

Brahmavart P.G. College, Mandhana, Kanpur

Department of Botany

Question Bank

Section-A

B.Sc. 5th Semester

*Title: Molecular Biology
&
Bioinformatics*

Course Code: B040502T

*Prepared By:
Dr. Divya Bhadauria*

Brahmavart P.G. College, Mandhana, Kanpur

Department of Botany
B.Sc. 5th Semester Paper 2nd

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Question Bank (Section-A)

Q. 1. Describe Griffith's transformation experiment and its significance.

Ans: Griffith's Transformation Experiment

Frederick Griffith, in **1928**, performed an important experiment on the bacterium *Streptococcus pneumoniae* to study the cause of pneumonia in mice. His experiment provided the first strong evidence for the existence of a “**transforming principle**” capable of transferring hereditary characteristics from one bacterial cell to another.

Bacterial Strains Used

Griffith used two strains of *S. pneumoniae*:

1. **S strain (Smooth):**
 - Has a polysaccharide capsule.
 - Forms smooth colonies.
 - Is **virulent** and causes disease in mice.
2. **R strain (Rough):**
 - Lacks the capsule.
 - Forms rough colonies.
 - Is **non-virulent**.

Experimental Steps

Experiment	Material injected into mouse	Result
1	Live S strain	Mouse died
2	Live R strain	Mouse survived
3	Heat-killed S strain	Mouse survived
4	Live R strain + heat-killed S strain	Mouse died

In the fourth experiment, living **R-type bacteria were transformed into virulent S-type bacteria**. Live S-type bacteria were subsequently recovered from the dead mouse.

Explanation

The results showed that some substance from the **heat-killed S bacteria** entered the living R bacteria and changed their hereditary characteristics. Griffith called this substance the “**transforming principle**.”

The transformation can be represented as:

Heat-killed S bacteria + Live R bacteria → Transformed S bacteria → Virulent bacteria

Significance of Griffith's Experiment

1. **Evidence for transformation:**

It demonstrated that bacteria can acquire new hereditary characteristics from other bacterial cells.

2. **Evidence for a transferable genetic factor:**

The experiment indicated that a stable substance could carry hereditary information from one cell to another.

3. **Foundation for molecular genetics:**

Griffith's findings stimulated further research into the chemical nature of the transforming principle.

4. **Led to Avery, MacLeod and McCarty's work:**

Later, in **1944**, Avery and his colleagues demonstrated that the transforming principle was **DNA**.

5. **Importance in understanding DNA as genetic material:**

Griffith's experiment was an important early step toward establishing the role of **DNA in heredity**.

Griffith's transformation experiment demonstrated that a hereditary characteristic could be **transferred from one bacterial strain to another**. Although Griffith did not identify the chemical nature of the transforming principle, his work provided a crucial foundation for the discovery that **DNA is the genetic material**.

Q. 2. Describe the Hershey-Chase experiment and its significance.

Ans: Hershey–Chase Experiment:

The **Hershey–Chase experiment**, performed by **Alfred Hershey and Martha Chase in 1952**, provided strong evidence that **DNA is the genetic material**. They used the bacteriophage **T2**, a virus that infects the bacterium *Escherichia coli*.

Principle of the Experiment

A T2 bacteriophage consists mainly of:

- **DNA** – contains phosphorus (P) but normally no sulphur (S).
- **Protein coat** – contains sulphur (S) in amino acids such as cysteine and methionine but essentially no phosphorus.

Therefore, radioactive isotopes were used to distinguish DNA from protein:

- **^{32}P (radioactive phosphorus)** → labelled DNA.
- **^{35}S (radioactive sulphur)** → labelled protein.

Experimental Procedure

1. Labelling DNA:

T2 phages were grown in bacteria containing radioactive ^{32}P , so their DNA became radioactive.

2. Labelling protein:

Another group of T2 phages was grown in bacteria containing radioactive ^{35}S , so their protein coats became radioactive.

3. Infection of bacteria:

The labelled phages were allowed to infect *E. coli* cells.

4. Separation:

After infection, the phage coats were separated from the bacterial cells using a **blender**, followed by **centrifugation**.

Results

Label	Molecule labelled	Radioactivity found mainly in
^{32}P	DNA	Bacterial cells (pellet)
^{35}S	Protein	Phage coats (supernatant)

The ^{32}P -labelled DNA entered the bacterial cells, whereas the ^{35}S -labelled protein remained outside the cells.

Significance

1. DNA is the genetic material:

The experiment demonstrated that DNA, rather than protein, enters the bacterial cell during phage infection and directs the formation of new phages.

2. DNA carries hereditary information:

It provided strong experimental evidence that genetic information is contained in DNA.

3. Confirmed earlier findings:

The experiment supported the conclusions emerging from **Griffith's transformation experiment** and the work of **Avery, MacLeod and McCarty**.

4. Foundation of molecular biology:

It helped establish DNA as the central molecule responsible for the storage and transmission of genetic information.

The Hershey–Chase experiment demonstrated that during T2 bacteriophage infection, **DNA enters the bacterial cell and carries the genetic information**, while the protein coat remains outside. Thus, it provided strong evidence that **DNA is the genetic material**.

Q. 3. Explain the historical development of the concept of genetic material.

Ans: Explain the Historical Development of the Concept of Genetic Material

The concept of **genetic material** developed gradually through a series of important experiments. Scientists initially believed that proteins were the genetic material, but later experiments established **DNA as the hereditary material**.

1. **Gregor Mendel (1866)**
Mendel proposed the concept of **hereditary factors (genes)** through his experiments on pea plants. He showed that characters are inherited from parents to offspring.
2. **Chromosome Theory of Inheritance – Sutton and Boveri (1902–1903)**
Sutton and Boveri proposed that **chromosomes carry hereditary factors (genes)**. This provided a connection between genes and chromosomes.
3. **Griffith's Transformation Experiment (1928)**
Frederick Griffith worked with *Streptococcus pneumoniae* and discovered **bacterial transformation**. He showed that a substance from dead virulent bacteria could transform harmless bacteria into virulent forms. He called this substance the “**transforming principle**.”
4. **Avery, MacLeod and McCarty (1944)**
They demonstrated that the transforming principle identified by Griffith was **DNA**. This was the first strong experimental evidence that DNA is genetic material.
5. **Hershey and Chase (1952)**
Using bacteriophages, Hershey and Chase showed that **DNA enters bacterial cells and directs the production of new viruses**, whereas protein does not. This provided strong evidence that DNA is the genetic material.
6. **Watson and Crick (1953)**
Watson and Crick proposed the **double-helical structure of DNA**, explaining how DNA can store and faithfully transmit genetic information.

Thus, the concept of genetic material progressed from **Mendel's hereditary factors → chromosomes → transforming principle → DNA**, with the experiments of Avery, MacLeod, McCarty, and Hershey and Chase providing decisive evidence that **DNA is the genetic material in most organisms**.

Q. 4. Describe the important features of the DNA double helix.

Ans: Describe the Important Features of the DNA Double Helix

The **DNA double helix** was proposed by **James Watson and Francis Crick in 1953**, based on experimental evidence including X-ray diffraction studies. The important features are:

1. **Double-stranded structure:**
DNA consists of **two polynucleotide strands** twisted around each other to form a right-handed double helix.

2. **Antiparallel strands:**

The two strands run in opposite directions. One strand runs $5' \rightarrow 3'$, while the other runs $3' \rightarrow 5'$.

3. **Sugar-phosphate backbone:**

The backbone of each strand consists of alternating **deoxyribose sugar and phosphate groups**. It lies on the outside of the helix.

4. **Complementary base pairing:**

Nitrogenous bases are present inside the helix and pair specifically:

- **Adenine (A) pairs with Thymine (T)** through two hydrogen bonds.
- **Guanine (G) pairs with Cytosine (C)** through three hydrogen bonds.

5. **Dimensions of the helix:**

The diameter of DNA is approximately **2 nm (20 Å)**. One complete turn contains about **10 base pairs** and measures approximately **3.4 nm (34 Å)**.

6. **Major and minor grooves:**

The twisting of the two strands produces **major and minor grooves**, which provide sites for interaction with proteins and other molecules.

7. **Stability:**

The double helix is stabilized mainly by **hydrogen bonding between complementary bases** and **base-stacking interactions** between adjacent bases.

8. **Genetic information:**

The **sequence of bases** along DNA stores genetic information. Complementary base pairing also provides the basis for **accurate DNA replication**.

Thus, the DNA double helix is a **stable, complementary and antiparallel structure** capable of storing and transmitting genetic information accurately from one generation to the next.

Q. 5. Describe the different types of DNA.

Ans: Describe the Different Types of DNA

DNA occurs in different structural forms depending on environmental conditions such as **hydration, salt concentration, and base sequence**. The three major forms are **A-DNA, B-DNA and Z-DNA**.

Feature	A-DNA	B-DNA	Z-DNA
Helix	Right-handed	Right-handed	Left-handed
Diameter	~2.6 nm	~2.0 nm	~1.8 nm
Base pairs/turn	~11	~10	~12
Pitch/turn	~2.8 nm	~3.4 nm	~4.5 nm
Condition	Low	Normal physiological	High salt/alternating purine-pyrimidine

Feature	A-DNA	B-DNA	Z-DNA
	hydration	conditions	sequences
Shape	Short and broad	Long and slender	Thin and elongated

1. A-DNA

- A-DNA is a **right-handed helix**.
- It is formed under conditions of **reduced hydration**.
- It has approximately **11 base pairs per turn**.
- It is shorter and broader than B-DNA.
- **RNA-DNA hybrids** and double-stranded RNA may adopt A-type structures.

2. B-DNA

- B-DNA is the **most common form of DNA under normal physiological conditions**.
- It is a **right-handed double helix**.
- It has approximately **10 base pairs per turn**.
- The diameter is about **2 nm** and one complete turn is about **3.4 nm**.
- It has well-defined **major and minor grooves** and is considered the classical Watson–Crick form.

3. Z-DNA

- Z-DNA is a **left-handed double helix**.
- Its sugar-phosphate backbone has a characteristic **zig-zag pattern**, giving it the name Z-DNA.
- It commonly occurs in DNA sequences containing **alternating purines and pyrimidines**, particularly GC-rich sequences.
- It may play a role in **regulation of gene expression** and changes in DNA structure.

The three important structural forms of DNA are **A-DNA, B-DNA and Z-DNA**. Among them, **B-DNA is the predominant form under normal cellular conditions**, while A- and Z-DNA occur under specific structural or environmental conditions.

Q. 6. Describe the different types of RNA.

Ans: Describe the Different Types of RNA

RNA (ribonucleic acid) is generally a **single-stranded nucleic acid** involved in the expression and regulation of genetic information. The three major types of RNA are **mRNA, tRNA and rRNA**.

1. Messenger RNA (mRNA)

- mRNA carries **genetic information from DNA to ribosomes** for protein synthesis.
- It is formed by the process of **transcription**.

- Its nucleotide sequence determines the **amino acid sequence of a protein**.
- In eukaryotes, mRNA undergoes processing such as **5' capping, poly-A tail addition and splicing**.

2. Transfer RNA (tRNA)

- tRNA carries specific **amino acids to the ribosome** during protein synthesis.
- It contains an **anticodon** that pairs with the complementary codon on mRNA.
- It has a characteristic **cloverleaf secondary structure**.
- Each tRNA is specifically attached to its corresponding amino acid.

3. Ribosomal RNA (rRNA)

- rRNA is a major structural and functional component of **ribosomes**.
- It combines with proteins to form ribosomal subunits.
- It helps in the **binding of mRNA and tRNA** during translation.
- rRNA also has **catalytic activity** and plays an important role in peptide-bond formation.

Other Important Types of RNA

Apart from the three major types, cells contain several regulatory RNAs, such as:

- **snRNA**: involved in RNA splicing.
- **snoRNA**: involved mainly in modification and processing of rRNA.
- **miRNA**: regulates gene expression by inhibiting translation or promoting mRNA degradation.
- **siRNA**: participates in gene silencing.
- **lncRNA**: involved in various aspects of gene regulation.

Thus, **mRNA, tRNA and rRNA** are the three principal types of RNA and are essential for **gene expression and protein synthesis**, while several other RNAs perform important regulatory and processing functions.

Q. 7. Explain the structure and functions of tRNA.

Ans: **Explain the Structure and Functions of tRNA**

Transfer RNA (tRNA) is a small, single-stranded RNA molecule that acts as an **adapter molecule during protein synthesis**. It carries specific amino acids to the ribosome according to the codons present on mRNA.

Structure of tRNA

1. **Size:**
tRNA is usually about **70–90 nucleotides** long.
2. **Cloverleaf structure:**
Its secondary structure resembles a **cloverleaf**, consisting of several arms and loops.
3. **Acceptor arm:**
 - It is formed by base pairing between the ends of the tRNA.

- The **3' end contains the CCA sequence**, where the specific amino acid is attached.
- 4. **Anticodon arm:**
 - It contains a three-nucleotide **anticodon**.
 - The anticodon pairs with the complementary **codon of mRNA** during translation.
- 5. **Dihydrouridine (D) arm:**
This arm contains **dihydrouridine** and contributes to the proper structure and function of tRNA.
- 6. **TΨC arm:**
It contains the conserved **T, pseudouridine (Ψ), and C sequence** and is involved in interaction with the ribosome.
- 7. **Variable loop:**
A variable region occurs between the anticodon and TΨC arms. Its size differs among different tRNAs.

Functions of tRNA

- **Carries amino acids:** Each tRNA transports a specific amino acid to the ribosome.
- **Recognizes codons:** Its anticodon recognizes the appropriate codon on mRNA.
- **Acts as an adaptor:** It connects the nucleotide sequence of mRNA with the corresponding amino acid sequence of a protein.
- **Participates in translation:** tRNA delivers amino acids in the correct order for polypeptide formation.
- **Maintains accuracy:** Specific charging of tRNA with its corresponding amino acid helps ensure accurate protein synthesis.

tRNA is an essential **adapter molecule of translation**. Its characteristic cloverleaf structure, **3'-CCA amino acid attachment site**, and **anticodon region** enable it to decode mRNA and deliver the correct amino acids during protein synthesis.

Q. 8. Differentiate between DNA and RNA.

Ans: Differentiate Between DNA and RNA

DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid) are nucleic acids, but they differ in their structure and functions.

Feature	DNA	RNA
Full name	Deoxyribonucleic acid	Ribonucleic acid
Sugar	Contains deoxyribose sugar	Contains ribose sugar
Nitrogen bases	Adenine, Guanine, Cytosine and Thymine	Adenine, Guanine, Cytosine and Uracil

Feature	DNA	RNA
Strandedness	Usually double-stranded	Usually single-stranded
Structure	Usually forms a double helix	Usually forms a single chain that may fold into complex structures
Location	Mainly present in the nucleus ; also in mitochondria and chloroplasts	Present in the nucleus and cytoplasm , particularly in ribosomes
Main function	Stores and transmits genetic information	Mainly involved in gene expression and protein synthesis
Types	Generally one principal genetic material	Major types include mRNA, tRNA and rRNA , along with several regulatory RNAs
Stability	Relatively more stable	Relatively less stable because of the 2'-OH group
Replication	Capable of self-replication	Generally synthesized from DNA by transcription
Base pairing	A pairs with T; G pairs with C	A pairs with U; G pairs with C

DNA mainly serves as the long-term storage of genetic information, whereas RNA performs diverse roles in the expression, regulation and utilization of that information, especially in protein synthesis.

Q. 9. Write a short note on the significance of DNA structure.

Ans: The Significance of DNA Structure

The **double-helical structure of DNA**, proposed by **Watson and Crick in 1953**, is highly significant because its structure explains how genetic information is stored, copied, and transmitted.

- 1. Storage of genetic information:**

The specific **sequence of nitrogenous bases** (A, T, G and C) stores genetic information.

- 2. Complementary base pairing:**

Adenine pairs with thymine and guanine pairs with cytosine. This complementary relationship allows DNA to maintain accurate genetic information.

- 3. Basis of DNA replication:**

During replication, each DNA strand acts as a **template for the formation of a new complementary strand**. Thus, genetic information can be accurately copied.

- 4. Transmission of hereditary information:**

The stable structure of DNA allows genetic information to be **passed from parent cells to daughter cells** and from one generation to the next.

5. **Role in protein synthesis:**

The sequence of bases in DNA determines the sequence of **RNA and ultimately the amino acids in proteins** through gene expression.

6. **DNA repair and stability:**

The complementary strands provide a mechanism for **recognizing and repairing damaged or incorrectly paired bases**, helping maintain genetic stability.

7. **Molecular basis of heredity:**

The structure explains how DNA can function as the **genetic material**, controlling inherited characteristics and cellular activities.

The structure of DNA is significant because it provides a **stable system for storing, replicating, transmitting and expressing genetic information**, forming the molecular basis of heredity.

Q. 10. Explain the semi-conservative mode of DNA replication.

Ans: The Semi-Conservative Mode of DNA Replication

DNA replication is the process by which DNA produces an identical copy of itself before cell division. The replication of DNA is **semi-conservative**, meaning that each daughter DNA molecule contains **one parental strand and one newly synthesized strand**.

The semi-conservative nature of DNA replication was experimentally demonstrated by **Meselson and Stahl (1958)** using *E. coli*.

Mechanism of Semi-Conservative Replication

1. **Unwinding of DNA:**

The double-stranded DNA unwinds with the help of the enzyme **DNA helicase**, which breaks the hydrogen bonds between complementary bases.

2. **Formation of replication fork:**

Unwinding produces a **replication fork**, where the two parental strands separate and act as templates.

3. **Primer formation:**

The enzyme **primase** synthesizes a short RNA primer that provides a free 3'-OH group for DNA synthesis.

4. **Formation of new strands:**

DNA polymerase adds nucleotides to the growing strand according to complementary base pairing:

- A pairs with T
- G pairs with C

5. **Leading and lagging strands:**

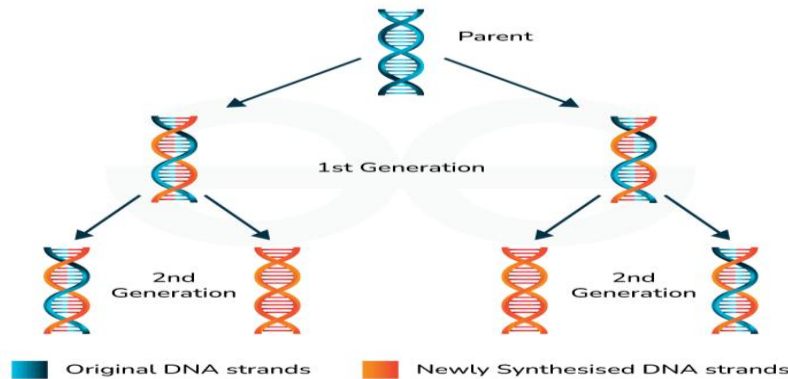
- The **leading strand** is synthesized continuously.
- The **lagging strand** is synthesized discontinuously as short **Okazaki fragments**.

6. Joining of fragments:

RNA primers are removed and replaced with DNA. The Okazaki fragments are joined by **DNA ligase**.

Result

At the end of replication, two identical DNA molecules are formed:



Thus, each daughter DNA molecule contains one original (parental) strand and one newly synthesized strand.

Experimental Evidence – Meselson and Stahl Experiment

Meselson and Stahl grew *E. coli* in a medium containing ^{15}N and then transferred the bacteria to a ^{14}N medium. After one generation, DNA showed an intermediate density, and after two generations, both intermediate and light DNA were observed. These results supported the **semi-conservative model**.

The semi-conservative mechanism ensures **accurate duplication and transmission of genetic information** from one generation of cells to the next.

Q. 11. Describe DNA replication in prokaryotes.

Ans: DNA Replication in Prokaryotes

DNA replication in prokaryotes is a **semi-conservative, bidirectional and semi-discontinuous process**. In bacteria such as *E. coli*, replication usually begins at a single origin called **oriC** and proceeds in both directions.

Steps of DNA Replication

1. Initiation

- Replication begins at the **oriC** region of the bacterial chromosome.
- **DnaA** proteins recognize and bind to the origin.
- **DNA helicase (DnaB)** unwinds the DNA double helix by breaking hydrogen bonds.
- **Single-strand binding (SSB) proteins** stabilize the separated strands.
- **DNA gyrase/topoisomerase** relieves the tension produced by unwinding.

- **Primase** synthesizes short RNA primers.
- 2. **Elongation**
 - **DNA polymerase III** adds nucleotides to the 3' end of the primer in the **5' → 3' direction**.
 - The **leading strand** is synthesized continuously toward the replication fork.
 - The **lagging strand** is synthesized discontinuously away from the replication fork as **Okazaki fragments**.
- 3. **Primer Removal and Joining**
 - **DNA polymerase I** removes RNA primers and replaces them with DNA.
 - **DNA ligase** joins the Okazaki fragments by forming phosphodiester bonds.
- 4. **Termination**
 - Replication continues until the two replication forks meet in the termination region.
 - The newly formed DNA molecules are separated, producing **two identical daughter DNA molecules**.

Important Enzymes

Enzyme/Protein	Function
DnaA	Recognizes the origin of replication
Helicase	Unwinds DNA
SSB proteins	Stabilize single DNA strands
Gyrase/Topoisomerase	Relieves supercoiling
Primase	Synthesizes RNA primers
DNA polymerase III	Main enzyme for DNA synthesis
DNA polymerase I	Removes primers and fills gaps
DNA ligase	Joins Okazaki fragments

Thus, prokaryotic DNA replication is a **semi-conservative and bidirectional process** involving coordinated action of several enzymes. It produces two DNA molecules, each containing **one parental strand and one newly synthesized strand**.

Q. 12. Explain the role of important enzymes involved in DNA replication.

Ans: DNA replication is a complex process involving several enzymes and proteins. Each enzyme performs a specific function to ensure accurate duplication of DNA.

Enzyme/Protein	Role in DNA Replication
Helicase	Unwinds the DNA double helix by breaking the hydrogen bonds

Enzyme/Protein	Role in DNA Replication
	between complementary bases.
Topoisomerase	Relieves tension and supercoiling that develops ahead of the replication fork during DNA unwinding.
Primase	Synthesizes a short RNA primer that provides a free 3'-OH group for DNA synthesis.
DNA Polymerase III	Main DNA-synthesizing enzyme in prokaryotes; adds nucleotides in the 5' → 3' direction and performs proofreading.
DNA Polymerase I	Removes RNA primers and replaces them with DNA nucleotides.
DNA Ligase	Joins Okazaki fragments on the lagging strand by forming phosphodiester bonds .
Exonucleases	Remove primers or damaged/incorrect nucleotides during replication and repair.
Single-strand binding proteins (SSB)	Prevent separated DNA strands from re-forming the double helix and protect them from degradation.

Functions in Sequence

Helicase → Topoisomerase → SSB proteins → Primase → DNA Polymerase → Primer removal → DNA Ligase

The coordinated action of these enzymes allows DNA to **unwind, copy accurately, proofread and join newly synthesized DNA strands**, ensuring faithful transmission of genetic information to daughter cells.

Q. 13. Explain the role of RNA primers in DNA replication.

Ans: An **RNA primer** is a short sequence of RNA nucleotides synthesized by the enzyme **primase** during DNA replication. It provides the starting point for the synthesis of a new DNA strand.

Role of RNA Primers

- Provides a starting point:**
DNA polymerase cannot start DNA synthesis on its own. It requires a pre-existing **free 3'-OH group**, which is provided by the RNA primer.
- Initiates DNA synthesis:**
Primase synthesizes the RNA primer complementary to the DNA template. DNA polymerase then adds DNA nucleotides to the **3' end of the primer**.
- Role on the leading strand:**
Generally, **one RNA primer** is required to initiate continuous synthesis of the leading strand.

4. **Role on the lagging strand:**

Multiple RNA primers are required because the lagging strand is synthesized discontinuously. Each primer initiates an **Okazaki fragment**.

5. **Primer removal:**

After DNA synthesis, RNA primers are removed. In prokaryotes, **DNA polymerase I** removes the primers and replaces them with DNA.

6. **Joining of DNA fragments:**

After primer replacement, **DNA ligase** joins the adjacent DNA fragments by forming phosphodiester bonds.

Simple Sequence

Template DNA → Primase → RNA primer → DNA polymerase → New DNA strand

RNA primers are essential for DNA replication because they provide the **3'-OH starting point required by DNA polymerase**. They ensure that DNA synthesis can begin on both the leading and lagging strands.

Q. 14. Differentiate between prokaryotic and eukaryotic DNA replication.

Ans: Differentiate Between Prokaryotic and Eukaryotic DNA Replication

DNA replication is semi-conservative in both prokaryotes and eukaryotes, but the process differs in several important ways.

Feature	Prokaryotic DNA Replication	Eukaryotic DNA Replication
1. Location	Occurs in the cytoplasm/nucleoid region because there is no true nucleus.	Occurs mainly in the nucleus .
2. DNA molecule	Usually has a single circular chromosome .	Chromosomes are linear .
3. Origin of replication	Usually has one origin of replication (e.g., <i>oriC</i> in <i>E. coli</i>).	Each chromosome has many origins of replication .
4. Replication speed	Relatively faster .	Relatively slower .
5. Replication forks	Two replication forks generally arise from the single origin.	Multiple replication forks arise from multiple origins.
6. Main DNA polymerases	DNA polymerase III is the major replicative polymerase; DNA polymerase I removes primers and fills gaps.	DNA polymerases α, δ and ϵ have major roles in nuclear DNA replication.
7. Okazaki fragments	Relatively longer (about 1,000–2,000 nucleotides).	Relatively shorter (about 100–200 nucleotides).
8. Telomeres	Generally absent because bacterial	Present at the ends of linear

Feature	Prokaryotic DNA Replication	Eukaryotic DNA Replication
	chromosomes are usually circular.	chromosomes and require telomerase for maintenance.
9. DNA packaging	DNA is associated with nucleoid proteins but lacks typical eukaryotic nucleosomes.	DNA is packaged into nucleosomes with histone proteins .
10. Cell-cycle control	Not restricted to a distinct S phase as in eukaryotic cells.	Mainly occurs during the S phase of the cell cycle.

Similarities

Both processes:

- Are **semi-conservative**.
- Require a **template DNA strand**.
- Synthesize new DNA in the **5' → 3' direction**.
- Involve enzymes such as **helicase, primase, DNA polymerase and ligase**.
- Produce complementary daughter DNA molecules.

Prokaryotic DNA replication is generally **simpler and faster**, with a single circular chromosome and usually one origin, whereas eukaryotic replication is **more complex**, involving multiple origins, linear chromosomes, histone-associated DNA, and telomeres.

Q. 15. Define transcription and explain its major steps.

Ans: Transcription is the process by which the genetic information present in DNA is copied into RNA. It is carried out by the enzyme RNA polymerase. The RNA produced may be mRNA, tRNA, rRNA or other types of RNA.

Major Steps of Transcription

1. Initiation

- Transcription begins when **RNA polymerase** recognizes and binds to a specific region of DNA called the **promoter**.
- The DNA double helix locally unwinds, exposing the template strand.
- RNA polymerase begins RNA synthesis at the transcription start site.

2. Elongation

- RNA polymerase moves along the **template DNA strand in the 3' → 5' direction**.
- It synthesizes the RNA strand in the **5' → 3' direction**.
- RNA nucleotides are added according to complementary base pairing:
 - DNA A → RNA U
 - DNA T → RNA A
 - DNA G → RNA C
 - DNA C → RNA G

3. Termination

- RNA polymerase reaches a specific **termination sequence** in the DNA.
- Transcription stops and the newly synthesized RNA molecule is released.
- The DNA strands then reassociate.

In Eukaryotes

The initial RNA transcript, particularly pre-mRNA, undergoes processing before becoming mature mRNA:

- **5' capping**
- **3' poly-A tail addition**
- **RNA splicing**, in which introns are removed and exons are joined.

Simple Flow

DNA → Initiation → Elongation → Termination → RNA

Transcription is an essential step in **gene expression** through which information stored in DNA is transferred to RNA, providing the RNA molecules needed for protein synthesis and other cellular functions.

Q. 16. Describe the role of RNA polymerase in transcription.

Ans: RNA polymerase is the key enzyme responsible for transcription, the process by which genetic information in DNA is copied into RNA. It synthesizes RNA using one strand of DNA as a template.

Major Roles of RNA Polymerase

1. **Recognition of the promoter:**
RNA polymerase recognizes and binds to the **promoter region** of a gene, with the help of associated transcription factors or initiation factors.
2. **Unwinding of DNA:**
After binding to the promoter, RNA polymerase helps form a local **transcription bubble** by separating the two DNA strands.
3. **Initiation of RNA synthesis:**
RNA polymerase starts RNA synthesis at the **transcription start site**. Unlike DNA polymerase, it does not require a pre-existing primer.
4. **Elongation of RNA:**
RNA polymerase moves along the template DNA strand in the **3' → 5' direction** and adds complementary ribonucleotides to the growing RNA strand in the **5' → 3' direction**.
5. **Base pairing:**
It follows complementary base pairing:
 - DNA A → RNA U
 - DNA T → RNA A
 - DNA G → RNA C
 - DNA C → RNA G

6. Termination:

When RNA polymerase encounters an appropriate **termination signal**, transcription stops and the newly synthesized RNA is released.

RNA Polymerases in Eukaryotes

RNA Polymerase	Major function
RNA Polymerase I	Synthesizes most rRNA
RNA Polymerase II	Synthesizes mRNA and several other RNAs
RNA Polymerase III	Synthesizes tRNA , 5S rRNA and some other small RNAs

RNA polymerase performs the central functions of **promoter recognition, DNA opening, RNA initiation, elongation and termination**, making it essential for the expression of genetic information.

Q. 17. Write a note on the promoter and terminator regions.

Ans: Promoter and terminator regions are important regulatory sequences of a gene that control the beginning and end of transcription, respectively.

1. Promoter Region

A **promoter** is a specific DNA sequence located **upstream of a gene** where RNA polymerase and associated factors bind to initiate transcription.

Important features:

- It determines the **starting point and direction of transcription**.
- It helps recruit **RNA polymerase** to the gene.
- In bacteria, common promoter elements include the **–10 region (Pribnow box)** and **–35 region**.
- In many eukaryotes, promoters may contain elements such as the **TATA box**, located upstream of the transcription start site.
- Promoter sequences also contribute to the **regulation of gene expression**.

2. Terminator Region

A **terminator** is a DNA sequence that signals the **termination of transcription**.

Important features:

- It causes RNA polymerase to stop transcription and release the newly synthesized RNA.
- In bacteria, termination may occur through **rho-dependent** or **rho-independent (intrinsic)** mechanisms.
- In eukaryotes, termination mechanisms differ among RNA polymerases. For example, **RNA polymerase II** transcribes beyond the polyadenylation signal, after which the RNA is cleaved and the polymerase eventually dissociates.

Difference Between Promoter and Terminator

Promoter	Terminator
Marks the beginning of transcription.	Marks the end of transcription.
Usually located upstream of the transcription unit.	Located toward the end of the transcription unit.
Provides a binding site for RNA polymerase and transcription machinery.	Provides signals that lead to termination and RNA release.
Controls initiation of transcription.	Controls termination of transcription.

The **promoter initiates and regulates transcription**, while the **terminator signals its completion**. Together, they ensure that transcription occurs at the correct location and produces the appropriate RNA molecule.

Q. 18. Explain the important features of genetic code.

Ans: The genetic code is the set of rules by which the sequence of nucleotides in mRNA determines the sequence of amino acids in a protein. A group of three nucleotides is called a codon, and each codon specifies an amino acid or a stop signal.

Important Features of Genetic Code

- Triplet code:**
The genetic code is a **triplet code**. Each codon consists of three nucleotides. Since there are four bases, $4^3 = 64$ codons are possible.
- Specific and unambiguous:**
Each codon specifies **only one particular amino acid**. For example, AUG codes for methionine.
- Degenerate:**
The code is **degenerate**, meaning that more than one codon can specify the same amino acid. For example, leucine is specified by six different codons.
- Nearly universal:**
The genetic code is **nearly universal**, meaning that the same codons generally specify the same amino acids in most organisms. Some exceptions occur, particularly in **mitochondrial and chloroplast genetic codes**.
- Non-overlapping:**
In a normal reading frame, one nucleotide belongs to only **one codon**. Thus, codons are read sequentially without overlap.
- Commaless:**
Codons are read **continuously**, without punctuation or gaps between successive codons.

7. **Start codon:**
AUG generally acts as the start codon and specifies **methionine** (formylmethionine in bacterial initiation).
8. **Stop codons:**
UAA, UAG and UGA are stop codons. They do not code for amino acids and signal termination of protein synthesis.
9. **Polarity:**
The mRNA is read in the **5' → 3' direction**, and the corresponding polypeptide is synthesized from the **N-terminal to the C-terminal end**.

The genetic code provides a precise relationship between **nucleotide sequences in mRNA and amino acid sequences in proteins**. Its triplet, degenerate and nearly universal nature is fundamental to gene expression and protein synthesis.

Q. 19. Explain the different stages of protein synthesis.

Ans: Protein synthesis is the process by which cells produce proteins using the genetic information encoded in DNA. It occurs mainly through two major stages: transcription and translation. Translation itself involves initiation, elongation and termination.

1. Transcription

- Transcription is the formation of **RNA from a DNA template**.
- **RNA polymerase** binds to the promoter and synthesizes an RNA molecule.
- In eukaryotes, the primary transcript is processed by **5' capping, poly-A tail addition and splicing** to form mature mRNA.
- The mature mRNA moves to the cytoplasm and associates with ribosomes.

2. Translation

Translation is the process by which the information in mRNA is used to synthesize a **polypeptide chain**.

A. Initiation

- The **ribosome binds to mRNA**.
- The start codon, usually **AUG**, is recognized.
- An initiator tRNA carrying methionine binds to the start codon.
- The complete initiation complex is formed.

B. Elongation

- tRNAs bring specific amino acids to the ribosome according to the **codons on mRNA**.
- The ribosome forms **peptide bonds** between adjacent amino acids.
- The ribosome moves along the mRNA in the **5' → 3' direction**.
- The polypeptide chain gradually increases in length.

C. Termination

- When the ribosome reaches a **stop codon (UAA, UAG or UGA)**, no tRNA binds to it.

- Release factors promote the release of the newly synthesized polypeptide.
- The ribosome separates from the mRNA.

3. Post-translational Modifications

After translation, the newly formed polypeptide may undergo:

- **Folding** into its functional three-dimensional structure.
- Removal or addition of specific chemical groups.
- **Cleavage** into active protein products.
- Transport to the appropriate cellular location.

Simple Flow

DNA → Transcription → mRNA → Translation → Polypeptide → Functional Protein

Protein synthesis is a coordinated process involving **transcription, translation and post-translational processing**. It converts the genetic information stored in DNA into functional proteins essential for cellular structure and activities.

Q. 20. Write a note on initiation, elongation and termination of translation.

Ans: Translation is the process by which the nucleotide sequence of mRNA is decoded by ribosomes to synthesize a polypeptide chain. It occurs in three major stages: initiation, elongation and termination.

1. Initiation

- The **small ribosomal subunit** binds to the mRNA.
- The ribosome recognizes the **start codon (AUG)**.
- An **initiator tRNA** carrying methionine binds to the AUG codon by complementary base pairing.
- The **large ribosomal subunit** then joins the complex, forming a complete ribosome.
- The initiator tRNA occupies the **P site** of the ribosome.

2. Elongation

- A charged tRNA carrying the next amino acid enters the **A site** according to the next mRNA codon.
- The ribosome catalyzes the formation of a **peptide bond** between the amino acids.
- The ribosome moves one codon forward along the mRNA in the **5' → 3' direction**. This is called **translocation**.
- The empty tRNA leaves through the **E site**.
- This cycle repeats, causing the polypeptide chain to grow.

3. Termination

- Elongation continues until a **stop codon (UAA, UAG or UGA)** enters the A site.
- Stop codons do not code for amino acids and are recognized by **release factors**.
- The completed polypeptide chain is released from the ribosome.

- The ribosomal subunits, mRNA and other components separate.

Summary

Initiation → Elongation → Termination

Start codon → Peptide-chain growth → Stop codon → Protein release

Initiation establishes the correct reading frame, elongation adds amino acids in the correct sequence, and termination releases the completed **polypeptide chain**. Together, these stages ensure accurate protein synthesis.

Q. 21. Explain the lac operon model.

Ans: **The lac operon model was proposed by François Jacob and Jacques Monod in 1961 to explain the regulation of gene expression in bacteria. It controls the utilization of lactose in *E. coli* and is a classic example of an inducible operon.**

Components of the Lac Operon

1. **Regulator gene (lacI):**

It produces the **repressor protein**, which regulates the operon.

2. **Promoter (P):**

It is the site where **RNA polymerase binds** to initiate transcription.

3. **Operator (O):**

It is the regulatory region to which the **repressor protein** binds.

4. **Structural genes:**

The lac operon contains three structural genes:

- **lacZ** – codes for **β-galactosidase**, which breaks down lactose.
- **lacY** – codes for **permease**, which facilitates the entry of lactose into the cell.
- **lacA** – codes for **transacetylase**.

Working of the Lac Operon

A. In the Absence of Lactose

- The **lacI gene** produces an active repressor.
- The repressor binds to the **operator**.
- RNA polymerase cannot effectively transcribe the structural genes.
- Therefore, the **lacZ, lacY and lacA genes remain largely inactive**.

No lactose → Repressor active → Operator blocked → Transcription OFF

B. In the Presence of Lactose

- Lactose is converted to **allolactose**, which acts as the inducer.
- Allolactose binds to the repressor and changes its shape.
- The repressor can no longer bind effectively to the operator.
- RNA polymerase transcribes **lacZ, lacY and lacA**.

- The resulting proteins help the cell take up and metabolize lactose.

Lactose → Allolactose → Repressor inactivated → Transcription ON

Role of Glucose

The lac operon is also influenced by glucose. When **glucose is low**, cellular **cAMP levels increase**, and the **CAP–cAMP complex** enhances transcription of the lac operon. Thus, the strongest expression occurs when **lactose is available and glucose is scarce**.

The lac operon demonstrates how bacteria regulate gene expression according to nutrient availability. It ensures that the enzymes required for lactose utilization are produced mainly **when they are needed**.

Q. 22. What is Southern blotting? Explain its principle and applications.

Ans: Southern blotting is a molecular biology technique used to **detect and identify a specific DNA sequence** in a complex mixture of DNA fragments. The technique was developed by Edwin Southern in 1975.

Principle

Southern blotting is based on the **complementary base pairing (hybridization)** between a specific, labelled **DNA probe** and its complementary target DNA sequence.

Major Steps

1. DNA isolation and digestion:

DNA is isolated and cut into fragments using **restriction endonucleases**.

2. Gel electrophoresis:

The DNA fragments are separated according to their size by **agarose gel electrophoresis**.

3. Denaturation:

The double-stranded DNA fragments are treated with an alkaline solution to convert them into **single-stranded DNA**.

4. Transfer (blotting):

The separated DNA fragments are transferred from the gel onto a **nylon or nitrocellulose membrane**.

5. Hybridization:

The membrane is treated with a labelled **DNA probe** that is complementary to the sequence of interest. The probe binds specifically to the target DNA.

6. Detection:

The bound probe is detected by **autoradiography** or other suitable detection methods, revealing the position of the target DNA fragment.

Applications

- **Detection of specific DNA sequences** in a genome.

- **Identification of genes** and analysis of their organization.
- **DNA fingerprinting** and genetic identification.
- Detection of certain **genetic mutations or gene rearrangements**.
- **RFLP analysis** (Restriction Fragment Length Polymorphism).
- **Genetic mapping** and analysis of genomic structure.
- Identification of **transgenes** in genetically modified organisms.
- Studying **gene copy number** and related genomic changes.

Simple Flow

DNA → Restriction digestion → Gel electrophoresis → Denaturation → Transfer to membrane → Probe hybridization → Detection

Southern blotting is an important technique for **identifying specific DNA sequences**. Its principle of DNA–DNA hybridization has made it useful in **genetic analysis, molecular diagnostics, gene mapping and genome studies**.

Q. 23. Explain Northern blotting and its applications.

Ans: Northern blotting is a molecular biology technique used to **detect and study specific RNA molecules**, particularly mRNA, in a sample. It helps determine the **presence, size and relative abundance of a particular RNA transcript**.

Principle

Northern blotting is based on **complementary base pairing (nucleic acid hybridization)** between a labelled probe and the specific RNA sequence of interest.

Major Steps

1. **RNA isolation:**
Total RNA is extracted from the cells or tissue.
2. **Gel electrophoresis:**
RNA molecules are separated according to their size using a **denaturing agarose gel**.
3. **Transfer:**
The separated RNA molecules are transferred from the gel onto a **nylon or nitrocellulose membrane**.
4. **Hybridization:**
The membrane is treated with a labelled **DNA or RNA probe** complementary to the target RNA sequence.
5. **Detection:**
The probe bound to the target RNA is detected using **autoradiography or other detection methods**.

Applications

- **Study of gene expression:** Determines whether a particular gene is being transcribed.
- **Measurement of RNA abundance:** Allows comparison of the relative amount of a specific mRNA under different conditions.

- **Determination of transcript size:** Helps identify the approximate length of an RNA transcript.
- **Study of alternative RNA processing:** Can reveal different transcript sizes produced from the same gene.
- **Analysis of developmental regulation:** Used to study changes in gene expression during different stages of development.
- **Study of plant responses:** Useful for investigating gene expression in response to **drought, salinity, temperature and pathogens.**
- **Detection of specific RNA transcripts:** Helps identify particular genes or transcripts in complex RNA samples.

Southern vs Northern Blotting

Southern Blotting	Northern Blotting
Detects DNA	Detects RNA
Studies DNA sequences	Studies RNA transcripts/gene expression
Uses DNA probes	Uses complementary DNA or RNA probes

Northern blotting is a useful technique for studying **RNA transcripts and gene expression**. It provides information about the **presence, size and relative abundance of specific RNA molecules** in a biological sample.

Q. 24. What is DNA fingerprinting? Explain its principle.

Ans: **DNA fingerprinting**, also called **DNA profiling**, is a molecular technique used to identify an individual by analyzing specific regions of their DNA that show variation between individuals. The technique was developed by **Sir Alec Jeffreys in 1984**.

Principle

DNA fingerprinting is based on the presence of **variable DNA sequences**, particularly **short tandem repeats (STRs)** or other polymorphic regions, that differ among individuals.

Although most human DNA is similar, these variable regions produce a **characteristic DNA profile** for an individual. DNA inherited from both parents contributes to this profile.

Basic Principle and Procedure

1. **Collection of DNA:**
DNA is obtained from biological samples such as blood, saliva, hair roots or other cells.
2. **DNA extraction:**
DNA is isolated and purified from the sample.
3. **Analysis of polymorphic regions:**
Specific variable regions, commonly **STRs**, are amplified using **PCR**.

4. **Separation and detection:**

The amplified DNA fragments are separated according to size and detected to produce a DNA profile.

5. **Comparison:**

The DNA profile is compared with that of another sample to determine whether the profiles are consistent.

Applications

- **Forensic identification** and comparison of biological evidence.
- **Paternity and kinship testing.**
- Identification of **missing persons** and human remains.
- **Population and genetic studies.**
- Wildlife and conservation genetics.
- Establishing biological relationships in research and legal investigations.

DNA fingerprinting is based on **DNA sequence variation among individuals**. Analysis of highly variable regions such as STRs produces a characteristic **DNA profile**, which can be compared between samples for identification and relationship studies.

Q. 25. Write a note on molecular DNA markers.

Ans: **Molecular DNA markers** are identifiable variations in DNA sequences that can be used to **distinguish individuals, varieties, species or populations**. They are important tools in genetics, plant breeding and molecular biology because they provide information about genetic variation directly at the DNA level.

Important Types of Molecular DNA Markers

1. **RFLP (Restriction Fragment Length Polymorphism):**
Detects differences in the lengths of DNA fragments produced by restriction enzymes.
2. **RAPD (Random Amplified Polymorphic DNA):**
Uses short random primers in PCR to amplify different regions of DNA and detect polymorphism.
3. **AFLP (Amplified Fragment Length Polymorphism):**
Combines restriction digestion and PCR amplification to detect DNA variation.
4. **SSR or Microsatellites (Simple Sequence Repeats):**
Consist of short, repeated DNA sequences. They are highly polymorphic and widely used for genetic analysis.
5. **SNP (Single Nucleotide Polymorphism):**
Represents a variation at a **single nucleotide position** in the DNA sequence.
6. **ISSR (Inter-Simple Sequence Repeat):**
Uses primers based on microsatellite regions to detect variation between individuals.

Applications of Molecular DNA Markers

- **Plant breeding:** Identification and selection of desirable traits.
- **Genetic diversity studies:** Assessment of variation among plant populations and species.
- **Gene mapping:** Locating genes associated with particular characters.
- **Variety identification:** Identification and characterization of plant varieties.
- **Marker-assisted selection (MAS):** Selection of plants carrying desirable genes.
- **Phylogenetic studies:** Understanding relationships among species.
- **Conservation genetics:** Assessment of genetic diversity in endangered plant populations.
- **DNA fingerprinting:** Identification of individuals or biological samples.

Advantages

- They are generally **not affected by environmental conditions**.
- They can detect variation at the **DNA level**.
- Many markers can provide high levels of **polymorphism**.
- They are useful for studying genetic relationships even when visible traits are similar.

Molecular DNA markers are valuable tools for detecting **genetic variation and relationships**. They have wide applications in **plant breeding, taxonomy, genetic mapping, biodiversity studies and conservation biology**.

Q. 26. Explain the principle and applications of RFLP.

Ans: **RFLP (Restriction Fragment Length Polymorphism)** is a molecular DNA marker technique used to detect **variations in DNA sequences** by comparing the lengths of DNA fragments produced after digestion with restriction enzymes.

Principle of RFLP

The principle of RFLP is based on the fact that differences in DNA sequences can **create or abolish restriction enzyme recognition sites**, or alter the distance between such sites. As a result, DNA from different individuals or varieties may produce restriction fragments of different lengths.

Major Steps

1. **DNA isolation:**
DNA is extracted from the biological sample.
2. **Restriction digestion:**
DNA is cut into fragments using specific **restriction endonucleases**.
3. **Gel electrophoresis:**
The resulting DNA fragments are separated according to their size.
4. **Southern blotting:**
The separated fragments are transferred from the gel to a membrane.
5. **Probe hybridization:**
A labelled DNA probe complementary to the region of interest is used to identify specific fragments.

6. **Detection:**

The hybridized fragments are detected, producing a characteristic **RFLP pattern**.

Applications of RFLP

- **Genetic mapping:** Used to locate genes and determine their positions on chromosomes.
- **DNA fingerprinting:** Used for identification of individuals and biological samples.
- **Paternity and relationship analysis:** Helps determine genetic relationships.
- **Plant breeding:** Used to identify and track desirable genes or traits.
- **Variety identification:** Helps distinguish different plant varieties or strains.
- **Disease and mutation analysis:** Can detect DNA variations associated with certain genetic conditions.
- **Population genetics:** Used to study genetic diversity and relationships among populations.
- **Evolutionary studies:** Helps investigate genetic relationships among species.

Advantages

- RFLP markers are generally **highly reproducible**.
- They can detect DNA variation without requiring knowledge of the complete gene sequence.
- They are useful for **genetic linkage and mapping studies**.

RFLP is based on differences in the **length of restriction fragments resulting from DNA sequence variation**. It has been widely used in **genetic mapping, DNA fingerprinting, plant breeding, population studies and molecular genetics**.

Q. 27. Explain the importance of molecular markers in plant science.

Ans: **Molecular markers** are specific and detectable variations in DNA that are used to identify and study **genetic differences among plants**. Examples include **RFLP, RAPD, AFLP, SSR and SNP markers**.

Importance of Molecular Markers in Plant Science

1. **Assessment of genetic diversity**
Molecular markers help determine the **genetic variation** among plant varieties, populations and species.
2. **Plant breeding**
They help breeders identify plants carrying **desirable genes or traits**, such as disease resistance, improved yield or stress tolerance.
3. **Marker-assisted selection (MAS)**
Molecular markers can be linked to useful traits and allow breeders to select desired plants at an **early stage**, even before the trait becomes visible.

4. **Gene mapping**

Markers are used to locate genes and determine their positions on **chromosomes**. They help construct genetic linkage maps.

5. **Variety identification**

Molecular markers can distinguish between closely related **cultivars, varieties and strains**, helping in their identification and characterization.

6. **Phylogenetic and evolutionary studies**

They provide molecular evidence for determining **relationships among plant species** and studying their evolutionary history.

7. **Conservation of plant genetic resources**

Molecular markers help assess genetic diversity within populations and assist in the **conservation of rare and endangered plant species**.

8. **Detection of genetic purity**

They can be used to check the **genetic purity of seeds and breeding materials**.

9. **Study of important traits**

Markers help identify genomic regions associated with traits such as **drought tolerance, salinity tolerance, disease resistance and quality characteristics**.

Molecular markers provide a **reliable method for studying genetic variation at the DNA level**. They have become important tools in **plant breeding, genetic mapping, taxonomy, biodiversity assessment and conservation**, thereby contributing significantly to modern plant science.

Q. 28. Define PCR and explain its principle.

Ans: PCR (Polymerase Chain Reaction) is an **in vitro molecular technique used to amplify a specific DNA sequence**, producing millions of copies of the target DNA from a small starting amount.

The technique was developed by **Kary Mullis** in the 1980s.

Principle of PCR

PCR is based on the **repeated cycles of DNA denaturation, primer annealing and DNA synthesis**. A thermostable DNA polymerase, commonly **Taq DNA polymerase**, synthesizes new DNA strands using primers and nucleotides.

Major Steps of PCR

1. **Denaturation**

- The reaction mixture is heated to about **94–98°C**.
- Double-stranded DNA separates into two single strands by breaking hydrogen bonds.

2. **Annealing**

- The temperature is lowered, usually to about **50–65°C**.

- Short DNA primers bind to their **complementary sequences** on the template DNA.

3. Extension

- The temperature is usually raised to about **72°C**, the optimum temperature for Taq polymerase.
- DNA polymerase adds nucleotides to the primers and synthesizes new DNA strands in the **5' → 3' direction**.

These three steps are repeated for **25–40 cycles**, resulting in exponential amplification of the target DNA.

Requirements of PCR

- Template DNA
- Two specific **primers**
- **Taq DNA polymerase**
- Four deoxyribonucleotide triphosphates (**dNTPs**)
- Suitable buffer and **Mg²⁺ ions**
- Thermal cycler

Applications

- **DNA amplification** for molecular studies
- Detection of **pathogens**
- Genetic and mutation analysis
- **DNA fingerprinting and forensic analysis**
- Detection of genetically modified organisms
- **DNA sequencing** and cloning
- Molecular identification of plants and microorganisms

Simple Flow

Denaturation → Annealing → Extension → Repetition → Millions of DNA copies

PCR is a rapid and highly sensitive technique for **amplifying specific DNA sequences**. Its ability to produce large amounts of DNA from a very small sample makes it an important tool in **molecular biology, genetics, biotechnology and plant science**.

Q. 29. Explain the role of different components used in PCR.

Ans: PCR (Polymerase Chain Reaction) is an in-vitro technique used to amplify a specific DNA sequence. A PCR reaction requires several essential components, each performing a specific function.

Component	Role in PCR
------------------	--------------------

- | | |
|------------------------|--|
| 1. Template DNA | Contains the target DNA sequence that is to be amplified. |
|------------------------|--|

Component	Role in PCR
2. Primers	Short, single-stranded DNA sequences that bind to complementary regions on either side of the target DNA. They provide the starting point for DNA synthesis .
3. Taq Polymerase	A thermostable enzyme that synthesizes new DNA strands by adding nucleotides to the primers. It remains active after repeated heating cycles.
4. dNTPs	The four DNA building blocks— dATP, dTTP, dGTP and dCTP —used to synthesize new DNA strands.
5. Buffer	Provides the appropriate pH and ionic conditions required for DNA polymerase activity.
6. Mg ²⁺ ions	Usually supplied as MgCl ₂ ; Mg ²⁺ acts as an essential cofactor for DNA polymerase and is required for efficient DNA synthesis.
7. Water	Provides the solvent and brings all components to the required reaction volume.

How the Components Work Together

1. **Template DNA** provides the sequence to be copied.
2. **Primers** identify the boundaries of the target sequence.
3. During extension, **Taq polymerase** uses **dNTPs** to synthesize new DNA.
4. **Mg²⁺ and buffer** provide the conditions necessary for efficient enzyme activity.
5. Repeated cycles of **denaturation** → **annealing** → **extension** result in exponential amplification of the target DNA.

The success of PCR depends on the coordinated action of **template DNA, primers, thermostable DNA polymerase, dNTPs, Mg²⁺ ions and buffer**. Together, these components allow specific DNA sequences to be amplified rapidly and efficiently.

Q. 30. What is gene editing? Explain its significance.

Ans: Gene editing is a molecular biotechnology technique used to **make precise changes in the DNA sequence of an organism**. It can involve inserting, deleting, replacing or modifying specific DNA sequences. Modern gene-editing systems include **CRISPR-Cas9**, TALENs and zinc-finger nucleases.

Principle

Gene editing generally involves:

1. **Targeting** a specific DNA sequence.
2. Making a **targeted change or break** in the DNA.
3. Allowing the cell's DNA-repair mechanisms to produce the desired genetic modification.

For example, in **CRISPR-Cas9**, a guide RNA directs the Cas9 enzyme to a complementary DNA sequence, where Cas9 makes a targeted DNA cut. The cell then repairs the DNA, which can result in a specific genetic change.

Significance of Gene Editing

1. **Crop improvement**
Gene editing can be used to develop plants with desirable characteristics such as **disease resistance, improved nutritional quality and tolerance to drought or salinity**.
2. **Functional genomics**
It helps researchers determine the **function of specific genes** by selectively modifying or disrupting them.
3. **Disease research**
Gene editing can be used to study genes involved in diseases and develop experimental models for understanding disease mechanisms.
4. **Development of improved varieties**
Specific genetic changes can potentially improve **yield, quality and stress tolerance** in crop plants.
5. **Basic biological research**
It provides a powerful method for studying **gene regulation, development and cellular processes**.
6. **Biotechnology**
Gene editing can be used to modify microorganisms, plants and animals for research and industrial applications.
7. **Conservation biology**
In research settings, gene-editing approaches may help investigate genetic factors important for **adaptation and conservation**.

Gene editing is an important modern biotechnology that allows **targeted modification of DNA**. In plant science, it has significant potential for **crop improvement, functional genomics, stress tolerance and disease resistance**, making it an important tool in modern genetic research.

Q. 31. Define recombinant DNA technology and explain its significance.

Ans: Recombinant DNA (rDNA) technology is a set of techniques used to **combine DNA molecules from different sources** and introduce the resulting recombinant DNA into a suitable host cell for replication or expression.

It is also called **genetic engineering** and is an important branch of modern biotechnology.

Basic Steps

1. **Isolation of DNA:** The desired gene and suitable vector DNA are isolated.
2. **Cutting of DNA:** Restriction enzymes cut the DNA at specific recognition sites.
3. **Joining of DNA:** The desired DNA fragment is joined to a vector using **DNA ligase**, forming recombinant DNA.
4. **Introduction into host:** The recombinant DNA is introduced into a suitable host cell.
5. **Selection and screening:** Cells containing the desired recombinant DNA are identified.
6. **Expression:** If appropriate, the inserted gene can be expressed to produce the desired protein or trait.

Significance of Recombinant DNA Technology

1. **Production of useful proteins:**

It is used to produce important proteins such as **human insulin and growth hormone**.

2. **Agricultural improvement:**

It helps develop genetically modified crops with desirable characteristics such as **pest resistance, improved nutritional quality and stress tolerance**.

3. **Gene cloning:**

Specific genes can be **isolated, copied and studied** in large quantities.

4. **Production of vaccines:**

Recombinant DNA methods are used in the development and production of several **recombinant vaccines**.

5. **Medical applications:**

It helps in studying genes associated with diseases and developing **diagnostic and therapeutic approaches**.

6. **Research in molecular biology:**

It allows scientists to study **gene structure, function and regulation**.

7. **Industrial biotechnology:**

Genetically modified microorganisms can be used to produce **enzymes, pharmaceuticals and other useful biological products**.

Recombinant DNA technology enables scientists to **isolate, modify, combine and express specific DNA sequences**. It has wide applications in **agriculture, medicine, research and biotechnology**, making it an important tool of modern biological science.

Q. 32. Write a note on the cDNA library.

Ans: A **cDNA library (complementary DNA library)** is a collection of cloned DNA fragments that represent the **mRNA molecules expressed in a particular cell, tissue, or organism at a specific time or under specific conditions**. Since cDNA is synthesized from mRNA, a cDNA library mainly contains **expressed genes**.

Construction of a cDNA Library

1. **Isolation of mRNA:**

mRNA is isolated from the selected cells or tissue. Mature mRNA is generally rich in a **poly-A tail**.

2. **Synthesis of first-strand cDNA:**

The enzyme **reverse transcriptase** uses mRNA as a template to synthesize a complementary DNA strand.

3. **Removal of RNA:**

The RNA template is removed or degraded, commonly using RNase activity.

4. **Synthesis of second DNA strand:**

A second DNA strand is synthesized to produce **double-stranded cDNA**.

5. **Insertion into a vector:**

The cDNA fragments are inserted into suitable cloning vectors such as **plasmids or bacteriophages** using DNA ligase.

6. **Transformation and storage:**

Recombinant vectors are introduced into suitable host cells, where they are multiplied and stored as a library.

Important Features

- Contains genes that are **actively expressed** in the source tissue.
- Generally **lacks introns**, because it is produced from processed mRNA.
- Does not usually contain promoter or other genomic regulatory sequences.
- The composition of the library depends on the **tissue, developmental stage, and environmental conditions** from which mRNA was obtained.

Applications

- Identification and isolation of **expressed genes**.
- Study of **gene expression**.
- Production of recombinant proteins.
- Cloning of eukaryotic genes for expression in bacteria.
- Study of tissue-specific and developmental genes.
- Functional and molecular studies of genes.

cDNA Library vs Genomic Library

cDNA Library	Genomic Library
Made from mRNA	Made from genomic DNA
Represents expressed genes	Represents the entire genome
Usually lacks introns	Contains introns and other non-coding regions
Depends on tissue and developmental stage	Generally represents the whole genome
Useful for studying gene expression	Useful for studying genome organization

A **cDNA library is a collection of DNA copies of expressed mRNAs**. It is an important molecular biology tool for identifying, cloning, and studying expressed genes, particularly genes that encode proteins.

Q. 33. Explain electroporation as a method of direct gene transfer.

Ans: Electroporation is a **direct gene transfer method** in which short, high-voltage electrical pulses are applied to cells or protoplasts to create **temporary pores in the plasma membrane**. Foreign DNA can enter through these pores and may subsequently become expressed or integrated into the genome.

Principle

The electrical pulse temporarily increases the permeability of the cell membrane. The temporary pores allow **DNA molecules** present in the surrounding medium to enter the cell. After the electrical pulse, the membrane repairs itself and returns to its normal state.

Steps of Electroporation

1. **Preparation of cells/protoplasts:**

Healthy plant cells or protoplasts are prepared and suspended in an appropriate solution.

2. **Addition of foreign DNA:**

The desired DNA or recombinant DNA is mixed with the cells.

3. **Application of electric pulse:**

A short, high-voltage electrical pulse is applied using an electroporator.

4. **Formation of temporary pores:**

The electric field causes temporary pores to form in the plasma membrane.

5. **Entry of DNA:**

The foreign DNA enters the cell through these temporary pores.

6. **Membrane recovery:**

After the pulse, the membrane reseals and the cells are allowed to recover.

7. **Selection and regeneration:**

Transformed cells are selected using suitable markers and, in plants, may be regenerated into complete plants under appropriate culture conditions.

Advantages

- Simple and relatively rapid method.
- Does not require a biological vector.
- Can transfer DNA directly into **plant protoplasts and cells**.
- Can be used for transient as well as stable transformation.
- Useful for introducing DNA, RNA, and other molecules into cells.

Limitations

- High electrical voltage can cause **cell damage or death**.
- Transformation efficiency depends on cell type and electrical conditions.
- Plant regeneration from transformed protoplasts may be difficult in some species.
- Requires specialized equipment.

Applications

- Genetic transformation of plant protoplasts.
- Introduction of genes for **plant improvement**.
- Transient gene expression studies.
- Functional analysis of genes.
- Production of genetically modified plant cells.

Electroporation is an important direct gene-transfer technique in which an electrical pulse temporarily permeabilizes the cell membrane, allowing foreign DNA to enter the cell. It is

particularly useful in plant biotechnology for the transformation of **protoplasts and cultured cells**.

Q. 34. Explain microinjection as a method of gene transfer.

Ans: Microinjection is a **direct gene transfer method** in which foreign DNA is introduced directly into a cell using a very fine glass needle or micropipette under a microscope.

Principle

The technique is based on the **direct physical delivery of DNA into the cytoplasm or nucleus** of a target cell. Since the DNA bypasses the cell membrane, no biological vector is required.

Steps of Microinjection

1. **Preparation of DNA:**
The desired gene or recombinant DNA is purified and prepared in a suitable solution.
2. **Selection of target cells:**
Healthy cells, protoplasts, embryos, or other suitable cells are selected.
3. **Preparation of micropipette:**
A very fine glass micropipette is used to hold and inject the DNA.
4. **Cell positioning:**
The target cell is placed under a microscope and held in position using a holding pipette.
5. **DNA injection:**
The micropipette is carefully inserted into the cell or nucleus, and a small amount of DNA is injected.
6. **Recovery of cells:**
The micropipette is withdrawn carefully, and the cell is allowed to recover.
7. **Selection and regeneration:**
Transformed cells are identified using suitable markers. In plants, selected cells may be regenerated into complete plants through **tissue culture**.

Advantages

- DNA is delivered **directly into the target cell or nucleus**.
- No vector is required.
- Precise control over the amount of DNA introduced.
- Can be used for **transient or stable gene expression**.
- Useful for studying gene function and cellular processes.

Limitations

- It is a **laborious and technically demanding** technique.
- Requires specialized equipment and skilled operators.
- Only a limited number of cells can be treated at a time.
- Injection may damage or kill some cells.
- Regeneration of whole plants from transformed cells may be difficult in some species.

Applications

- Introduction of foreign genes into plant cells and protoplasts.
- Gene expression studies.
- Functional analysis of genes.
- Genetic transformation of embryos and other cells.
- Production and study of genetically modified organisms.

microinjection is a **direct and precise method of gene transfer** in which foreign DNA is physically injected into the cytoplasm or nucleus of a cell. It is particularly useful when controlled delivery of DNA into individual cells is required.

Q. 35. Write a short note on transgenic plants.

Ans: Transgenic plants are plants that contain and express a **foreign gene (transgene)** introduced into their genome using genetic engineering techniques. The introduced gene may come from another species or may be a modified gene designed to provide a desired trait.

Production of Transgenic Plants

The general steps involved are:

1. **Selection of desired gene:** A gene responsible for the required trait is identified and isolated.
2. **Gene cloning:** The desired gene is inserted into a suitable vector along with necessary regulatory sequences.
3. **Gene transfer:** The recombinant DNA is introduced into plant cells using methods such as **Agrobacterium-mediated transformation, gene gun, electroporation, or microinjection.**
4. **Selection of transformed cells:** Cells carrying the transgene are selected using suitable selectable markers.
5. **Regeneration:** Selected cells are cultured through tissue culture to produce complete plants.
6. **Confirmation:** Molecular techniques are used to confirm the presence and expression of the introduced gene.

Examples

- **Bt cotton:** Contains *cry* genes from *Bacillus thuringiensis* and provides resistance against certain insect pests.
- **Golden Rice:** Developed to produce **β-carotene**, a precursor of vitamin A, in the grain.
- **Herbicide-tolerant crops:** Carry genes that provide tolerance to particular herbicides.

Applications

- Development of **insect- and disease-resistant crops.**
- Improvement of nutritional quality.

- Development of herbicide-tolerant crops.
- Improvement of tolerance to environmental stresses such as drought and salinity.
- Production of useful **pharmaceutical and industrial products**.
- Research on gene function and plant molecular biology.

Advantages

- Can introduce specific desirable traits.
 - Helps improve crop productivity and quality.
 - Provides useful traits that may be difficult to obtain through conventional breeding.
-

Q. 36. Explain the importance of Bt cotton as a transgenic crop.

Ans: Importance of Bt Cotton as a Transgenic Crop

Bt cotton is a genetically modified (transgenic) cotton crop that contains a gene from the bacterium *Bacillus thuringiensis* (Bt). The introduced **Bt gene** produces a protein that is toxic to certain insect pests, particularly **bollworms**, which are major pests of cotton.

Importance of Bt Cotton

1. **Insect resistance**
Bt cotton provides effective protection against major cotton pests such as American bollworm, tobacco bollworm, and pink bollworm.
2. **Reduced use of chemical insecticides**
Since the plant itself produces an insecticidal Bt protein, farmers may need fewer insecticide applications for susceptible pests. This can reduce chemical exposure and production costs.
3. **Improved crop yield**
Better protection from insect damage helps plants retain more squares and bolls, which can contribute to higher and more stable cotton yields under suitable conditions.
4. **Better quality of cotton**
Reduced boll damage results in healthier and better-developed cotton bolls, improving the quality of the harvested fibre.
5. **Economic benefits to farmers**
Lower expenditure on insecticides and reduced crop losses can improve the economic returns from cotton cultivation.
6. **Environment-friendly approach**
Reduced insecticide use can decrease contamination of soil and water and can reduce the harmful effects of broad-spectrum pesticides on non-target organisms.
7. **Important example of genetic engineering**
Bt cotton demonstrates how **genetic engineering can introduce a useful trait—pest resistance—into a crop** without relying solely on conventional breeding.

Limitations

Bt cotton is not resistant to all insects, and pests can develop **resistance to Bt toxins** if the technology is not properly managed. Therefore, refuge strategies, appropriate pest management, and monitoring are important.

Bt cotton is an important transgenic crop because it provides genetic resistance to major insect pests, can reduce dependence on chemical insecticides, protect yield, and provide economic benefits to farmers. It is a major example of the practical application of biotechnology in crop improvement.

Q. 37. Explain Flavr Savr tomato.

Ans: Flavr Savr Tomato

Flavr Savr tomato was one of the first genetically modified (GM) crops developed for commercial use. It was produced by **Calgene** using recombinant DNA technology. The main aim was to **delay fruit softening and prolong shelf life**.

Principle

Fruit softening occurs mainly due to the action of the enzyme **polygalacturonase (PG)**, which breaks down pectin in the cell wall. In Flavr Savr tomato, the expression of the **polygalacturonase gene was reduced using antisense RNA technology**.

Production

- A modified copy of the **polygalacturonase (PG) gene** was introduced into tomato cells.
- The introduced antisense gene produced RNA complementary to the normal PG mRNA.
- The antisense RNA paired with the PG mRNA and **reduced its translation**.
- As a result, less PG enzyme was produced.
- Pectin degradation was slowed, so the tomato **softened more slowly**.

Importance

1. **Delayed ripening and softening** of fruits.
2. **Extended shelf life** after harvesting.
3. Allowed tomatoes to remain firm during **transport and storage**.
4. Reduced losses caused by premature softening.
5. It demonstrated the practical application of **antisense technology in crop improvement**.

Flavr Savr tomato is an important example of a **transgenic crop developed through genetic engineering**. By suppressing the polygalacturonase gene, fruit softening was delayed, resulting in a tomato with improved storage and transport characteristics.

Q. 38. Differentiate between direct and indirect gene transfer methods.

Ans: Difference between Direct and Indirect Gene Transfer Methods

Gene transfer is the process of introducing a desired gene into the cells of an organism. In plants, gene transfer methods are broadly classified into **direct** and **indirect methods**.

Basis	Direct Gene Transfer	Indirect Gene Transfer
Definition	DNA is introduced directly into plant cells without using a biological vector.	DNA is introduced into plant cells with the help of a biological vector.
Vector	Generally, no biological vector is required.	A biological vector, mainly <i>Agrobacterium tumefaciens</i> , is commonly used.
Methods	Particle bombardment (gene gun), electroporation, microinjection, PEG-mediated transfer, etc.	<i>Agrobacterium</i> -mediated transformation.
Cell requirement	DNA can be delivered directly into protoplasts or intact cells, depending on the method.	Requires suitable interaction between the plant cells and <i>Agrobacterium</i> .
DNA integration	Integration may occur randomly in the plant genome.	T-DNA from the Ti plasmid is transferred and can integrate into the plant genome.
Efficiency	Efficiency varies with the technique and plant species.	Often highly effective in many plant species, although it depends on genotype.
Applications	Useful for plants that are difficult to transform using <i>Agrobacterium</i> and for chloroplast transformation in some systems.	Widely used for production of transgenic plants.

Examples

Direct methods:

- Electroporation
- Microinjection
- Gene gun/particle bombardment
- PEG-mediated gene transfer

Indirect method:

- *Agrobacterium tumefaciens*-mediated gene transfer

Direct gene transfer introduces DNA into plant cells by physical or chemical techniques, whereas **indirect gene transfer** uses a biological vector such as *Agrobacterium*. Both methods are important tools in **plant genetic engineering and the production of transgenic plants**.

Q. 39. Explain the concept of totipotency.

Ans: Concept of Totipotency

Totipotency is the ability of a living plant cell to **develop into a complete, mature plant** under suitable conditions. The concept was proposed by **Gottlieb Haberlandt in 1902**.

Main Features

1. **Cellular potential:** A plant cell contains the complete genetic information required for the development of the whole plant.
2. **Differentiation:** Under suitable conditions, differentiated cells can lose their specialized state and form actively dividing cells.
3. **Cell division:** These cells undergo repeated mitotic divisions and form a mass of cells called a **callus**.
4. **Organ formation:** With appropriate growth regulators, the callus can develop **shoots and roots** through organogenesis.
5. **Complete plant formation:** The regenerated organs can develop into a complete plant through **plant tissue culture**.

Importance

- It forms the **basic principle of plant tissue culture**.
- It is useful for **micropropagation and rapid multiplication** of plants.
- It helps in the production of **disease-free plants**.
- It is important in **genetic transformation and production of transgenic plants**.
- It is also used for **conservation of rare and valuable plant species**.

Thus, **totipotency is the inherent capacity of a plant cell to regenerate into a complete plant under suitable cultural conditions**, making it a fundamental principle of plant biotechnology and tissue culture.

Q. 40. Describe the major steps involved in plant tissue culture.

Ans: Major Steps Involved in Plant Tissue Culture

Plant tissue culture is the technique of growing plant cells, tissues, or organs under **sterile and controlled conditions** on a suitable nutrient medium.

The major steps are:

1. **Selection of Explant**
A healthy plant part such as a **leaf, stem, root, bud, or meristem** is selected as the explant.
2. **Surface Sterilization**
The explant is treated with suitable sterilizing agents to remove microorganisms and then washed with sterile water.

3. **Preparation of Culture Medium**

A suitable nutrient medium containing **mineral salts, vitamins, sugars, plant growth regulators, and water** is prepared and sterilized.

4. **Inoculation**

The sterilized explant is placed on the culture medium under **aseptic conditions**.

5. **Callus Formation and Growth**

Under suitable conditions, the explant may produce an undifferentiated mass of cells called **callus**. The callus is maintained on an appropriate medium for growth.

6. **Organogenesis or Embryogenesis**

By adjusting plant growth regulators, the cultured cells develop into **shoots and roots** or form somatic embryos.

7. **Plantlet Development**

The regenerated shoots and roots develop into complete **plantlets**.

8. **Hardening and Acclimatization**

The plantlets are gradually adapted to external environmental conditions and then transferred to **soil or field conditions**.

Flow Chart

Selection of explant → Surface sterilization → Preparation of medium → Inoculation → Callus formation → Shoot and root development → Plantlet formation → Hardening → Transfer to soil

Plant tissue culture involves the **in-vitro culture and regeneration of plant cells or tissues under sterile conditions**. It is widely used for **rapid multiplication, production of disease-free plants, conservation, and plant improvement**.

Q. 41. What is callus culture? Explain its importance.

Ans: Callus Culture and Its Importance

Callus culture is a plant tissue culture technique in which an **unorganized, undifferentiated mass of actively dividing cells called callus** is grown under sterile, controlled conditions on a suitable nutrient medium.

Formation of Callus

1. A suitable **explant** such as leaf, stem, root, or meristem is selected.
2. The explant is **surface sterilized** to remove microorganisms.
3. It is placed on a suitable nutrient medium containing **auxins and cytokinins**.
4. The cells of the explant undergo repeated cell division and form an **undifferentiated callus**.
5. The callus can subsequently be transferred to a suitable medium for **shoot and root formation**, resulting in a complete plant.

Importance of Callus Culture

1. **Plant regeneration:** Callus can be used to regenerate complete plants through organogenesis or somatic embryogenesis.
2. **Micropropagation:** It helps in the rapid multiplication of selected plants.
3. **Genetic improvement:** Callus cultures are useful for **genetic transformation and selection of useful variants**.
4. **Production of secondary metabolites:** They can be used for the production of valuable compounds such as **alkaloids, pigments, and medicinal substances**.
5. **Disease-free plants:** Callus culture, particularly when combined with suitable meristem culture techniques, can contribute to the production of healthy planting material.
6. **Germplasm conservation:** Callus and related in-vitro cultures can be used for the **conservation of valuable plant material**.

Thus, callus culture is an important technique of plant tissue culture in which **undifferentiated cells are grown in vitro**. It has applications in **plant regeneration, crop improvement, genetic transformation, secondary metabolite production, and conservation**.

Q. 42. Explain the process of micropropagation.

Ans: Process of Micropropagation

Micropropagation is the technique of **rapid multiplication of plants through in-vitro culture of small plant parts** such as shoot tips, axillary buds, or meristems under sterile and controlled conditions. It produces genetically similar plants in large numbers.

Major Steps of Micropropagation

1. **Selection of Explant**
A healthy and disease-free plant is selected, and a suitable explant such as a **shoot tip, axillary bud, or meristem** is taken.
2. **Surface Sterilization**
The explant is carefully sterilized to remove microorganisms and prevent contamination.
3. **Establishment of Culture**
The sterilized explant is placed on a suitable **nutrient medium** containing mineral salts, vitamins, sugar, and plant growth regulators.
4. **Multiplication**
The cultured explant produces multiple shoots through **axillary bud proliferation or adventitious shoot formation**. The newly formed shoots are repeatedly subcultured to increase their number.
5. **Root Formation**
The shoots are transferred to a rooting medium, usually containing an appropriate concentration of **auxins**, where roots develop.

6. **Hardening and Acclimatization**

The rooted plantlets are gradually adapted to external environmental conditions by maintaining them under controlled humidity and light.

7. **Transfer to Soil**

After successful hardening, the plantlets are transferred to **pots or field conditions** for further growth.

Flow Chart

Selection of explant → Sterilization → Culture establishment → Shoot multiplication → Rooting → Hardening → Transfer to soil

Importance

- Rapid production of a large number of plants.
- Production of **uniform and healthy planting material**.
- Useful for propagation of plants that are difficult to multiply by conventional methods.
- Helps in the **conservation of rare and valuable plants**.
- Useful for commercial production of crops and ornamental plants.

Micropropagation is an important application of **plant tissue culture** that enables the rapid and large-scale production of plants under controlled conditions.

Q. 43. Explain the advantages and applications of micropropagation.

Ans: Advantages and Applications of Micropropagation

Micropropagation is the rapid multiplication of plants through the **in-vitro culture of small plant parts**, such as shoot tips, axillary buds, or meristems, under sterile and controlled conditions.

Advantages of Micropropagation

1. **Rapid multiplication:** A large number of plants can be produced from a small amount of starting material within a short period.
2. **Uniform plants:** Plants produced from a selected parent are generally genetically similar and maintain desirable characteristics.
3. **Disease-free planting material:** Meristem-based micropropagation can be used to produce healthy and virus-free planting material.
4. **Year-round production:** Plant multiplication can be carried out throughout the year under controlled laboratory conditions.
5. **Propagation of difficult plants:** It is useful for plants that are difficult to propagate through seeds or conventional vegetative methods.
6. **Efficient use of space:** Large numbers of plants can be produced in relatively small laboratory areas.

Applications of Micropropagation

1. **Commercial plant production:** Widely used for large-scale production of ornamental, horticultural, and agricultural plants.
2. **Production of disease-free plants:** Used for producing healthy planting material of crops such as **potato, banana, and strawberry**.
3. **Conservation of rare plants:** Helps in the multiplication and conservation of **rare, endangered, and valuable plant species**.
4. **Propagation of elite varieties:** Selected plants with desirable characteristics can be multiplied rapidly.
5. **Germplasm conservation:** In-vitro techniques can help maintain valuable genetic resources.
6. **Plant improvement:** Tissue culture provides material for **genetic transformation, mutation selection, and other crop improvement programmes**.

Micropropagation is an important application of **plant tissue culture** because it enables the **rapid, large-scale and controlled multiplication of plants**. It has major applications in agriculture, horticulture, forestry, conservation, and plant biotechnology.

Q. 44. Define somatic hybridization and explain its importance.

Ans: Somatic Hybridization and Its Importance

Somatic hybridization is a technique in which **two different plant protoplasts are fused to form a hybrid cell**, which can subsequently regenerate into a complete hybrid plant. It is also called **parasexual hybridization**.

Process of Somatic Hybridization

1. **Isolation of protoplasts:** Protoplasts are isolated from plant cells by removing their cell walls using enzymes such as **cellulase and pectinase**.
2. **Fusion of protoplasts:** Protoplasts from two different plants are fused using chemical agents such as **PEG (polyethylene glycol)** or by electrofusion.
3. **Selection of hybrid cells:** Hybrid cells are identified and selected from the mixture of fused and unfused protoplasts.
4. **Culture and regeneration:** The selected hybrid cells are cultured on a suitable nutrient medium. They form a cell mass and subsequently regenerate into **shoots, roots, and complete plants**.
5. **Hybrid plant formation:** The regenerated plant contains genetic material from both parental plants and is called a **somatic hybrid**.

Importance of Somatic Hybridization

1. **Overcomes sexual incompatibility:** It allows hybridization between plants that cannot be crossed by conventional sexual methods.
2. **Combines desirable traits:** Useful characteristics from two different species or genera can be combined in a single plant.

3. **Production of novel hybrids:** It can produce hybrids with new combinations of genetic characters.
4. **Transfer of cytoplasmic traits:** Cytoplasmic genes and organelles such as **chloroplasts and mitochondria** can be combined or transferred.
5. **Crop improvement:** It can be used to introduce traits such as **disease resistance, stress tolerance, and improved quality**.
6. **Useful in distant hybridization:** It provides a method for combining genetic material from distantly related plants.

Somatic hybridization is an important **plant biotechnology technique** that involves the fusion of protoplasts from different plants. It helps overcome barriers of sexual reproduction and is useful for **crop improvement and the development of novel plant hybrids**.

Q. 45. What are synthetic seeds? Explain their preparation and applications.

Ans: Synthetic Seeds

Synthetic seeds are artificially prepared structures in which **somatic embryos or other viable plant propagules** are encapsulated in a protective coating or gel, usually **sodium alginate**. They can be handled and sown like natural seeds and are capable of developing into complete plants under suitable conditions.

Preparation of Synthetic Seeds

The main steps are:

1. **Production of somatic embryos:** Somatic embryos are produced from suitable plant cells or tissues through tissue culture.
2. **Selection of embryos:** Healthy and well-developed embryos, preferably at the appropriate developmental stage, are selected.
3. **Encapsulation:** The embryos are mixed with a suitable **sodium alginate solution**.
4. **Complex formation:** The alginate-coated embryos are dropped into a solution of **calcium chloride (CaCl_2)**. Calcium ions react with alginate and form a firm gel coating around the embryo.
5. **Maturation and storage:** The encapsulated embryos are allowed to develop and can be stored or directly transferred to a suitable culture medium or growing conditions.
6. **Plant regeneration:** Under suitable conditions, the encapsulated embryo germinates and develops into a **complete plantlet**.

Applications of Synthetic Seeds

1. **Rapid propagation:** They can be used for large-scale multiplication of desirable plants.
2. **Easy handling and transport:** Encapsulated propagules are easier to handle, transport, and sow than conventional tissue cultures.

3. **Conservation:** They can help in the conservation and exchange of valuable or rare plant germplasm.
4. **Propagation of seedless plants:** They provide a method for multiplying plants that do not produce viable seeds.
5. **Uniform plant production:** Plants derived from selected somatic embryos can maintain desirable characteristics.
6. **Germplasm storage:** Synthetic seeds may be useful for short- or medium-term storage of selected plant material.

Synthetic seeds are **encapsulated plant propagules, mainly somatic embryos**, prepared through plant tissue culture. They have important applications in **mass propagation, conservation, storage, transport, and distribution of valuable plant material**.

Q. 46. Explain the role of computers in biological research.

Ans: Role of Computers in Biological Research

Computers play an important role in modern biological research by helping researchers **store, analyse, interpret, and communicate large amounts of biological data**. The use of computational methods in biology is known as **bioinformatics** when applied to biological information.

Major Roles of Computers

1. **Data storage and management**
Computers are used to store large amounts of biological information such as **DNA sequences, protein sequences, gene expression data, and experimental results**.
2. **Sequence analysis**
Computer programs help in comparing **DNA, RNA, and protein sequences**, identifying similarities, mutations, and conserved regions.
3. **Bioinformatics databases**
Researchers can access databases containing genomic and protein information for analysis and research purposes.
4. **Statistical analysis**
Computers help in performing statistical calculations and analysing experimental data quickly and accurately.
5. **Molecular modelling**
Computer-based tools are used to study the **three-dimensional structures of proteins, DNA, and other biomolecules**.
6. **Genome analysis**
Computers assist in **genome sequencing, gene identification, genome annotation, and comparative genomics**.

7. **Data visualization**

Complex biological data can be represented through **graphs, charts, molecular structures, and other visual forms**, making interpretation easier.

8. **Research communication**

Computers facilitate preparation of research papers, presentations, databases, and sharing of scientific information.

Applications in Biology

Computers are widely used in **genomics, proteomics, molecular biology, biotechnology, ecology, plant breeding, drug discovery, and bioinformatics**.

Thus, computers have become an essential tool in biological research. They enable **rapid data processing, sequence analysis, modelling, statistical analysis, and interpretation of complex biological information**, thereby improving the efficiency of modern biological research.

Q. 47. Write a note on the use of PowerPoint in scientific presentations.

Ans: Use of PowerPoint in Scientific Presentations

Microsoft PowerPoint is a presentation software widely used by scientists, teachers, and students to present scientific information in a clear and organized manner. It allows text, images, graphs, tables, diagrams, and videos to be combined in a single presentation.

Uses of PowerPoint in Scientific Presentations

1. **Organized presentation of information**

Scientific content can be arranged systematically into slides, such as **introduction, objectives, methodology, results, discussion, and conclusion**.

2. **Presentation of data**

Experimental results can be displayed using **tables, graphs, charts, and diagrams**, making data easier to understand.

3. **Use of images and illustrations**

Microscopic images, photographs, molecular structures, flowcharts, and biological diagrams can be included to explain concepts effectively.

4. **Explanation of complex concepts**

Animations and diagrams can help explain processes such as **DNA replication, photosynthesis, cell division, and genetic engineering**.

5. **Research communication**

PowerPoint is commonly used for **seminars, conferences, classroom teaching, project presentations, and research meetings**.

6. **Improved audience understanding**

Well-designed slides highlight important points and provide visual support to the speaker.

7. **Easy editing and updating**

Scientific presentations can be easily modified when new experimental results or information become available.

Guidelines for Effective Scientific Presentations

- Use **short and clear points** rather than lengthy paragraphs.
- Use readable fonts and appropriate figures.
- Present data accurately and provide necessary references.
- Avoid excessive animations and unnecessary effects.
- Maintain a logical sequence from **introduction to conclusion**.

PowerPoint is an effective tool for **communicating scientific information visually and systematically**. It helps researchers and students present experimental data, concepts, and conclusions clearly during academic and scientific presentations.

Q. 48. Define bioinformatics and explain its scope.

Ans: Bioinformatics and Its Scope

Bioinformatics is an interdisciplinary field that combines **biology, computer science, mathematics, and statistics** to store, manage, analyse, and interpret biological data, particularly data related to **DNA, RNA, proteins, and genomes**.

Scope of Bioinformatics

1. **Genomics**

Bioinformatics is used to analyse genome sequences, identify genes, and compare genomes of different organisms.

2. **Proteomics**

It helps in studying **protein sequences, structures, functions, and interactions**.

3. **Sequence analysis**

DNA, RNA, and protein sequences can be compared to identify similarities, mutations, conserved regions, and evolutionary relationships.

4. **Biological databases**

Bioinformatics is used to develop and maintain databases containing **genomic, protein, structural, and other biological information**.

5. **Structural bioinformatics**

Computational tools are used to predict and analyse the **three-dimensional structures of proteins and other biomolecules**.

6. **Drug discovery**

Bioinformatics helps identify potential drug targets and study interactions between **drug molecules and biological targets**.

7. **Phylogenetic analysis**

It is used to study **evolutionary relationships** among organisms using molecular sequence data.

8. **Plant biotechnology and agriculture**

Bioinformatics helps in **gene identification, crop genome analysis, molecular marker development, and crop improvement.**

Bioinformatics is an essential component of modern biological research. Its scope extends from **genome and protein analysis to drug discovery, evolutionary studies, agriculture, and biotechnology**, making it an important interdisciplinary field.

Q. 49. Write a note on NCBI.

Ans: NCBI

NCBI (National Center for Biotechnology Information) is a major biological information resource established in **1988** as part of the **National Library of Medicine (NLM)** of the United States. It provides access to a wide range of biological databases and computational tools used in biomedical and biological research.

Major Functions and Resources of NCBI

1. **GenBank**

NCBI maintains **GenBank**, a large public database of nucleotide sequences submitted by researchers worldwide.

2. **Sequence analysis**

NCBI provides tools such as **BLAST (Basic Local Alignment Search Tool)** for comparing DNA, RNA, and protein sequences.

3. **Protein databases**

It provides information about **protein sequences, structures, and functions.**

4. **PubMed**

NCBI provides access to **PubMed**, a major database for searching scientific literature in biomedical and life sciences.

5. **Genome databases**

NCBI provides genome-related information for numerous organisms, including **plants, animals, fungi, and microorganisms.**

6. **Taxonomy database**

The NCBI Taxonomy database provides information about the **classification and relationships of organisms.**

7. **Research applications**

NCBI resources are widely used in **genomics, molecular biology, genetics, biotechnology, bioinformatics, and evolutionary studies.**

Importance

NCBI provides researchers with **free access to biological data and computational tools**, facilitating sequence comparison, gene identification, genome analysis, literature searches, and biological research.

NCBI is an important **bioinformatics resource** that integrates biological databases, scientific literature, and computational tools. It plays a major role in modern **genomic and molecular biological research**.

Q. 50. Write a short note on DDBJ/JDBD.

Ans: DDBJ/JDDB – Short Note

DDBJ (DNA Data Bank of Japan) is a major international database that collects and provides access to **DNA and nucleotide sequence data**. It is operated by the **National Institute of Genetics (NIG), Japan**.

DDBJ is a member of the **International Nucleotide Sequence Database Collaboration (INSDC)** along with **GenBank (NCBI, USA)** and **ENA (Europe)**. These databases exchange sequence data regularly, so the information submitted to one database becomes available through the others.

Major Functions

1. **Storage of nucleotide sequences:** DDBJ stores DNA sequence data submitted by researchers.
2. **Data submission:** Scientists can submit newly determined nucleotide sequences to the database.
3. **Sequence retrieval:** Researchers can search and download nucleotide sequence information.
4. **Bioinformatics research:** It supports **genome analysis, gene identification, comparative genomics, and evolutionary studies**.
5. **International data sharing:** It promotes worldwide exchange of biological sequence information.

Importance

DDBJ is an important resource for **molecular biology, genetics, genomics, biotechnology, and bioinformatics**. It provides researchers with reliable access to large-scale nucleotide sequence data.

Q. 51. What is Genetic code?

Ans: Genetic Code

The **genetic code** is the set of rules by which the information present in the nucleotide sequence of **mRNA** is translated into the sequence of amino acids in a protein.

Important Features of Genetic Code

1. **Triplet:** Each amino acid is specified by a sequence of **three nucleotides**, called a **codon**.
2. **64 codons:** There are **64 possible codons**. Of these, **61 code for amino acids** and 3 are stop codons.
3. **Start codon:** **AUG** acts as the start codon and generally codes for methionine.
4. **Stop codons:** **UAA, UAG, and UGA** are stop codons and terminate protein synthesis.
5. **Degenerate:** Most amino acids are represented by more than one codon.
6. **Unambiguous:** Each codon specifies only **one particular amino acid**.
7. **Nearly universal:** The genetic code is largely the same in most organisms, with some exceptions, particularly in certain organelles.

Thus, the genetic code provides the **molecular language for converting genetic information into proteins**, and it is essential for gene expression and protein synthesis.

Q. 52. Explain the dot-matrix method of sequence alignment.

Ans: Dot-Matrix Method of Sequence Alignment

The **dot-matrix method** is a simple graphical technique used to compare two DNA, RNA, or protein sequences and identify regions of **similarity, repeats, insertions, deletions, and mutations**.

Principle

In a dot-matrix plot, one sequence is written along the **horizontal axis** and the other along the **vertical axis**. A dot is placed at a position where the residues in the two sequences match. Continuous matches form **diagonal lines**, indicating sequence similarity.

Procedure

1. Write the first sequence along the horizontal axis and the second sequence along the vertical axis.
2. Compare each residue of one sequence with every residue of the other sequence.
3. Place a **dot** wherever the residues match.
4. Examine the resulting pattern of dots.
5. A continuous diagonal line generally represents a region of **good alignment or similarity**.

Interpretation

- **Main diagonal:** Indicates high similarity between the two sequences.
- **Broken or interrupted diagonal:** May indicate substitutions, insertions, or deletions.
- **Parallel diagonal lines:** May indicate **repeated sequences**.
- **Reverse diagonal patterns:** Can indicate **inverted or reverse-complementary sequences**.

Applications

- Comparison of **DNA, RNA, and protein sequences**.
- Identification of conserved regions.
- Detection of **repeated sequences**.
- Identification of insertions, deletions, and mutations.

- Study of similarities between genes or proteins.

The dot-matrix method provides a **visual representation of sequence similarity** and is useful as an initial tool for studying relationships between biological sequences.

Q. 53. Describe the principle and applications of BLAST.

Ans: BLAST: Principle and Applications

BLAST (Basic Local Alignment Search Tool) is a bioinformatics tool used to compare a **DNA, RNA, or protein sequence** with sequences present in biological databases. It identifies regions of **local similarity** between sequences.

Principle of BLAST

BLAST works on the principle of **local sequence alignment**.

1. The **query sequence** is divided into short subsequences or words.
2. These words are compared with sequences in a database to find **short matching regions**.
3. Matching regions are extended in both directions to produce longer **local alignments**.
4. The resulting matches are scored according to sequence similarity.
5. The results are ranked using statistical measures such as the **E-value**, which indicates the likelihood of obtaining a match by chance.

Applications of BLAST

1. **Identification of unknown sequences:** Helps identify an unknown DNA or protein sequence by comparing it with known sequences.
2. **Gene identification:** Helps determine whether a sequence corresponds to a known gene.
3. **Protein function prediction:** Similarity to proteins of known function can provide clues about the function of an unknown protein.
4. **Sequence similarity analysis:** Used to find similar or conserved regions among sequences.
5. **Evolutionary studies:** Helps compare genes and proteins from different organisms and study their evolutionary relationships.
6. **Detection of mutations:** Can help identify differences such as substitutions, insertions, and deletions.
7. **Genome annotation:** Used in identifying and annotating genes in newly sequenced genomes.

BLAST is a powerful sequence-comparison tool based on local alignment. It is widely used in **genomics, molecular biology, genetics, biotechnology, and bioinformatics** for sequence identification, functional analysis, and comparative studies.

Q. 54. Explain FASTA and its applications.

Ans: FASTA and Its Applications

FASTA is a **sequence comparison and alignment program** used to search for similarities between a query **DNA, RNA, or protein sequence** and sequences stored in biological databases. It is also commonly associated with the **FASTA file format**, which represents biological sequences in a simple text form.

Principle of FASTA

FASTA identifies regions of similarity by comparing **short subsequences** between the query sequence and database sequences. It first detects matching regions and then extends these matches to obtain a good local alignment. The similarity between sequences is evaluated using alignment scores.

Applications of FASTA

1. **Sequence similarity search:** Used to find similar DNA, RNA, or protein sequences in databases.
2. **Identification of unknown sequences:** Helps identify an unknown sequence by comparing it with known sequences.
3. **Protein function prediction:** Similarity with proteins of known functions can provide clues about the function of an unknown protein.
4. **Gene identification:** Helps determine whether a nucleotide sequence corresponds to a known gene.
5. **Evolutionary studies:** Sequence similarities can be used to study relationships among organisms.
6. **Comparative analysis:** Useful for comparing genes or proteins from different organisms.
7. **Database searching:** FASTA can search large biological sequence databases to identify significant sequence matches.

FASTA Format

The **FASTA file format** represents a biological sequence beginning with a header line starting with the symbol >, followed by the nucleotide or amino-acid sequence.

FASTA is an important bioinformatics tool for **sequence comparison and similarity searching**. It has applications in **gene identification, protein analysis, functional prediction, comparative genomics, and evolutionary studies**.

Q. 55. Define phylogenetic tree and explain its importance.

Ans: Phylogenetic Tree and Its Importance

A **phylogenetic tree** is a branching diagram that represents the **evolutionary relationships and common ancestry** among different organisms, genes, or proteins. The branches indicate evolutionary relationships, while the nodes generally represent **common ancestors**.

Main Components

- **Root:** Represents the common ancestral lineage of the organisms being studied.
- **Branches:** Represent evolutionary lineages.
- **Nodes:** Represent common ancestors or points of divergence.
- **Tips/Terminals:** Represent the organisms, genes, or sequences being compared.

Importance of Phylogenetic Trees

1. **Study of evolutionary relationships:** They help determine relationships among different organisms based on shared characteristics or molecular sequences.
2. **Identification of common ancestors:** They provide information about possible common ancestral lineages.
3. **Classification of organisms:** Phylogenetic analysis supports the classification of organisms according to their evolutionary relationships.
4. **Molecular evolution:** DNA, RNA, and protein sequences can be compared to study molecular changes during evolution.
5. **Species identification:** Molecular phylogenetic methods can help distinguish closely related species.
6. **Comparative genomics:** Phylogenetic trees help compare genes and genomes across different organisms.
7. **Study of gene families:** They can reveal relationships among related genes and help trace gene duplication and divergence.

A phylogenetic tree is an important tool in **evolutionary biology and bioinformatics**. It provides a graphical representation of evolutionary relationships and helps in understanding **common ancestry, divergence, classification, and molecular evolution**.

Q. 56. Differentiate between rooted and unrooted trees.

Ans: Difference between Rooted and Unrooted Phylogenetic Trees

Phylogenetic trees are diagrams used to represent relationships among organisms, genes, or protein sequences. They may be **rooted** or **unrooted**.

Basis	Rooted Tree	Unrooted Tree
Definition	Shows evolutionary relationships along with the direction of evolution from a common ancestor.	Shows relationships among organisms without indicating the direction of evolution.
Root	Has a clearly defined root representing the common ancestor.	Has no defined root .
Evolutionary direction	Indicates the direction of evolutionary change.	Does not indicate the direction of evolutionary change.
Common ancestor	The common ancestral lineage can be identified from the root.	A common ancestor cannot be located directly from the tree.

Interpretation	Shows both relationships and the possible sequence of divergence.	Shows only the relative relationships or branching pattern.
Construction	Usually requires information or assumptions that allow the tree to be rooted, often using an outgroup.	Can be constructed directly from measures of similarity or genetic distance without specifying an ancestral root.
Use	Useful for studying evolutionary history and ancestral relationships .	Useful for showing relationships and similarity when the ancestral direction is unknown.

Example

Rooted tree:

Common ancestor $\rightarrow A \rightarrow B$ and C

Unrooted tree:

$A - B - C$

(No evolutionary direction is indicated.)

A **rooted phylogenetic tree** provides information about the **direction and history of evolution**, whereas an **unrooted tree** represents relationships among taxa without specifying their evolutionary direction or common ancestor.

Q. 57. Explain cladogram and phylogram.

Ans: Cladogram and Phylogram

Both **cladogram** and **phylogram** are types of phylogenetic trees used to represent relationships among organisms, genes, or protein sequences. However, they differ in what the branches represent.

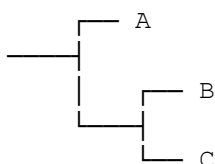
1. Cladogram

A **cladogram** is a branching diagram that represents relationships based on **shared derived characters (synapomorphies)**. It shows the pattern of branching but does not necessarily represent the amount of evolutionary change.

Features:

- Shows relationships based on shared derived characters.
- Branch lengths generally **do not represent evolutionary distance**.
- Each branching point represents a division of lineages.
- Used mainly in **cladistic classification**.

Example:



2. Phylogram

A **phylogram** is a phylogenetic tree in which the **length of each branch represents the amount of evolutionary change or genetic distance**.

Features:

- Branch lengths are proportional to the amount of evolutionary change.
- Longer branches indicate greater sequence or character change.
- It is commonly constructed using **molecular sequence data**.
- Useful for comparing the degree of genetic divergence among organisms.

Difference between Cladogram and Phylogram

Basis	Cladogram	Phylogram
Purpose	Shows relationships based on shared derived characters.	Shows relationships and amount of evolutionary change.
Branch length	Usually has no quantitative meaning.	Represents evolutionary change or genetic distance.
Data used	Mainly morphological or molecular characters.	Often molecular sequence or quantitative character data.
Evolutionary change	Not directly represented by branch length.	Directly represented by branch length.
Main use	Cladistic classification.	Studying genetic divergence and evolutionary change.

A **cladogram** mainly shows the **pattern of relationships**, whereas a **phylogram** shows both the relationships and the **amount of evolutionary change represented by branch lengths**.

Q. 58. What is hogness box & where is it located?

Ans: Hogness Box

The **Hogness box** is a short, conserved DNA sequence present in the **promoter region of eukaryotic genes**. It is also known as the **TATA box** because it commonly contains the sequence **TATAAA**.

Location

- It is located approximately **25–30 base pairs upstream of the transcription start site (+1)**.
- It is found in the **5' regulatory region** of many eukaryotic genes.

Function

The Hogness box serves as a **binding site for transcription factors and RNA polymerase II-associated transcription machinery**. It helps in the **proper initiation and positioning of transcription**.

In short:

Hogness box = TATA box → promoter region → about 25–30 bp upstream of the transcription start site → involved in transcription initiation.

Q. 59. Nitrogen Base.

Ans: Nitrogenous Bases

Nitrogenous bases are organic compounds containing **nitrogen atoms** that form an important component of **nucleotides**, the building blocks of DNA and RNA.

Types of Nitrogenous Bases

They are mainly divided into two groups:

1. **Purines** – have a **double-ring structure**:
 - **Adenine (A)**
 - **Guanine (G)**
2. **Pyrimidines** – have a **single-ring structure**:
 - **Cytosine (C)**
 - **Thymine (T)** – found mainly in DNA
 - **Uracil (U)** – found mainly in RNA

Base Pairing

In DNA:

- **Adenine pairs with Thymine (A–T)** through two hydrogen bonds.
- **Guanine pairs with Cytosine (G–C)** through three hydrogen bonds.

In RNA, **Uracil replaces Thymine**, so adenine pairs with uracil (A–U).

Importance

Nitrogenous bases are important because they:

- Carry **genetic information**.
 - Participate in **DNA replication and transcription**.
 - Help determine the **amino-acid sequence of proteins** through the genetic code.
 - Form part of energy-carrying molecules such as **ATP and GTP**.
-

Q. 60. Short notes on blotting technique.

Ans: Blotting Techniques

Blotting techniques are molecular biology methods used to **detect and identify specific DNA, RNA, or protein molecules** from a mixture. The separated molecules are transferred from a gel onto a membrane and then detected using a specific probe or antibody.

Major Types of Blotting

1. **Southern Blotting**

- Used for detection and identification of **specific DNA sequences**.
 - DNA is separated by gel electrophoresis and transferred to a membrane.
 - A labelled complementary DNA probe is used for detection.
2. **Northern Blotting**
- Used for detection of **specific RNA molecules**.
 - It is useful for studying **gene expression and RNA size**.
3. **Western Blotting**
- Used for detection of **specific proteins**.
 - Proteins are separated by electrophoresis and transferred to a membrane.
 - Specific antibodies are used to detect the target protein.

General Steps

Sample preparation → Gel electrophoresis → Transfer to membrane → Probe/antibody treatment → Detection

Applications

- Detection of specific **genes, RNA transcripts, and proteins**.
- Study of **gene expression**.
- Identification of mutations or genetic changes.
- Analysis of proteins and their expression.
- Used in **molecular biology, genetics, biotechnology, and medical research**.

Blotting techniques are important molecular biology tools that allow researchers to identify specific **DNA, RNA, and proteins** from complex biological samples. The three major techniques are **Southern, Northern, and Western blotting**.
