SREE SIDDAGANGA COLLEGE
OF ARTS, SCIENCE and COMMERCE
B.H. ROAD, TUMKUR

(AFFILIATED TO TUMKUR UNIVERSITY)

BOTANY PAPER-VII

III BSC VI SEMESTER

STUDY MATERIAL

Unit-1; **Molecular biology 16 Hrs**

Genetic material: Introduction, identification of genetic material (Griffith's and Avery's experiments, Hershey-Chase experiment), **Chemical nature of genetic material**: Nucleotides, nucleosides,DNA: structure, replication (Semi-Conservative and Rolling circle model).RNA: Structure, genetic RNA and non genetic RNA (mRNA, rRNA, tRNA), **Biosynthesis of proteins**: Genetic code, Transcription, RNA Splicing, Translation and Polysomes.**Regulation of gene expression**: Prokaryotes: (Lac- Operon) and Eukaryotes (Britten and Davidson's model).

Unit-2: Bio-molecular Techniques 6 Hrs

Blotting techniques: Northern, Southern and Western Blotting,
DNA finger printing; DNA sequencing (Sanger's method), PCR

Unit -3: **Genetic engineering**: **12Hrs**

A concise account of methods used in recombinant DNA technology. Tools of rDNA technology: Plasmids (PBR322, PUC 18, Ti-plasmid), Restriction endonuclease, DNA ligase, and Bioreactor. Genomic and cDNA libraries, screening of genomic library. Applications of Genetic Engineering technology in agriculture .(Transgenic plants- Bt-cotton, golden rice), in medicine (insulin synthesis, gene therapy), in environment (bioremediation and bio-mining).

Unit-4: Bioinformatics 5 Hrs

Introduction, Aim and scope .Biological Databases: DNA database and protein databases.
A brief account of NCBI, DNA Data Bank of Japan (DDBJ) and Protein Information Resource (PIR)

Unit 5; **Biotechnology: 6 Hrs**

Fermentation technology: Production of Ethyl alcohol, production of antibiotics (Penicillin), production of single cell protein (*Spirulina)* :

Environmental technology: Waste water treatment process: primary, secondary and advanced treatment of sewage (domestic waste water),

Unit-1; **Molecular biology (16 Hrs)**

Genetic material: Introduction, identification of genetic material (Griffith's and Avery's experiments, Hershey-Chase experiment), Chemical nature of genetic material: Nucleotides, nucleosides
DNA: structure, replication (Semi-Conservative and Rolling circle model) ,RNA: Structure, genetic RNA and non genetic RNA (mRNA, rRNA, tRNA), Biosynthesis of proteins: Genetic code, Transcription, RNA Splicing, Translation and Polysomes. Regulation of gene expression: Prokaryotes: (Lac- Operon) and Eukaryotes (Britten and Davidson's model).

The branch of Biology that deals with the study of **structure and functions of Bio molecules in the living body** is called **Molecular Biology**. In 1945williamAstbury coined the term molecular biology. Organic components of the cell such as Proteins, Carbohydrates, Lipids and Nucleic acids form Bio molecules.

Identification of Genetic material

A genetic material has to be stable in its structure and have self replicating capacity to transfer the biological information from one generation to the next. DNA posses all these characteristics .Hence DNA is regarded as genetic material.

DNA as Genetic material

Griffth 's experiment:

Fredrich griffth , a British medical officer in 1928 while working on pathogenicity of streptococcus pneumonia (it Cause pneumonia in mammals) observed in two strains of bacteria. They are:

1) **Smooth strain(S)**:It has polysaccharide capsule.
2) **Rough strains(R):** It lacks polysaccharide capsule. it produce rough colonies in culture and it is nonpathogenic.

In his experiments, he first injected it to two separate groups of mice. The mice that were infected with the S-strain die from pneumonia. **'S'** strains are the virulent strains causing pneumonia. The mice that were infected with the R-strain do not develop pneumonia and they live.

S-strain (virulent strain) → Inject into mice → Mice die

In the **next set of experiments**, Griffith killed the bacteria by heating nd injected to mice, these did not die but lived, whereas the mice that were injected a mixture of heat-killed S-strain and live R-strain bacteria, died due to unexpected symptoms of pneumonia.

S-strain (heat killed) → Inject into mice → Mice live

S-strain (heat killed) + R-strain (live) → Inject into mice → Mice die

Griffith concluded that the live **R-strain bacteria were transformed** by the heat-killed **S-strain bacteria.**

He proved that there was some '**transforming principle'** that was transferred from the heat-killed S-strain, which helped the R-strain bacteria to synthesise a smooth polysaccharide coat and thus, become virulent(pathogenic). That was due to the transfer of the genetic material. He was not able to define the biochemical nature of genetic material from his experiments.

Avery experiment

Oswald Avery, Colin MacLeod and Maclyn McCarty (1933-44) worked to determine the biochemical nature of '**transforming principle'** in Griffith's experiment in an in vitro system on mice with Streptococcus pneumonia as follows :-

1) A culture of **R strain** produced rough colonies .When it was injected into mouse it did not cause pneumonia .
2) A culture **of S strain** produced smooth colonies when injected into a mice it caused pneumonia .
3) A culture of heat killed **S strain** did not form any colony.
4) A culture of **heat killed' S' strain mixed with living' R' strain** produced smooth colonies of' S' strain instead of' R' strain colonies,
5) Heat killed **S strain were treated with** an enzyme **a protease (**Protein-digesting enzymes) and then cultured with' R'strain ,S strain colonies were formed .
6) Heat **killed S strain were treated with** an enzyme **RNAase** (RNA-digesting enzymes) and then cultured with R strain 'S'strain colonies were formed,
7) Heat **killed S strain were treated with** an enzyme **DNAase** (DNA-digesting enzyme) then cultured with 'R 'strain, only'R'colonies were formed**, Transformation was absent.**
8) This proved that the 'transforming substance' was neither the protein nor RNA, But **the Transformation principle is DNA**.
   Avery's experiment suggests that the DNA caused the transformation and concluded that **DNA is the hereditary material**

Hershey and Chase Experiment:

Alfred Hershey and Martha Chase (1952) carried out experiments with **bacteriophages** (**viruses that infect bacteria)** to prove that **DNA is a genetic material.**

When bacteriophage gets attached to the bacteria, the genetic material enters the bacterial cell, it replicates and with the help of protein synthetic machinery of bacteria to synthesise viral protein .DNA and viral protein assemble to form new bacteriophages.

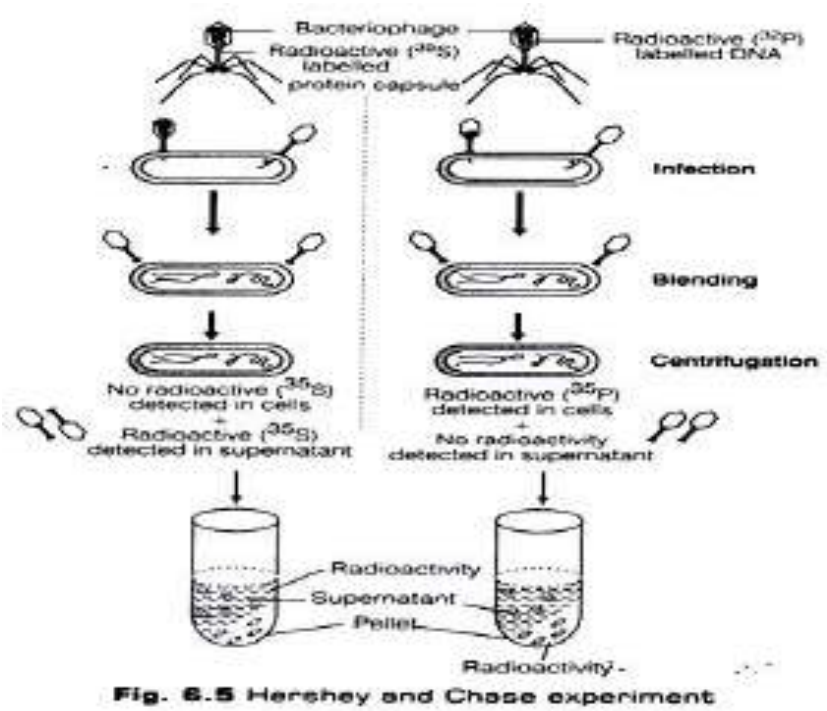


Fig. 6.5 Hershey and Chase experiment

Hershey and Chase experimented to find out **it was protein or DNA from the virus that had entered into the bacteria.**

(i)They took two separate media to grow bacteriophages.

(ii) **One medium** contained **radioactive phosphorus** and the **other medium** contained **radioactive sulphur**.

(a) The viruses grown in the **presence of radioactive phosphorus (^{32}P) contained radioactive DNA**; because phosphorus is present only DNA and not in protein.

(b) The viruses **grown in the medium containing radioactive sulphur (^{35}S)** contained **radioactive protein**, because DNA does not contain sulphur.

(iii) These radioactive bacteriophages were then allowed to attach to bacteria (E. coli). As the process of infection with virus continued, the **bacteria were agitated in a blender** and the viral coats of the bacteria were removed.

(iv) They were centrifuged; virus particles were separated from the bacteria and observed that the **bacteria that were infected with virus containing radioactive DNA were radioactive**, whereas the bacteria that were infected with radioactive proteins were not radioactive.

(v) This indicates that **only DNA** entered the bacterial cell and it is passed from virus to bacteria is DNA. This proves that DNA is a genetic material.

From the Hershey and Chase experiment, proves that **DNA acts as a genetic material**. In some viruses (e.g., Tobacco Mosaic Viruses, Influenza virus, Polio virus) RNA is the genetic material.

Deoxy Ribose Nucleic acid

DNA is a molecule of heredity .It functions as genes in all organisms except few Viruses. In Eukaryotic nucleus, DNA is in the form of double helix . In Bacteria , Mitochondria and Plastids DNA molecules are circular.

CHEMICAL COMPOSITION OF DNA

DNA is made up of 3 chemical components .namely Sugar, Phosphoric acid, Nitrogenous bases.

- **SUGAR:-**The sugar present in DNA is De oxy Ribose. It is a pentose sugar .It is a 5 carbon sugar . At carbon no 2 position one oxygen atom atom is less than Ribose sugar .

- **Phosphoric acid :-**Phosphoric acid is an inorganic acid .In nucleic acid it is alternately arranged with pentose sugar . 'p' is linked to 3 'c' atom of one pentose sugar and 5 'C' atom of another pentose sugar by phosphor di ester bonds .
  1) **NITROGEN BASES:-** These are Nitrogen containing organic compounds .They are of 2 types namely:-**Purines 2)Pyramidines**
     1)Purines:- These are two ringed nitrogen compounds. They are Adenine and Guanine.
     2) Pyramidines:- These are single ringed Nitrogen compounds .They are Thymine, Cytosine and Uracil . Uracil is absent in DNA.

Nucleotide and Nucleoside

1. **Nucleotide** :- Nucleotides are made up of 3 components. They are Nitrogen base ,pentose sugar and phosphoric acid .

Nitrogen base may be purines like Adenine ,Guanine and Pyramidines like Guanine , Cytosine ,Uracil. Sugar may be Deoxy ribose or Ribose. Accordingly they are grouped into deoxy ribose nucleotide and ribonucleotide. Nucleotides are named according to Purines and pyramidines as follows:-

Nitrogen base	Nucleotide
Adenine	Adenilic acid or Adeninosine monophosphate
Thymine	Thymidilic acid or Thymidine Monophosphate
Cytosine	Cytidilic acid or cytocineMonophosphate
Uracil	Uridylic acid or uridine Monophosphate .

Nucleotides may occur in tissues freely as ADP(Adenosine di phosphate) and ATP(Adenosine tri phosphate).

Significance of Nucleotides

1. Nucleotides form main components of Nucleic acids.
2. The deoxyribonucleotides of DNA functions as genetic material.
3. Nucleotides are source of high enrgy .Ex: **ATP**
4. Some Nucleotides functions as co- enzymes Ex: **FMN .**
5. Some nucleotides functions as vitamins . Ex: **FAD,NAD** .

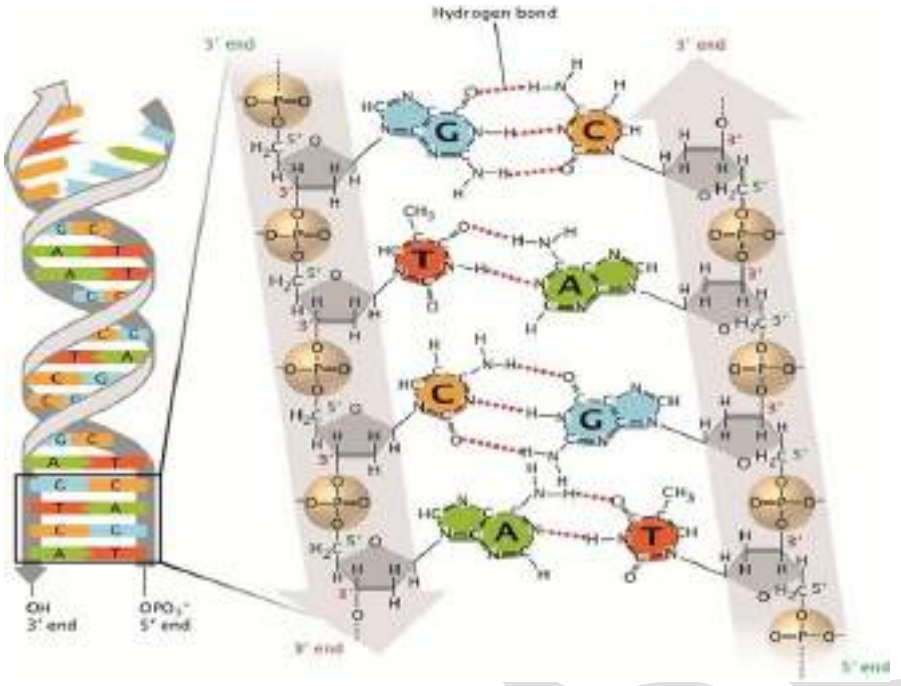
2. **NUCLEOSIDES:-**Nucleosides are composed of 2 types Nitrogen bases linked to pentose sugar. Nitrogen bases are of 2 types. They are Purines like Adenine ,Guanine and Pyramidines like Thymine, Cytosine , Uracil.

Pentose sugar is of 2 types .they are deoxy ribose sugar that forms Deoxy ribose nucleoside and Ribose sugar that forms Ribose nucleoside .

STRUCTURE OF DNA :

Duplex model OR Double helix model OR Watson and Crick model OR' B' form DNA

James D Watson and F H C Crick in 1953 with the available data provided by **wilkins** proposed the duplex of DNA. In 1962 they were awarded Noble prize.According to Duplex model DNA shows fallowing structure:-



1. DNA consists of two polynucleotide strands. These run antiparallel to each other and are twisted in right handed direction.
2. The 2 strands are made up of alternately arranged Deoxy Ribose sugar and phosphate.
3. Phophate group is connected to third carbon atom of one sugar and fifth carbon atom of other sugar by phospho di ester bond.
4. The two strands are interconnected by cross bars made up of nitrogen bases.
5. The Nitrogen molecule is joined to the first carbon atom of the sugar by Glycosidic bond.
6. Purines of one strand always with Pyramidine of other strand.ie.,Adenine pair withThymine by 2 Hydrogen bonds and __.This specific base pairing is called" **Base complementality" or " Complementary base pairing ".**
7. The amount of Adenine is equal to the amount of Thymine and amount of Guanine is equal to the amount of cytosine. This is known as **"ChargoffS rule".**
8. The helix has diameter of 20 A^o .
9. DNA helix makes a full turn at every 34 A ^{o}. along this distance there is one deep major groove and one shallow minor groove.
10. Along the length of 34 A ^{o}there are 10 nucleotides. The distance between each nucleotide is 3.4 A^o .

Alternative forms of DNA

The commonly and naturally occurring DNA described by Watson and crick is called **"B" form DNA.** This double helical structure is found to exist in other forms. They are : A & Z form DNA .

- **A form DNA :** DNA can undergo reversible conformational changes and give rise to **'A' form** upon dehydration. It shows fallowing features:

1. A form DNA is formed at 75% relative humidity, high salt ionic strength.
2. The diameter of helix is **23 A^o** .
3. The rotation per base is **32.7 A ^{o}** and is right handed**;** it has a major groove and a minor groove.
4. 11 base pairs are present per turn**. 5.** The vertical rise per base is **2.56 A^o** .

- **Z DNA** : Z DNA was discovered by A wary and Alexander rich. It shows fallowing features:

1. Z DNA is found in light salt concentration, can also found in solution of high ionic strength.
2. The diameter of helix is **18 A^o** .
3 .The 2 antiparallel polynucleotide chains have left handed rotation.

4. 12 base pairs are present per turn. 5. One complete turn of helix **is 45 A⁰** .

DNA Replication

Replicaiton or Duplication of DNA molecule means to make exact copies of its own structure .It takes place inside the chromosome during "S" phase of interphase.

TYPES OF DNA REPLICATION: There are 3 types of DNA replication. They are:-

1) **Semi conservative method**
2) Conservative method
3) Dispersive method.

SEMICONSERVATIVE METHOD

This method was proposed by Watson and Crick. In 1958 it was proved by M. Meselson and F. W. Sthal.

In Semi conservative method of **DNA replication** 2 daughter molecules are formed each **with one parental strand and a new strand.**

Semi conservative method of **DNA replication** takes place as follows:

Enzymes involved in DNA replication:-

1. **Helicase**(DNA unwinding protein)- **unwind DNA helix.**
2. **polymerase enzyme:** synthesis **RNA primer** a short polynucleotide chain.
3. **DNA polymerase III:** addition of nucleotides to 3-oH group of RNA primer in 5- 3 direction .
4. **DNA Ligase:** joins okazaki fragments.
5. **DNA polymerase I**: Degrades RNA primer and simultaneously catalyses the synthesis of **short DNA fragment** to replace RNA primer.

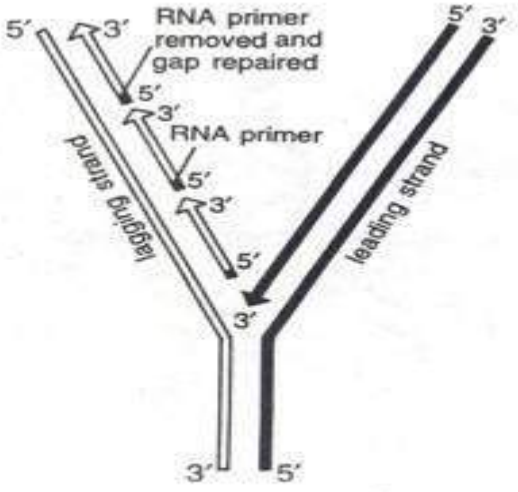


Fig. 8. DNA replication is continuous in one strand, and discontinuous on the other.

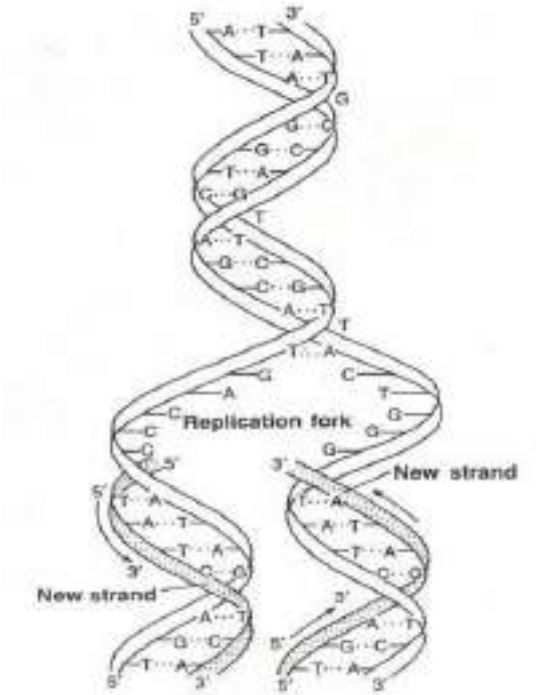


Fig. 6. DNA replication–unwinding of two strands of double helix and then each strand acts as a template for newly synthesized molecules.

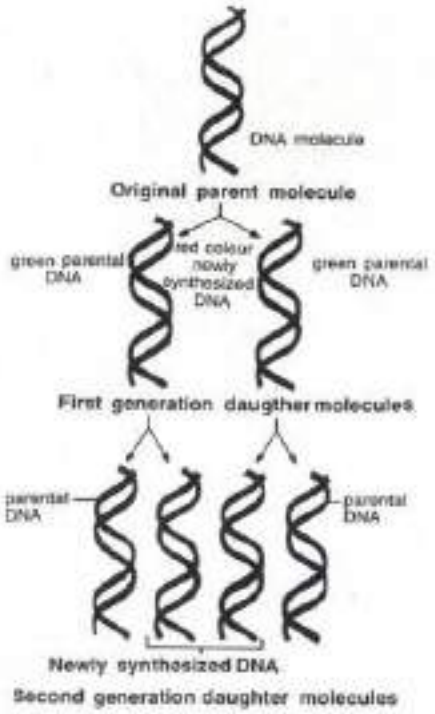


Fig. 7. Schematic diagram of semiconservative replication.

1. DNA Replication takes place at a specific point called **ORI site or origin site**.

2.The **DNA helix unwind** with the help of DNA unwinding protein Helicase , the 2 strands separate from each other after breakage of hydrogen bonds between base pairs. Single strand binding protein extend along single strand and stabilize it.

3. This results in **Y shaped replication fork**. The strain caused by unwinding is relieved by super helix relaxing protein.

4. Initiation of DNA synthesis requires **RNA primer**. It is a short polynucleotide chain synthesized by DNA template in presence of **polymerase enzyme**. The separated strands act as **Templates.**

5 .synthesis of new DNA strand takes place by addition of nucleotides to 3-oH group of RNA primer in 5-3 direction in presence of **DNA polymerase III** .It takes place continuously and it is referred **as Leading strand.**

6 .The other strand is synthesized in short fragments **on Lagging strand** .These are called **Okazaki fragments**. Named after the discoverer Okazaki .

8. **DNA Ligase joins okazaki fragments** into long polynucleotide chain.

9. **DNA polymerase I** degrades RNA primer and simultaneously catalyses the synthesis of **short DNA fragment** to replace RNA primer . This segment is joined to main DNA strand by DNA ligase.

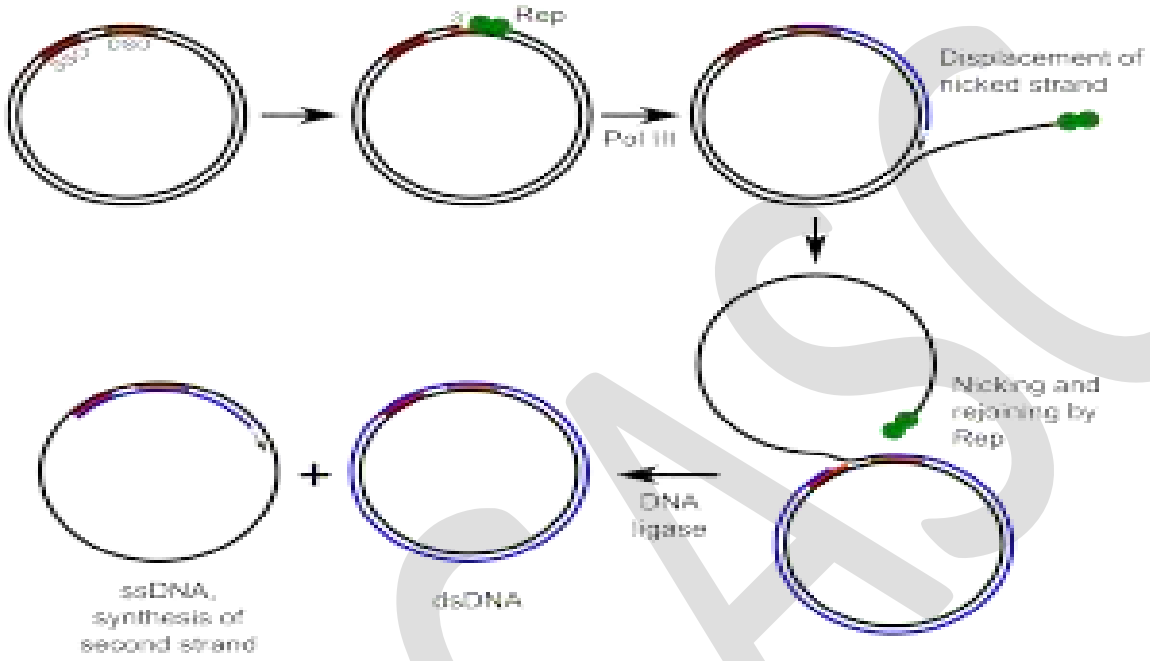
10. The newly formed daughter strands are complementary to their template and undergo coiling with it.

11.Thus 2 daughter DNA molecules are formed .**Each daughter DNA** has **one parental strand and another new strand .**Hence this method of DNA replication is called **Semiconservative method.**12. DNA duplication is a complex process carried by multienzyme complex called **Replisome.** The segment of DNA under replication with its origin to termination is called **"Replicon".**

DNA replication in prokaryotes

Invitro DNA replication has been extensively studied in E. coli and phages .Various models have been proposed to explain the DNA replication. They are : 1) Carins model. 2) Theta model. 3) Rolling circle model.

ROLLING CIRCLE MODEL:- Rolling circle model of DNA Replication takes place in Bacteria and certain Virus .



The process of rolling circle model of DNA replication involves fallowing steps:-:-

1 .DNA replication takes place at **specific point called ORI site**. Protein A recognizes the site.
2. **Nick is formed in one strand** generating free 3-oH end.
3. Replication fork is generated by the influence of **Helicase and SSB protein**.
4.**3-oH end serve as primer** for the synthesis of new strand by the action of **DNA polymerase.**
5. Newly synthesized strand **replaces the original** strand.
6. Long displaced strand **appear as tail joined to a circular structure**. It resemble Greek letter Sigma.
7. Displaced strand may be cut at unit length giving rise to **several single stranded DNA** which circularize and inserted inside phage particle.
8. **Single strand may synthesise another strand** to give rise to Duplex DNA which can be **circularized.**
9. Each circle replicate by Rolling circle model.

FUNCTIONS OF DNA

1. DNA acts as a carrier of genetic information from generation to generation.
2. DNA is a stable macro molecule in almost all living organisms and it is immortal.
3 .DNA controls all biological activities of a cell.
4. It consists of Genetic code for protein synthesis.
5 .DNA serves as a Template for synthesis of RNA.

Ribose nucleic Acid(RNA)

1) RNA is a nucleic acid more **than 90%of RNA is present in the cytoplasm** and rest in the nucleus.

2) RNA is **a single stranded polynucleotide** produced from DNA by the process of Transcription.
3) Each nucleotide is composed of Ribose sugar, phosphate and Nitrogen bases.
4) The nitrogen bases are of two types purines like **Adenine, Guanine and Pyramidines like cytosine, uracil.**
5) Single stranded **RNA fold upon itself forming hairpin loops** which show complementary base pairs.
6) Base composition **does not follow chargoff's rule**.

Kinds of RNA:-

There are two types of RNA they are: 1)**Genetical** RNA 2) **Non-Genetical** RNA

1) **Genetical RNA**; The RNA which constitutes the genetic material is called '**Genitical RNA'.** Ex; HIV,TMV,Polio myelitis,Influenza virus.
2) **Non-Genitical RNA :** The RNA which **do not constitute the genetic material** but play major **role in genetic code and protein synthesis** is called' **Non-Genitical RNA'**.It is present in all living organisms from bacteria to man. These are of three types they are
    1) Ribosomal RNA(r- RNA or in Soluble RNA)
    2) Transfer RNA (t- RNA or Soluble RNA or Adoptar RNA)
    3) Messenger RNA (m- RNA**)**

Ribosomal RNA [r RNA] or Soluble RNA

- Ribosomal RNA is the most stable kind of Ribonucleic acid associated with ribosomes. It constitutes about **80% of the cellular RNA.**
- It is a **single stranded polynucleotide chain folded upon itself** in some regions.
- The **folded region shows base pairing**. Complementary base pairs are joined by hydrogen bonds.
- The **unfolded regions do not show base pairing**. Thus purine and pyramidine equality is absent.
- In Eukaryotes it is formed at nucleolar organizer region of chromosome.

Functions of r-RNA:-

1) During protein synthesis it helps to **recognize and bind m–RNA and t-RNA**

2) **It establishes peptidal binding site** (P site) Amino acyl binding site (A site)in ribosome.

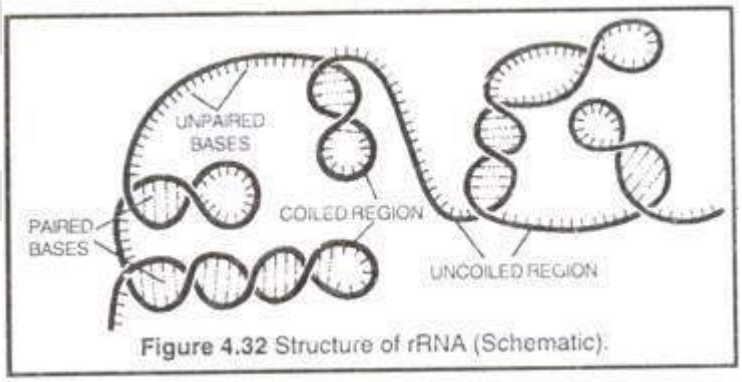
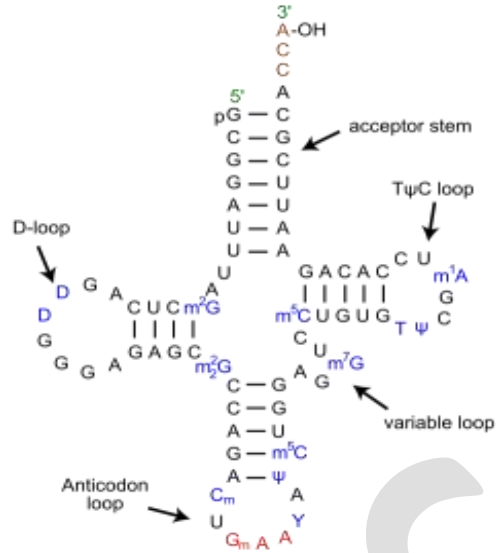


Figure 4.32 Structure of rRNA (Schematic).

Transfer RNA (t-RNA) or soluble RNA

The t –RNA are single stranded polynucleotide chain made up of 70-90 nucleotides. They form 10-20 % of total RNA of the cell. They are synthesized by tRNA genes of DNA by Transcription .The first formed tRNA transcript undergo several levels of processing to become mature 3 dimensional tRNA
The structure of mature tNA is 3 dimentional, when folded into **"L"** shaped molecule. It can also be in two dimensions **as' Clover leaf model'**. It was proposed by **Holley.**



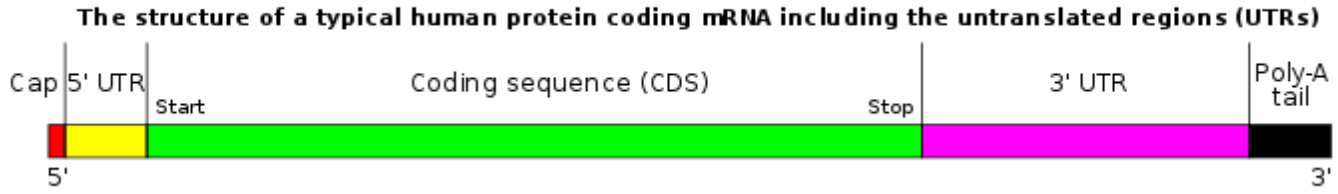
- The t-RNA is a Ribonucleic acid that transfer the activated amino acids to the ribosome to synthesise proteins.
- t-RNA is very small and remains in the supernatant during centrifugation. Hence it is also called as **Soluble RNA**
- t-RNA serve as an adaptor molecule to attach amino acids .Hence it is also called **as Adaptor RNA.**
- t-RNA constitutes 10 to 15% of the total weight of RNA of the cell. It is single stranded with 3' and 5 'ends which is folded on itself.
-

In 1965 Holley proposed "Clover leaf model " to explain secondary structure of t-RNA. According to this model **t–RNA has 5 arms** . They are :-a)Acceptor armb) D- arm c) Anti codon arm d) Variable arm e) T arm.The **D,T arm and Anticodon arm** shows stem and a loop. In stem there is base pairing , in loop there is no base pairing .

1. **Acceptor Arm :-**This is 3' end of the strand with CCA unpaired base .with the help of an enzyme it recognize the activated amino acid and bind to it by covalent bond to form Amino acyl t-RNA
2. **D Arm (DHU ARM):-** It contain Dihydroxy uridine nucleotide hence it is **called D arm**. In presence of t-RNA synthetase it recognize activated amino acid .
3. **Anticodon arm:-**It contains 5 paired bases in stem and 7 unpaired bases in loop. **3 bases of the loop form Anticodon** which recognize codon of mRNA and bind to it by hydrogen bonds.
4. **Variable arm :-**It is a small arm with unpaired bases.
5. **T arm :-**It is a Ribosome identifying site and helps in binding t-RNA to it.

MESSENGER RNA(m-RNA)

Messenger RNA carries genetic information for Protein synthesis from DNA to Cytoplasm, it constitutes 3 to 5% of the total RNA of the cell. Volkin discovered it in E. coli .Jacob and Monad coined the term m-RNA .m-RNA is an un coiled polynucleotide with 5'-3' end. It shows fallowing structural features:-



1. **Cap: -** In Eukaryotes cap is present at 5' end. In Prokaryotes it is absent . It helps the m-RNA to bind with ribosome.
2. **Non coding region I (leader sequence**):- It fallows cap. It is formed of 10 – 100 nucleotides ,rch in Adenine and uracil.
3. **Initiator codon** :- It fallows leader sequence .AUG is the initiator codon . It initiates the processes of protein synthesis .
4. **Coding region**: - It fallows initiator codon. It contain 1500 nucleotideswhich code for amino acids .
5. **Terminator codon** :- It fallows Coding region .**3 Terminator codons namely UAA ,UAG,UGA** stops the process of protein synthesis .
6. **Non coding region II** :- It fallows Terminator . It contain **50-150 nucleotides** which never code any amino acid .
7. **Poly A sequence** :- In Eukaryotes it is present at 3' end, In Prokaryotic m-RNA it is absent . It consists of 200 to 250 Adenylate sequences.

FUNCTIONS

1. M-RNA carries message from DNA in the form of codons to Ribosomes .
2. Single M-RNA can accommodate many ribosomes to synthesise multiple copies of same protein (Poly ribosome).

GENETIC CODE

DNA is the genetic material. The Nitrogen bases in DNA convey the message through m-RNA , to code for specific sequence of Amino acids to form proteins . This is called **Genetic code** .**OR Triplet code.**
The sequence of 3 nitrogen bases (Nucleotide) in m-RNA molecule which contains the information for the synthesis of protein molecule is called **Genetic code** .
F.H.C crick gave the idea of triplet code Marshal Nirenberg gave experimental proof for existence of genetic code. Hargobind khorana established Genetic code dictionary.
Properties of genetic code:- A Genetic code shows fallowing characteristics:-
1**. The code is Triplet**: - Genetic code is a Triplet code .it is represented by a sequence of 3 nitrogen bases determining an amino acid .
2**. Universal**:-Genetic code is universal. A particular code determines the same amino acid in all organisms . Ex: UUU and UUC codes phenyl alanine in virus, bacteria plants and Animals .
3. Co linearity and Polarity:-Codes in polypeptide chain have linear arrangement. It has polarity. It is read in one direction 5' end to 3'.
4. Comma less:-There are no punctuation marks between codons .
5. Non overlapping:-co dons are read in units of 3' nitrogen bases without overlapping.
6. Initiator codon:- AUG is the initiator codon . It initiates protein synthesis and codes for amino acid methionine. If AUG is absent GUG is the initiator codon.
7.Terminator codon:- Among 64 codons , 3 codons namely UAA,UAG,UGA terminate the synthesis of polypeptide chain. These are not read by t-RNA, but are recognized by releasing factors .these 3 codons do not code for any amino acids. Hence they are **called 'Nonsense codons'.**
8. Degeneracy: - An amino acid is coded by one or more codons . This is called 'Degenerate 'or 'Redundant codon'. Ex: Alanine is coded by GCU, GCC, GCA, and GCG.
9. Non-Ambiguous:-Codons are sensible and code for specific amino acid. There is no ambiguity.

WOBBLE HYPOTHESIS

The ability of t-RNA to recognize more than one codon by unusual pairing with third nitrogen base of m-RNA is called '**Wobble hypothesis'** .This is due to the non specificity of the third base of the codon.

In 1965 F.H.C Crick proposed wobble hypothesis. According to them the hydrogen bonding between anticodon and codon for first 2 bases fallow complementary pairing, but base pairing of third base of codon allow several types .This hypothesis is widely accepted because of experimental evidence.

Base in Anticodon	3rd Base in Codon
G	U or C
C	G
A	U
U	AorG

CODE:-A sequence of 3 unpaired Nitrogen bases found in DNA.

Codon:- A sequence of 3 Nitrogen bases found in m-RNA which are complementary to codes of DNA.

Anticodon:- A sequence of 3 Nitrogen bases found in t-RNA which are complementary to codons of m-RNA .

Codes of DNA AAA ACA CGA TGC

Codons of m-RNA UUU UGC GCU ACG

Anticodons of t-RNA AAA ACA CGA UCG

Polypeptide chain Phenyl alanine-Cystine—Alanine—Serine

RNA Splicing

Editing of pre m- RNA transcript in which **introns** are **removed a**nd **exons** are **joined together is called RNA splicing**. It takes place with in the nucleus either co-transcriptionally or immediately after transcription**. (**A eukaryotic gene that contains introns splicing is required to create m RNA molecule that can be translated into protein).

- Several methods of RNA splicing occur in nature.
- The type of splicing depends on the structure spliced, intron and catalyst required for splicing ..
- splicosomal introns are with in the eukaryotic protein coding genes.
- With in intron Donar site GU sequence (in 5' to 3' direction) Acceptor site AG sequence,(3' end of intron) branch site (located 20 to 50 bases upstream of acceptor site) consensus sequence CU(AG) A (C/U) A is conserved to all genes, plays critical role in enzymztic reactions.

Mechanism : It involves 5 small nuclear ribonucleo protein sub units termed Uridine rich small nucleo proteins (U1, U2, U4, U5, U6 SnRNPs)associated with Sn RNPs splicing factors form large complex (60s) called Spliceosome. In 2 steps enzymatic reaction introns are removed, 2 neighbouring exons are joined.U3 RNPs is involve din processing of Pre m-RNA.After 2 steps enzymatic reaction , intron is removed,, 2 neighbouring exons are joined .

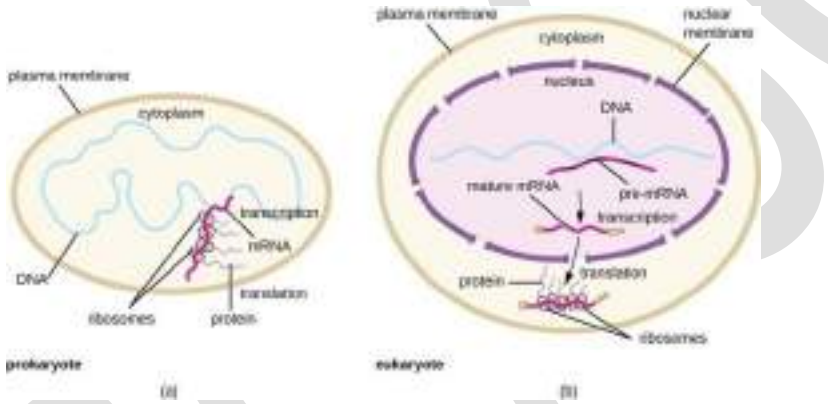
I STEP: 2' Hydroxyl group of conserved Adenosine with in branching site attach conserved Guanine of splicing site at exon1, intron junction. Unusal 2'-5' phosphodiester bond is made between both residues and exon-1 intron junction is cleaved. Products are 2'-5' phosphor diester RNA lariant structure and free 3'-OH and upstream exon.

II STEP: 3'OH end of released exon 1 attaches the scissile phosphodiester bond of conserved Guanine of 3' splicing site at the intron exo2' junction. The exon 2 is then split.

3'-OHof the intron liberates free lariant and spliced exons, 2 exons sequences are joined together, introns are released as lariant structure which degrades in nucleus,SnRNPs will be recycled..

PROTEIN SYNTHESIS

Proteins are **macro molecules** formed of **20 types of amino acids linked by peptide bonds** in different combinations. It is a complex biological activity that takes place in the Ribosomes and they regulate biochemical reactions in the body.



The central dogma of protein synthesis

The unidirectional flow of genetic information from DNA to RNA to Protein is called ' **Central dogma'**.

DNA by transcription form RNA. M-RNA in Ribosome with association of t-RNA, r-RNA and enzymes by translation synthesize Proteins. This can be represented as fallows:-

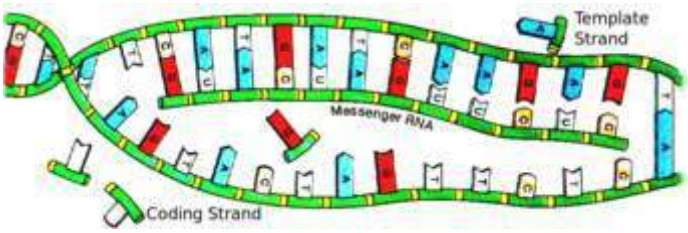
DNA Transcription **RNA** Translation **Proteins.**

Mechanism of Protein synthesis:-The process of protein synthesis involves 2 steps .They are: -

Transcription **2. Translation**

I.TRANSCRIPTION

The synthesis of RNA on DNA is called '**Transcription'** .Steps involved in Transcription is as follows:-



M- RNA synthesis on sense strand of DNA

1. Transcription starts at Promoter region on DNA in presence of an enzyme RNA polymerase.

2. **Sigma factor** of RNA polymerase recognize the Promotor site and gets attached to it .This site is called **"Pribnow box or TATAA box.**
3. In this region DNA unwinds .one of the strand acts as template to synthesise RNA .It is called' **Sense strand**'. The other strand is called **'Antisense strand'**.
4. After initiation of sigma factor is released from core enzyme chain elongation takes place in 5' to 3' direction by addition of nucleotides through RNA primer .Base pairing is complementary ie Adenine pair with uracil and Guanine pair with cytosine.
5. Addition of nucleotides stops when RNA polymerase reaches particular site called **" termination site '.or "Pause site".**
6. 'Rho factor ' or 'sizing factor recognize the termination site.

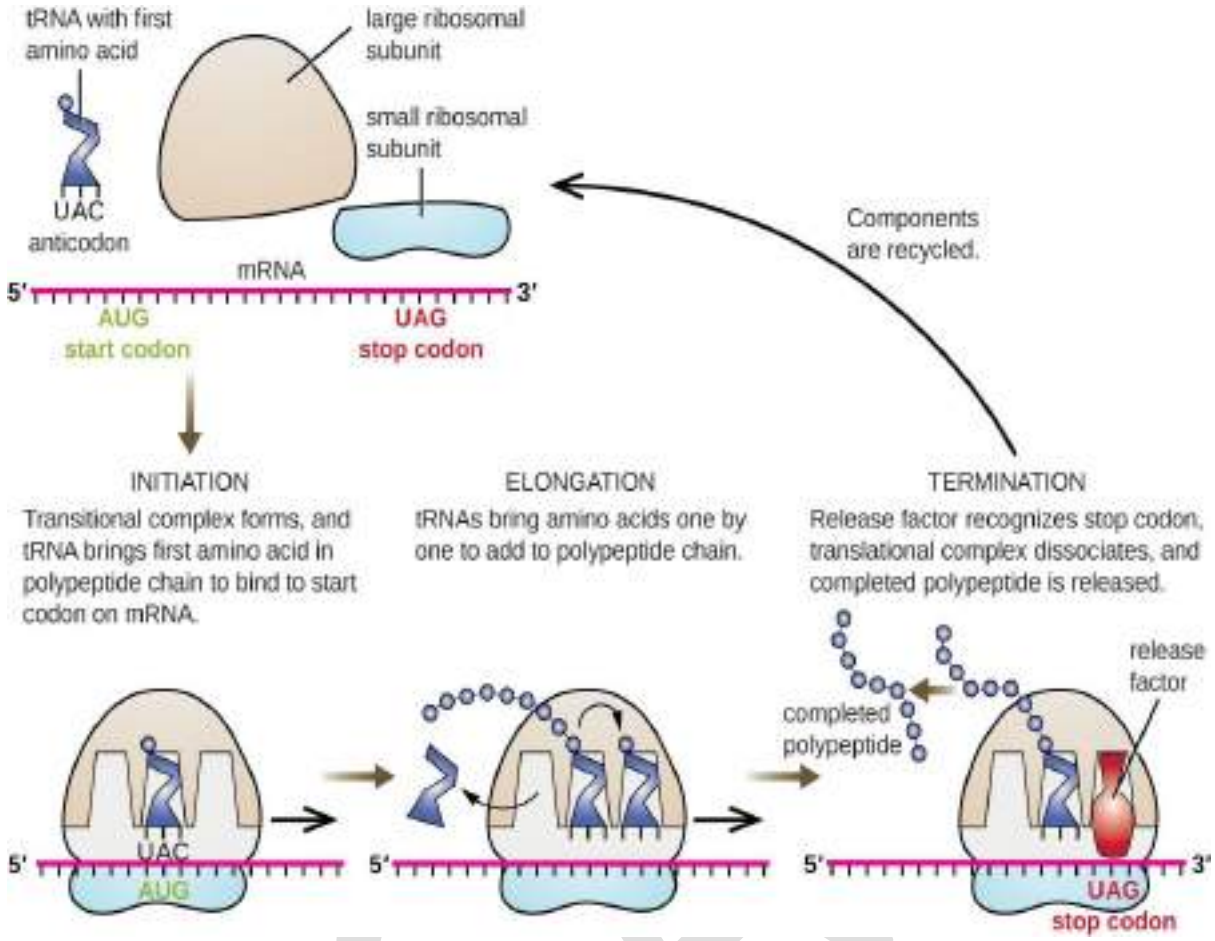
7 .**NUS A (**Nitrogen utilization factor –A) helps in release of new RNA molecules. DNA under go coiling.

II. TRANSLATION

The synthesis of polypeptide chain from Amino acids using coded information in m-RNA is called Translation. Steps involved in the process of translation are as fallows; -

1**) Activation** of amino acids
2**) Initiation** of polypeptide chain
3) Chain **elongation**
4) **Termination**

Diagramatic representation of Translation



1. <u>Activation of amino acids:-</u>

20 types of amino acids present in the cytoplasm are <u>activated by ATP and specific amino acyl synthetase</u> enzyme to form <u>amino acyl adenylate</u>.

Activated amino acid molecule <u>bind to acceptor arm of t-RNA at CCA</u> 3' to form amino **acyl t-RNA complex.**

2. <u>Initiation of polypeptide chain</u> :-

t-RNA molecule with **n-formyl methionine** binds to **initiator codon**. AUG on m-RNA at 30 s sub unit of ribosome in presence <u>of Guonsine triphosphate</u> and initiation factors <u>IF1,IF2,IF3.</u> This is <u>called **pre initiation complex**</u>.50 s sub unit ribosome joins with 30s sub unit to form <u>functional 70s ribosome</u> this is <u>called Initiation Complex.</u>AA1is**at' P' Site** (Peptidal Site) of 70 s ribosome.

3 <u>.Elongation of Polypeptide Chain:-</u>

t-RNA with AA2 occupy' <u>A 'Site</u> (Aminoacyl site) peptide bond develops between AA1 and A,in presence <u>of peptidal transferase enzyme</u>.

<u>Methionine and t-RNA are separated</u> by an enzyme <u>t-RNA deacytalase</u>. T-RNA is released into cytoplasm for further amino acylation.

<u>Ribosome moves on codons of m-RNA Amino acid molecules</u> are added linked by peptide bonds thus <u>polyptide chain elongates in presence of EF-Ts and EF-TU</u>.

4.<u>Termination of polyptide chain:-</u>

When ribosome reaches **terminator codons on m-RNA like UAA, UAC,UGA** <u>synthesis of polyptide chain stops.</u>

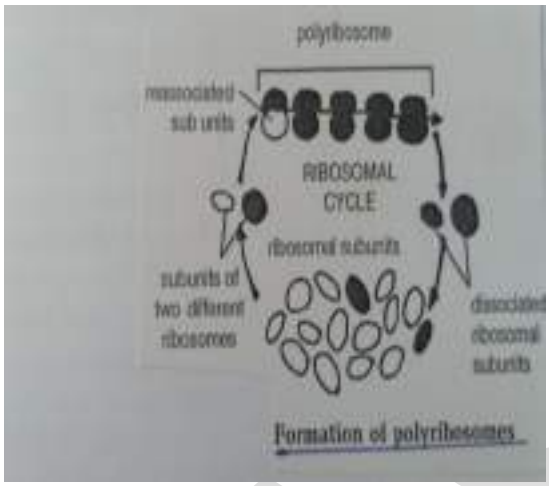
Releasing factors **RF1or RF2 interacts** with terminator codon to form <u>RF- Terminator codon- ribosome complex</u> which block further elongation. **2 subunits of ribosome dissociates**

Polypeptide chain is released a specific protein.

<u>POLY RIBOSOME</u>

Single m-RNA with many Ribosomes to synthesise multiple copies of same protein is **called Poly ribosome'.**

After ribosome has translated about 25 codons the 5' end of m-RNA is free. The second ribosome form initiation complex moves along m-RNA to produce second polypeptide chain. It is fallowed by third, fourth, fifth and so on. Thus series of Ribosomes on m-RNA are involved in synthesis of same type of protein.



GENE REGULATION

F. Jacob and Monad in 1961 proposed **Operon model of gene regulation** in Prokaryotes.Ex: E. coli. They were awarded noble prize.

Operon is a unit of bacterial gene expression and regulation. It consists of Structural genes, Control genes, and Regulator genes and effector molecule.

1. **Strctural genes** :-

Structural genes include **Cistrons** that codes for m-RNA to produce specific enzymes .in Lac operon, it include **Z,Y,A genes**. **Z gene** transcribe for B-Galactosidase enzyme , enzyme ,**Y gene** for permease ,**A gene** for transacetylase enzyme.

2. **Controlling elements** :- Controlling genes include **promoto**r and Operator genes . These regulates function of structural genes.

Promotor gene is a specific gene sequence in DNA, recognized by RNA polymerase and initiate Transcription.

Operator gene is a specific base sequence in DNA present between promotor and structural gene. It interacts with regulator protein to promote or prevent transcription.

3,Regulator protein:- Regulator gene produce a protein called **Repressor Protein**(Regulator protein).It has potentiality to specifically bind to controlling elements due to their 3 dimensional structure. they may be linked or unlinked to structural genes.

4.Effector molecules:-

Effector molecules are called **Inducer or co repressor** .These may be sugar, Amino acids that bind to regulator protein. In prokaryotes operon is of 2 types, namely

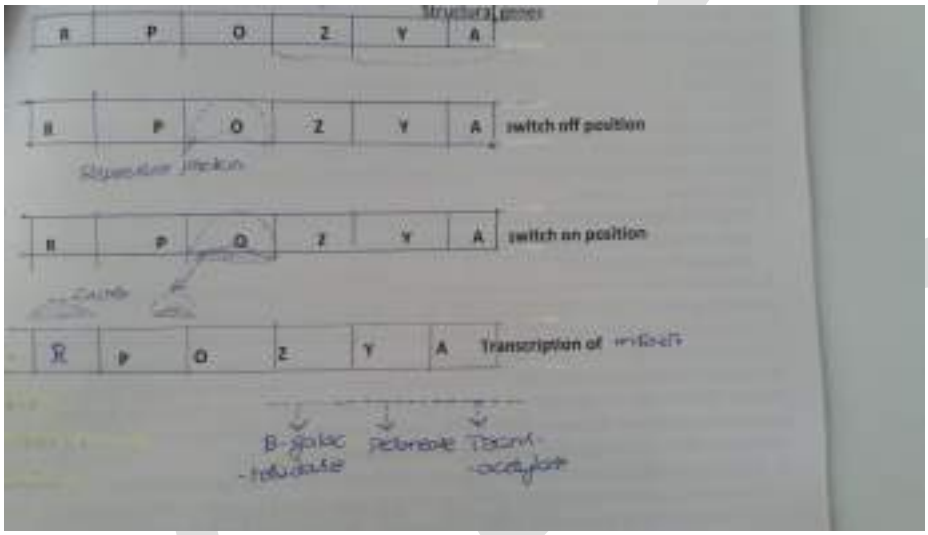
a) **Inducible operon,**

b) Repressible operon.

A) <u>Inducible operon</u>:- Inducible operon functions in presence of inducer like Lactose . Hence it is **called Lac operon.**

<u>In absence of Inducer Lactose in the cell</u>, the regulator gene produce <u>repressor protein. It binds to operator gene</u>, then **switch is off** .This is called **Repressed stage.** The <u>Promotor gene prevents transcription of m-RNA.</u> Thus enzymes are not produced for Lactose utilisation.

<u>In presence of lactose in the cell</u>, lactose binds to repressor protein produced by regulator gene, switch is on. This is called derepressed stage. The promotor gene promotes transcription of m-RNA by structural genes. <u>Thus enzymes essential for lactose utilization are produced</u>.



GENE REGULATION IN EUKARYOTES

<u>Introduction :-</u>

Gene regulation refers t**o the control of rate in which a gene is expressed.** (**Gene regulation** is the process by which the <u>cell determines, through interactions among DNA, RNA, proteins</u>, and other substances, when and where genes will be activated and how much gene product will be produced).

Gene expression is controlled by a complex of numerous regulatory genes and regulatory proteins. The gene regulation is studied in eukaryotes.

Britten and Davidson Model Related to Gene Regulation:

In Eukaryotes Gene regulation is studied by Britten and Davidson model is also known as **'Gene battery model'.** In this model four types of genes are involved **in gene regulation**. They are:-

(1) **Producer gene (2) Receptor gene, (3) Integrator gene, and (4)**. **Sensor gene**

A brief description of these genes in relation to gene regulation is presented below.

1. **Producer Gene**: It is comparable with structural gene of prokaryotic operon. **This gene is located just near the receptor gene.** This gene controls the transcription of mRNA from DNA and also synthesis of specific proteins**. The producer gene starts transcription after receiving signal from receptor gene.**

Each producer gene may have several receptor sites, each responding to one activator.

Thus, a single activator can recognize several genes; different activators may activate the same gene at different times.

2.Receptor Site (Gene): It is comparable to the operator in bacterial operon. One such receptor site is always assumed to be present adjacent to each producer gene.

At least one receptor site is present adjacent to each producer gene. It provides a link between integrator gene and producer gene. The activator RNA binds with receptor gene.

The receptor gene activates the producer gene as soon as it receives signal from the integrator gene through activator RNA. A specific receptor site is activated when a specific activator RNA or an activator protein, a product of integrator gene, binds with it.

The receptor sites and integrator genes may be repeated a number of times so as to control the activity of a large number of genes in the same cell. Repetition of receptor ensures that the same activator recognizes all of them and in this way several enzymes of one metabolic pathway are simultaneously synthesized.

3. **Integrator Gene: Integrator gene is comparable to regulator gene.** It is responsible for the **synthesis of an activator RNA molecule that may or may not give rise to proteinsbefore it activates the receptor site**

At least one integrator gene is present adjacent to each sensor site. **The main function of integrator gene is to start transcription after receiving signal from sensor gene.**

The RNA which is transcribed by the integrator gene is known as **activator RNA**. This activator RNA is a link between integrator and receptor genes.

Britten and Davidson proposed that the integrator gene products are **activator RNAs that interact directly with the receptor genes to trigger the transcription** of the continuous **producer genes. Transcription of the same gene may be needed in different developmental stages. This is achieved by the multiplicity of receptor sites and integrator genes.**

4. **Sensor Gene (Site):** Sensor site regulates the activity ofintegrator gene, which can be transcribed only when the sensor site is activated by agents like harmones , proteins, changes the pattern of gene expression. In this model genes (Producer and integrator gene)are involved in RNA synthesis where as Receptor and Sensor site are those sequences which help only in recognition without taking part in RNA synthesis.

These are signal receiving genes. These genes are very sensitive for specific signals from the cell and its environment. Sensor genes are activated by various cellular substances like enzymes, hormones and metabolites. Whenever these genes receive signals, they pass on the message to the adjacent gene (integrator) for initiation of transcription

According to the Britten Davidson model, specific sensor genes represent sequence-specific binding sites that respond to a specific signal(similar to CAP-cAMP binding site in the E. coil).

When sensor genes receive the appropriate signals, they activate the transcription of the adjacent integrator genes. The integrator gene products will then interact in a sequence specific manner with receptor genes.

A **set of structural genes controlled by one sensor site** is termed as a **battery**. When major changes are needed, it is necessary to activate several sets of genes. If one sensor site is associated with several integrators, it may cause transcription of all integrators simultaneously thus causing transcription of several producer genes, through receptor sites.

The repetition of integrator genes and receptor sites states that sufficient repeated DNA occurs in the eukaryotic cells. The most **attractive features of the Britten and Davidson model** is that it provides a plausible reason for moderately repetitive DNA sequences and single copy DNA sequences. Direct evidence indicates that most structural genes are indeed single copy DNA sequences.

Merits and Demerits of Britten and Davidson Model:

Merits:

1. This model is widely accepted for gene regulation in eukaryotes.
2. The mRNA is stable in eukaryotes which is unstable in prokaryotes.

Demerits:

1. This is only a theoretical model and lacks sound practical proof.
2. The mechanism of this model is more complex than operon model of gene regulation in prokaryotes.
3. This model is lesser understood than operon model of gene regulation in prokaryotes

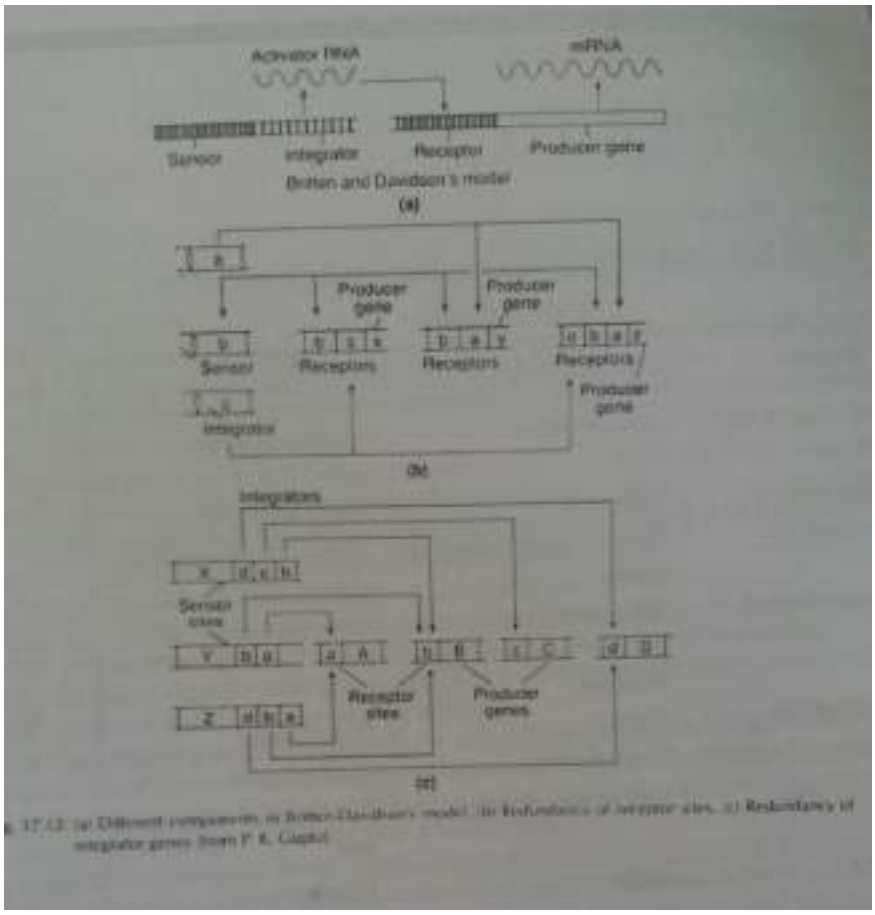


TABLE 26.1. Comparison of gene regulation model of prokaryotes and eukaryotes

S.No.	Particulars	Prokaryotes	Eukaryotes
1.	Gene regulation model developed by	Jacob and Monod	Britten and Davidson
2.	Model known as	Operon model	Battery model
3.	Genes involved	Structural, operator, promoter and regulator	Sensor, integrator, receptor and producer
4.	Enzymes involved	beta-galactosidase, galactosidase permease, transacetylase and RNA polymerase.	mRNA polymerase
5.	Gene regulation	Simple	More complex
6.	Perception of mechanism	Better understood	Lesser understood
7.	Basis of model	Based on empirical results	It is a theoretical model without proof
8.	mRNA	Unstable	Stable
		Polycistronic	Monocistronic
9.	Matching gene	Structural genes,	Producer gene
		Operator gene	Receptor gene
		Promoter gene	Sensor gene
		Regulator gene	Integrator gene
10.	Popularity	Widely accepted	Widely accepted

.

2 MARKS QUESTIONS

1. Differentiate between leading and lagging strand. 1+1
2. Name any four enzymes invoved in D.N.A replication. ½ each
3. What is Palindromic sequence? Mention its importance. 1+1
4. What are nonsense codons? Mention any two of them. 1+1
5. Mention any 4 characteristics of RNA. ½ mark each.
6. What are Nucleotides? Mention their types. 1+1
7. De fine initiator codon. Name the amino acid it codes . 1+ 1
8. Differentiate between Nucleoside and Nucleotide. 1+1
9. Name the enzymes involved in DNA replication. ½ each
10. What is chargaff's rule? Mentin its function. 1+1
11. Mention the different types of RNA and their importance. 1+1
12. List any 4 functions of DNA. ½ each
13. Differentiate between 'A' and 'Z'Form DNA. 1+1
14. Write central dogma of protein synthesis.
15. what is Poly ribosome? Mention its significance.
16. Mention Merits and Demerits of Britten and Davidson Model.

5 MARKS QUESTIONS

1. Griffth 's experiment: S stran-1, R-1, Explanation-3 marks
2. Give an account of Avery experiment Def-2, explanation-3
3. Write note on Hershey and Chase Experiment. **Fig-2, explanation-3**
4. Give an account of chemical composition of DNA. 1+4
5. Explain Nucleotides and Nucleosides. Definition-1 , Explanation-4
6. Explain Rolling circle model of DNA. Fig-2, explanation-3
7. Draw a neat labeled diagram of DNA Fig-1, explain-4
8. RNA splicing. Definition-1, Mechanism -4
9. Describe regulation of gene in Eukaryotes Fig-2, explanation-3.
10. Explain the transcription in Protein synthesis. Fig-1, Explain-4
11. With neat labeled diagram explain the structure of m-RNA. Fig-2, exp-3
12. Describe the structure of t-RNA. Fig-1, explanation-4
13. Describe the properties of Genetic code. ½ each
14. Explain Lac operon Concept. Fig-2, Explanation-3

10 MARKS QUESTIONS

1)Explain the semi-conservative method of DNA replication. Fig-4, explain-6

2)Explain Translation in protein synthesis. 4 steps 11/2 each, Fig -3 marks
3)Explain Watson and Crick model of DNA with neat labeled diagram. Fig-4,expl-6.
4) Describe Gene regulation in Eukaryote / Britten and Davidson model/Battery model.

Unit-2: Bio-molecular Techniques (6 Hrs)

Padmashree.M.S. SSCASC,

Blotting techniques: Northern, Southern and Western Blotting, DNA finger printing; DNA sequencing (Sanger's method), PCR .

Southern Blotting

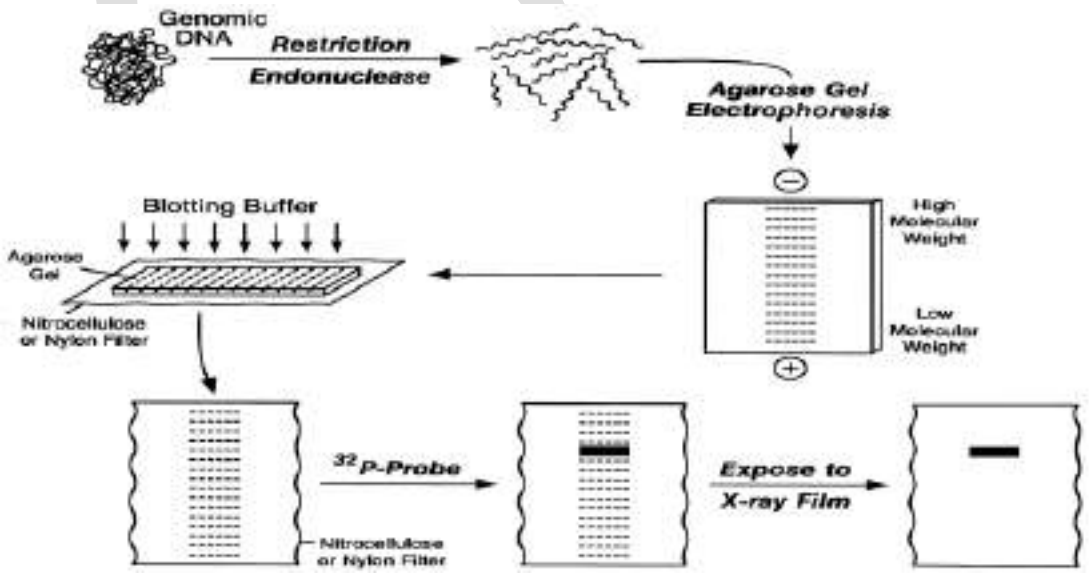
Southern blotting is a hybridization technique for identification of particular size of DNA from the mixture of other similar molecules.). It was developed by Edward M. Southern (1975).

Southern blotting is an example of **RFLP** (restriction fragment length polymorphism. This technique is based on the **principle of separation of DNA fragments by gel electrophoresis and identified by labelled probe hybridization**.

The DNA fragments are **separated on the basis of size and charge** during **electrophoresis**. DNA fragments are separated after transferring on **nylon membrane**; the desired DNA is **detected using specific DNA probe** that is complementary to the desired DNA. (A hybridization probe is a short (100-500bp), single stranded DNA.).The probes are labeled with a marker so that they can be detected after hybridization.

Procedure for Southern Blotting

1. **Restriction digest**: by RE enzyme and amplification by PCR
2. **Gel electrophoresis**: SDS gel electrophoresis
3. **Denaturation**: Treating with HCl and NaOH
4. **Blotting**
5. **Baking and Blocking** with casein in BSA
6. **Hybridization** using labelled probes
7. **Visualization** by autoradiogram



Step I: Restriction digest

- The DNA is fragmentized by using suitable restriction enzyme. RE cuts the DNA at specific site generating fragments
- The number of fragments of DNA obtained by restriction digest is amplified by PCR

Step II: Gel electrophoresis

- The desired DNA fragments is separated by gel electrophoresis
-

Step III: Denaturation

- The SDS gel after electrophoresis is then soaked in alkali (NaOH) or acid (HCl) to denature the double stranded DNA fragments.
- DNA strands get separated

Step IV: Blotting

- The separated strands of DNA are then transferred to positively charged membrane nylon membrane (Nitrocellulose paper) by the process of blotting.

Step V: Baking and blocking

- After the DNA of interest bound on the membrane, it is baked on autoclave to fix in the membrane.
- The membrane is then treated with casein or Bovine serum albumin (BSA) which saturates all the binding site of membrane

Step VI: Hybridization with labelled probes

- The DNA bound to membrane is then treated with labelled probe
- The labelled probe contains the complementary sequences to the gene of interest
- The probe bind with complementary DNA on the membrane since all other non-specific binding site on the membrane has been blocked by BSA or casein.

Step VII: Visualization by Autoradiogram

- The membrane bound DNA labelled with probe can be visualized under autoradiogram which give pattern of bands.

Application of Southern blotting:

1. Southern blotting technique is used to **detect DNA** in given sample.
2. DNA finger printing is an example of southern blotting.
3. Used for **paternity testing, criminal identification**, victim identification.
4. **To isolate and identify** desire gene of interest.
5. Used in restriction fragment length polymorphism (**RFLP).**
6. To identify **mutation** or gene rearrangement in the sequence of DNA.
7. Used in **diagnosis of disease** caused by genetic defects.
8. Used to **identify infectious agents.**

Northern Blotting

Northern Blotting is a technique used for the study of **gene expression**. It is done by **detection of particular RNA** (or isolated mRNA). This method reveals the **identity, number, activity, and size of the particular gene**. The northern blot technique was developed in 1977 by **James Alwine, David Kemp and George Stank** at Stanford University. The technique got its name due to the similarity of the process with

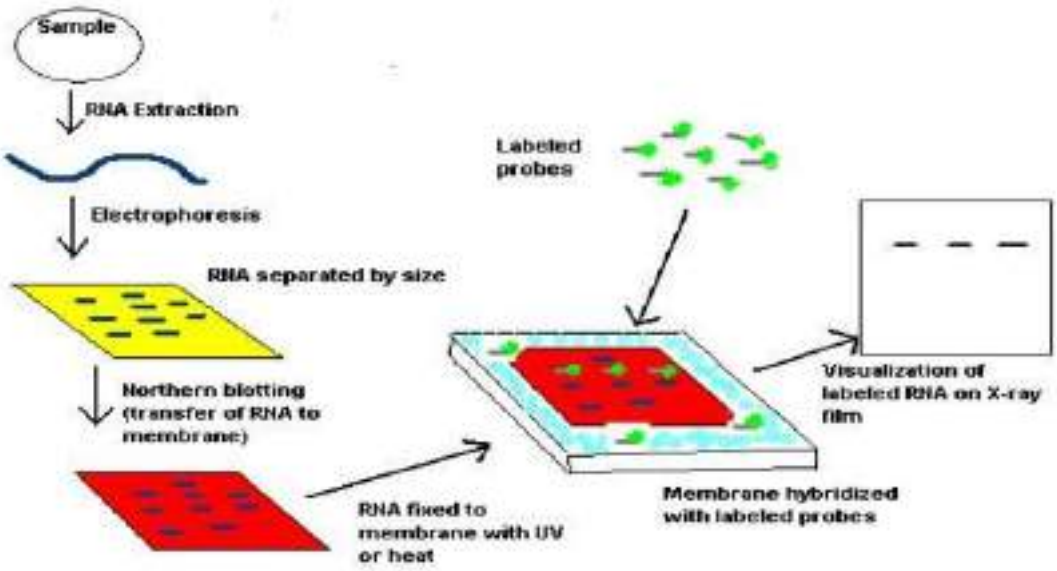
Southern blotting. The primary difference between these two techniques is that northern blotting concerns only about RNA.

Principle:-

Northern blotting starts with the electrophoresis to separate RNA samples by size. Electrophoresis separates the RNA molecules based on the charge of the nucleic acids. The charge in the nucleic acids is proportional to the size of the nucleic acid sequence. Thus the electrophoresis membrane separates the Nucleic acid sequence according to the size of the RNA sequence.

Procedure

1. The tissue or culture sample collected is **homogenized**. The samples may be different types of culture for comparison or it can be for the study of different stages of growth inside the culture.
2. The RNA sequence is **separated** in the electrophoresis unit using agarose gel.
3. The separated RNA sequence is **transferred** to the nylon membrane by two mechanisms capillary action and the ionic interaction.
4. The transfer is done by keeping the gel in the following order. First, the **agarose gel** is placed on the bottom of the stack, followed by the **blotting membrane**. On top of these paper **towels a mild weight** (glass plate) is placed. The entire setup is kept in a beaker containing transfer buffer.
5. RNA transferred to the nylon membrane is **then fixed using UV radiation**.
6. The fixed nylon membrane is then **mixed with probes**. The probes are specifically designed for the gene of interest, so that they will hybridize with RNA sequences on the blot corresponding to the sequence of interest.
7. The blot membrane is **washed to remove** unwanted probe
8. **Labeled probe is detected** by chemiluminescence or autoradiography. The result will be **dark bands** in x ray film.



Application of Northern blotting:

1. **Detecting** a specific mRNA in a sample
2. Used in the **screening** of recombinants by detecting the mRNA produced by the transgene
3. In gene **expression** studies.
4. This blotting technique is be used for the **growth of a tissue** or organism.
5. Using these technique different stages of **differentiation and morphogenesis**, the abundance of an RNA changes can be identified.
6. It also aids in the **identification of abnormal, diseased or infected** condition at the molecular level.

(Additional information :- Probes

The RNA samples are separated using agarose gels using formaldehyde. In small RNA or micro RNA sequences, polyacrylamide sequences with urea as a denaturing agent also can be used. Ethidium bromide is be used as a staining agent. An RNA ladder and ribosomal subunit are used for the identification of the size of the RNA sequences. Probes can be complementary to the whole or part of the RNA of interest. They can be RNA, DNA or oligonucleotides of 25 complementary basepairs to the target RNA.

In case of RNA probes, invitro produced probes are used as invivo probes can denature due to the rigorous washing. In case of cDNA, the probes are labeled with radioactive isotopes, alkaline phosphatase or horseradish peroxidase in case of chemiluminescence.)

Western blotting technique:

Principle:

Western blotting technique is used for identification of **particular protein** from the mixture of protein.

In this method **labeled antibody** against particular protein is used **identify the desired protein,** so it is a specific test. Western blotting is also known **as immunoblotting** because it uses antibodies to detect the protein.

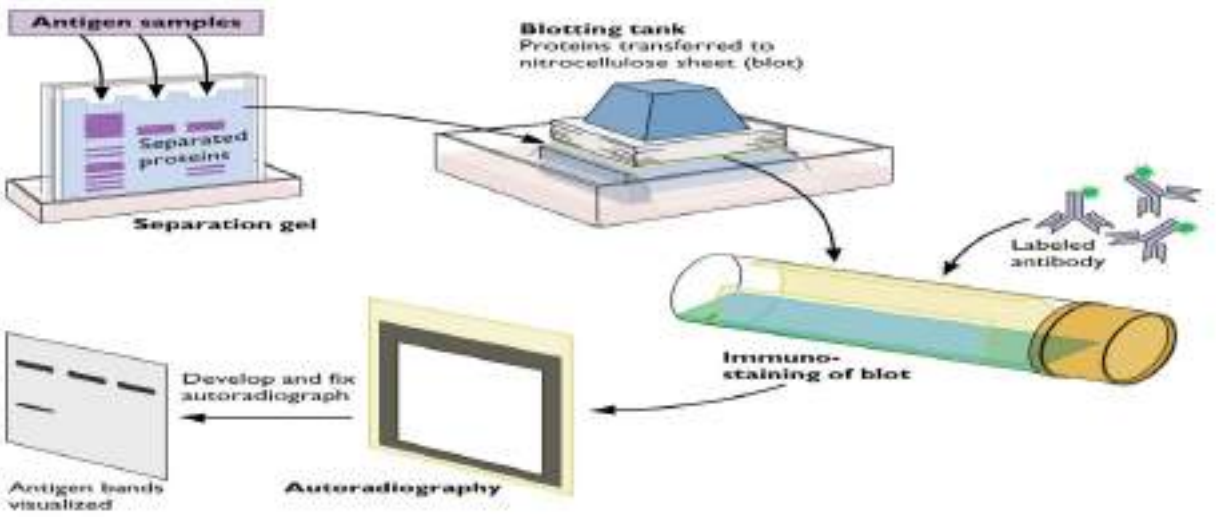
Procedure for Western blotting technique:

1. **Extraction** of protein

2. Gel electrophoresis-**separation and analysis of macromolecules** (DNA, RNA and proteins) and their fragments, based on their size and charge. It is used in clinical chemistry to separate proteins
3. Electrical or capillary **blotting**
4. Blocking-method of **transferring proteins**, DNA or RNA, onto a carrier (for example, a nitrocellulose, polyvinylidene Treatment with primary antibody
5. **Treatment** with secondary **antibody** (enzyme labelled anti Ab)
6. **Treatment with** specific substrate; if **enzyme is alkaline phosphatase**, substrate is p-nitro phenyl phosphate which give color.

Uses

- It is most **sensitive and specific test** for **determining size and amount of protein** present in any material.
- The **confirmatory HIV test** employs a western blot to detect anti-HIV antibody in a human serum sample.
- A western blot is also used as the **definitive test for Creutzfeldt-Jakob Disease**, Lyme disease, Hepatitis B infection and HSV-2 (Herpes Type 2) infection.



DNA Fingerprinting

INTRODUCTION:-

A DNA fingerprint is an ideal way to distinguish an individual. It is **same for every cell, tissue and organ of a person and it cannot be changed by any known treatment**. **Alec Jeffreys** (1984) invented the **DNA fingerprinting** technique at Leicester University, United Kingdom. **Dr. V.K. Kashyap and Dr. Lalji Singh started the DNA fingerprinting technology in India** at CCMB (Centre for Cell and Molecular Biology) Hyderabad.

DNA-fingerprinting is also called **DNA typing or DNA profiling.** It is a technique of **determining nucleotide sequences of certain areas of DNA** which are **unique to each individual**. **Each person has a unique DNA fingerprint**.

Principle of DNA Fingerprinting:-

Human genome possesses **small noncoding but inheritable sequences of bases** which are **repeated** many times .These occur near telomere, centromeres, Y chromosome and heterochromatic area. The area with same sequence of bases repeated several times is called '**Repetitive DNA'.** They can be separated as

satellite from the bulk DNA during density gradient centrifugation and hence called **satellite DNA**. In satellite DNA, repetition of bases is in tandem.

Depending upon length, base composition and numbers of tandemly repetitive units, satellite DNAs have subcategories like microsatellites and mini-satellites.

Variations occur due to mutations which produce alleles with different expressions. Mutations in noncoding repetitive DNA has no immediate impact. These have piled up with time and form the basis of DNA polymorphism (variation at genetic level arises due to mutations). **DNA polymorphism is the basis of genetic mapping of human genome as well as DNA finger printing.**

Technique for DNA Fingerprinting

1. The DNA is extracted from the nuclei of white blood cells or of spermatozoa or of the hair follicle cells.
2. The DNA molecules are first broken with the help of enzyme restriction endonuclease into fragments. The fragments of DNA also contain the variable number of tandem repeats VNTRs.
3. The fragments are separated according to size by gel electrophoresis.
4. Fragments of a particular size having VNTRs are multiplied through PCR technique. They are treated with alkaline chemicals to split them into single stranded DNAs.
5. The separated fragments of single stranded DNA are transferred onto a nylon membrane.
6. Radioactive DNA probes having repeated base sequences complementary to possible VNTRs are poured over the nylon membrane. Some of them will bind to the single stranded VNTRs. (Southern Blotting), The nylon membrane is washed to remove extra probes.
7. An X-ray film is exposed to the nylon membrane to mark the places where the radioactive DNA probes have bound to the DNA fragments. These places are marked as dark bands when X-ray film is developed. This is known as autoradiography.
8. The **dark bands on X-ray film** represent the '**DNA fingerprints'** (= DNA profiles).

Applications of DNA Fingerprinting:

- **Individuality:** DNA finger printing can help to distinguish one human being from another with exception of monozygotic twins,
- **Paternity/Maternity Disputes**:DNA finger printing can identify the real genetic mother, father and the offspring,
- **Human Lineage**:DNA from various probables is being studied to find out human lineage,
- **Hereditary Diseases:** The technique is being used to identify genes connected with hereditary diseases,
- **Forensics:** DNA finger printing is very useful in the detection of crime and legal pursuits.
- **Sociology:** It can identify racial groups, their origin, historical migration and invasions. Genography is the study of migratory history of human species.

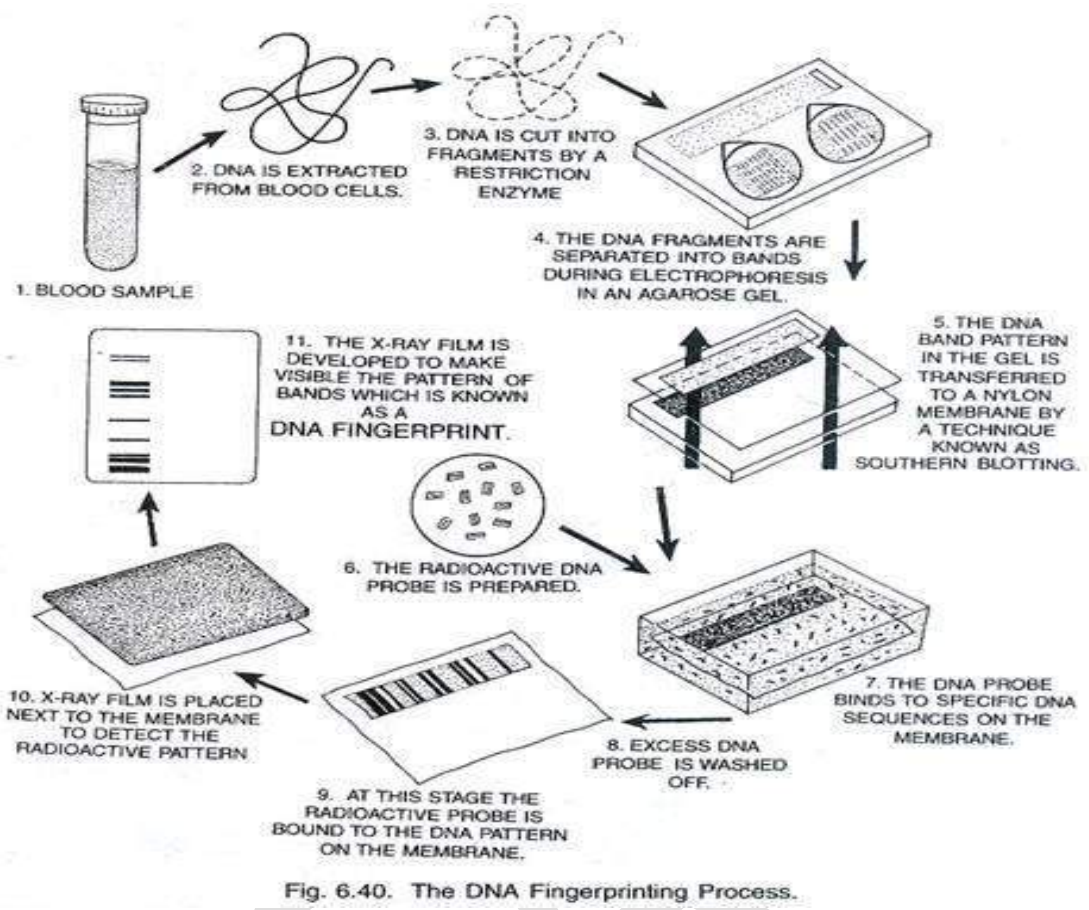


Fig. 6.40. The DNA Fingerprinting Process.

Minisatellites:-

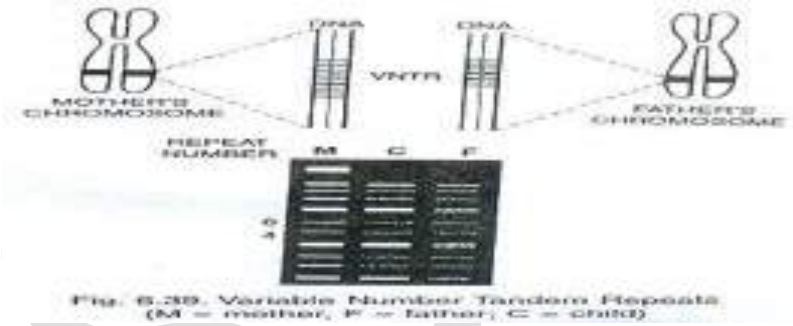


Fig. 6.39. Variable Number Tandem Repeats (M = mother; F = father; C = child)

Short nucleotide repeats in the DNA are very specific in each individual and vary in number from person to person but are inherited. These are the 'Variable Number Tandem Repeats' (VNTRs). These are also called "minisatellites". Each individual inherits these repeats from his/her parents which are used as genetic markers in a personal identity test. For example, a child might inherit a chromosome with six tandem repeats from the mother and the same tandem repeated four times in the homologous chromosome inherited from the father. One half of VNTR alleles of the child resemble that of the mother and other half with that of the father.

Polymerase chain reaction (PCR)

INTRODUCTION

Polymerase chain reaction (PCR) is a method used to **make many copies of a specific DNA segment**. Using PCR, a single copy of a **DNA sequence is exponentially amplified to generate thousands to millions of more copies of that particular DNA segment**. PCR was developed **by Kary Mullis** in 1983. He was awarded the Nobel Prize in Chemistry in 1993 (along with Michael Smith) for his work.

Principle: - It is a chain reaction; a small fragment of the DNA section of interest is identified. It serves as the template for producing the primers to initiate the reaction. One DNA molecule is used to produce two copies, then four, then eight and so forth.

Requirements:-

1. Template DNA: - A PCR template for replication can be of any DNA source, such as genomic DNA (gDNA), complementary DNA (cDNA), and plasmid DNA.

2. Taq polymerase :- PCR requires a DNA polymerase enzyme that makes new strands of DNA, using existing strands as templates. **The DNA polymerase typically used in PCR is called *Taq* polymerase** isolated from heat-tolerant bacterium *Thermus aquaticus*.

3. PCR primers:- PCR primers are **short pieces of single-stranded DNA**, (usually around 2020 nucleotides in length). Two primers are used in each PCR reaction . The primers bind to the template by complementary base pairing.

4. Deoxynucleoside triphosphates (dNTPs):-dNTPs consist of four basic nucleotides—dATP, dCTP, dGTP, and dTTP—as building blocks of new DNA strands. These four nucleotides are typically added to the PCR reaction for optimal base incorporation.

5. Magnesium ion (Mg^{2+}):- Magnesium ion (Mg^{2+}) functions as a cofactor for activity of DNA polymerases by enabling incorporation of dNTPs during polymerization.

6. **Buffer:-**PCR is carried out in a buffer that provides a suitable chemical environment for activity of DNA polymerase. The buffer pH is usually between 8.0 and 9.5 and is often stabilized by Tris-HCl.

The steps of PCR

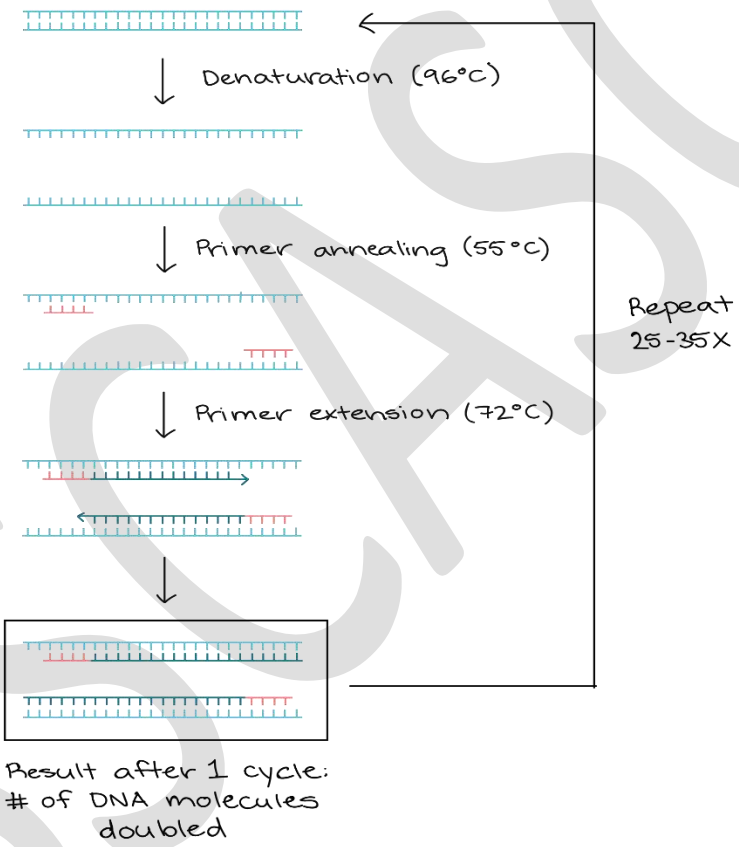
The key ingredients of a PCR reaction *Taq* polymerase, primers, template DNA, and nucleotides are assembled in a tube, along with cofactors needed by the enzyme, and are put through repeated cycles of heating and cooling that allow DNA to be synthesized.

The basic steps are:-

- **Initialization:** It consists of heating the reaction chamber to a temperature of 94–96 °C, or 98 °C if extremely thermostable polymerases are used, which is then held for 1–10 minutes.
- **Denaturation:** This is the first regular cycling event and consists of heating the reaction chamber to 94–98 °C for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.
- **Annealing:** In this step, the reaction temperature is lowered to 50–65 °C for 20–40 seconds, allowing annealing of the primers to each of the single-stranded DNA templates. Two different primers are included in the reaction mixture: one for each of the two single-stranded complements containing the target region
- **Extension/elongation:** In this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free dNTPs from the reaction mixture that are complementary to the template in the 5'-to-3' direction.

(The temperature at this step depends on the DNA polymerase used; the optimum activity temperature for the thermostable DNA polymerase. Taq polymerase is approximately 75–80 °C, though a temperature of 72 °C is commonly used with this enzyme).

- **Final elongation**: This single step is optional, but is performed at a temperature of 70–74 °C for 5–15 minutes; after the last PCR cycle to ensure that any remaining single-stranded DNA is fully elongated.
- **Final hold**: The final step cools the reaction chamber to 4–15 °C for an indefinite time, and can be employed for short-term storage of the PCR products.



Applications of Polymerase chain reaction (PCR)

1. **Selective DNA isolation:-**PCR allows isolation of DNA fragments from genomic DNA by selective amplification of a specific region of DNA.
2. **Amplification and quantification of DNA :-**PCR amplifies the regions of DNA that it targets PCR can be used to analyze extremely small amounts of sample.
   PCR may also be used in the analysis of ancient DNA (Thousands of years old-successfully used on animals, such as a forty-thousand-year-old mammoth, and also on human DNA, in the analysis of Egyptian mummies).
3. **Medical and diagnostic applications:-**Prospective parents can be tested for being genetic carriers, or their children might be tested for being affected by a disease.

- DNA samples for prenatal testing can be obtained by amniocentesis, chorionic villus sampling, or even by the analysis of rare fetal cells circulating in the mother's bloodstream.
- PCR analysis is essential to preimplantation genetic diagnosis, where individual cells of a developing embryo are tested for mutations.
- Used as part of a sensitive test for tissue typing, vital to organ transplantation.
- PCR permits early diagnosis of malignant diseases such as leukemia and lymphomas.

4. **Infectious disease applications:-**PCR allows for rapid and highly specific diagnosis of infectious diseases, caused by bacteria or viruses.
   - PCR permits identification of non-cultivatable or slow-growing microorganisms such as mycobacteria, anaerobic bacteria, or viruses .
   - PCR technique helps in Quick detection of Diseases . Ex: **pertussis** (or whooping cough) respiratory infection , Epedemic diseases like **H1N1, Dengue**, etc.

5. **Forensic applications:-**Forensic DNA typing is an effective way of identifying criminal suspects due to analysis evidence discovered at a crime scene.
   - PCR with DNA fingerprinting can help in DNA paternity testing.

2 MARKS QUESTIONS

1Mention Applications of Southern blotting. Any 4 ½ marks each

2. What is Northern Blotting? Mention its application. Definition-1, any 2 **½ marks each**

3. What is the Principle of Western Blotting?

4. Mention Applications of DNA Fingerprinting. **Any 4 ½ marks each**

5 MARKS QUESTIONS

1. Expand PCR? Mention its significance. Definition-1,significance-1

2.Describe Southern Blotting Technique.

3.Explain Northern Blotting? Mention its application.

4.Describe Western blotting technique.

10 MARKS QUESTIONS

1.Give an account of DNA Fingerprinting. Add a note on its application.

2. Describe Polymerase chain reaction (PCR)

Unit -3: Genetic engineering: 12Hrs

Syllabus

A concise account of methods used in recombinant DNA technology. Tools of rDNA technology: Plasmids (PBR322, PUC 18, Ti-plasmid), Restriction endonuclease, DNA ligase, and Bioreactor. Genomic and cDNA libraries, screening of genomic library. Applications of Genetic Engineering technology in agriculture. (Transgenic plants- Bt-cotton, golden rice), in medicine (insulin synthesis, gene therapy), in environment (bioremediation and bio-mining).

GENETIC ENGINEERING

The alteration of genotype of an organism using in vitro techniques to produce products and services of human benefit is called **'Genetic engineering** '.

- First rDNA molecule was made by Paul berg in 1972 by combining DNA from monkey Virus SV40 with Lambda virus.
- First genetically modified organism Bacterium was generated by Herbert boyer and Stanley Cohen in 1973.
- Genetically enginnered Humulin was commercialized in 1982.
- Genetically modified food was sold in1994 with the release of Flavr savr Tomato which has longer shelf life.
- GLO fish, first genetically modified organism designed pet sold in Dec.2003 in U.S.A.

In 1973 Stanly Cohen (Stanford University, USA) Hebert bayer (University of California USA) reported that desired gene can be isolated and joined into the plasmid of another organism using enzymes.

Tools used in genetic engineering : The biological tools used in genetic engineering are : Vector , Enzymes ,desired gene ,Host cell, Bio reactor.

1] Vector : Vectors are DNA molecules that can carry a foreign DNA fragment inserted into them without any disturbance in their expression .. It is also called as '**Cloning vehicle ' or'Vehicle DNAs'.** It may be Plasmid ,Bacteriophage ,transpons.

2] Plasmid:-Plasmids are extrachromosomal, self replicating, double stranded, circular DNA molecules present in Bacterial cell. In 1973 Cohen et al., reported cloning DNA using Plasmid as Vector. Plasmids are used as cloning vehicles when they posses fallowing features:-

Properties of Plasmids*:*

A Plasmid can be considered as a suitable cloning vehicle if it shows following feature: -

1) Plasmid can be easily isolated from the cells. They are specific to one or a few particular bacteria.
2) They replicate independently of the bacterial chromosome and they code for their own transfer.
3) It contain single restriction site for one or more restriction enzyme. It must possess marker gene such as Resistance for Tetracycline, kanamycin , Ampicillin
4) Insertion of foreign gene should not alter replication properties of plasmids.
5) It can be reintroduced into bacterial cell and shows normal activity in the parental cell.

Types of Plasmids:- Various types of plasmids naturally occur in bacterial cells. They are classified based on their main functions encoded by their own genes. Following are the main type of plasmids.

1. F-plasmid (or F-factor):

F-plasmid or F-factor **("F" stands for fertility**) is a circular dsDNA molecule of 99,159 base pairs. It plays a major role in conjugation in bacteria E. Coli and confers '**maleness'** on the bacterial cells; the term '**sex-factor'** is also used to refer to F-plasmid.

One region of the plasmid contains genes involved in regulation of the DNA replication (rep genes), the **other region** contains **transposable elements** (IS3, Tn 1000, IS3 and IS2 genes) involved in its ability to function as an episome, and the **third large region, the tra region, consists of tra genes and possesses ability to promote transfer of plasmids during conjugation**. Example F-plasmid of E. coli.

2. R-plasmids:

R-plasmids confer resistance to antibiotics and various other growth inhibitors. Hence called **resistant plasmids**.

R- Plasmids have genes that code for **enzymes able to destroy and modify antibiotics**. Many R-plasmids are conjugative and possess drug- resistant genes as transposable elements

Some R-plasmids possess only a single resistant gene or eight genes. For example Plasmid **R 100**, is a 94.3 kilo base-pair plasmid that carries resistant genes for sulphonamides, streptomycin and spectinomycin, chloramphenicol, tetracycline etc and also carries genes resistance to mercury.

they play an important role in medical microbiology as their spread through natural populations can have profound consequences in the treatment of bacterial infections.

3. Virulence-plasmids:

Virulence-plasmids **confer pathogenesity** on the host bacterium. They make the bacterium more pathogenic as the bacterium is better able to resist host defence or to produce toxins.

For example, **Ti-plasmids** of Agrobacterium tumefaciens induce **crown gall** disease of angiosperm plants; E. coli (entertoxigenic strains) causes **traveller's diarrhoea**.

4. Col-plasmids:

Col-plasmids carry genes that confer **ability to the host bacterium to kill other bacteria by secreting bacteriocins(** a type of proteins**). Col plasmids of some E.coli** code for the synthesis of bacteriocin, namely cloacins that kill Enterobacter species. Bacteriocins act only **against closely related strains.**

For example,. **Col E1** plasmid of E. coli code for the synthesis of bacteriocin called **colicins** which **kill other susceptible strains of E. coli**.

Lactic acid bacteria produce bacteriocin **NisinA** which strongly **inhibits the growth** of a variety of **gram-positive bacteria** and is used as a preservative in the food industry.

5. Metabolic plasmids:

Metabolic plasmids (also called degradative plasmids) possess genes **to code enzymes** that **degrade unusual substances** such as **toluene** (aromatic compounds), **pesticides** (2, 4-dichloro- phenoxyacetic acid), and s**ugars** (lactose).

For example **TOL (= pWWO)** plasmid of Pseudomonas putida . some metabolic plasmids occurring in certain strains of **Rhizobium induce nodule formation in legumes** and carry out fixation of atmospheric nitrogen.

Plasmids which occur naturally do not possess all the characteristics to use as cloning vector. Hence plasmids are constructed by inserting the genes for replication and antibiotic resistance.

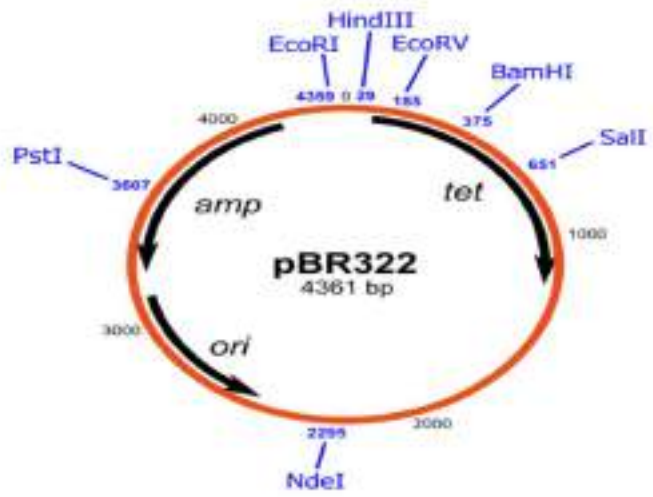
PBR 322 :-

The PBR 322 is the first artificial cloning vector developed in 1977 by Boliver and Rodrigueg .It is derived from naturally occurring Plasmid from Escherichia coli.

- It is double stranded circular DNA with 4362 base pair length.
- It contains **gene bla** for resistance to **ampicillin, gene tetA for Tetracycline,**and can be amplified with **Chlorumphenical.** Molecular weight is $2.83x10^6$ daltons.
- It contains **origin of replication PMB, & rop gene** which encodes for restrictor of plasmid copy number.

- PBR 322 contains origin of replication.
- Two selectable marker genes conferring resistance to antibiotics like Ampicillin, Tetracyclin.
- It has unique restriction sites for 40 restriction enzymes.11 of these4 lie in tet^R gene , HIND III and Lac with promote 6 restriction in Amphycillin.

Fig



PUC 18

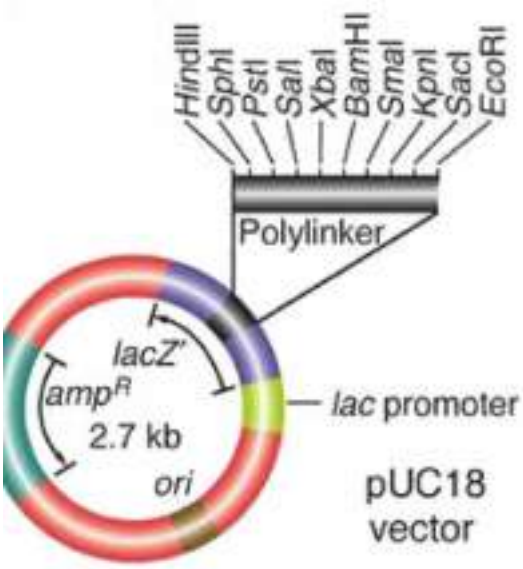
In 1983 Messings and co workers developed PUC vectors at the University of California. They are PUC8, PUC 9, PUC 12, PUC 13, PUC 18, and PUC19.These consists of ori gene, Amp R, Lac z gene. The lac z gene consists of multiple cloning sites.

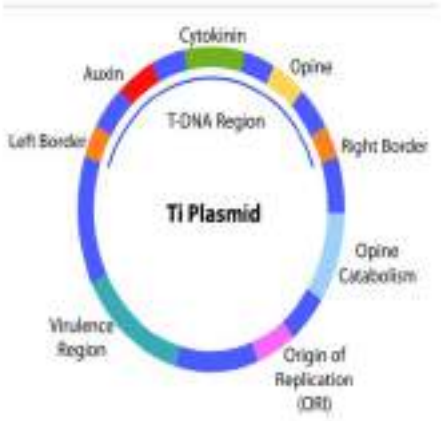
PUC 18 is an artificial plasmid genetically engineered to include

1. A gene for **antibiotic resistance to Ampicillin.**
2. A gene for **Beta-galactosidase(LacZ).**
3. **Polylinker** region with series of **unique restriction sites** found nowhere else in the plasmid**.**

Digestion with any one of these endonucleases will **make a single cut, that linearizes the circular plasmid DNA and** allow to recombine with **foreign DNA that has been cut with same endonuclease.**

Fig





Ti-plasmid

1. Agro bacterium tumificans is a Rod shaped, Gram +ve , soil bacterium which has 200kb plasmid called ' **Ti plasmid'**.
2. Ti-plasmid is a double stranded, circular DNA .It infects damaged plant tissues, induces formation of a Plant tumor called **'Crown goll'.**
3. **Ti plasmid is lost when** Agro bacterium **grown above 28ºC.**
4. Ti-plasmid has 4 major regions. **T-DNA region** (Transfer DNA) has genes for Auxin, Cytokinin and Opine and it is integrated into plant genome. **A left border repeats and Right border repeats, an origin of replication. Virulence region** has genes that mediate T DNA transfer.

Ti plasmid is important in genetic engineering.

- The bacterium transfers Ti plasmid into plants inducing tumors, so as to modify the plant metabolism to produce certain substances which are required by bacterium.
- Ti Plasmid is classified based on the type of Opine produced by their genes. (Octopine, nopaline, Succinamopine, Leucinopine).
- Ti plasmid 196 genes that code for 195 proteins.
- It has one structural RNA and Plasmid is 206.479 nucleotides long.
- Guanine and cytocine content is 56% & 81% of the material is coding genes. There are no Pseudogenes.

II ENZYMES

In genetic engineering Enzymes are used as Biological tools to cut and link DNA molecules. Some of them are as follows: -

Exonuclease : It act upon the genetic material , cleaves the 5' or 3' end of DNA.

Endonuclease: It act upon the genetic material, cleaves one strand of the double stranded DNA at any points except ends.

1.Restriction Endonuclease (REN): In 1970 Hamilton discovered REN in the bacterium Haemophilus influenza.

REN recognize specific DNA sequence on both the strands and cut within the same recognition site which are palindromic (Palindromic nucleotides are sequences that reads same forward and backward). REN are also called '**Molecular scissors'**. REN cleave the DNA at recognition points. The cut ends posses' short

single stranded free ends called sticky ends. It can join with similar complementary ends of DNA from any source. The REN are named based on following principles: REN is given three letter code written in italics.

- First letter is written in capital and it refers to genus name of bacteria from which it is obtained.
- Later two letters represent species name.
- Next letter indicate strain.
- Roman numerals represent different number of REN derived from same organism. Ex:
  1) **E Co R I =** **E**=Escherichia, **Co**=Coli, R= Strain, **I**= number.
  2) **E Co R II** = **E=**Escherichia, **Co**=Coli, R= Strain**, II=** second enzyme from same bacterium
  3) **H in d III** = **H=** Haemophilous, **in**= Influenza, **d=**strain**, III**=3rd enzyme

Ligase: (DNA Ligase) : Ligase is a common joining enzyme obtained from Escherichia coli. These join any two cut ends of DNA, hence, they are commonly called 'Molecular Glue' or Molecular stichers'. In genetic engineering Ligase is used to join the desired gene to the DNA of the Vector.

III . DESIRED GENE

The gene of interest like 'Nif 'gene, 'Bt' gene for growth of plant hormone , insulin secretion etc. that produce desired product is called " desired gene". It is identified, isolated, inserted into the plasmid. It forms the recombinant DNA.

IV. HOST CELL

The commonly used host cell for cloning desired gene is Bacteria Escherichia coli. It is prokaryotic, Gram--ve, non-spore producing, non capsulated bacillus form. They are found in soil, water, mouth, gut and help to protect intestinal track from infection. They form small amount of vitamin B 12 and K.E. coli can be cultured in nutrient medium at 37degree C. Yeast can also be used as host.

V. BIO REACTOR

.Bioreactor is a vessel used for cell cultivation of microbial plant or animal cells under sterile condition & controlled environmental conditions e.g., pH, Temperature, Dissolved oxygen etc. either in aerobic or anaerobic condition.**Objective** : To obtained High productivity, high product yield and high product concentration of plant tissue process development. .

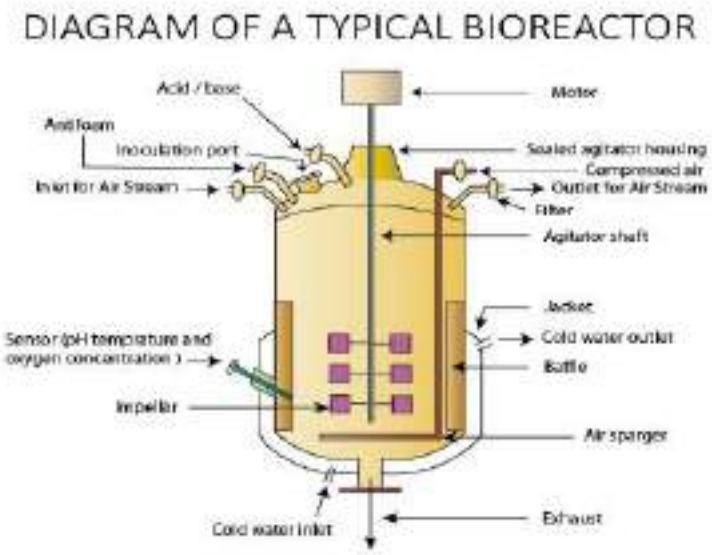
- **The bioreactors are cylindrical tanks made of stainless steel, ranging in size from litres to cubic metres,**
- A typical bioreactor consists of following parts:
- **Agitator –** used for the mixing of the contents of the reactor which keeps the "cells" in the perfect homogenous condition for better transport of nutrients and oxygen to the desired product(s)..
- **Baffle** – used to break the vortex formation in the vessel, which is usually highly undesirable as it changes the center of gravity of the system and consumes additional power.
- **Sparger** – In aerobic cultivation process, the purpose of the sparger is to supply adequate oxygen to the growing cells.
- **Jacket** – The jacket provides the annular area for circulation of constant temperature of water which keeps the temperature of the bioreactor at a constant value
- **Operational stages in a bio-process**

  A bioprocess is composed of three stages — **upstream processing, bio reaction, and downstream processing** to convert raw material to finished product.

After upstream processing step, the resulting feed is transferred to one or more. **In Bio reaction stage** bioreactors form the base of the Bioreaction step. This step is mainly consists of three operations namely, **production of biomass, metabolize biosynthesis and biotransformation. In downstream processing** is to convert it into more useful form. It consists **of physical separation operations** which includes, solid liquid separation, adsorption, liquid-liquid extraction, distillation, drying etc

- **BIOREACTORS TYPES** :Variety of bioreactor types for providing growth and expression of bioactive substances for plant cell and tissue cultures.some of them are:**1.Continuous Stirred Tank Bioreactors**

2. Bubble Column Bioreactors 3. Airlift Bioreactors 4. Fluidized Bed Bioreactors 5. Packed Bed Bioreactors 6. Photo-Bioreactors.



RECOMBINANT DNA TECHNOLOGY (r DNA)

The technology by which **desired DNA fragment inserted** into **a plasmid is transferred into a host and activates it to produce desired product is called "Recombinant DNA technology".** The process of rDNA technology involves following steps:-

- **Isolation of desired gene from the donor.**
- **Insertion of desired gene into plasmid to form r plasmid.**
- **Transfer of r DNA (r plasmid) into a host.**
- **Culture of transformed cells to synthesize desired gene product.**

Isolation of desired Gene :- The gene that produce desired product is identified, isolated by refrigerated centrifuge technique. If desired gene is Eukaryotic, gene is obtained by "c DNA technique" (reverse transcription).invitro synthesis of DNA from m-RNA by action of reverse transcriptase is called '**Reverse transcription'**. In 1970 Temin, Dulbecco, Baltimore discovered reverse transcriptase in retro virus. In 1975 they were awarded Nobel Prize.

The process of synthesis of cDNA from m-RNA involves following steps:-

1. m-RNA is passed through an oligo-dI cellulose affinity column. It binds to poly-A-tail and provide free OH site for Reverse Transcription.
2. Reverse transcriptase add complementary deoxy ribose nucleotide (dNTPs) one by one to 3'-oH site and form single stranded DNA.
3. Terminal transferase, dCTP synthesize a short hair pin loop at 3' of cDNA.
4. On hydrolysis using alkaline sucrose m-RNA separates from cDNA.
5. CDNA acts as a Template and synthesizes double stranded DNA in presence of DNA polymerase .
6. SI nuclease cleaves hair pin loop. Thus double stranded cDNA is formed.

2. Insertion of Desired gene into a plasmid

Plasmid is a circular, double stranded DNA found in bacterial cell which is used as vector for cloning desired gene. It is isolated from bacterial cell by treating with lysozyme(rupture cell wall) and centrifugation.(separate from other cell components).

Insertion of desired gene into plasmid involves following steps:-

- Plasmid is cut with REN .It produces DNA fragment with sticky or blunt ends.
- The plasmid and desired gene are mixed together. Sticky end of plasmid and desired gene get linked by complementary base pairs.

- Enzyme Ligase seals the nick between plasmid and desired gene. Thus Recombinant DNA (r DNA) is obtained.

3. Transfer of rDNA into the host cell

There are several methods to transfer rDNA into host cell. The method depends on type of vector and host cells. Few methods are as follows:-Transformation, Electroporation, Shot gun method etc. In Transformation strains of E.coli bacteria are pretreated with calcium chloride at low temperature and r DNAmixed up with it. rDNA migrate into E.coli.

4. **Culture of transformed cells**

Host cells with recombinant DNA are identified and separated. This process is called Selection or Screening. Selected cells are grown with suitable nutrient media in Bioreactor .It produce large number of identical organisms or molecules. This is called 'Cloning'. The cloned gene rDNA produces desired product in an organisms.

GENE LIBRARY

The collection of different DNA sequence from an organism, where each gene is cloned into a vector for purification, Storage & analysis is called "Gene Library".

Based on the source of DNA used, Gene library is of 2 types. They are as follows**:-**

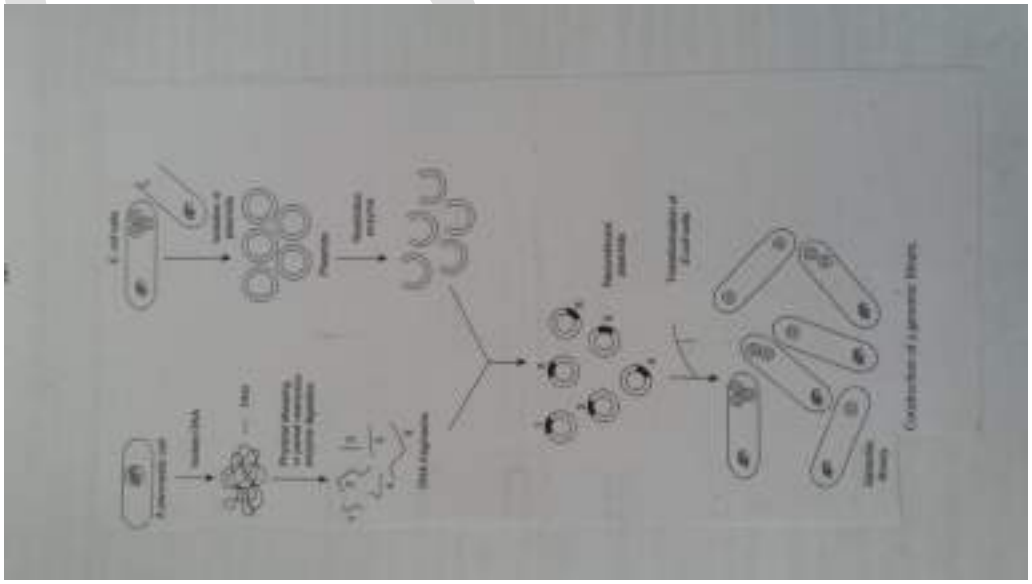
- **Genomic Library**
- **cDNA library**

Genomic Library

Collection of all genes of an organism is called **'Genomic library'. Or 'Gene bank'**. It is obtained by '**Shot gun cloning technique'**. It involves following steps:-

- **Isolation and fragmentation of genome of a cell:-**A cell of an organism contains all genes of an organism. The DNA of the cell is isolated and cut into fragments by addition of REN.
- **Isolation of Vector:-**Plasmid vector present in bacteria is isolated, cut with endonuclease.
- **Insertion of Donor DNA into plasmid:-**DNA fragments are inserted into plasmid, fused with it by ligase to produce r DNA.
- **Insertion of rDNA into Host cell:-**rDNA are introduced into host cell E.coli by bacterial transformation.
- **Cloning the host cells:-**Host cells are cultured in an agar plate. Each colony contain particular gene of donor organism.
- **Identification of cloned genes;-**Cloned genes are identified, selected from colonies by immunochemical or colony hybridization technique.

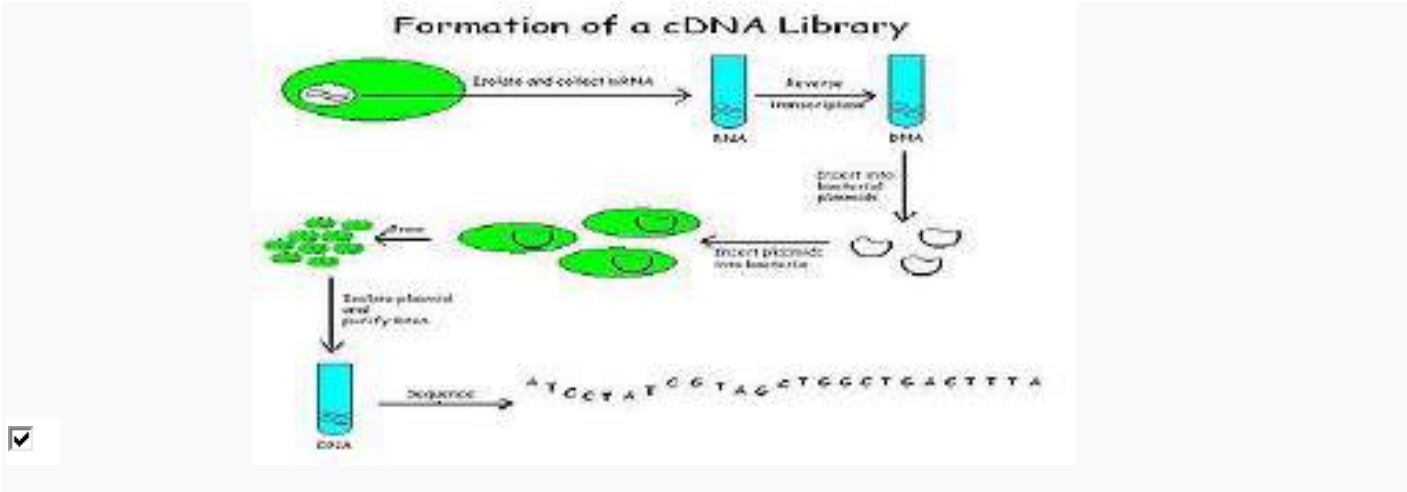
  Thus identified and isolated genes form **genomic library of particular organism**.



cDNA Library or DNA Library

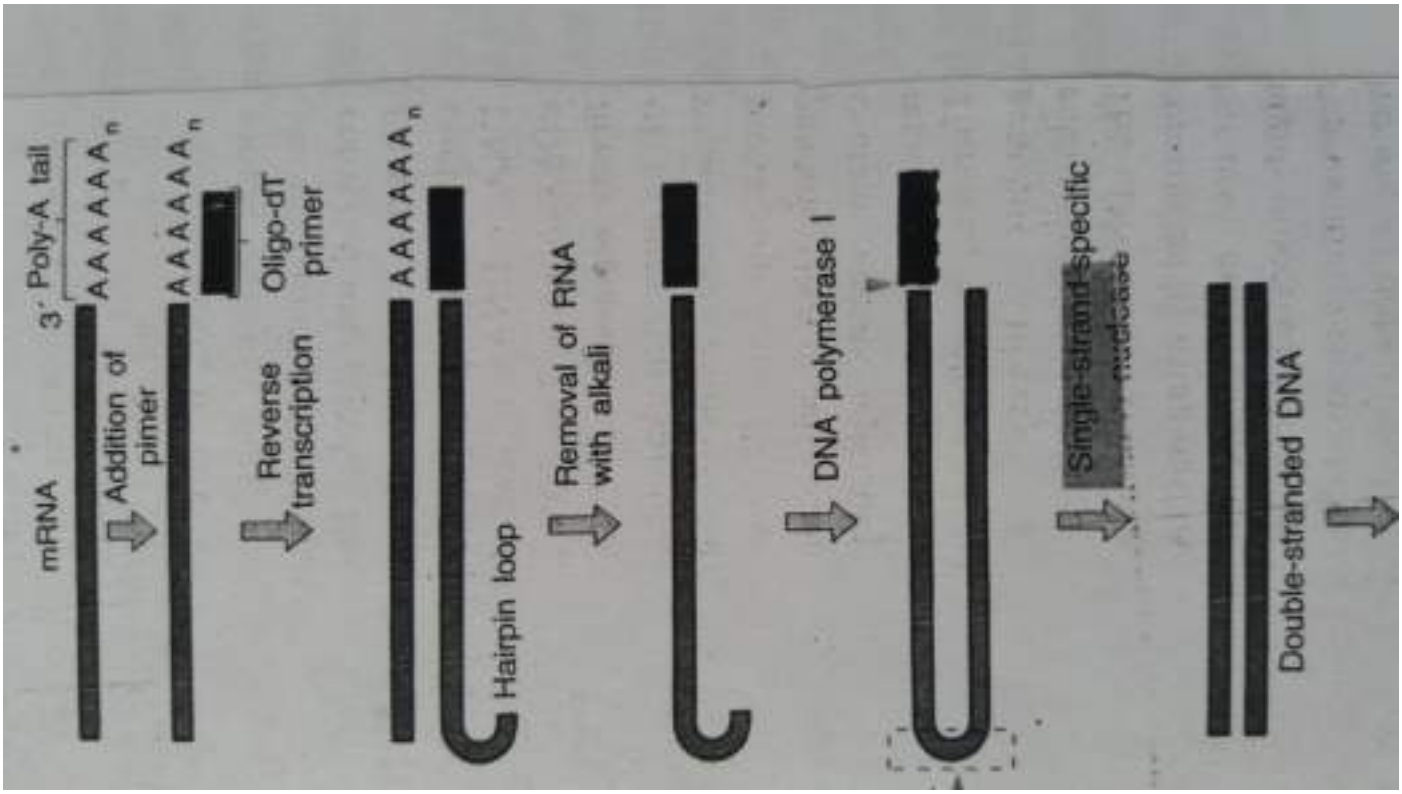
A collection of cDNA molecules for different characters made from m-RNA by the Reverse transcription is known as '**cDNA'(Complementary library) 'DNA library'**. It represents DNA of only Eukaryotes.

Introduction:-In Eukaryotes heterogenous RNA is produced which contain coding sequence called '**Exons'**, and non coding regions **'Introns'**. From this m-RNA, cDNA is synthesized by reverse transcriptase, Deoxyribo nucleotides. Etc. In Eukaryotic cells, more number of m-RNA are involved in expression of many characters. Hence cDNA molecules for different characters can be obtained. This constitutes DNA Library.



Fomation of cDNA Library

cDNA is created from a mature mRNA **from a eukaryotic cell with the use of** reverse transcriptase. In eukaryotes, a poly-(A) tail, distinguishes mRNA from tRNA and rRNA and can be used as a primer site for reverse transcription.



1.M RNA extraction

- **mRNA is obtained and purified from the rest of the RNAs** by several methods such as trizol**extraction and** column purification.
- **Column purification** is done by using **oligomeric dT nucleotide** coated resins .where only the mRNA having the poly-A tail will bind and rest of the RNAs are eluted out.
- The mRNA is eluted by using eluting buffer and heat to separate the mRNA strands from oligo-dT(a short sequence of deoxy-thymidine nucleotides).

2.cDNA construction

- After mRNA is purified, oligo-dT) is tagged as a complementary primer which binds to the poly-A tail providing a free 3'-OH end ,that can be extended by reverse transcriptase to create the complementary DNA strand.
- Then mRNA is removed by using a RNAse enzyme leaving a single stranded cDNA (sscDNA). This sscDNA is converted into a double stranded DNA with the help of DNA polymerase.
- For DNA polymerase to synthesize a complementary strand a free 3'-OH end is needed. This is provided by the sscDNA itself by generating a hairpin loop at the 3' end by coiling on itself.
- The polymerase extends the 3'-OH end and later the loop at 3' end is opened by the scissoring action of S_1 nuclease**.** Restriction endonucleases and DNA ligase are then used to clone the sequences into bacterial plasmids.
- The cloned bacteria are selected through the use of antibiotic selection.
- Selected, stocks of the bacteria are grown and sequenced to compile the **cDNA library.**

cDNA Library uses

1. **cDNA libraries** are commonly used **for reproducing eukaryotic genomes**,.
2. cDNA libraries are used to **express eukaryotic genes in prokaryotes** as cDNA does not have introns . (Prokaryotes do not have introns in their DNA and therefore do not possess any enzymes that cut it out during transcription process.

3.cDNA is produced from fully transcribed mRNA found in the nucleus .hence, contains only the expressed genes of an organism. (Tissue-specific cDNA libraries can be produced).

4. cDNA libraries are most useful in reverse genetics where the additional genomic information is of less use.
5. cDNA libraries are used in functional cloning to identify genes based on the encoded protein's function.
6. Information in cDNA libraries is a **powerful and useful tool** since gene products are easily identified. (The libraries lack information about enhancers, introns, and other regulatory elements found in a genomic DNA library).

APPLICATION OF GENETIC ENGINEERING IN AGRICULTURE, IN MEDICINE, IN ENVIRONMENT

I. Applications of Genetic Engineering technology in agriculture. (Transgenic plants- Bt-cotton, golden rice),

The process of production of desired plants for goods and services of mankind by incorporating desired genes through genetic engineering methods is called 'Tran geneses'. The plants with inserted foreign genes are called '**Transgenic plants'.**

Plant scientists have developed transgenic plants in Agriculture, Horticulture and floriculture with the objective as follows:

- **To increase value addition to crop.**
- **Disease, Herbicide, insect, Virus resistant plants.**
- **Alter flower colors.**
- **Ability to fix Nitrogen by non-leguminous plants.**
- **Production of value added crops:**

1. **Golden Rice:-Vitamin-A rich Rice (**Golden rice) by transferring of Vitamin A producing genes. Vitamin A, deficiency, every year is kill 670,000 children under the age of 5 and cause an 500,000 cases of irreversible childhood blindness.

Golden rice is a variety of rice (Oryza sativa) produced through genetic engineering to bio-synthesize beta-carotene, a precursor of vitamin A, in the edible parts of rice.(23 times more beta-carotene than the original rice).

Golden rice was created by transforming rice with two beta –carotene biosynthesis genes psy(phytoene synthase) from Daffodil (Narcissus pseudonarcissus) crtl(phytoenedesaturase) from soil bacterium Erwinia uredovora. Incorporation of these genes allows the rice plant to modify certain metabolic pathways in its cells to produce precursors of Vitamin A.

2. **Bt cotton**:- Strains of the bacterium *Bacillus thuringiensis* produce over 200 different Bt toxins, Bt toxins are insecticidal to the larvae of moths and butterflies, beetles, cotton bollworms and ghtu flies but are harmless to other forms of life.

The gene coding for **Bt toxin** has been **inserted into cotton** as a transgene, causing it to produce this **natural insecticide in its tissues**. The main pests in commercial cotton are lepidopteran larvae.These are killed by the BT protein in the genetically modified cotton they eat. This eliminates the need to use large amounts of insecticides to kill lepidopteran pests.

3. Genetic engineered **French bean** with storage protein phaseolin.
4. **Transgenic potato** with Starch and amino acid. By transferring AMAI protein cDNA of Amaranthus.
5. Gnetically engineered **Rape seed** rich in Serates and oleic acid (by transferring 9 stearoyl ACP desaturase .(It has stability during frying and no cholesterol).
6. Genetically engineered **Soya bean** yield cocoa oil. It is made by transferring of genes for enzymes responsible for cocoa oil.
7. **Transgenic tomato** (Flavo servu) is Brusie resistant and ripen slowly is achieved by transfer of antisense producing gene for polyalacturonase,
8. **Transgenic pea** contain Sulphur rich amino acids
9. **Transgenic cereals** contain high essential amino acids such as Lysine.
7. **Trnasgenic plants** contain gene for antibody producton and yield edible vaccine .Ex: edible vaccine for hepatitis-B from Banana, Spinach or Tobacco for rabies.
8. **Production of resistant plants:** Herbicide resistant transgenic plant produced by introducing herbicide tolerant gene is ecofriendly, environmentally safe, detoxify herbicide and tolerate the effects.
9. Virus resistant transgenic plants developed from by introducing gene that code for viral protein .Ex: Transgenic Tobacco is resistant to TMV Genetic engineered Potato resistant to Potato virus x, y. Genetic engineered Papaya resistant to papaya ring spot virus.
10. Transgenic plants with attractive colors are produced by introducing genes involved in falconoid metabolism or antisense RNA producing genes .Ex: petunia.

II. APPLICATION IN MEDICINE (INSULIN SYNTHESIS, GENE THERAPY),

INSULIN SYNTHESIS:- Attempts to produce insulin by recombinant DNA starts in late 1970s. In July 1980 seventeen diabetic volunteers were first administered recombinant insulin at Guys hospital.London.In 1980 Eli lilly company received approval to market insulin under **'HUMULIN'.** Shreya Life sciences ,Pune (India) Co., has started producing the second generation rDNA based Insulin. Recombinant DNA technology (rDNA technology) made it possible to insert human gene into genetic material of E.coli. Thus recombinant microorganisms could produce protein encoded by Human gene.

Insulin is secreted by islets of langerhans of pancreas which catalizes glucose in blood.precursor of Insulin is pre pro insulin.which is 109 acids long. Insulin consists of 2 polypeptide chains, **chain A** with 21 amino acids and chain **B 31** amino acids long.it is connected with chain C with 35 amino acids.
mRNA for pre –proinsulin are isolated from Islets of langerhans of pancreas **or** chemically synthesized DNA sequence (Itakura-1977) for A and B chains of insulin, inserted into PBR ^{322} plasmids by side of B-galactosidase gene. Then the recombinant plasmids were separately transferred into E.coli cells which secreted fused B-galactisidase- A chain and B- galactosidase B-chain separately. These chains were isolated from galactosidase in pure form (10mg/24g of healthy transformed cells).
Extra methionine is added at the N terminus of each gene for A and B chain to detach pro insulin. Then A and B chain are joined invitro to reconstitute Insulin by sulphonating two chains with Sodium disulpite.

Gene therapy: -

Gene therapy is an **experimental technique** that uses **genes to treat or prevent disease.** In future this technique may allow doctors to treat a disorder by inserting a gene into patient's cells instead of using drugs or Surgery. **(Gene therapy** is the therapeutic delivery of nucleic acid into a patient's cell as a drug to treat disease).

First attempt at modifying human DNA was done in 1980 by Martin cline, but successfully approved by National institute of health in May 1989.

Researchers are testing several approaches to gene therapy. They are as follows:-

1. **Replacing a mutated gene that causes disease** with a **healthy** copy of the gene**.**
2. **Inactivating a mutated gene** that is functioning **improperly.**
3. **Introducing a new gene** into the body to **help fight disease.**

Although the technique is promising treatment for number of diseases, it remains risky and is still under study to make sure that it is safe and effective**.**

III. APPLICATION IN ENVIRONMENT (BIOREMEDIATION AND BIO-MINING).

BIOREMEDIATION:-

Bioremediation is a process used to treat contaminated Soil, water and sub surface material, by altering environmental conditions to stimulate growth of microorganism and degrade the target pollutants. Additional nutrients, Vitamins, Minerals and pH buffers are added to optimise conditions for microorganism.

Ex: - 1) Degradation of pesticides, insecticides and fungicides in-situ (in soil) by microbes Pseudomonas A3, P .putida, P .aeruginosa and Serratia marinorubra.

2) Heavy metal detoxification and biosorption by employing the bacterium bacillus species YW.

3) The removal of less toxic trivalent chromim through biosorption using the EPS Azotobacter species.

Significance

1. It is less expensive and more sustainable.

2. It involves oxidation reduction reactions, where either an electron acceptor is added to stimulate oxidation of reduced pollutant (hydrocarbons)or an electron donor is added to reduce oxidized pollutants(such as Nitrate, perchlorate, oxidized metals, chlorinated solvents, explosives)

BIO-MINING:-

Bio mining is a Technique of **extracting metals from ores and other solid materials** typically using **Prokaryotes or Fungi.** It was discovered in 1900.These organisms secrete different organic compounds that chelate metals from the environment and bring it back to the cell where, they are typically used to co ordinate electrons.

Micro organisms use metals in the cellssome microbes can use stable metalssuch as Iron, Copper, Zinc and Gold as well as unstable atomssuch as Uranium and Thorium.

Significance:-

1. Biomining is an environmental friendly technique as microbes releases only metabolites and gases that they secrete where as mining releases Pollutants.
2. In Bio mining bacteria mine for metals to clean oil spills, Purify gold and use radioactive elements for energy.
3. Bio mining can be used for bioremediation model where Bacteria can be inoculated into environment contaminated with metals, Oils and Toxic components which clean environment by absorbing these toxic compounds to create energy in the cell.

To alter Flower color,Transgenic plants as Bioreactors: Transgenic plants are used as plant Bioreactors for large scale production of valuable products like vaccines, interferons, Biodegradable plastics.

2 MARKS QUESTIONS

1. What is Plasmid? Give an example. 1+1
2. What is C DNA library? Mention its significance. Definition-1, one use-1
3. What are Molecular scissors? Give an example. 1+1
4. What is Vector? Give an example. 1+1
5. What is Bioremediation? Mention ts significance. 1+1
6. What is Bio Mining? Mention ts significance. 1+1
7. What is Gene therapy? Mention its significance. 1+1
8. What is Bioremediation? Give examples. 1+1
9. What is Ti Plasmid? Mention its importance. 1+1

5 MARKS QUESTIONS

1. Explain Vector Definition-1, fig-1, explain-3
2. Give an account of gene libraries. Definition-1, Fig-1+explanation-3 marks.

3. Explain the applications of genetic engineering in agriculture, 3+2
4. Give an account of Plasmids. Definition-1,types-4
5. Explain PBR 322. Definition-1, fig-1, explain-3
6. Describe PUC 18. Definition-1, fig-1, explain-3
7. Give an account of Ti-plasmid. Definition-1, fig-1, explain-3
8. Write a note on REN. Define+ explanation-3, Naming-1, example-1
9. Explain application in medicine
10. Describe Gene therapy. Define+ explanation-3, example-2
11. Describe cDNA Library. Definition-1, Fig-1+explanation-3 marks
12. Explain Tools used in genetic engineering. 5
13. Write a note on Bioreactor. Definition-1, fig-1, explain-3

10 MARKS QUESTIONS

1. Give an account of Recombinant DNA / Genetic engineering. Def-1mk, ste-6,fi-3.
2. Explain Applications of Genetic Engineering technology in agriculture

3.Give an account of application of Genetic engineering in bioremediation and bio-mining.

UNIT-4 Bioinformatics (6 Hrs)

Introduction

Bioinformatics is an interdisciplinary field involving molecular biology and genetics, computer science, mathematics, and statistics. biotechnology, statistics and engineering. Data intensive, large-scale biological problems such as modeling biological processes at the molecular level and making inferences from collected data. are addressed from a computational point of view. A bioinformatics solution involves the following steps: Collect statistics from biological data. Build a computational model. Solve a computational modeling problem. Test and evaluate a computational algorithm.

Definition of Bioinformatics

Bioinformatics is an application of computational technology to handle the rapidly growing information related to molecular biology.

Aims of Bioinformatics

The basic aims of bioinformatics are:-

1. **To store the biological data organized in form of a database**. To organize vast reams of molecular biology data in an efficient manner; to develop tools that aid in the analysis of such data; and to interpret the results accurately and meaningfully the databases must also be able to correlate between different hierarchies of information. For example: GenBank for nucleotide and protein sequence information, Protein Data Bank for 3D macromolecular structures, etc.
2. **To develop tools and resources that aid in the analysis of data**. For example: *BLAST* to find out similar nucleotide/amino-acid sequences, *ClustalW* to align two or more nucleotide/amino-acid sequences, Primer3 to design primers probes for PCR techniques, etc.

3. To exploit these computational tools to analyze the biological data interpret the results in a biologically meaningful manner.

Scope of bioinformatics(Applications of Bioinformatics)

1. **Bioinformatics** has specific career opportunities in areas as Bio-analytics, Clinical pharmacologist, Computational chemist, Database design and maintenance, Proteomics, Pharmacogenomics, Pharmacology, Sequence assembly, Sequence analysis, Informatics developer, etc.
2. **It has application in areas, such as gene sequencing, gene expression studies and drug discovery**. For example, in medicine, bioinformatics can be used to identify links between specific diseases and the gene sequences that cause them.
3. Pharmacogenomics uses bioinformatics data to **tailor medical treatments** to the patients who take them, **based on their DNA.**
4. Bioinformatics is also used to **develop more effective vaccines** through the development of new, stronger antibodies**.**
5. It is useful for **managing and analyzing large sets of data**, such as those generated by the fields of genomics and proteomics.

Biological data base:

Bioinformatics can be used in any system where information can be represented digitally; it can be applied across the entire spectrum of living organisms, from single cells to complex ecosystems.

The rapid rise of bioinformatics is due to the massive increases in computing power and laboratory technology. These **advances have made possible to process and analyze the digital information – DNA, genes and** genomes

Data bases

An organized collection of data like schemes, tables, queries, reports, images, and other objects **is referred to as database.**

Ex: - To get an idea of data and information (Bioinformatics), consider the human genome. A genome is an organism's complete set of DNA. DNA molecules are made of two twisting, paired strands, and each strand is made of nucleotide bases – adenine (A), thymine (T), guanine (G), and cytosine (C). The human genome contains about 3 billion of these base pairs. Genome sequencing involved figuring out the exact order of all 3 billion of these DNA nucleotides; it would not have been possible without mass **Data bases.**

In the databases an access to information is provided by an integrated set of computer software, which is referred to as a "**database management system" (DBMS**).The DBMS allows users **to access all of the data contained in the databases.** It has general functions for data definition, entry, storage, update, administration, and retrieval of large quantities of information in an organized way that requires modeling clustering, query languages and query optimization, and visualization algorithms.

Development of data bases, is dependent on bioinformatics tools, advances, research, and applications.

There are large number of databases available, Some are DNA database, Protein data base, EMBL, DDJB.

DNA data base

A DNA database or DNA databank is a database **of** DNA profiles **which can be used in the analysis of** genetic diseases**,** genetic fingerprinting **for** criminology**, or** genetic genealogy**. DNA databases may be public or private, the largest ones being** national DNA databases.

- **National DNA database: National DNA database is a DNA database maintained by the government for storing DNA profiles of its population.** Each DNA profile is based on PCR and uses STR (Short Tandem Repeats) analysis. They are generally used for forensic purposes which include searching and matching of DNA profiles of potential criminal suspects. **When a match is made from a national DNA database to link a crime scene to a person whose DNA profile is stored on a database, that link is often referred to as a *cold hit*.** A cold hit is of particular value in linking a specific person to a crime scene, but is of less evidential value than a DNA match made

without the use of a DNA database. **Research shows that DNA databases of criminal offenders reduce crime rates.**

- **Forensic**:-A centralised DNA database for storing DNA profiles of individuals that enables searching and comparing of DNA samples collected from a crime scene against stored profiles. The important function of the **forensic** database is to produce matches between the suspected individual and crime scene bio-markers, and then provides evidence to support criminal investigations, and also leads to identify potential suspects in the criminal investigation. Majority of the National DNA databases are used for forensic purposes.
- **The Interpol DNA database:** It is used in criminal investigations. **Interpol maintains an automated DNA database called DNA Gateway** that contains DNA profiles submitted by member countries collected from crime scenes, missing persons, and unidentified bodies. The DNA Gateway was established in 2002, and at the end of 2013, it had more than 140,000 DNA profiles from 69 member countries. DNA Gateway is only **used for information sharing and comparison, it does not link a DNA profile to any individual**, and the physical or psychological conditions of an individual are not included in the database.

  A national or forensic DNA database are not available for non-police purposes.
- **Genealogical**: GenBank is a **public genetic genealogy database** that stores **genome sequences submitted** by many genetic genealogists. These databases are mainly **obtained from individual laboratories or large-scale sequencing projects.** The files stored in GenBank are divided into different groups, such as BCT (bacterial), VRL (viruses), PRI (primates)...etc. People can access GenBank from NCBI's retrieval system, and then use "BLAST" function to identify a certain sequence within the GenBank or to find the similarities between two sequences.Until now, GenBank has contained large number of DNA sequences gained from more than 140,000 registered organizations, and is updated every day to ensure a uniform and comprehensive collection of sequence information.
- **Medical: Medical DNA database is a DNA database of medically relevant genetic variations**. It collects an individual's DNA which can **reflect their medical records and lifestyle details**. Through recording DNA profiles, scientists may find out the interactions between the genetic environment and occurrence of certain diseases (such as cardiovascular disease or cancer), and thus **finding some new drugs or effective treatments in controlling these diseases**. It is often collaborated with the National Health Service.

[1)In 2009 Interpol reported there were 54 police national DNA databases in the world at the time and 26 more countries planned to start one.

2)In Europe Interpol reported there were 31 national DNA databases and six more planned.

3)The European Network of Forensic Science Institutes (ENFSI) DNA working group made 33 recommendations in 2014 for DNA database management and guidelines for auditing DNA databases.

4) Other countries have adopted privately developed DNA databases, such as Qatar, which has adopted Bode dbSEARCH].

Protein Data base

Protein database maintains the text record for individual protein sequences, derived from many different resources such as NCBI Reference Sequence (RefSeq) project, GenBank, PDB and UniProtKB/SWISS-Prot. Protein records are present in different formats including FASTA and XML and are linked to other NCBI resources.

Protein provides the relevant data to the users such as genes, DNA/RNA sequences, biological pathways, expression and variation data and literature. It also provides the pre-determined sets of similar and identical proteins for each sequence as computed by the BLAST. The Structure database of NCBI contains 3D coordinate sets for experimentally-determined structures in PDB that are imported by NCBI. The Conserved Domain database (CDD) of protein contains sequence profiles that characterize highly conserved domains within protein sequences. It also has records from external resources like SMART and Pfam. **Protein Clusters database** contains sets of proteins sequences that are clustered according to the maximum alignments between the individual sequences as calculated by BLAST.

National Center for Biotechnology Information (NCBI)

1. The NCBI was founded in 1988 by Senator Claude Pepper. NCBI is located in Bethesda, Maryland. It is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health (NIH).
2. NCBI has had responsibility for making available the GenBank DNA sequence database since 1992. GenBank coordinates with individual laboratories and other sequence databases such as those of the European Molecular Biology Laboratory (EMBL) and the DNA Data Bank of Japan (DDBJ)
3. The NCBI houses a series of databases relevant to biotechnology and biomedicine and is an important resource for bioinformatics tools and services.

4. Major databases include GeneBank **for DNA sequences,** PubMed**,** a **bibliographic database** for the biomedical literature**,** NCBI Epigenomics database. All these databases are available online through the Entrez search engine.

5. NCBI was directed by David Lipman, One of the original authors of the BLAST sequence alignment program.(BLAST is a sequence similarity searching program. BLAST can do sequence comparisons against the GenBank DNA database in less than 15 seconds).

6. NCBI provides Gene, Online Mendelian Inheritance in Man, the Molecular Modeling Database (3D protein structures), dbSNP (a database of single-nucleotide polymorphisms), a map of the human genome, and a taxonomy browser, and coordinates with the National Cancer Institute to provide the Cancer Genome Anatomy Project. The NCBI assigns a unique identifier (taxonomy ID number) to each species of organism.

7. The "NCBI Bookshelf is a collection of freely accessible, downloadable, on-line versions of selected biomedical books. The Bookshelf covers a wide range of topics including molecular biology, biochemistry, cell biology, genetics, microbiology, disease states from a molecular and cellular point of view, research methods, and virology. Some of the books are online versions of previously published books.

8. Others, such as Coffee Break, are written and edited by NCBI staff. The Bookshelf is a complement to the Entrez PubMed repository of peer-reviewed publication.

9. Abstracts in that Bookshelf contents provide established perspectives on evolving areas of study and a context in which many disparate individual pieces of reported research can be organized

10. BLAST is an algorithm used for calculating sequence similarity between biological sequences such as nucleotide sequences of DNA and amino acid sequences of proteins.

The DNA Data Bank of Japan (DDBJ) (DDBJ, http://www.ddbj.nig.ac.jp)

DNA Data Bank of Japan Center (DDBJ) **maintains and provides public archival, retrieval and analytical services for biological information**. The contents of the DDBJ databases are shared with the US National Center for Biotechnology Information (NCBI) and the European Bioinformatics Institute (EBI) within the framework of the International Nucleotide Sequence Database Collaboration (INSDC). Since 2013, the DDBJ Center has been operating the Japanese Genotype-phenotype Archive (JGA) in collaboration with the National Bioscience Database Center (NBDC) in Japan. In addition, the DDBJ Center develops semantic web technologies for data integration and sharing in collaboration with the Database Center for Life Science (DBCLS) in Japan. This paper briefly reports on the activities of the DDBJ Center over the past year including submissions to databases and improvements in our services for data retrieval, analysis, and integration.

It is a biological database that collects DNA sequences.[1][2] It is located at the National Institute of Genetics (NIG) in the Shizuoka prefecture of Japan. It is also a member of the International Nucleotide Sequence Database Collaboration or INSDC. It exchanges its data with European Molecular Biology Laboratory at the European Bioinformatics Institute and with GenBank at the National Center for Biotechnology Information on a daily basis. Thus these three databanks contain the same data at any given time.

DDBJ began data bank activities in 1986 at NIG and remains the only nucleotide sequence data bank in Asia. Although DDBJ mainly receives its data from Japanese researchers, it can accept data from contributors from any other country. DDBJ is primarily funded by the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT). DDBJ has an international advisory committee which consists of nine members, 3 members each from Europe, US, and Japan. This committee advises DDBJ about its maintenance, management and future plans once a year. Apart from this DDBJ also has an international collaborative committee which advises on various technical issues related to international collaboration and consists of working-level participants.

The Protein Information Resource (PIR),

- Protein Information Resource (PIR) is an integrated public resource of protein informatics that supports genomic and proteomic research and scientific discovery.
- **PIR** located at Georgetown University Medical Center (GUMC), is an integrated public bioinformatics resource to support genomic and proteomic research, and scientific studies.It contains protein sequences databases .
- PIR was established in 1984 by the **National Biomedical Research Foundation** (NBRF) as a resource to assist researchers and customers in the identification and interpretation of protein sequence information.
- PIR maintains the Protein Sequence Database (PSD), an annotated protein database containing over 283 000 sequences covering the entire taxonomic range.
- **Family classification** is used for **sensitive identification**, consistent annotation, and detection of annotation errors. The **superfamily** curation defines **signature domain architecture and categorizes** memberships to **improve automated classification**. To increase the amount of **experimental annotation.**
- The PIR has developed a bibliography system for literature searching, mapping, and user submission, and has conducted retrospective attribution of citations for experimental features.

- PIR also maintains NREF, a non-redundant reference database, and iProClass, an integrated database of protein family, function, and structure information.

- PIR-NREF provides a timely and comprehensive collection of protein sequences, currently consisting of more than 1 000 000 entries from PIR-PSD, SWISS-PROT, TrEMBL, RefSeq, GenPept, and PDB.

- The PIR web site (http://pir.georgetown.edu) connects data analysis tools to underlying databases for information retrieval and knowledge discovery, with functionalities for interactive queries, combinations of sequence and text searches, and sorting and visual exploration of search results.

- The FTP site provides free download for PSD and NREF biweekly releases and auxiliary databases and files.

Unit 5; Biotechnology: 6 Hrs

Fermentation technology: Production of Ethyl alcohol, production of antibiotics (Penicillin), production of single cell protein (*Spirulina)* :
Environmental technology: Waste water treatment process: primary, secondary and advanced treatment of sewage (domestic waste water)

PRODUCTION OF ENZYMES –ETHANOL

Louis Pasteur demonstrated the fermentation of sugar by micro organisms. In 1815 Gay-Llussac formulated conversion of Glucose to ethanol. Recently recombinant DNA technology has helped micro –organisms used in industrial process. India is the third largest producer of fermentation ethanol.

Ethanol is used as Solvent, extract, Antifreeze, as substrates for synthesis of Dyes, Lubricants, Pharmaceuticles, detergents, Pesticides, Explosives, Resins, Plasticizers, manufacture of synthetic fiber, as liquid fuel in the name of "Gasohol" and as Alcoholic beverage.

Micro organisms used : Bacteria such as Clostridium acetobutylum, klebsiella pneumonia Fungi such as Aspergillus oryzae, Schharomyces cerevisiae (yeast).

Fermentation of ethanol is carried out in a large fermentor. The inoculums of micro organisms are maintained at optimum growth conditions like Temperature, pH, Oxygen, concentration of substrate, carbohydrate.

Preperation of inoculums

Fermentation process requires huge quantity of microbial culture. Medium has to be agitated and aerated for production of large amount of cell mass. PH range is 4.8 to 5 is optimum & temperature range between 20 to 30 degrees is optimum.

Preparation of inoculums for yeast culture involves following steps:

- Take 25 ml of sterile culture medium in a test tube, add culture of yeast, and incubate at 28 to 30 degrees C for appropriate time.
- Transfer the culture to conical flask containing 250 ml of sterile medium; incubate at 28 to 30 degrees C for appropriate time to get required quantity of culture.
- Transfer the culture to large container to accommodate 5 liters of culture medium, incubate at 28 to 30 degrees for suitable time to obtain hug ecell mass.
- Transfer the culture to a small tank that contain 50 to 200 liters of culture medium, incubate and then transfer to actual fermentor by pumping or gravity. The method of adding inoculums to the fermentor is called Pitching.

Raw materials required for fermentation

Plant based substrates which contain starch or Sugar are used as raw materials to begin fermentation. They can be grouped as follows:

- Roots, Tubers or Grains such as Potato Starch, Corn starch, Wheat flour, Cereals like oats, Barley etc.
- Sugary materials such as Molasses, or Juice from Sugar cane, Sugar beet etc.
- Wood or Waste products from processed wood.

Media preparation for fermentation

In India Molasses, Substrates containing sugars, Starch and cellulose are used as raw materials. Yeast do not contain Amylase, hence starch has to be hydrolysed (Saccharification) to form Glucose and Maltose. [In Saccharification Starchy roots are ground, squeezed, sun dried. Starch is liqified, put under pressure, hydrolysed by adding enzymes. For fermentation 10 to 18 percent concentration of sugars is essential. It can be measured with Balling hydrometer.] In modern plants liquefaction and Saccarification is carried out by using steam injection and vaccum suction.

The following conditions are to be controlled to produce optimum quantity of Ethanol.

- Nitrogen is very important .As nitrogen source Ammonium sulphate is added in the quantity of 0.15 g per 15 liters of molasses. Excess of nitrogen will inhibit fermentation.
- Carbon compounds of 10 to 18 percent is satisfactory . high concentration affects growth of yeast and low conc. Reduces rate of fermentation.
- pH: pH range of 4.8 to 5 is required. Higher ph increases contamination and lower pH reduces content of ethanol.
- Temperature: Temperature of 72 to 80 F is preferable. Fermentation increases temp. To reduce temperatures cooling coils or cold waer spray are used.
- Agitation: Agitation of the medium uniformly cools the medium.
- Time: Fermentation begins few hours after addition of yeast culture. Production of Ethanol begins after 32 to 72 hours after initial process. When specified gravity of fermenting liquid called Wash becomes constant, it contains 6 to 8 percent of ethanol .5 liters of Molasses contains 2 liters of ethanol.

Recovery of Ethanol

After fermentation Wash is allowed to settle down, and then subjected to fractional distillation in analyzer and rectifier columns to obtain ethyl alcohol.

Byproducts of ethanol fermentation

Apart from Ethanol 3 important byproducts are formed. They are:

Cabon di oxide: It is liquefied or converted into Dry ice, used commercially.

Distillary effluents: Alcohols produced as byproducts, after refining, used in perfumes and other industries. By esterification various types of acetates (n- propyl acetate, isopropyl acetate, amyl acetate) which are used in paint and liquor industry are formed. Yeast cell mass : It is a valuable fodder .

Alcoholic beverages:- Percentage of Alcohol differs in different alcoholic beverages. Ethanol is used in Alcoholic beverages.

Wine: Alcohol percentage is 10.22.European drink produced from juice of fresh grapes.
Brandy (Fortified wine): Alcoholic percentage is 20. Prepared from addition of extra ethanol to wine.
Beer: Alcoholic percentage is 4 to 8. Produced after fermentation of mixture of Barley, malt and starchy solution by yeast.
Rum: Alcohol percentage is 51. It is a distilled product of culture fluid. (Culture medium is prepared from Black strap molasses containing 12 to 14 % fermentable sugar, Ammonium Sulphate, Phosphates. After fermentation, culture fluid is distilled to remove alcohol which is used as Rum).
Whisky: Alcohol percentage is 51 to 59. It is prepared through fermentation of grain mash. Culture fluid contains Alcohol, esters.

PRODUCTION OF ANTIBIOTICS -Penicillin

Penicillin is an Antibiotic extracted from penicillium notatum by Alexander fleming in 1929. Benzyl penicillin and penicillin v are natural antibiotics effective against Gram +ve bacteria. as they inhibit bacterial cell wall synthesis. But these are ineffective against microbes that produce B- lactanase, since they hydrolyse penicillin. the yield of pencillin from p.notatum is very low and it could be easily destroyed by acid and heat.

To overcome such problems semisynthetic penicillins are developed and used against gram—ve bacteria .Hence now a day's Penicillium chrysogenum is used for large scale production of pencillin by fallowing steps:

- **Selection of strain:** High yielding varieties of p. chrysogenum are selected. They are genetically instable, hence maintained and stored carefully in frozen state in liquid nitrogen or fine suspension of spore, mixed with inert material like soil or sand and kept under desiccation.
- **Preparation of inoculum :** Pure inoculum of p.chrysogenum is developed to initiate fermentation using moyer & Corgill culture medium ,Nitrogen source is ammonium acetate, Ammonium sulphate , corn steep liquor supply potassium, dihydrogen phosphate, Magnesium sulphate.
- **Incubation ;** production tank is incubated using pressure to push 10% of inoculum into fermentation tank at 25- 26 c for 3-5 days. Periodic survey is done to check contamination if any and to determine quantity.
- **Harvest and recovery:** aseptically remove mycelium using Rotatory vacuum filter. extract penicillium using counter current solvent extraction .Adjust PH of filtrate to acidic state (2.5) using sulphuric acid. It is back extracted into an alkaline buffer with PH 7- 7.5 .Thus crude penicillin is obtained .It is further treated with aqueous sodium hydroxide fallowed by charcoal to eliminate

pyrogens and then filtered by Seitz filter to eliminate bacteria. For medicinal purpose dry powder is stored in vials.

Single cell Protein (SCP)

The dried cells of Micro-organisms like layer Algae, bacteria, Fungi used as food are collectively known as Microbial proteins. In 1967 in the first international conference on "Microbial protein" was held at Massachusetts. The Microbial protein was replaced by **'Single cell protein' (SCP).**

Spirulina is a blue-green algae .Predominant species in India namely Spirulina platensis, Spirulina fusiformis and Spirulina maxima.

This filamentous cyanobacterium accumulates natural resources such as proteins, minerals, and a few vitamins. Spirulina is eaten since 16th century in Mexico.Due to its increasing popularity as an abundant nutritional biological resource, it is being used in poultry, aquaculture, animal feed, wastewater treatment and agriculture and also supplementing human food requirements

Benefits of Spirulina

- **Rich in Vitamins and Minerals:** It is s low in calories, carbohydrates and fat. It's rich in many vitamins and minerals, such as vitamins B1, B2, B3, iron, copper, protein, magnesium, manganese and potassium. It's one of the most nutritious foods to eat.
- **An Antioxidant: Spirulina** have antioxidant properties. The main active component is phycocyanin, which can fight free radicals. Phycocyanin has anti-inflammatory properties.
- **Other Health Benefits:** Spirulina helps to relieve allergies such as allergic rhinitis, lower triglycerides and LDL cholesterol, which is considered bad cholesterol. It may simultaneously raise HDL cholesterol, the good cholesterol.

Production/ Cultivation of Single cell protein (SCP). :-Spirulina is a unicellular, microscopic, and filamentous; Cyanobacteria It is longed and coiled. Several methods are attempted to cultivate single cell proteins such as **closed system, outdoor open tubes with paddle wheel, and photo-reactor system**.

Photosynthetic pigment, phycocyanin, adaptability, plasticity and its asexual reproduction by binary fission helps to get good yield. (Yield of 35 tonnes of Spirulina per hectare per year from a commercial pond was achieved in the Siam Algae Site near Bangkok).

Protocol to culture Spirulina in Lab:-

1. Obtain the mother culture of Spirulina from Culture labs Ltd.
2. Mix 1.50 ml mother culture with 200 ml designed culture medium. (medium composition NaCl:2.0 g., $NaHCO_3$:16.0 g., $NaNO_3$:2.0 g., K_2SO_4: 1.0 g., KH_2PO_4:0.5 g., $FeSO_4$).
3. 100 mg per litre water (boiled and chilled) with pH value of 10.5.
4. Keep the culture in an orbital shaker with natural illumination for 7 days and observe.

5. The intensity of green colour through UV spectrophotometer at 540 nm. Once, the OD value 0.8 is reached, the initial culture is to be transfered to 10.0 L volume plastic tubs for mass culture in 1:4 proportions (initial grown culture: medium).
6. Allow the tubs with mass culture under direct sunlight for 7 days and observe the growth of Spirulina interms of intensity of green colour.
7. Replenish with 2 L fresh medium on 4th day. Harvest at the end of the 7th day using a muslin cloth.
8. Centrifuge the slurry mass and then take into a syringe barrel with the help of the plunger.
9. The semisolid Spirulina mass pushed through a narrow nozzle of the syringe and allowed to collect on a clean porcelain tiled surface, de-moisturized under ceiling fan initially and later dried under sunlight.
10. Dried Spirulina fragments appeared like green fragile needles which were ready for consumption.

2.Artificially built cultivation system:- Based on the water quality and the nutrient status artificially built cultivation system can be grouped into 2 types. They are:

a)Clean water system b) Waste water system.

a).Clean water system :-In clean water system artificial cultivation farm is constructed. These have shallow race way ponds circulated with paddle and high quality nutrients. To promote growth of algae NaNo3 and NaHCO3 is added. PH is initially maintained at 8.5. Spirulina is self pH adjusting alga which elevates pH between 10 to 10.5 at which there will be no contamination. India has 2 centers. Namely

- Central food technological research institute (CFTRI) at Mysore.
- Murugappa chettiar research center at Chennai. It produces about 75 tonnes of Spirulina annually.

b).Waste water system:- In waste water system Human, Animal waste and sewage are used for growth of Spirulina.This can be applicable to highly populated countries where high quantities of wastes are generated and pose environmental problems.
In this system waste water is first added to digester which helps in setting of solid particles. Liquid effluents, NaNo3, NaHCO 3 are added as source of nutrition to artificially constructed ponds .Then Spirulina is added to this water. After sufficient growth, it is harvested from the pond, added to aquaculture to feed fish, or dried in small solar drier for human food.

Growth requirements for spirulina:

- **Algal tank**: Circular or Rectangular cement tank with depth of 25 cm are constructed.
- **Light:** Low light intensity is required to avoid photolysis.
- **Temperature**: For optimum growth temperature should be 35 to 40 C
- **pH** : pH ranging from 8.5 to 10.5
- **Agitation**: Agitation is necessary for good quality, better yield. The culture is agitated by brush, paddle power, rotators. Wind power, pipe pumps.
- **Harvesting**: Spirulina form thick mat over water surface. It can be harvested by fine mesh screen, Nylon or cotton cloth.
- **Drying**: Sun drying gives good results.
- **Yeild**: 8- 12 gms/ day is obtained in India. It is equal to 20 tonnes / annum.
- **Avoid contamination**: Dried powder is packed in Aluminium bags or sealed in bottles and sent to market.

Spirulina Farming at Home

One can grow fresh spirulina at home to save money. Fresh spirulina is three times more potent than the dry form. It can be grown indoors near a window or using artificial light. It can also be grown outdoors in direct sunlight in a tank, basin or even a pond.

Requirements

Tank or basin (An average-sized), large spoon for stirring and aeration,mesh cloth or net, drying racks.

Culture Medium

The culture medium is water mixed with the nutrients. This consists of sodium bicarbonate or direct CO2, magnesium sulfate, potassium nitrate, citric acid, salt, urea, calcium chloride, iron sulfate and ammonium sulfate. spirulina seeds to start your culture.

Water

The purity of the water is essential for healthy spirulina growth(Use bottled or filtered water). Spirulina thrives best at a temperature of 77 to 100 degrees Fahrenheit. It can tolerate lower temperatures, but this will affect the rate at which it grows and multiplies, so warmer temperatures are ideal.

Mother Culture

To begin growing spirulina, a mother culture is essential. The spirulina medium can be bought in a starter kit. It can also be made from spirulina seeds. From there, when placed in the culture medium, spirulina multiplies quite rapidly.

Agitation

Spirulina needs frequent agitation to make sure its filaments are getting adequate sunlight and carbon dioxide. Agitation and aeration can be created by frequent stirring. For optimum spirulina production, add an electric air pump tank can be used.

Harvesting

Spirulina takes approximately two to three weeks to grow. At this point, it is ready to be harvested. Allow the excess water to drain from the spirulina on drying racks. Once the excess water is gone.

Spirulina can be eaten fresh. It can also be dried and ground up into a powder, which can be added to smoothies, sprinkled on foods or added to a salad.

Conclusion

Blue-green algae, also referred to as cyanobacteria, is a tiny organism. These microbes conduct photosynthesis using sunlight, water and carbon dioxide to produce oxygen.

Harvesting our own spirulina can be a fun and rewarding way to optimize our health. adding spirulina to diet forms superfood for us.

Environmental technology: Waste water treatment process: primary, secondary and advanced treatment of sewage (domestic waste water),

Sewage Treatment

Introduction:-

Human health and environment are mainly affected by direct disposal of industrial and human effluents into the natural resources without any treatment. The sewage treatment is necessary to reduce the toxicity of sewage and maintain a safe and healthy environment, and to promote human welfare.

OBJECTIVES

- Decreasing waste and pollution by improving the behavior of human production and consumption.
- Development of clean alternative technologies to replace the methods proven negative impact on health and pollute the environment.
- Set up economical models to implement and commercialize related innovations by encouraging the creation of jobs and novel careers in the field.

Sewage contains a huge amount of organic matters which are toxic. Microorganisms are used in the sewage treatment plant for removing these toxic organic matters. Sewage or wastewater treatment plant consists of two stages.

Sewage water treatment (Wastewater treatment**)** refers to the process of removing contaminants and undesirable components from domestic, industrial and polluted waters to safely return it to the environment for drinking, irrigation, industrial, and other uses.

1. **Primary Treatment:-**It involves the removal of large or small sized components in the wastewater through physical processes.

2. **Biological Treatment**:- Aerobic microorganisms are inoculated into the sewage treatment plant. These microbes utilize the organic components of the sewage and reduce the toxicity. This can be measured by BOD (Biological oxygen demand).

After the biological treatment, the sludge is pumped from the treatment plant into a large tank. This consists of anaerobic bacteria that digest sludge. During digestion, biogas is produced and it is used as an energy source. Hence, Sewage treatment plant design and sewage management play a crucial role in the maintenance of human welfare.Sewage Treatment Process

The increase in ecological awareness and enhanced government regulation has made technologies, implemented to clean alternatives for wastewater treatment purposes.

Several steps are basically employed during any wastewater treatment process.

1. The first consists of **separating the solids from the liquid water**. This is achieved through gravity as solids are heavier than the liquid water.

2. **Other solid components like oils and woods** which are less dense than liquid water could be removed from the water surface through separation.
3. Then **liquid wastewater is subjected to filtration** processes to dispose of any colloidal suspensions of fine solids, Chemicals particulates and impurities.
4. The resulting filtered water is finally exposed to **oxidation to reduce or eliminate the toxicity** of any remaining pollutants and disinfect the wastewater before release it to the environment.

Advanced Sewage water treatment

Numbers of advanced methods are tested and used for wastewater treatment either alone or in combination with other conventional methods. Some of them are as follows:-

1. **Bioreactors:-.Bioreactor is a device containing bacteria and microorganisms placed in/on a** moving bed bio film reactor.

Bioreactors have separators linked to sequential tanks and a mechanical separator to accelerate the split of liquid water from the bio solids. They also contain aerators for oxygen supply to speed up the biochemical reactions by the living microorganisms which lead **to a transformation of pollutants to less or non-toxic forms**.

In metal-containing wastewater, bioreactors are inoculated with sulphate reducing bacteria (SRB) to produce hydrogen sulphide which precipitates the dissolved metals as insoluble metal sulphides that are recovered as valuable by-products.

2. **Biofiltration:- In Biofiltration** method, some selected species of bacteria and microorganisms are grown on a biofilter to form a biofilm. The wastewater is then passed through the biofilm either upflow or down flow in a continuous or discontinuous manner. During this process, living microorganisms speed up the degradation of organic matter and pollutants present in the wastewater.

Parameters like activity of the microorganisms, age of the biofilm, oxygen levels, temperature and water composition play key roles in the performance of the biofilm, and thus the quality of the resulting treated wastewaters.,

3. **Bioremediation: - In** bioremediation living microorganisms are used to remove and neutralize pollutants and hazardous species from contaminated wastewater sites to yield less toxic or nontoxic materials. In this process living microorganisms are directly added to contaminated sites during *in-situ* remediation processes and contaminated sites are treated elsewhere during *ex-situ* remediation.

All contaminants are not cleaned through bioremediation or bio filtration. Examples heavy metals like copper, nickel, cadmium, lead, and mercury, are treated with other advanced technologies like including emew electro winning and electrocoagulation have been developed.

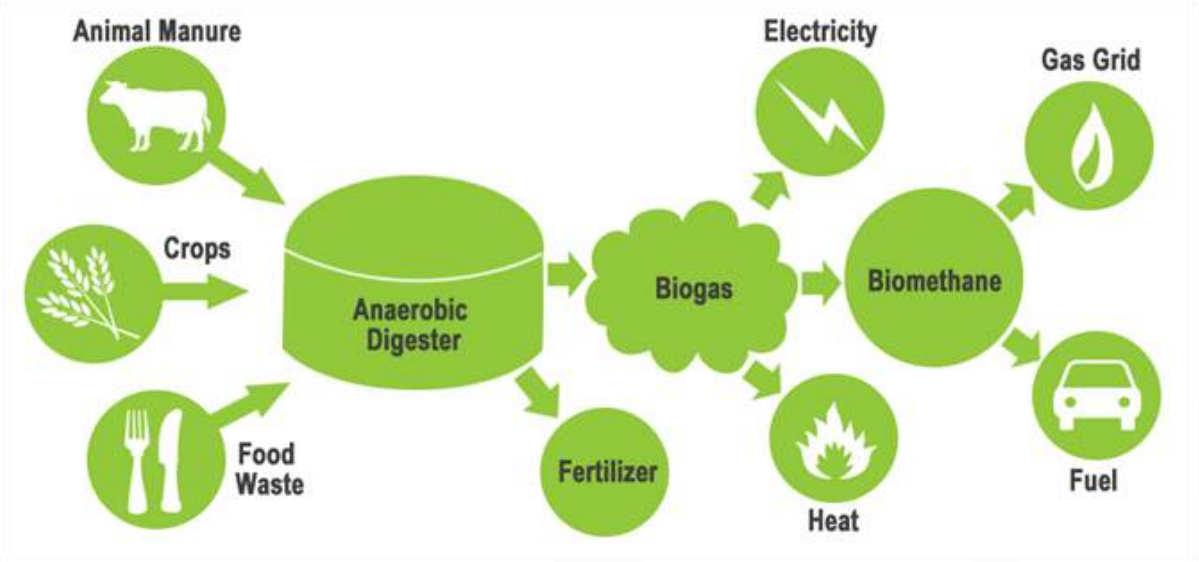
4. **Electrowinning:-** In electrowinning, a current is passed between two electrodes (cathode and anode) immersed in an electrolyte solution. Metals are then electroextracted from their oxidized forms (dissolved cations) to deposit on the cathode. Thus, heavy metals including copper, nickel, silver, gold, cadmium, bismuth, cobalt and others can be recovered from wastewaters through electrowinning.

5. **Electrocoagulation:- T**he electrocoagulation uses an electric current to remove contaminants from wastewaters**.** Cations generated at the sacrificial anode set off physical and chemical reactions that can be divided into three successive stages:

(1) By applying an electric current, hydrated cations are produced at the sacrificial iron or aluminum anode;
(2) The released cations neutralize the charges of pollutant particles which become unstable forming micro-flocculants;

(3) These destabilized particles begin to coagulate as macroscopic flocs that can be easily separated from the water.

The l mechanism is a combination of ionization, electrolysis, hydrolysis, and free-radical formation which modifies not only the physical but also chemical properties of the wastewater resulting in a net removal of pollutant species and production of a clear treated water suitable for discharge.



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ADVANTAGES OF ADVANCE WASTEWATER TREATMENT.

1. **Bio filters** are less susceptible by changes or intermittent loading to hydraulic shock, a prerequisite for industrial wastewater treatment plants. Also, their operational costs are often lower than those of other methods such as activated sludge.
2. **Bioremediation** methods are also advantageous in terms of cost effectiveness because they can be utilized without excavation, incineration, or need for cleaning strategies like "pump and treat" used for groundwater remediation. For ex: contaminated groundwater with hydrocarbon spills or chlorinated solvents might be treated by adding microorganisms to reduce or eliminate levels of undesirable contaminants.
3. Advanced technologies break down contaminants to the molecular level instead of storage or chemical dispersion. electro coagulation can produce clear, colourless, odourless water suitable for discharge.

2 MARKS QUESTIONS

1. Mention microbes involved in production of pencillin. Any 2 from pencillin
2. Mention the microbes involved in production of Ethanol. 1+1
3. Name the microbes involved in pencillin and ethanol production. 1+1
4. Mention OBJECTIVES of water treatment 1+1
5. What is Sweage treatment?Mention its types. 1+1
6. Explain microbial production of Pencillin. Microbes-1, Steps-4

5 MARKS QUESTIONS

1. Explain Single cell protein structure- ½ , significance-4 ½ ,
2. Explain the production of Ethanol. Microbes-1, steps -4.
3. Explain Single cell protein structure- ½ , significance-4 ½ ,
4. Explain the production of Ethanol. Microbes-1, steps -4.
5. Explain Sewage treatment. Microbes-1, steps -4.
6. Describe the steps involved in waste water treatment. Steps -5.
7. Explain the role of microbes in agriculture

8. Describe Advanced Sewage water treatment . Steps -5.

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