UNIT – 4

a) Immuno-Blotting Techniques - ELISA, Western Blotting, Southern Blotting

ELISA (Enzyme-Linked Immunosorbent Assay):

- **Principle:** Detection of specific proteins or antibodies using enzyme-labelled antibodies and colorimetric or fluorescent substrates.
- **Applications:** Diagnosis of infections (HIV, hepatitis), detection of antibodies, and quantification of proteins.

Western Blotting:

- **Principle:** Separation of proteins by electrophoresis, transfer to a membrane, and detection using specific antibodies.
- **Applications:** Protein identification, quantification, and analysis of post-translational modifications.

Southern Blotting:

- **Principle:** Transfer of DNA fragments from a gel to a membrane, followed by hybridization with a labelled probe.
- **Applications:** DNA fragment analysis, gene mapping, and identification of specific DNA sequences.

b) Genetic Organization of Eukaryotes and Prokaryotes

Eukaryotes:

1. **Nucleus:** Contains linear DNA organized into chromosomes.
2. **Membrane-Bound Organelles:** Endoplasmic reticulum, Golgi apparatus, mitochondria, etc.
3. **Introns and Exons:** Presence of non-coding introns within genes.
4. **Multiple Linear Chromosomes:** Typically more than one chromosome per cell.

Prokaryotes:

1. **Nucleoid Region:** Circular DNA without a true nucleus.
2. **No Membrane-Bound Organelles:** Lack of endoplasmic reticulum, mitochondria, etc.
3. **Operons:** Clusters of genes with related functions often organized into operons.
4. **Single Circular Chromosome:** Typically one chromosome per cell.

c) Microbial Genetics - Transformation, Transduction, Conjugation, Plasmids, and Transposons

Transformation:

- **Process:** Uptake of foreign DNA from the environment by a cell.

- **Mechanism:** Incorporation of the foreign DNA into the recipient cell's genome.
- **Example:** Uptake of DNA by competent bacteria.

Transduction:

- **Process:** Transfer of genetic material from one bacterium to another by a bacteriophage.
- **Mechanism:** The phage carries bacterial DNA from one host to another.
- **Example:** Generalized and specialized transduction.

Conjugation:

- **Process:** Direct transfer of genetic material between bacterial cells.
- **Mechanism:** Conjugative plasmids or sex pili facilitate the transfer of plasmids or genomic DNA.
- **Example:** Fertility (F) plasmids in E. coli.

Plasmids:

- **Nature:** Circular DNA molecules separate from the chromosomal DNA.
- **Functions:** Carry accessory genes, such as antibiotic resistance or toxin production.
- **Example:** Fertility (F) plasmids, R plasmids.

Transposons:

- **Nature:** Mobile genetic elements capable of changing their position in the genome.
- **Mechanism:** Cut and paste or copy and paste mechanisms.
- **Functions:** Contribute to genetic diversity and can cause mutations.
- **Example:** Insertion sequences (IS elements).

d) Introduction to Microbial Biotransformation and Applications

Microbial Biotransformation:

- **Definition:** Conversion of one chemical compound into another by microbial activity.
- **Organisms Involved:** Bacteria, yeast, and fungi.
- **Applications:** Production of pharmaceuticals, chemicals, and biodegradation of pollutants.

Applications:

1. **Pharmaceutical Industry:** Production of antibiotics, steroids, and other therapeutic compounds.
2. **Chemical Industry:** Synthesis of specialty chemicals and intermediates.
3. **Environmental Cleanup:** Biodegradation of pollutants and waste.

e) Mutation: Types of Mutation/Mutants

Types of Mutations:

1. **Point Mutation:**
    - **Substitution:** Replacement of one nucleotide with another.
    - **Insertion:** Addition of one or more nucleotides.
    - **Deletion:** Removal of one or more nucleotides.
2. **Frameshift Mutation:**
    - Insertion or deletion of nucleotides, altering the reading frame of the gene.
3. **Missense Mutation:**
    - Point mutation leading to the substitution of one amino acid in the protein.
4. **Nonsense Mutation:**
    - Point mutation causing the formation of a premature stop codon.
5. **Silent Mutation:**
    - Point mutation that does not alter the amino acid sequence due to redundancy in the genetic code.

Mutants:

- **Definition:** Organisms carrying a mutation in their genetic material.
- **Types:**
    - **Wild Type:** The typical, naturally occurring form of an organism.
    - **Mutant Type:** Organisms with one or more mutations.
- **Use in Research:** Mutants are often used to study gene function, genetic pathways, and cellular processes.

Understanding these concepts in molecular biology and genetics is fundamental for researchers, clinicians, and professionals working in various scientific disciplines. They provide insights into the molecular basis of diseases, biotechnological applications, and microbial physiology.