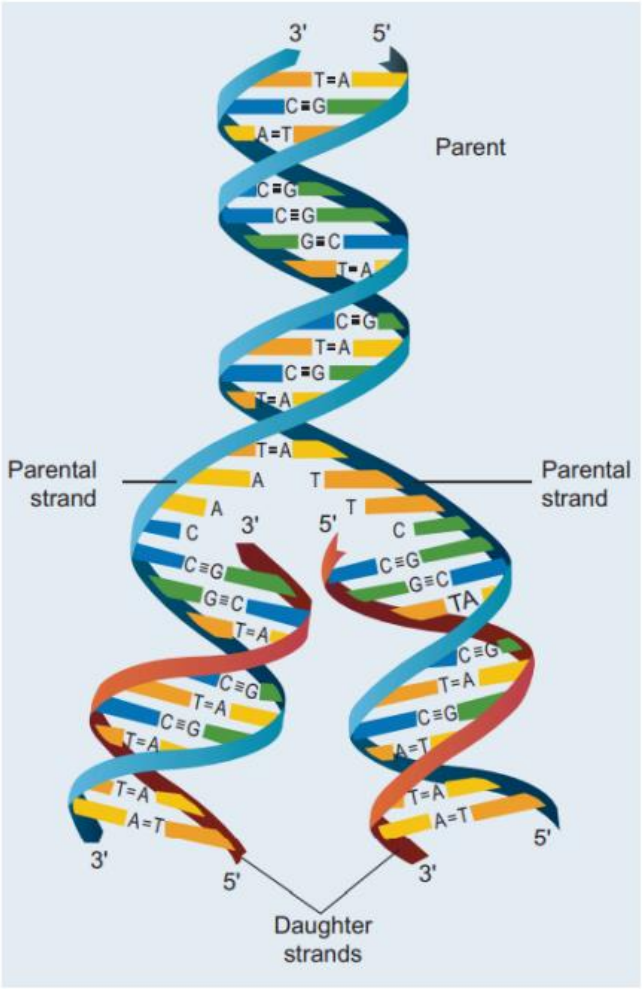
nature of chromosome structure. Additional set of proteins are required for packing of chromatin at higher level and are referred to as non-histone chromosomal proteins (NHC). In a typical nucleus, some regions of chromatin are loosely packed (lightly stained) and are referred to as euchromatin. The chromatin that is tightly packed (stained darkly) is called heterochromatin. Euchromatin is transcriptionally active and heterochromatin is transcriptionally inactive.

**DNA Replication:**

Replication of DNA takes place during the S phase of cell cycle. During replication, each DNA molecule gives rise to two DNA strands, identical to each other as well as to the parent strand. Three hypotheses of DNA replication have been proposed. They are conservative replication, dispersive replication, and semiconservative replication.

In conservative replication, the original double helix serves as a template. The original molecule is preserved intact and an entirely new double stranded molecule is synthesized. In dispersive replication, the original molecule is broken into fragments and each fragment serves as a template for the synthesis of complementary fragments. Finally two new molecules are formed which consist of both old and new fragments.

Semi-conservative replication was proposed by Watson and Crick in 1953. This mechanism of replication is based on the DNA model. They suggested that the two polynucleotide strands of DNA molecule unwind and start separating at one end. During this process, covalent hydrogen bonds are broken. The separated single strand then acts as template for the synthesis of a new strand. Subsequently, each daughter double helix carries one polynucleotide strand from the parent molecule that acts as a template and the other strand is newly synthesised and complementary to the parent strand.

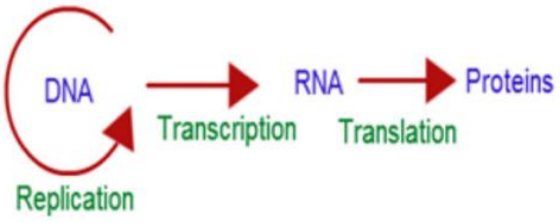



**Transcription:**

Francis Crick proposed the Central dogma in molecular biology which states that genetic information flows as follows:

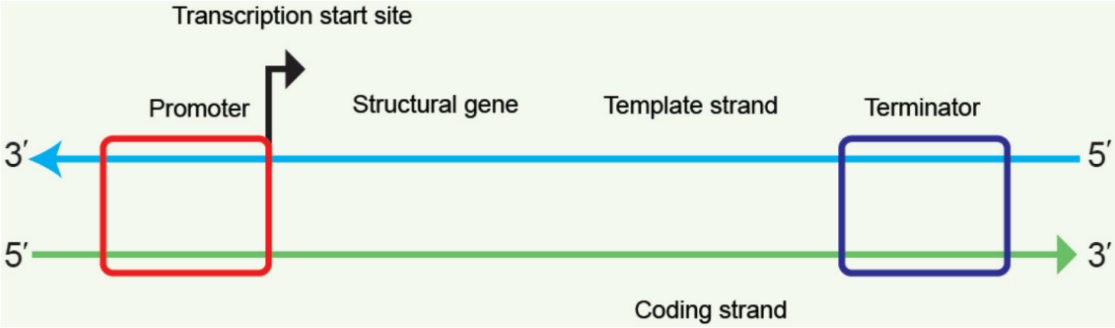
The process of copying genetic information from one strand of DNA into RNA is termed transcription. This process takes place in presence of DNA dependent RNA polymerase.

In some retroviruses that contain RNA as the genetic material (e.g, HIV), the flow of information is reversed. RNA synthesizes DNA by reverse transcription, then transcribed into mRNA by transcription and then into proteins by translation.



For a cell to operate, its genes must be expressed. This means that the gene products, whether proteins or RNA molecules must be made. The RNA that carries genetic information encoding a protein from genes into the cell is known as messenger RNA (mRNA). For a gene to be transcribed, the DNA which is a double helix must be pulled apart temporarily, and RNA is synthesized by RNA polymerase. This enzyme binds to DNA at the start of a gene and opens the double helix. Finally, RNA molecule is synthesized. The nucleotide sequence in the RNA is complementary to the DNA template strand from which it is synthesized.

Both the strands of DNA are not copied during transcription for two reasons. 1. If both the strands act as a template, they would code for RNA with different sequences. This in turn would code for proteins with different amino acid sequences. This would result in one segment of DNA coding for two different proteins, hence complicate the genetic information transfer machinery. 2. If two RNA molecules were produced simultaneously, double stranded RNA complementary to each other would be formed. This would prevent RNA from being translated into proteins.

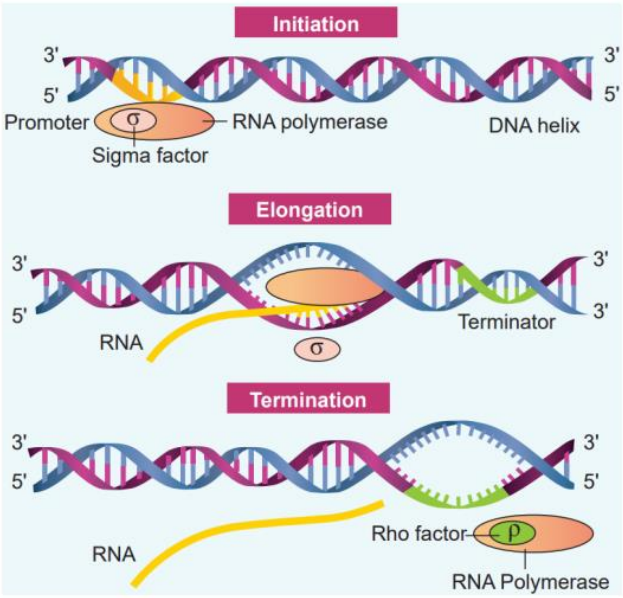



**Process of transcription:**

In prokaryotes, there are three major types of RNAs: mRNA, tRNA, and rRNA. All three RNAs are needed to synthesize a protein in a cell. The mRNA provides the template, tRNA brings amino acids and reads the genetic code, and rRNAs play structural and catalytic role during translation. There is a single DNA-dependent RNA polymerase that catalyses transcription of all types of RNA. It binds to the promoter and initiates transcription (Initiation). The polymerase binding sites are called promoters. It uses nucleoside triphosphate as substrate

and polymerases in a template depended fashion following the rule of complementarity. After the initiation of transcription, the polymerase continues to elongate the RNA, adding one nucleotide after another to the growing RNA chain. Only a short stretch of RNA remains bound to the enzyme, when the polymerase reaches a terminator at the end of a gene, the nascent RNA falls off, so also the RNA polymerase.

The question is, how the RNA polymerases are able to catalyse the three steps initiation, elongation and termination? The RNA polymerase is only capable of catalyzing the process of elongation. The RNA polymerase associates transiently with initiation factor sigma (σ) and termination factor rho (r) to initiate and terminate the transcription, respectively. Association of RNA with these factors instructs the RNA polymerase either to initiate or terminate the process of transcription.

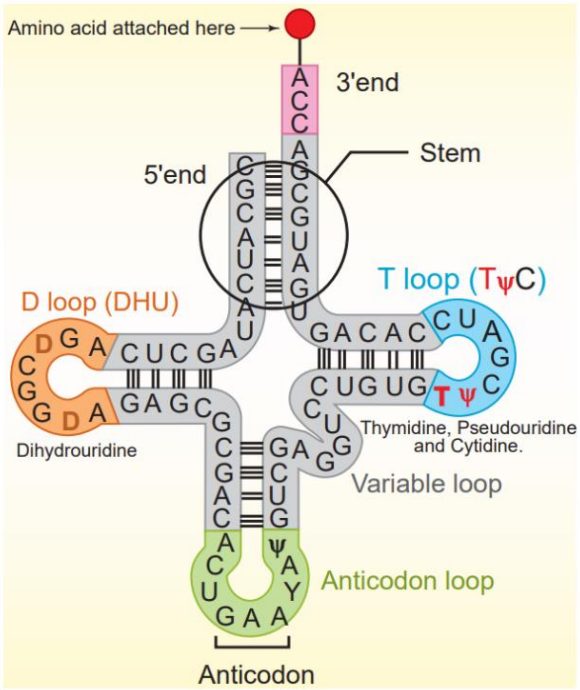



In bacteria, since the mRNA does not require any processing to become active and also since transcription and translation take place simultaneously in the same compartment (since there is no separation of cytosol and nucleus in bacteria), many times the translation can begin much before the mRNA is fully transcribed. This is because the genetic material is not separated from other cell organelles by a nuclear membrane consequently; transcription and translation can be coupled in bacteria.

**tRNA – the adapter molecule:**

The transfer RNA, (tRNA) molecule of a cell acts as a vehicle that picks up the amino acids scattered through the cytoplasm and also reads specific codes of mRNA molecules. Hence it is called an adapter molecule. This term was postulated by Francis Crick.

The two dimensional clover leaf model of tRNA was proposed by Robert Holley. The secondary structure of tRNA depicted in looks like a clover leaf. In actual structure, the tRNA is a compact molecule which looks like an inverted L. The clover leaf model of tRNA shows the presence of three arms namely DHU arm, middle arm and TΨC arm. These arms have loops such as amino acyl binding loop, anticodon loop and ribosomal binding loop at their ends. In addition it also shows a small lump called variable loop or extra arm. The amino acid is attached to one end (amino acid acceptor end) and the other end consists of three anticodon nucleotides. The anticodon pairs with a codon in mRNA ensuring that the correct amino acid is incorporated into the growing polypeptide chain. Four different regions of double-stranded RNA are formed during the folding process. Modified bases are especially common in tRNA. Wobbling between anticodon and codon allows some tRNA molecules to read more than one codon.



The process of addition of amino acid to tRNA is known as aminoacylation or charging and the resultant product is called aminoacyl- tRNA (charged tRNA). Without aminoacylation tRNA is known as uncharged tRNA. If two such tRNAs are brought together peptide bond formation is favoured energetically. Numbers of amino acids are joined by peptide bonds to form a polypeptide chain. This aminoacylation is catalyzed by an enzyme aminoacyl – tRNA synthetase. This is an endothermic reaction and is associated with ATP hydrolysis. 20 different aminoacyl – tRNA synthetases are known. The power to recognize codon on the mRNA lies in the tRNA and not in the attached amino acid molecule.

The tRNA charged with amino acid serves as an adapter molecule to decode the information on mRNA. This is achieved by the interaction of tRNA with mRNA. The tRNA molecule has a region that contains complementary bases (anticodon) to the codon on the mRNA. For initiation, there is another specific tRNA that is referred to as initiator tRNA. There are no tRNAs for stop codons.

**Translation:**

Translation refers to the process of polymerization of amino acids to form poly peptide chain. The decoding process is carried out by ribosomes that bind mRNA and charged tRNA molecules. The mRNA is translated, starting at the 5' end. After binding to mRNA, the ribosomes move along it, adding new amino acids to the growing polypeptide chain each time it reads a codon. Each codon is read by an anticodon on the corresponding tRNA. Hence the order and sequence of amino acids are defined by the sequence of bases in the mRNA.

**Mechanism of Translation:**

The cellular factory responsible for synthesizing protein is the ribosome. The ribosome consists of structural RNAs and about 80 different proteins. In inactive state, it exists as two subunits; large subunit and small subunit. When the subunit encounters an mRNA, the process of translation of the mRNA to protein begins. The prokaryotic ribosome (70 S) consists of two subunits, the larger subunit (50 S) and smaller subunit (30 S). The ribosomes of eukaryotes (80 S) are larger, consisting of 60 S and 40 S sub units. 'S' denotes the

sedimentation efficient which is expressed as Svedberg unit (S). The 30 S subunit of bacterial ribosome contains 16Sr RNA and 50 S subunit contains 5Sr RNA molecules and 23 S RNA and 31 ribosomal proteins. The larger subunit in eukaryotes consist of a 23 S RNA and 5Sr RNA molecule and 31 ribosomal proteins. The smaller eukaryotic subunit consist of 18Sr RNA component and about 33 proteins.

One of the alternative ways of dividing up a sequence of bases in DNA or RNA into codons is called reading frame. Any sequence of DNA or RNA, beginning with a start codon and which can be translated into a protein is known as an Open Reading Frame (ORF). A translational unit in mRNA is the sequence of RNA that is flanked by the start codon (AUG) and the stop codon and codes for polypeptides. mRNA also have some additional sequences that are not translated and are referred to as Untranslated Regions (UTR). UTRs are present at both 5' end (before start codon) and at 3' end (after stop codon). The start codon (AUG) begins the coding sequence and is read by a special tRNA that carries methionine (met). The initiator tRNA charged with methionine binds to the AUG start codon. In prokaryotes, N - formyl methionine ($f^{met}$) is attached to the initiator tRNA whereas in eukaryotes unmodified methionine is used. The 5' end of the mRNA of prokaryotes has a special sequence which precedes the initial AUG start codon of mRNA. This ribosome binding site is called the Shine – Dalgarno sequence or S-D sequence. This sequences base-pairs with a region of the 16Sr RNA of the small ribosomal subunit facilitating initiation. The subunits of the ribosomes (30 S and 50 S) are usually dissociated from each other when not involved in translation.

Initiation of translation in E. coli begins with the formation of an initiation complex, consisting of the 30S subunits of the ribosome, a messenger RNA and the charged N-formyl methionine tRNA (fmet – t RNA $f^{met}$), three proteinaceous initiation factors (IF1, IF2, IF3), GTP(Guanine Tri Phosphate) and Mg $2^{+}$.

The components that form the initiation complex interact in a series of steps. IF3 binds to the 30S and allows the 30S subunit to bind to mRNA. Another

initiation protein (IF2) then enhances the binding of charged formyl methionine tRNA to the small subunit in response to the AUG triplet. This step 'sets' the reading frame so that all subsequent groups of three ribonucleotides are translated accurately.

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Elongation is the second phase of translation. Once both subunits of the ribosomes are assembled with the mRNA, binding sites for two charged tRNA molecules are formed. The sites in the ribosome are referred to as the aminoacyl site (A site), the peptidyl site (P site) and the exit site (E site). The charged initiator tRNA binds to the P site. The next step in prokaryotic translation is to position the second tRNA at the 'A' site of the ribosome to form hydrogen bonds between its anticodon and the second codon on the mRNA (step1). This step requires the correct transfer RNA, another GTP and two proteins called elongation factors (EF-Ts and EF-Tu).

Once the charged tRNA molecule is positioned at the A site, the enzyme peptidyl transferase catalyses the formation of peptide bonds that link the two amino acids together (step 2). At the same time, the covalent bond between the amino acid and tRNA occupying the P site is hydrolyzed (broken). The product of this reaction is a dipeptide which is attached to the 3' end of tRNA still residing in the A site. For elongation to be repeated, the tRNA attached to the P site, which is now uncharged is released from the large subunit. The uncharged tRNA moves through the 'E' site on the ribosome.

The entire mRNA-tRNA-aa1-aa2 complex shifts in the direction of the 'P' site by a distance of three nucleotides (step 3). This step requires several elongation factors (EFs) and the energy derived from hydrolysis of GTP. This results in the third triplet of mRNA to accept another charged tRNA into the A site (step 4).

The sequence of elongation is repeated over and over (step 5 and step 6). An additional amino acid is added to the growing polypeptide, each time mRNA advances through the ribosome. Once a polypeptide chain is assembled, it emerges out from the base of the large subunit.

Termination is the third phase of translation. Termination of protein synthesis occurs when one of the three stop codons appears in the 'A' site of the ribosome. The terminal codon signals the action of GTP – dependent release factor, which cleaves the polypeptide chain from the terminal tRNA releasing it from the translational complex (step 1). The tRNA is then released from the ribosome, which then dissociates into its subunits (step 2).

**Human Genome Project (HGP):**

The international human genome project was launched in the year 1990. It was a mega project and took 13 years to complete. The human genome is about 25 times larger than the genome of any organism sequenced to date and is the first vertebrate genome to be completed. Human genome is said to have approximately $3\times10^9$ bp. HGP was closely associated with the rapid development of a new area in biology called bioinformatics.

**Goals and methodologies of Human Genome Project:**

1. Identify all the genes (approximately 30000) in human DNA.
2. Determine the sequence of the three billion chemical base pairs that makeup the human DNA.
3. To store this information in databases.
4. Improve tools for data analysis.
5. Transfer related technologies to other sectors, such as industries.
6. Address the ethical, legal and social issues (ELSI) that may arise from the project.

The methodologies of the Human Genome Project involved two major approaches. One approach was focused on identifying all the genes that are expressed as RNA (ETSS – Expressed Sequence Tags). The other approach was sequence annotation. Here, sequencing the whole set of genome was taken, that

contains all the coding and non-coding sequences and later assigning different regions in the sequences with functions.

For sequencing, the total DNA from a cell is isolated and converted into random fragments of relatively smaller sizes and cloned in suitable hosts using specialized vectors. This cloning results in amplification of pieces of DNA fragments so that it could subsequently be sequenced with ease. Bacteria and yeast are two commonly used hosts and these vectors are called as BAC (Bacterial Artificial Chromosomes) and YAC (Yeast Artificial Chromosomes). The fragments are sequenced using automated DNA sequencers (developed by Frederick Sanger). The sequences are then arranged based on few overlapping regions, using specialized computer based programs. These sequences were subsequently annotated and are assigned to each chromosome. The genetic and physical maps on the genome are assigned using information on polymorphism of restriction endonuclease recognition sites and some repetitive DNA sequences, called microsatellites. The latest method of sequencing even longer fragments is by a method called Shotgun sequencing using super computers, which has replaced the traditional sequencing methods.

**Salient features of Human Genome Project:**

1. Although human genome contains 3 billion nucleotide bases, the DNA sequences that encode proteins make up only about 5% of the genome.
2. An average gene consists of 3000 bases, the largest known human gene being dystrophin with 2.4 million bases.
3. The function of 50% of the genome is derived from transposable elements such as LINE and ALU sequence.
4. Genes are distributed over 24 chromosomes. Chromosome 19 has the highest gene density. Chromosome 13 and Y chromosome have lowest gene densities.
5. The chromosomal organization of human genes shows diversity.
6. There may be 35000-40000 genes in the genome and almost 99.9 nucleotide bases are exactly the same in all people.
7. Functions for over 50 percent of the discovered genes are unknown.

8. Less than 2 percent of the genome codes for proteins.
9. Repeated sequences make up very large portion of the human genome. Repetitive sequences have no direct coding functions but they shed light on chromosome structure, dynamics and evolution (genetic diversity).
10. Chromosome 1 has 2968 genes whereas chromosome 'Y' has 231 genes.
11. Scientists have identified about 1.4 million locations where single base DNA differences (SNPs – Single nucleotidepolymorphism – pronounce as 'snips') occur in humans. Identification of 'SNIPS' is helpful in finding chromosomal locations for disease associated sequences and tracing human history.

**Applications and future challenges:**

The mapping of human chromosomes is possible to examine a person's DNA and to identify genetic abnormalities. This is extremely useful in diagnosing diseases and to provide genetic counselling to those planning to have children. This kind of information would also create possibilities for new gene therapies. Besides providing clues to understand human biology, learning about non-human organisms, DNA sequences can lead to an understanding of their natural capabilities that can be applied towards solving challenges in healthcare, agriculture, energy production and environmental remediation. A new era of molecular medicine, characterized by looking into the most fundamental causes of disease than treating the symptoms will be an important advantage.

1. Once genetic sequence becomes easier to determine, some people may attempt to use this information for profit or for political power.
2. Insurance companies may refuse to insure people at 'genetic risk' and this would save the companies the expense of future medical bills incurred by 'less than perfect' people.
3. Another fear is that attempts are being made to "breed out" certain genes of people from the human population in order to create a 'perfect race'.

**Applications of Biotechnology:**

**Applications of Biotechnology in Medicine:**

**Recombinant Human Insulin:**

The Human insulin is synthesized by the β cells of Islets of Langerhans in the pancreas. It is formed of 51 aminoacids which are arranged in two polypeptide chains, A and B. The polypeptide chain A has 21 amino acids while the polypeptide chain B has 30 amino acids. Both A and B chains are attached together by disulphide bonds. Insulin controls the levels of glucose in blood. It facilitates the cellular uptake and utilization of glucose for the release of energy. Deficiency of insulin leads to diabetes mellitus which is characterized by increased blood glucose concentration and a complex of symptoms which may lead to death, if untreated. A continuous program of insulin dependence is required to treat this deficiency.

In the early years, insulin isolated and purified from the pancreas of pigs and cows was used to treat diabetic patients. Due to minor differences in the structure of the animal insulin as compared to human insulin, it resulted in the occurrence of allergic reactions in some diabetic patients. Production of insulin by recombinant DNA technology started in the late 1970s. This technique involved the insertion of human insulin gene on the plasmids of E.coli. The polypeptide chains are synthesized as a precursor called pre-pro insulin, which contains A and B segments linked by a third chain (C) and preceded by a leader sequence. The leader sequence is removed after translation and the C chain is excised, leaving the A and B polypeptide chains.

Insulin was the first ever pharmaceutical product of recombinant DNA technology administered to humans. The approval to use recombinant insulin for diabetes mellitus was given in 1982. In 1986 human insulin was marketed under the trade name Humulin.

**Human Growth Hormone (HGH):**

At about the same time when recombinant insulin was first made in E. coli, other research groups worked on human growth hormones somatostatin and somatotropin. These are peptide hormones secreted by the pituitary gland that

helps in the growth and development by increasing the uptake of amino acids and promoting protein synthesis. Deficiency of human growth hormone causes dwarfism, which could be treated by injecting HGH extracted from the human pituitary glands.

Using recombinant DNA technology HGH can be produced. The gene for HGH is isolated from the human pituitary gland cells. The isolated gene is inserted into a plasmid vector and then is transferred into E. coli. The recombinant E. coli then starts producing human growth hormone.The recombinant E. coli are isolated from the culture and mass production of HGH is carried out by fermentation technology.

A recombinant form of human growth hormone called somatropin is used as a drug to treat growth disorders in children.

**Human Blood-Clotting Factor VIII:**

You would have studied in your earlier class that many factors are required for normal blood clotting process and the factor VIII is one of them. The genes for the formation of factor VIII is located in the X chromosome. A genetic defect in the synthesis of factor VIII results in Haemophilia A, a sex-linked disease characterized by prolonged clotting time and internal bleeding. Clotting factor VIII isolated from blood of normal human being was used in the treatment of Haemophilia A. Requirement of large quantities of blood for this purpose and the risk of transmission of infectious diseases like AIDS is a disadvantage. Recombinant DNA technology was used to produce Recombinant Factor VIII in the Chinese Hamster ovary and in the baby Hamster kidney cells. More recently a cell line of human origin has been used for the first time to produce human blood clotting factor VIII.

**Interferons:**

Interferons are proteinaceous, antiviral, species specific substances produced by mammalian cells when infected with viruses. Interferons were discovered by Alick Isaacs and Jean Lindemann in 1957. Based on the structure of interferons they are classified as α, β and γ interferons. They stimulate the cellular DNA to produce antiviral enzymes which inhibit viral replication and protect the cells.

Similar to factor VIII, interferons could be isolated from blood, but the amount of blood required for isolation of interferons is enormous and not practical. To overcome this issue interferons could be produced by rDNA technology. The yeast Saccharomyces cerevisiae is more suitable for production of recombinant interferons than E.coli, since E.coli does not possess the machinery for glycosylation of proteins. Interferons are used for the treatment of various diseases like cancer, AIDS, multiple sclerosis, hepatitis C and herpes zoster. In spite of the therapeutic applications interferons are not within the reach of the common man due to high cost for its production.

**Recombinant Vaccines:**

Recombinant DNA technology has been used to produce new generation vaccines.The limitations of traditional vaccine production could be overcome by this approach.

The recombinant vaccines are generally of uniform quality and produce less side effects as compared to the vaccines produced by conventional methods. Different types of recombinant vaccines include subunit recombinant vaccines, attenuated recombinant vaccines and DNA vaccines.

**i. Subunit recombinant vaccines**

Vaccines that use components of a pathogenic organism rather than the whole organism are called subunit vaccines; recombinant DNA technology is very suited for developing new subunit vaccines. It includes components like proteins, peptides and DNAs of pathogenic organisms. The advantages of these vaccines include their purity in preparation, stability and safe use.

**ii. Attenuated recombinant vaccines**

This includes genetically modified pathogenic organisms (bacteria or viruses) that are made nonpathogenic and are used as vaccines. It is now possible to genetically engineer the organisms (bacteria or viruses) and use them as live vaccines and such vaccines are referred to as attenuated recombinant vaccines.

**iii. DNA Vaccines**

Genetic immunisation by using DNA vaccines is a novel approach that came into being in 1990. The immune response of the body is stimulated by a DNA

molecule. A DNA vaccine consists of a gene encoding an antigenic protein, inserted onto a plasmid, and then incorporated into the cells in a target animal. DNA instructs the cells to make antigenic molecules which are displayed on its surfaces. This would evoke an antibody response to the free floating antigen secreted by the cells. The DNA vaccine cannot cause the disease as it contains only copies of a few of its genes. DNA vaccines are relatively easy and inexpensive to design and produce.

Vaccines produced by these new techniques have definite advantages like producing target proteins, long lasting immunity and trigger immune response only against specific pathogens with less toxic effects.

Recombinant hepatitis B vaccine as a subunit vaccine is produced by cloning hepatitis B surface antigen (HbsAg) gene in the yeast, Saccharomyces cerevisiae.

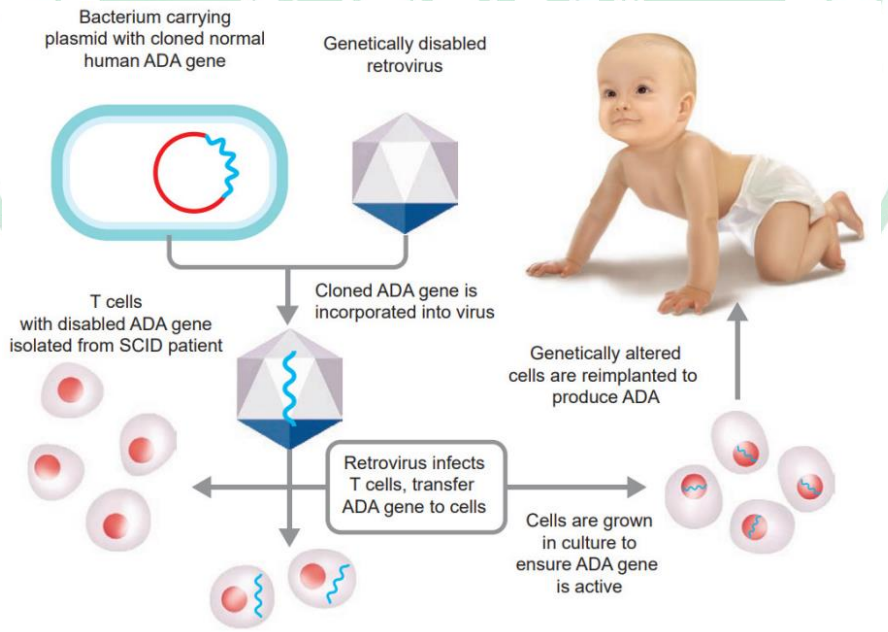
**Gene Therapy:**

If a person is born with a hereditary disease, can a corrective therapy be given for such disease? Yes, this can be done by a process known as gene therapy. This process involves the transfer of a normal gene into a person's cells that carries one or more mutant alleles. Expression of normal gene in the person results in a functional gene product whose action produces a normal phenotype. Delivery of the normal gene is accomplished by using a vector. The main thrust of gene therapy has been directed at correcting single gene mutations as in cystic fibrosis and haemophilia. At present most genetic diseases have no effective treatment and so gene therapy could offer hope for many people. There are two strategies involved in gene therapy namely; Gene augmentation therapy which involves insertion of DNA into the genome to replace the missing gene product and Gene inhibition therapy which involves insertion of the anti sense gene which inhibits the expression of the dominant gene.

The two approaches to achieve gene therapy are somatic cell and germ line gene therapy. Somatic cell therapy involves the insertion of a fully functional and expressible gene into a target somatic cell to correct a genetic disease permanently whereas Germline gene therapy involves the introduction of DNA into germ cells which is passed on to the successive generations. Gene therapy

involves isolation of a specific gene and making its copies and inserting them into target cells to make the desired proteins. It is absolutely essential for gene therapists to ensure that the gene is harmless to the patient and it is appropriately expressed and that the body's immune system does not react to the foreign proteins produced by the new genes.

| SOMATIC CELL GENE THERAPY | GERM LINE GENE THERAPY |
|---|---|
| Therapeutic genes transferred into the somatic cells. | Therapeutic genes transferred into the germ cells. |
| Introduction of genes into bone marrow cells, blood cells, skin cells etc., | Genes introduced into eggs and sperms. |
| Will not be inherited in later generations. | Heritable and passed on to later generations. |




**Stem Cell Therapy:**

Stem cells are undifferentiated cells found in most of the multi cellular animals. These cells maintain their undifferentiated state even after undergoing numerous mitotic divisions.

Stem cell research has the potential to revolutionize the future of medicine with the ability to regenerate damaged and diseased organs. Stem cells are capable of self renewal and exhibit 'cellular potency'. Stem cells can differentiate into all types of cells that are derived from any of the three germ layers ectoderm, endoderm and mesoderm.

In mammals there are two main types of stem cells – embryonic stem cells (ES cells) and adult stem cells. ES cells are pluripotent and can produce the three primary germ layers ectoderm, mesoderm and endoderm. Embryonic stem cells are multipotent stem cells that can differentiate into a number of types of cells. ES cells are isolated from the epiblast tissue of the inner cell mass of a blastocyst. When stimulated ES can develop into more than 200 cells types of the adult body. ES cells are immortal i.e., they can proliferate in a sterile culture medium and maintain their undifferentiated state.

Adult stem cells are found in various tissues of children as well as adults. An adult stem cell or somatic stem cell can divide and create another cell similar to it. Most of the adult stem cells are multipotent and can act as a repair system of the body, replenishing adult tissues.The red bone marrow is a rich source of adult stem cells.

The most important and potential application of human stem cells is the generation of cells and tissues that could be used for cell based therapies. Human stem cells could be used to test new drugs.

**Stem Cell Banks:**

Stem cell banking is the extraction, processing and storage of stem cells, so that they may be used for treatment in the future, when required. Amniotic cell bank is a facility that stores stem cells derived from amniotic fluid for future use. Stem cells are stored in banks specifically for use by the individual from whom such cells have been collected and the banking costs are paid. Cord Blood Banking is the extraction of stem cells from the umbilical cord during childbirth. While the umbilical cord and cord blood are the most popular sources of stem cells, the placenta, amniotic sac and amniotic fluid are also rich sources in terms of both quantity and quality.

**Molecular Diagnostics:**

Early diagnosis of infectious diseases or inherent genetic defects is essential for appropriate treatment. Early detection of the disease is not possible using conventional diagnostic methods like microscopic examinations, serum analysis and urine analysis.These laboratory techniques are indirect and not always specific. Scientists are continuously searching for specific, sensitive and simple diagnostic techniques for diagnosis of diseases. Recombinant DNA technology, Polymerase Chain Reactions (PCR) and Enzyme Linked Immunosorbent Assay (ELISA) are some of the techniques that are reliable and help in early diagnosis. Presence of pathogens like virus, bacteria, etc., is detected only when the pathogen produces symptoms in the patient. By the time the symptoms appear concentration of pathogen becomes very high in the body.However very low concentration of a bacteria or a virus, even when the symptoms of the disease does not appear, can be detected by amplification of their nucleic acid.

**Animal Cloning:**

Cloning is the process of producing genetically identical individuals of an organism either naturally or artificially. In nature many organisms produce clones through asexual reproduction.

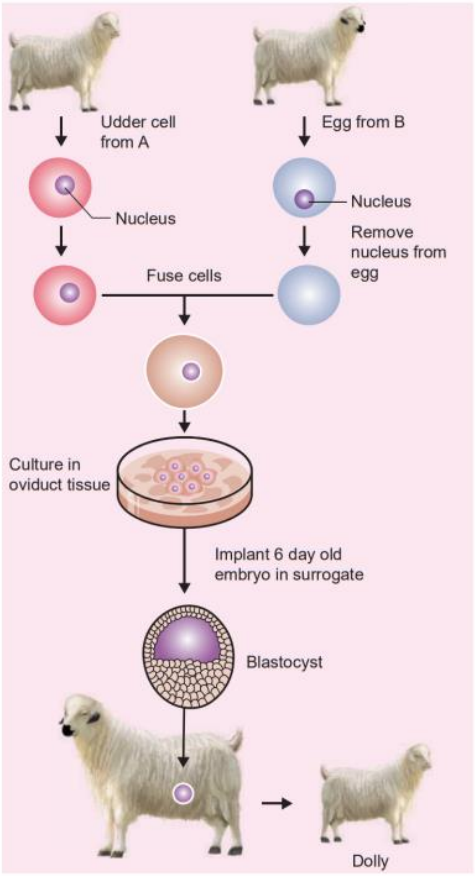
Cloning in biotechnology refers to the process of creating copies of organisms or copies of cells or DNA fragments (molecular cloning).

Dolly was the first mammal (Sheep) clone developed by Ian Wilmut and Campbell in 1997. Dolly, the transgenic clone was developed by the nuclear transfer technique and the phenomenon of totipotency. Totipotency refers to the potential of a cell to develop different cells, tissues, organs and finally an organism.

The mammary gland udder cells (somatic cells) from a donor sheep (ewe) were isolated and subjected to starvation for 5 days. The udder cells could not undergo normal growth cycle, entered a dormant stage and became totipotent. An ovum (egg cell) was taken from another sheep (ewe) and its nucleus was removed to form an enucleated ovum. The dormant mammary gland cell/udder

cell and the enucleated ovum were fused. The outer membrane of the mammary cell was ruptured allowing the ovum to envelope the nucleus.

The fused cell was implanted into another ewe which served as a surrogate mother. Five months later dolly was born. Dolly was the first animal to be cloned from a differentiated somatic cell taken from an adult animal without the process of fertilization.




**Advantages and Disadvantages of Cloning Animals:**

1. Offers benefits for clinical trials and medical research. It can help in the production of proteins and drugs in the field of medicine.
2. Aids stem cell research.
3. Animal cloning could help to save endangered species.
4. Animal and human activists see it as a threat to biodiversity saying that this alters evolution which will have an impact on populations and the ecosystem.
5. The process is tedious and very expensive.
6. It can cause animals to suffer.

7. Reports show that animal surrogates were manifesting adverse outcomes and cloned animals were affected with disease and have high mortality rate.
8. It might compromise human health through consumption of cloned animal meat.
9. Cloned animals age faster than normal animals and are less healthy than the parent organism as discovered in Dolly
10. Cloning can lead to occurrence of genetic disorders in animals.
11. More than 90% of cloning attempts fail to produce a viable offspring

**Ethical Issues:**

Biotechnology has given to the society cheap drugs, better friuts and vegetables, pest resistant crops, indigenious cure to diseases and lot of controversy. This is mainly because the major part of the modern biotechnology deals with genetic manipulations. People fear that these genetic manipulations may lead to unknown consequences. The major apprehension of recombinant DNA technology is that unique microorganisms either inadvertently or deliberately for the purpose of war may be developed that could cause epidemics or environmental catastrophies. Although many are concerned about the possible risk of genetic engineering, the risks are in fact slight and the potential benefits are substantial.

**Structure of DNA:**

DNA is the hereditary material as it contains the genetic information. It is the most important constituent of a chromosome. The most widely accepted model of DNA is the double helical structure of James Watson and Francis Crick. They proposed the three-dimensional model of DNA on the basis of X-ray diffraction studies of DNA obtained by Rosalind Franklin and Maurice Wilkins. In appreciation of their discoveries on the molecular structure of nucleic acids Watson, Crick and Wilkins were awarded Nobel Prize for Medicine in 1962.

**Chemical Composition of DNA molecule:**

DNA is a large molecule consisting of millions of nucleotides. Hence, it is also called a polynucleotide. Each nucleotide consists of three components.

1. A sugar molecules – Deoxyribose sugar.
2. A nitrogenous base.

   There are two types of nitrogenous bases in DNA. They are

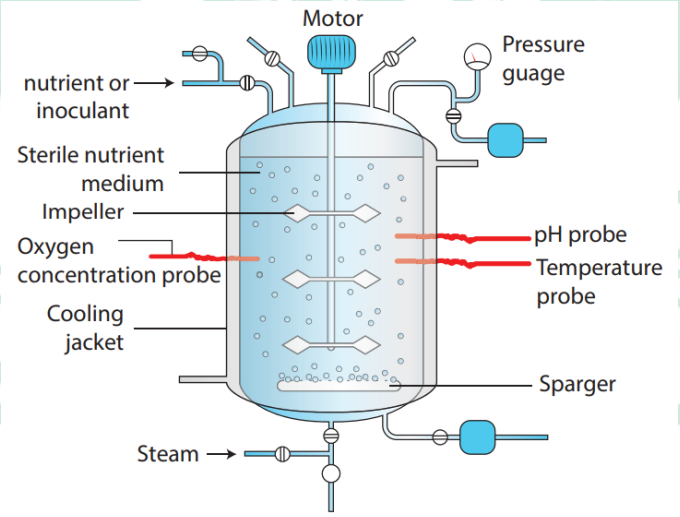
   a) Purines (Adenine and Guanine)
   b) Pyrimidines (Cytosine and Thymine)
3. A phosphate group

**Nucleoside and Nucleotide:**

1. Nucleoside = Nitrogen base + Sugar
2. Nucleotide = Nucleoside + Phosphate

**Bioreactor (Fermentor):**

Bioreactor (Fermentor) is a vessel or a container that is designed in such a way that it can provide an optimum environment in which microorganisms or their enzymes interact with a substrate to produce the required product. In the bioreactor aeration, agitation, temperature and pH are controlled. Fermentation involves two process namely upstream and downstream process.




**i. Upstream process**

All the process before starting of the fermenter such as sterilization of the fermenter, preparation and sterilization of culture medium and growth of the suitable inoculum are called upstream process.

**ii. Downstream process**

All the process after the fermentation process is known as the downstream process. This process includes distillation, centrifuging, filtration and solvent extraction. Mostly this process involves the purification of the desired product.

**Procedure of Fermentation**

1. Depending upon the type of product, bioreactor is selected.
2. A suitable substrate in liquid medicine is added at a specific temperature, pH and then diluted.
3. The organism (microbe, animal/plant cell, sub-cellular organelle or enzyme) is added to it.
4. Then it is incubated at a specific temperature for the specified time.
5. The incubation may either be aerobic or anaerobic.
6. Withdrawal of product using downstream processing methods

**Single Cell Protein (SCP):**

Single cell proteins are dried cells of microorganism that are used as protein supplement in human foods or animal feeds. Single Cell Protein (SCP) offers an unconventional but plausible solution to protein deficiency faced by the entire humanity. Although single cell protein has high nutritive value due to their higher protein, vitamin, essential amino acids and lipid content, there are doubts on whether it could replace conventional protein sources due to its high nucleic acid content and slower in digestibility. Microorganisms used for the production of Single Cell Protein are as follows:

1. Bacteria - Methylophilus methylotrophus, Cellulomonas, Alcaligenes
2. Fungi - Agaricus campestris, Saccharomyces cerevisiae (yeast), Candida utilis
3. Algae - Spirulina, Chlorella, Chlamydomonas

The single cell protein forms an important source of food because of their protein content, carbohydrates, fats, vitamins and minerals. It is used by Astronauts and Antarctica expedition scientists.

Spirulina can be grown easily on materials like waste water from potato processing plants (containing starch), straw, molasses, animal manure and even

sewage, to produce large quantities and can serve as food rich in protein, minerals, fats, carbohydrate and vitamins. Such utilization also reduces environmental pollution. 250 g of Methylophilus methylotrophus, with a high rate of biomass production and growth, can be expected to produce 25 tonnes of protein.

**Applications of Single-Cell Protein:**

1. It is used as protein supplement
2. It is used in cosmetics products for healthy hair and skin
3. It is used as the excellent source of protein for feeding cattle, birds, fishes etc.
4. It is used in food industry as aroma carriers, vitamin carrier, emulsifying agents to improve the nutritive value of baked products, in soups, in ready-to-serve-meals, in diet recipes
5. It is used in industries like paper processing, leather processing as foam stabilizers.

**Methods of Gene Transfer:**

The next step after a recombinant DNA molecule has been generated is to introduce it into a suitable host cell. There are many methods to introduce recombinant vectors and these are dependent on several factors such as the vector type and host cell.

For achieving genetic transformation in plants, the basic pre-requisite is the construction of a vector which carries the gene of interest flanked by the necessary controlling sequences, i.e., the promoter and terminator, and deliver the genes into the host plant.

**i. Direct or Vectorless Gene Transfer:**

In the direct gene transfer methods, the foreign gene of interest is delivered into the host plant without the help of a vector. The following are some of the common methods of direct gene transfer in plants.

1. **Chemical mediated gene transfer:** Certain chemicals like polyethylene glycol (PEG) and dextran sulphate induce DNA uptake into plant protoplasts.

2. **Microinjection:** The DNA is directly injected into the nucleus using fine tipped glass needle or micro pipette to transform plant cells. The protoplasts are immobilised on a solid support (agarose on a microscopic slide) or held with a holding pipette under suction.
3. **Electroporation Methods of Gene Transfer:** A pulse of high voltage is applied to protoplasts, cells or tissues which makes transient pores in the plasma membrane through which uptake of foreign DNA occurs.
4. **Liposome mediated method of Gene Transfer:** Liposomes the artificial phospholipid vesicles are useful in gene transfer. The gene or DNA is transferred from liposome into vacuole of plant cells. It is carried out by encapsulated DNA into the vacuole. This technique is advantageous because the liposome protects the introduced DNA from being damaged by the acidic pH and protease enzymes present in the vacuole. Liposome and tonoplast of vacuole fusion resulted in gene transfer. This process is called lipofection.
5. **Biolistics:** The foreign DNA is coated onto the surface of minute gold or tungsten particles (1-3 μm) and bombarded onto the target tissue or cells using a particle gun (also called as gene gun/micro projectile gun/shotgun). Then the bombarded cells or tissues are cultured on selected medium to regenerate plants from the transformed cells.

**ii. Indirect or Vector-Mediated Gene Transfer:**

Gene transfer is mediated with the help of a plasmid vector is known as indirect or vector mediated gene transfer. Among the various vectors used for plant transformation, the Ti-plasmid from Agrobacterium tumefaciens has been used extensively. This bacterium has a large size plasmid, known as Ti plasmid (Tumor inducing) and a portion of it referred as T-DNA (transfer DNA) is transferred to plant genome in the infected cells and cause plant tumors (crown gall). Since this bacterium has the natural ability to transfer T-DNA region of its plasmid into plant genome, upon infection of cells at the wound site, it is also known as the natural genetic engineer of plants.

The foreign gene (e.g. Bt gene for insect resistance) and plant selection marker gene, usually an antibiotic gene like npt II which confers resistance to antibiotic kanamycin are cloned in the T DNA region of Ti-plasmid in place of unwanted DNA sequences.

**Genome editing and CRISPR - Cas9:**

Genome editing or gene editing is a group of technologies that has the ability to change an organism's DNA. These technologies allow genetic material to be added, removed, or altered at particular locations in the genome. Several approaches to genome editing have been developed. A recent one is known as CRISPR-Cas9, which is short form of Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9. The CRISPR-Cas9 system has generated a lot of excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other existing genome editing methods.

Rice, was among the first plants to be used to demonstrate the feasibility of CRISPR-mediated targeted mutagenesis and gene replacement. The gene editing tool CRISPR can be used to make hybrid rice plants that can clone their seed. Imtiyaz Khand and Venkatesan Sundaresan and colleagues reported in a new study which clearly shows one can re-engineer rice to switch it from a sexual to an asexual mode.

**Transgenic Plants / Genetically Modified Crops (Gm Crops):**

**Herbicide Tolerant – Glyphosate:**

Weeds are a constant problem in crop fields. Weeds not only compete with crops for sunlight, water, nutrients and space but also acts as a carrier for insects and diseases. If left uncontrolled, weeds can reduce crop yields significantly.

Glyphosate herbicide produced by Monsanto, USA company under the trade name 'Round up' kills plants by blocking the 5-enopyruvate shikimate-3 phosphate synthase (EPSPS) enzyme, an enzyme involved in the biosynthesis of aromatic amino acids, vitamins and many secondary plant metabolites. There are several ways by which crops can be modified to be glyphosate-tolerant.

One strategy is to incorporate a soil bacterium gene that produces a glyphosate tolerant form of EPSPS. Another way is to incorporate a different soil bacterium gene that produces a glyphosate degrading enzyme.

**Advantages of Herbicide Tolerant Crops:**

1. Weed control improves higher crop yields;
2. Reduces spray of herbicide;
3. Reduces competition between crop plant and weed;
4. Use of low toxicity compounds which do not remain active in the soil; and
5. The ability to conserve soil structure and microbes.

**Herbicide Tolerant – Basta:**

Trade name 'Basta' refers to a non-selective herbicide containing the chemical compound phosphinothricin. Basta herbicide tolerant gene PPT (L-phosphinothricin) was isolated from Medicago sativa plant. It inhibits the enzyme glutamine synthase which is involved in ammonia assimilation. The PPT gene was introduced into tobacco and transgenic tobacco produced was resistant to PPT. Similar enzyme was also isolated from Streptomyces hygroscopicus with bar gene encodes for PAT (Phosphinothricin acetyl transferase) and was introduced into crop plants like potato and sugar-beet and transgenic crops have been developed.

**Insect resistance - Bt Crops:**

**i. Bt Cotton:**

Bt cotton is a genetically modified organism (GMO) or genetically modified pest resistant plant cotton variety, which produces an insecticide activity to bollworm.

Strains of the bacterium Bacillus thuringiensis produce over 200 different Bt toxins, each harmful to different insects. Most Bt toxins are insecticidal to the larvae of moths and butterflies, beetles, cotton bollworms and gatflies but are harmless to other forms of life.

The genes are encoded for toxic crystals in the Cry group of endotoxin. When insects attack and eat the cotton plant the Cry toxins are dissolved in the insect's stomach.

The epithelial membranes of the gut block certain vital nutrients thereby sufficient regulation of potassium ions are lost in the insects and results in the death of epithelial cells in the intestine membrane which leads to the death of the larvae.

**Advantages:**

1. Yield of cotton is increased due to effective control of bollworms.
2. Reduction in insecticide use in the cultivation of Bt cotton
3. Potential reduction in the cost of cultivation.

**Disadvantages:**

1. Cost of Bt cotton seed is high.
2. Effectiveness up to 120 days after that efficiency is reduced
3. Ineffective against sucking pests like jassids, aphids and whitefly.
4. Affects pollinating insects and thus yield.

**ii. Bt Brinjal:**

The Bt brinjal is another transgenic plant created by inserting a crystal protein gene (Cry1Ac) from the soil bacterium Bacillus thuringiensis into the genome of various brinjal cultivars. The insertion of the gene, along with other genetic elements such as promoters, terminators and an antibiotic resistance marker gene into the brinjal plant is accomplished using Agrobacterium- mediated genetic transformation. The Bt brinjal has been developed to give resistance against Lepidopberan insects, in particular the Brinjal Fruit and Shoot Borer (Leucinodes orbonalis).

**iii. Dhara Mustard Hybrid (DMH):**

DMH -11 is transgenic mustard developed by a team of scientists at the Centre for Genetic Manipulation of Crop Plants Delhi University under Government sponsored project. It is genetically modified variety of Herbicide Tolerant (HT) mustard. It was created by using "barnase/barstar" technology for genetic modification by adding genes from soil bacterium that makes mustard, a self-

pollinating plant. DMH -11 contains three genes viz. Bar gene, Barnase and Barstar sourced from soil bacterium. The bar gene had made plant resistant to herbicide named Basta.

**Virus Resistance:**

Many plants are affected by virus attack resulting in series loss in yield and even death. Biotechnological intervention is used to introduce viral resistant genes into the host plant so that they can resist the attack by virus. This is by introducing genes that produce resistant enzymes which can deactivate viral DNA.

**FlavrSavr Tomato:**

Agrobacterium mediated genetic engineering technique was followed to produce Flavr-Savr tomato, i.e., retaining the natural colour and flavor of tomato.

Through genetic engineering, the ripening process of the tomato is slowed down and thus prevent it from softening and to increase the shelf life. The tomato was made more resistant to rotting by Agrobacterium mediated gene transfer mechanism of introducing an antisense gene which interferes with the production of the enzyme polygalacturonase, which help in delaying the ripening process of tomato during long storage and transportation.

**Golden rice – Biofortification:**

Golden rice is a variety of Oryza sativa (rice) produced through genetic engineering of biosynthesized beta-carotene, a precursor of Vitamin-A in the edible parts of rice developed by Ingo Potrykus and his group. The aim is to produce a fortified food to be grown and consumed in areas with a shortage of dietary Vitamin-A. Golden rice differs from its parental strain by the addition of three beta-carotene biosynthesis genes namely ‘psy’ (phytoene synthase) from daffodil plant Narcissus pseudonarcissus and ‘crt-1’ gene from the soil bacterium Erwinia auredorora and ‘lyc’ (lycopene cyclase) gene from wild-type rice endosperm.

The endosperm of normal rice, does not contain beta-carotene. Golden-rice has been genetically altered so that the endosperm now accumulates Beta-carotene.

This has been done using Recombinant DNA technology. Golden rice can control childhood blindness - Xerophthalmia.

**GM Food – Benefits:**

1. High yield without pest
2. 70% reduction of pesticide usage
3. Reduce soil pollution problem
4. Conserve microbial population in soil

**Risks - believed to:**

1. Affect liver, kidney function and cancer
2. Hormonal imbalance and physical disorder
3. Anaphylactic shock (sudden hypersensitive reaction) and allergies.
4. Adverse effect in immune system because of bacterial protein.
5. Loss of viability of seeds seen in terminator seed technology of GM crops.

**Polyhydroxybutyrate (PHB):**

Synthetic polymers are non-degradable and pollute the soil and when burnt add dioxin in the environment which cause cancer. So, efforts were taken to provide an alternative eco-friendly biopolymers. Polyhydroxyalkanoates (PHAs) and polyhydroxybutyrate (PHB) are group of degradable biopolymers which have several medical applications such as drug delivery, scaffold and heart valves. PHAs are biological macromolecules and thermoplastics which are biodegradable and biocompatible.

Several microorganisms have been utilized to produce different types of PHAs including Gram-positive like Bacillus megaterium, Bacillussubtilis and Corynebacterium glutamicum, Gram-negative bacteria like group of Pseudomonas sp. and Alcaligenes eutrophus.

**Polylactic acid (PLA):**

Polylactic acid or polylactide (PLA) is a biodegradable and bioactive thermoplastic. It is an aliphatic polyester derived from renewable resources, such as corn starch, cassava root, chips or starch or sugarcane. For the production of PLA, two main monomers are used: lactic acid, and the cyclic

diester, lactide. The most common route is the ring-opening polymerization of lactide with metal catalysts like tin octoate in solution. The metal-catalyzed reaction results in equal amount of d and polylactic acid.

**Green Fluorescent Protein (GFP):**

The green fluorescent protein (GFP) is a protein containing 238 amino acid residues of 26.9 kDa that exhibits bright green fluorescence when exposed to blue to ultraviolet range (395 nm). GFP refers to the protein first isolated from the jellyfish Aequorea victoria. GFP is an excellent tool in biology due to its ability to form internal chromophore without requiring any accessory cofactors, gene products, enzymes or substrates other than molecular oxygen. In cell and molecular biology, the GFP gene is frequently used as a reporter of expression. It has been used in modified forms to make biosensors.

**Biopharming:**

Biopharming also known as molecular pharming is the production and use of transgenic plants genetically engineered to produce pharmaceutical substances for use of human beings. This is also called "molecular farming or pharming". These plants are different from medicinal plants which are naturally available. The use of plant systems as bioreactors is gaining more significance in modern biotechnology. Many pharmaceutical substances can be produced using transgenic plants. Example: Golden rice

**Bioremediation:**

It is defined as the use of microorganisms or plants to manage environmental pollution. It is an approach used to treat wastes including wastewater, industrial waste and solid waste. Bioremediation process is applied to the removal of oil, petrochemical residues, pesticides or heavy metals from soil or ground water. In many cases, bioremediation is less expensive and more sustainable than other physical and chemical methods of remediation. An eco-friendly approach and can deal with lower concentrations of contaminants more effectively. The strategies for bioremediation in soil and water can be as follows:

1. Use of indigenous microbial population as indicator species for bioremediation process.

2. Bioremediation with the addition of adapted or designed microbial inoculants.
3. Use of plants for bioremediation - green technology.

**Some examples of bioremediation technologies are:**

1. **Phytoremediation** - use of plants to bring about remediation of environmental pollutants.
2. **Mycoremediation** - use of fungi to bring about remediation of environmental pollutants.
3. **Bioventing** a process that increases the oxygen or air flow to accelerate the degradation of environmental pollutants.
4. **Bioleaching** use of microorganisms in solution to recover metal pollutants from contaminated sites.
5. **Bioaugmentation** a addition of selected microbes to speed up degradation process.
6. **Composting process** by which the solid waste is composted by the use of microbes into manure which acts as a nutrient for plant growth.
7. **Rhizofiltration** uptake of metals or degradation of organic compounds by rhizosphere microorganisms.
8. **Rhizostimulation** stimulation of plant growth by the rhizosphere by providing better growth condition or reduction in toxic materials.

**Limitations:**

1. Only biodegradable contaminants can be transformed using bioremediation processes.
2. Bioremediation processes must be specifically made in accordance to the conditions at the contaminated site.
3. Small-scale tests on a pilot scale must be performed before carrying out the procedure at the contaminated site.
4. The use of genetic engineering technology to create genetically modified microorganism or a consortium of microbes for bioremediation process has great potential.

**Biopiracy:**

Biopiracy can be defined as the manipulation of intellectual property rights laws by corporations to gain exclusive control over national genetic resources, without giving adequate recognition or remuneration to the original possessors of those resources. Examples of biopiracy include recent patents granted by the U.S. Patent and Trademarks Office to American companies on turmeric, 'neem' and, most notably, 'basmati' rice. All three products are indigenous to the Indo-Pak subcontinent.

**1. Biopiracy of Neem**

The people of India used neem and its oil in many ways to controlling fungal and bacterial skin infections. Indian's have shared the knowledge of the properties of the neem with the entire world. Pirating this knowledge, the United States Department of Agriculture (USDA) and an American MNC (Multi Nation Corporation) W.R.Grace in the early 90's sought a patent from the European Patent Office (EPO) on the "method for controlling of diseases on plants by the aid of extracted hydrophobic neem oil". The patenting of the fungicidal and antibacterial properties of Neem was an example of biopiracy but the traditional knowledge of the Indians was protected in the end.

**2. Biopiracy of Turmeric**

The United States Patent and Trademark Office, in the year 1995 granted patent to the method of use of turmeric as an antiseptic agent. Turmeric has been used by the Indians as a home remedy for the quick healing of the wounds and also for purpose of healing rashes. The journal article published by the Indian Medical Association, in the year 1953 wherein this remedy was mentioned. Therefore, in this way it was proved that the use of turmeric as an antiseptic is not new to the world and is not a new invention, but formed a part of the traditional knowledge of the Indians. The objection in this case US patent and trademark office was upheld and traditional knowledge of the Indians was protected. It is another example of Biopiracy.

**3. Biopiracy of Basmati**

On September 2, 1997, the U.S. Patent and Trademarks Office granted Patent on "basmati rice lines and grains" to the Texas-based company RiceTec. This broad patent gives the company several rights, including exclusive use of the term 'basmati', as well proprietary rights on the seeds and grains from any crosses. The patent also covers the process of breeding RiceTec's novel rice lines and the method to determine the cooking properties and starch content of the rice grains.

India had periled the United States to take the matter to the WTO as an infringement of the TRIPS agreement, which could have resulted in major embarrassment for the US. Hence voluntarily and due to few decisions take by the US patent office, Rice Tec had no choice but to lose most of the claims and most importantly the right to call the rice "Basmati". In the year 2002, the final decision was taken. Rice Tec dropped down 15 claims, resulting in clearing the path of Indian Basmati rice exports to the foreign countries. The Patent Office ordered the patent name to be changed to 'Rice lines 867'.

**Applications of Biotechnology:**

1. Biotechnology is one of the most important applied interdisciplinary sciences of the 21st century. It is the trusted area that enables us to find the beneficial way of life.
2. Biotechnology has wide applications in various sectors like agriculture, medicine, environment and commercial industries.
3. This science has an invaluable outcome like transgenic varieties of plants e.g. transgenic cotton (Bt-cotton), rice, tomato, tobacco, cauliflower, potato and banana.
4. The development of transgenics as pesticide resistant, stress resistant and disease resistant varieties of agricultural crops is the immense outcome of biotechnology.
5. The synthesis of human insulin and blood protein in E.coli and utilized for insulin deficiency disorder in human is a breakthrough in biotech industries in medicine.

6. The synthesis of vaccines, enzymes, antibiotics, dairy products and beverages are the products of biotech industries.
7. Biochip based biological computer is one of the successes of biotechnology.
8. Genetic engineering involves genetic manipulation, tissue culture involves aseptic cultivation of totipotent plant cell into plant clones under controlled atmospheric conditions.
9. Single cell protein from Spirulina is utilized in food industries.
10. Production of secondary metabolites, biofertilizers, biopesticides and enzymes.
11. Biomass energy, biofuel, Bioremediation, phytoremediation for environmental biotechnology.

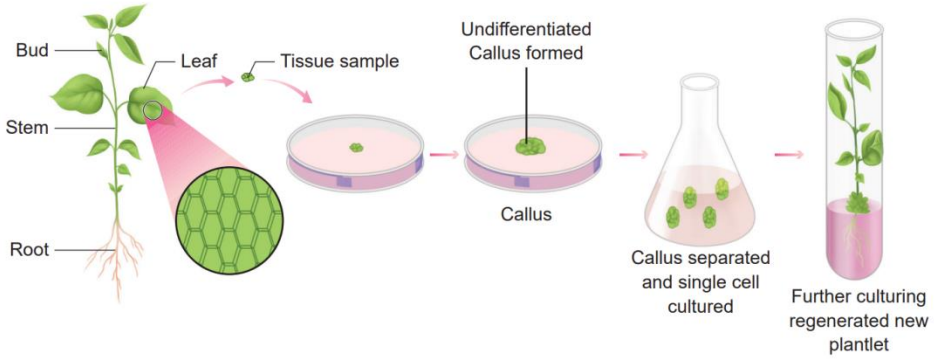
**Plant Tissue Culture (PTC):**

Plant tissue culture is used to describe the in vitro and aseptic growth of any plant part on a tissue culture medium.

**Laboratory Facilities for PTC:**

1. Washing facility for glassware and ovens for drying glassware.
2. Medium preparation room with autoclave, electronic balance and pH meter.
3. Transfer area sterile room with laminar air-flow bench and a positive pressure ventilation unit called High Efficiency Particulate Air (HEPA) filter to maintain aseptic condition.
4. Culture facility: Growing the explant inoculated into culture tubes at 22-28° C with illumination of light 2400 lux, with a photoperiod of 8-16 hours and a relative humidity of about 60%.

**Technique Involved in PTC:**




**Sterilization:**

Sterilization is the technique employed to get rid of microbes such as bacteria and fungi in the culture medium, vessels and explants.

Maintenance of Aseptic Environment: During in vitro tissue culture maintenance of aseptic environmental condition should be followed, i.e., sterilization of glassware, forceps, scalpels, and all accessories in wet steam sterilization by autoclaving at 15 psi (121°C) for 15 to 30 minutes or dipping in 70% ethanol followed by flaming and cooling.

Sterilization of culture room: Floor and walls are washed first with detergent and then with 2% sodium hypochlorite or 95% ethanol. The cabinet of laminar airflow is sterilized by clearing the work surface with 95% ethanol and then exposure of UV radiation for 15 minutes.

Sterilization of Nutrient Media: Culture media are dispensed in glass containers, plugged with non-absorbent cotton or sealed with plastic closures and then sterilized using autoclave at 15 psi (121°C) for 15 to 30 minutes. The plant extracts, vitamins, amino acids and hormones are sterilized by passing through Millipore filter with 0.2 mm pore diameter and then added to sterilized culture medium inside Laminar Airflow Chamber under sterile condition.

Sterilization of Explants: The plant materials to be used for tissue culture should be surface sterilized by first exposing the material in running tap water and then treating it in surface sterilization agents like 0.1% mercuric chloride, 70% ethanol under aseptic condition inside the Laminar Air Flow Chamber.

**Media Preparation:**

The success of tissue culture lies in the composition of the growth medium, plant growth regulators and culture conditions such as temperature, pH, light and humidity. No single medium is capable of maintaining optimum growth of all plant tissues. Suitable nutrient medium as per the principle of tissue culture is prepared and used.

MS nutrient medium (Murashige and Skoog 1962) is commonly used. It has carbon sources, with suitable vitamins and hormones. The media formulations available for plant tissue culture other than MS are B5 medium (Gamborg.et.al 1968), White medium (white 1943), Nitsch's medium (Nitsch & Nitsch 1969). A medium may be solid or semisolid or liquid. For solidification, a gelling agent such as agar is added.

**Culture condition:**

**i. pH**

The pH of medium is normally adjusted between 5.6 to 6.0 for the best result.

**ii. Temperature**

The cultures should be incubated normally at constant temperature of 25°C ± 2°C for optimal growth.

**iii. Humidity and Light Intensity**

The cultures require 50-60% relative humidity and 16 hours of photoperiod by the illumination of cool white fluorescent tubes of approximately 1000 lux.

**iv. Aeration**

Aeration to the culture can be provided by shaking the flasks or tubes of liquid culture on automatic shaker or aeration of the medium by passing with filter-sterilized air.

**Induction of Callus:**

Explant of 1-2 cm sterile segment selected from leaf, stem, tuber or root is inoculated (transferring the explants to sterile glass tube containing nutrient medium) in the MS nutrient medium supplemented with auxins and incubated at 25°C ± 2°C in an alternate light and dark period of 12 hours to induce cell

division and soon the upper surface of explant develops into callus. Callus is a mass of unorganized growth of plant cells or tissues in in vitro culture medium.

**Embryogenesis:**

The callus cells undergoes differentiation and produces somatic embryos, known as Embryoids. The embryoids are sub-cultured to produce plantlets.

**Hardening:**

The plantlets developed in vitro require a hardening period and so are transferred to greenhouse or hardening chamber and then to normal environmental conditions.

Hardening is the gradual exposure of in vitro developed plantlets in humid chambers in diffused light for acclimatization so as to enable them to grow under normal field conditions.

**Applications of Plant Tissue Culture:**

Improved hybrids production through somatic hybridization.

1. Somatic embryoids can be encapsulated into synthetic seeds (synseeds). These encapsulated seeds or synthetic seeds help in conservation of plant biodiversity.
2. Production of disease resistant plants through meristem and shoot tip culture.
3. Production of stress resistant plants like herbicide tolerant, heat tolerant plants.
4. Micropropagation technique to obtain large numbers of plantlets of both crop and tree species useful in forestry within a short span of time and all through the year.
5. Production of secondary metabolites from cell culture utilized in pharmaceutical, cosmetic and food industries.

**Intellectual Property Right (IPR):**

Intellectual property right (IPR) is a category of rights that includes intangible creation of the human intellect, and primarily consists of copyrights, patents, and trademarks. It also includes other types of rights, such as trade secrets, publicity rights, moral rights, and rights against unfair competition.

1. In biotechnology, the transformed microorganisms and plants and technologies for the production of commercial products are exclusively the property of the discoverer.
2. The discoverer has the full rights on his property. It should not be neglected by the others without legal permission.
3. The right of discoverer must be protected and it does by certain laws framed by a country.
4. The IPR is protected by different ways like patents, copyrights, trade secrets and trademarks, designs and geographical indications.

**Patents:**

It is a special right to the discoverer/inventor that has been granted by the government through legislation for trading new articles.

A patent is a personal property which can be licensed or sold by the person or organisation just like any other property. Patent terms give the inventor the rights to exclude others from making, using or selling his invention.

**Biosafety and Bioethics:**

Advances in biotechnology and their applications deals with genetic manipulation.

**Biosafety:**

Biosafety is the prevention of large-scale loss of biological integrity, focusing both on ecology and human health. These prevention mechanisms include conduction of regular reviews of the biosafety in laboratory settings, as well as strict guidelines to follow. Many laboratories handling biohazards employ an ongoing risk management assessment and enforcement process for biosafety. Failures to follow such protocols can lead to increased risk of exposure to biohazards or pathogens.

**Bioethics - Ethical, Legal and Social Implications (ELSI):**

Bioethics refers to the study of ethical issues emerging from advances in biology and medicine. It is also a moral discernment as it relates to medical policy and practice. Bioethicists are concerned with the ethical questions that arise in the

relationships among life sciences, biotechnology and medicine. It includes the study of values relating to primary care and other branches of medicine.

The scope of bioethics is directly related to biotechnology, including cloning, gene therapy, life extension, human genetic engineering, astroethics life in space, and manipulation of basic biology through altered DNA, RNA and proteins. These developments in biotechnology will affect future evolution, and may require new principles, such as biotic ethics, that values life and its basic biological characters and structures.

The Ethical, Legal, and Social Implications (ELSI) program was founded in 1990 as an integral part of the Human Genome Project. The mission of the ELSI program was to identify and address issues raised by genomic research that would affect individuals, families, and society. A percentage of the Human Genome Project budget at the National Institutes of Health and the U.S. Department of Energy was devoted to ELSI research.

**Genetic Engineering Appraisal Committee (GEAC):**

GEAC is an apex body under Ministry of Environment, Forests and Climate change for regulating manufacturing, use, import, export and storage of hazardous microbes or genetically modified organisms (GMOs) and cells in the country. It was established as an apex body to accord approval of activities involving large scale use of hazardous microorganisms and recombinants in research and industrial production. The GEAC is also responsible for approval of proposals relating to release of genetically engineered organisms and products into the environment including experimental field trials.