BIOTECHNOLOGY

UNIT 4 NOTES

- IMMUNOBLOTTING TECH.
- EUKARYOTES & PROKARYOTES
- MICROBIAL GENETICS
- MICROBIAL BIOTRANSFORMATION
- MUTATION

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BLOTTING TECHNIQUES

- Blotting techniques are laboratory methods used to detect specific biological molecules such as DNA , RNA & protiens.
- Blotting techniques are widely used in molecular biology , biotechnology , genetics & disease diagnosis.

TYPES OF BLOTTING TECHNIQUES

1. Southern Blotting (detect DNA)
2. Northern Blotting (detect RNA)
3. Western Blotting (detect profeins)

ELISA

- ELISA is a laboratory technique used to detect & measure antigens & antibodies in a sample.
- ELISA stands for Enzyme Linked Immunosorbent Assay.
- It is based on principle of antigen - antibody reaction.
- ELISA is highly sensitive, specific, rapid & widely used in diagnosis & research.
- It is commonly used for detection of infectious diseases, hormones & vaccines.

PRINCIPLE

- ELISA works on the specific binding blw antigen & antibody.
- An Enzyme - linked antibody reacts with a substrate to produce a coloured product, which helps in detection.

COMPONENTS OF ELISA

- Antigen or antibody
- Enzyme - linked antibody
- Solid surface or microtiter plate
- Washing buffer
- Substrate solution

TYPES OF ELISA

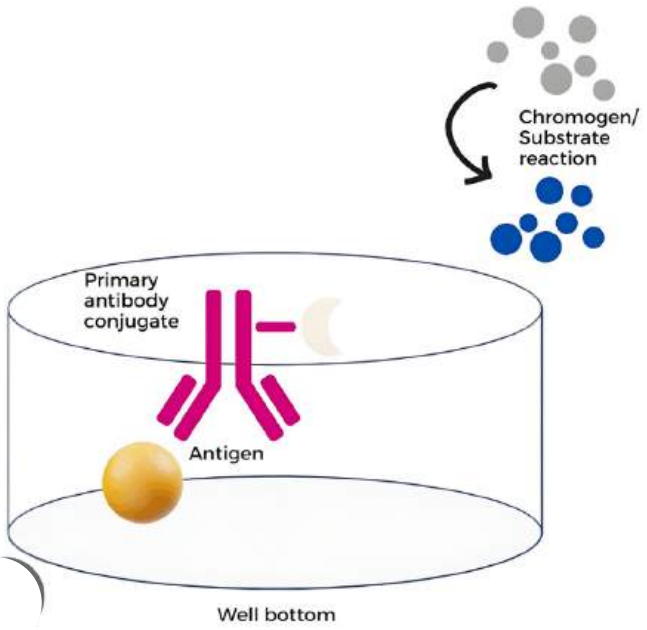
1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA
4. Competetive ELISA

① DIRECT ELISA

- Direct ELISA is a type of Enzyme - linked immunosorbent Assay used to detect antigens in a simple.
- In this method, an enzyme - linked primarily antibody directly binds to the target antigen.
- It is one of the simplest & fastest ELISA methods.
- Only one antibody is used for detection.

PRINCIPLE

- The antigen is attached to the microtiter plate & detected directly by an enzyme - linked primarily antibody.
- After addition of substrate, the enzyme produces a color change indicating the presence of antigen.



PROCEDURE

① Coating Of Antigen

- Sample containing the antigen is added into the wells of a microtiter plate.
- The antigen gets absorbed & attached to the surface of the plate.

② Blocking

- A blocking agent such as bovine serum albumin is added to cover the empty spaces of the plate.
- This step prevents non-specific binding of antibodies during the test.

③ Addition of Antibody

- An antibody specific to the antigen is added into the wells.
- The antibody directly binds with the antigen if is present in the sample.

④ Washing

- The wells are washed with buffer solution to remove unbound antibodies.
- Only antigen-antibody complexes remain attached to the plate surfaces.

⑤ Addition Of Substrate

- A suitable substrate is added which reacts with the attached enzyme.
- The enzyme converts the substrate into a coloured product.

⑥ Observation Of Result

- Development of colour indicates the presence of antigen in the sample.
- The intensity of color is proportional to the amout of antigen present.

APPLICATION

- Detection of antigens
- Diagnosis of infectious diseases
- Research laboratories
- Hormone & protein analysis

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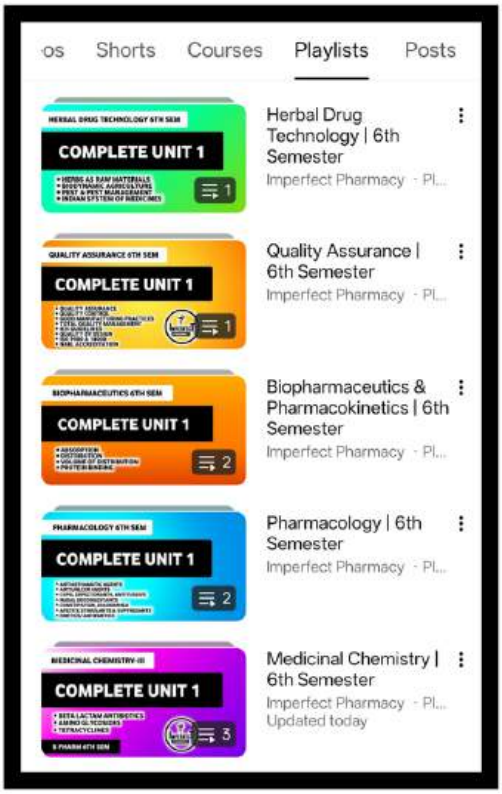

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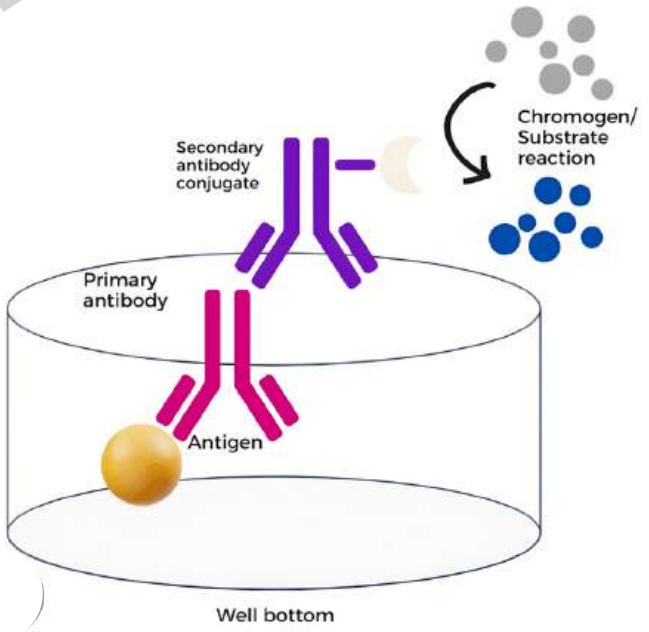
② INDIRECT ELISA

- Indirect ELISA is a type of Enzyme-linked Immunosorbent Assay used to detect Antibodies in a sample.
- In this method, the Primary Antibody binds to the antigen & an enzyme-linked secondary antibody binds to the primary antibody.
- This method is widely used in diagnosis & research laboratories.

PRINCIPLE

- A known antigen complementary to the Antibody to be known is attached to the microtiter plate.
- First, the primary antibody binds to the antigen, then an enzyme-linked secondary antibody binds to the primary antibody.
- After addition of substrate, the enzyme produces a color change indicating the presence of antibody.



PROCEDURE

① Coating of Antigen

- A known antigen is added to the microtiter plate.

② Blocking

- Unoccupied sites are blocked to prevent non-specific binding.

③ Addition of Primary Antibody

- Sample containing primary antibody (Antibody to be known) is added & binds to the antigen.

④ Washing

- Excess unbound primary antibody is removed.

⑤ Addition of Enzyme-linked Secondary Antibody

- Secondary antibody binds to the Primary Antibody.

⑥ Washing

- Excess secondary antibody is removed.

⑦ Addition of Substrate

- Enzyme reacts with substrate & produces colour

⑧ Observation

- Colour intensity indicates the presence & amount of antibody.

APPLICATION

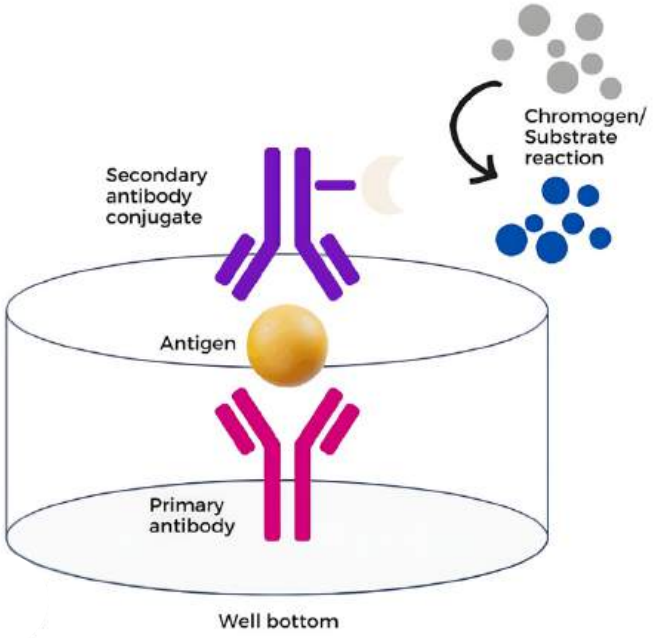
- Detection of antibodies in serum
- Diagnosis of HIV & Hepatitis
- Detection of viral & bacterial infections.

SANDWITCH ELISA

- Sandwitch ELISA is a type of Enzyme-linked Immunosorbent Assay used to detect antigens in a sample.
- In this method, the antigens are trapped b/w two antibodies: a capture antibody & a detection antibody.
- It is one of the most sensitive.

PRINCIPLE

- The antigen from the sample binds to the capture antibody, & then an enzyme-linked detection antibody binds to the antigen forming an
- Antibody-antigen-Antibody sandwitch.
  After addition of substrate, the enzyme produces a color change indicating the presence of antigen.



PROCEDURE

① Coating of Capture Antibody

- Capture antibody is attached to the microtiter plate.

② Blocking

- Unoccupied sites are blocked to prevent non-specific binding.

③ Addition of Sample

- Sample containing antigen is added & antigen binds to the capture antibody.

④ Washing

- Excess unbound substances are removed.

⑤ Addition of Enzyme-linked Detection Antibody

- Detection antibody binds to the antigen.

⑥ Washing

- Excess unbound detection antibody is removed.

⑦ **Addition of Substrate**

- Enzyme reacts with substrate & produces color.

⑧ **Observation**

- Color intensity indicates the presence & amount of antigen.

APPLICATIONS

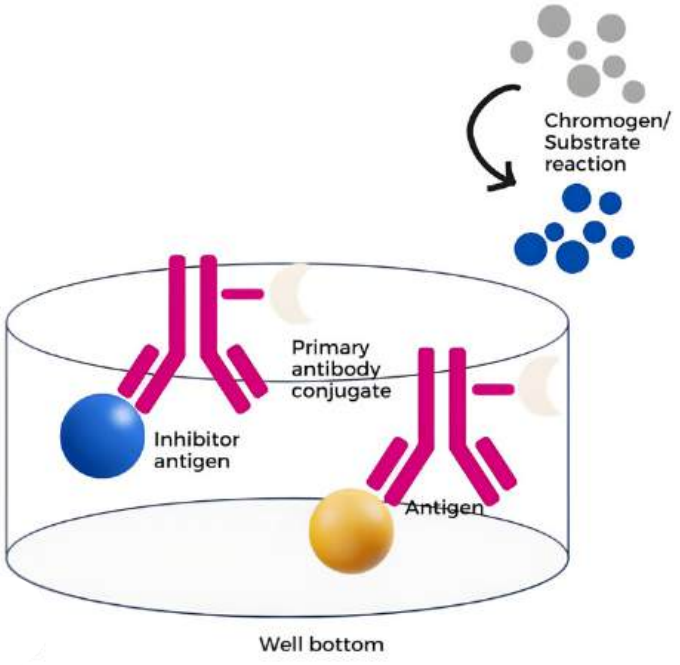
- Detection of hormones & cytokines.
- Diagnosis of infectious diseases.
- Detection of viral & bacterial antigens.

COMPETITIVE ELISA

- Competitive ELISA is a type of Enzyme-linked Immunosorbent Assay used to detect & measure antigens or antibodies in a sample.
- In this method, sample antigen competes with labeled antigen for binding to a specific antibody.
- It is commonly used for detection of small antigens & hormones.

PRINCIPLE

- In competitive ELISA, the antigen present in the sample competes with enzyme-labeled antigen for binding sites on a specific antibody.
- If more antigen is present in the sample, less labeled antigens binds to the antibody, resulting in less color formation after addition of substrate.



PROCEDURE

① Coating of Antibody

- Specific antibody is attached to the microtiter plate.

② Addition of Sample Antigen & Labeled Antigen

- Sample antigen & enzyme - labeled antigen are added together & compete for antibody binding sites.

③ Incubation

- Competition occurs b/w labeled & unlabeled antigen.

④ Washing

- Excess unbound substances are removed.

⑤ Addition of Substrate

- Enzyme reacts with substrate & produces color.

⑥ Observation

- Less color indicates higher concentration of antigen in the sample.

APPLICATION

- Detection of hormones
- Detection of toxins & drugs
- Measurement of small antigens
- Clinical diagnosis

SOUTHERN BLOTTING

- Southern Blotting is a laboratory technique used to detect a specific DNA sequence in sample.
- It was developed by scientist Edwin Southern.
- It helps scientists identify the presence of a particular gene or DNA fragments.

PRINCIPLE

- DNA fragments are first separated according to their size by gel electrophoresis & then transferred to a membrane.
- A labeled DNA probe binds to the matching DNA sequence, which helps in detection.

STEPS OF SOUTHERN BLOTTING

① Isolation of DNA

- DNA is extracted from the sample.

② Cutting of DNA

- Restriction enzymes cut DNA into small fragments.

③ Giel Electrophoresis

- DNA fragments are separated on agargose gel accoding to size.

④ Denaturation

- Double- stranded DNA is converted into sigle strands using alkaline soln.

⑤ Blotting

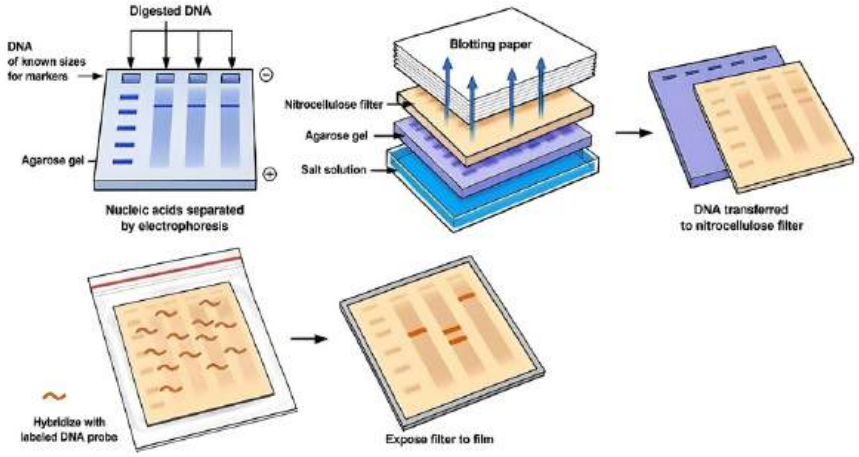
- DNA fragments are transferred from gel onto a nitrocellulose or nylon membrane.

⑥ Hybridization with Probe

- A labeled DNA probe is added. It binds to the complementary DNA sequence.

⑦ Detection

- The bound probe is detected by autoradiography or other detection methods.



APPLICATION

- Detection of specific genes
- DNA fingerprinting
- Diagnosis of genetic disorders
- Identification of mutations.

WESTERN BLOTTING

- Western Blotting is a laboratory technique used to detect specific proteins in a biological sample.
- It is also known as Immunoblotting.
- It is widely used in molecular biology & medical diagnosis.

PRINCIPLE

- Proteins are separated according to their size by gel electrophoresis & then transferred to a membrane.
- Specific antibodies binds to the target protein, which helps in its detection.

PROCEDURE

① Protien Separation

- Proteins are separated according to molecular size using SDS-PAGE electrophoresis.

② Blotting

- Separated proteins are transferred from gel onto Nitrocellulose or PVDF membrane.

③ **Blocking**

- Membrane is treated with blocking agents to prevent non-specific binding.

④ **Addition of Primary Antibody**

- Primary antibody specifically binds to the target protein.

⑤ **Addition of Secondary Antibody**

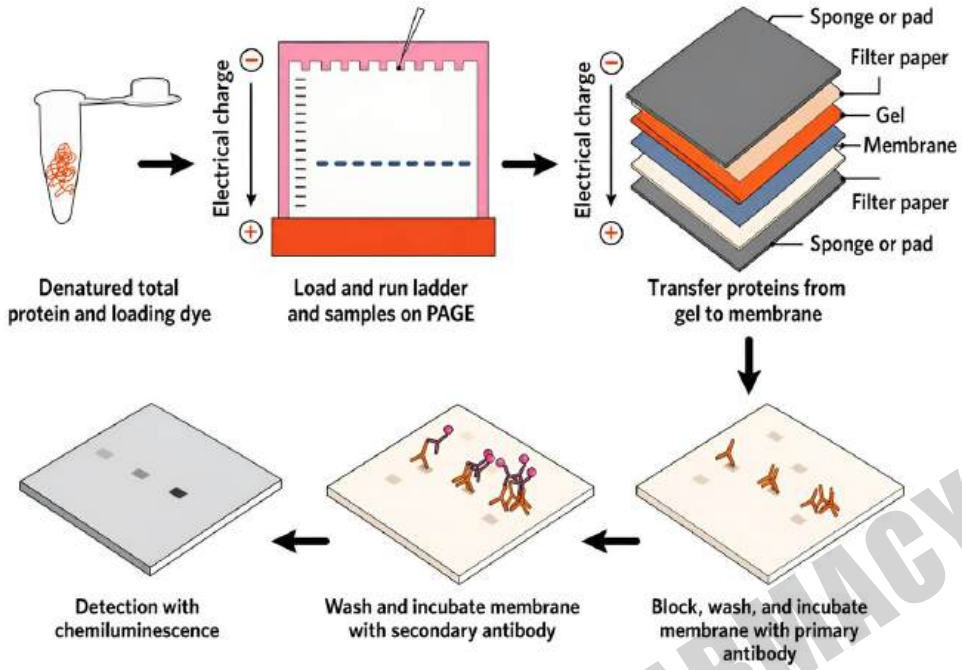
- Enzyme-linked secondary antibody binds to the primary antibody.

⑥ **Washing**

- Excess unbound antibodies are removed by washing.

⑦ **Detection**

- Substrate is added, Enzyme reacts with substrate & produces color which confirms the presence of the target protein.



APPLICATIONS

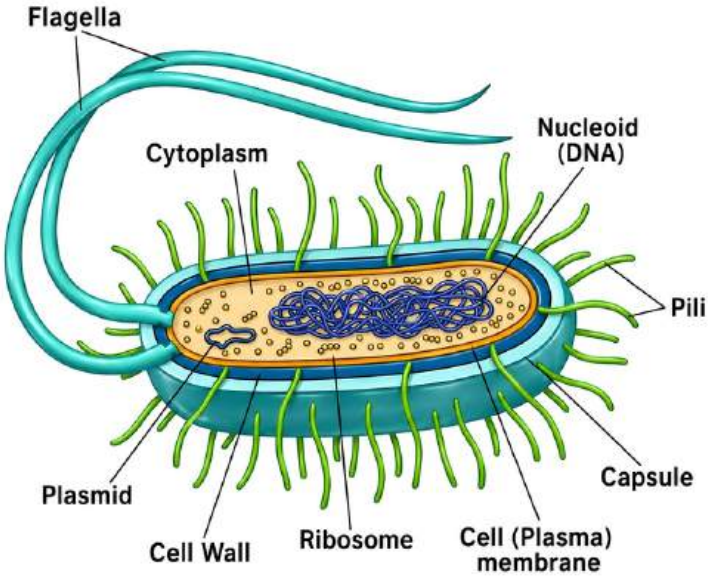
- Detection of specific proteins
- Diagnosis of diseases such as HIV
- Protein Aanlysis in research
- Detection of antibodies & antigens

Genetic Organization Of Prokaryotes & Eukaryotes

- Genetic Organization means how genetic material (DNA) is arranged & managed inside a cell.
- Prokaryotes & Eukaryotes have different types of genetic organization explained as follows:

Prokaryotes

- Prokaryotes are organism having Prokaryotic cells.
- Prokaryotic cell are simple & small cells that do not have a true nucleus or membrane-bound organelles.
- Their genetic material is present freely in the cytoplasm in a region called the Nucleoid.
- Example: bacteria



Genetic Organization In Prokaryotes

① Single Circular DNA

- Prokaryotes usually contain one cellular chromosome.
- The DNA is double-stranded & not enclosed by a nuclear membrane.

② Nucleoid Region

- DNA is present in a region called the Nucleoid
- It is not surrounded by a membrane.

③ Absence of Histone Protiens

- Most Prokaryotic DNA is not associated with histone proteins.

④ Plasmids

- Plasmids are small circular DNA molecules present outside the chromosome.
- They replicate independently.

⑤ Less Non-coding DNA

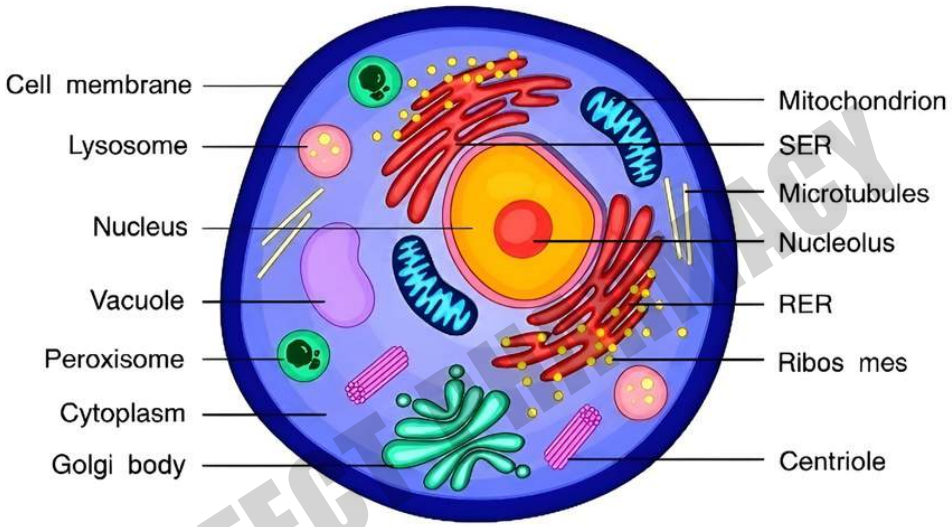
- Most of the DNA is coding DNA (exons).
- Interons (Non-coding DNA are generally absent).

⑥ Haploid Nature

- Prokaryotic Cells generally contain only one copy of each gene.

EUKARYOTES

- Eukaryotic Cell are larger & more complex cells that contain a true nucleus & membrane - bound organelles.
- Their genetic material is enclosed inside the nucleus.
- Examples: Plants, animals, fungi & Protozoa.



GENETIC ORGANIZATION IN EUKARYOTES

① Multiple Linear Chromosomes

- Eukaryotic cells contain multiple linear chromosomes.
- These chromosomes are present inside the nucleus.

② Nuclear Envelope

- The genetic material is enclosed within a double - layered nuclear membrane called the Nuclear envelope.

③ Presence of Histone Proteins

- Histone proteins help in the organization & packaging of DNA.
- DNA wraps around histones to form Nucleosomes.

④ Presence of Introns & Exons

- Eukaryotic genes contain both:
- Exons → coding regions
- Introns → non-coding regions

⑤ Diploid Nature

- Most eukaryotic organisms are diploid

DIFFERENCE IN GENETIC ORGANIZATION

FEATURE	PROKARYOTES	EUKARYOTES
Cell type	Simple	Complex
Nucleus	Present	Present
Chromosome type	Single Circular chromosome	Multiple linear chromosomes
Location of DNA	Nucleoid	Nucleus
Histone Proteins	Mostly absent	Present
Plasmids	Common	Rare
Introns	Usually absent	Present
Non-coding DNA	Less	Large amount
DNA Packaging	Simple	Highly organized
Size of genome	Small	Large

Microbial Genetics

- Microbial genetics is the branch of biology that studies the genes, heredity, & genetic variations in microorganisms such as:
- In bacteria, genes are usually present on a single circular chromosome & sometimes on extra chromosomal DNA called Plasmids.
- Microbial genes can be transferred from one microorganism to another through processes such as:

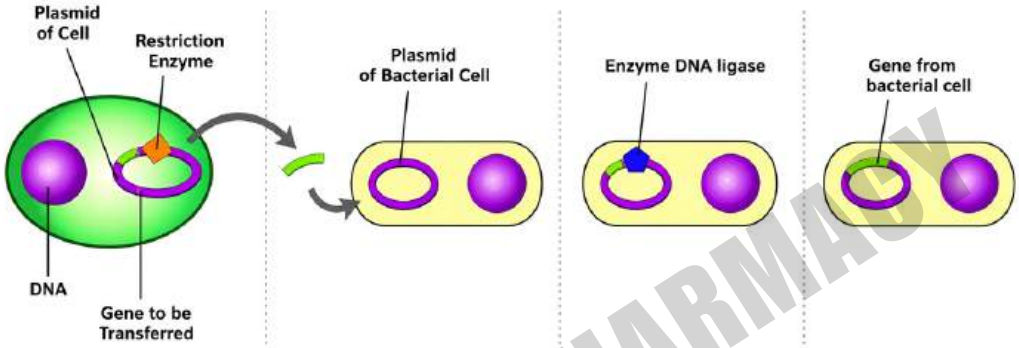
① Transformation
② Transduction
③ Conjugation

Transformation

- Transformation is a method of genetic transfer in which a bacterium takes up free or naked DNA from its sorrounding & incorporates it into its own genetic material.
- This process causes a change in the characteristics of the bacterium.
- The bacterium that recieves the DNA is called the Recipient Cell.
- The DNA released from another cell is known as Donor DNA.

Steps Of Transformation

- Release of DNA from donor bacterial cell
- Uptake of free DNA by recipient cell
- Integration of DNA into bacterial chromosome
- Expression of new character



Griffith's Transformation Experiment

- The transfosmation process was first discovered by Freedrick Griffith in 1928 while working with the bacterium Streptococcus pneumoniae.

Types Of Bacteria Used

① S-strain (Smoth strain)

- It has a polysaccharide capsule.
- It is Virulent (causes disease).

② R-strain (Rough Strain)

- It doesn't have capsule.
- It is non virulent (does not cause disease)

EXPERIMENT PROCEDURES

① Live S-strain injected into mice

- Mice died
- Because S-strain is virulent.

② Live -R-strain injected into mice

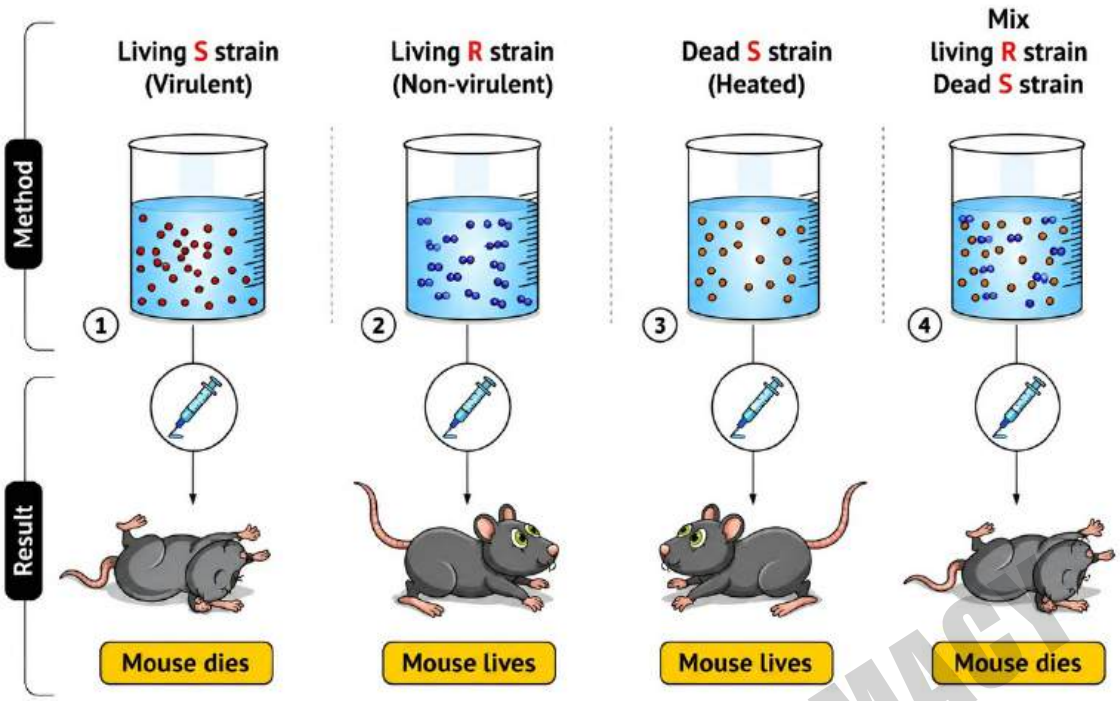
- Mice survived
- Because R-strain in non-virulent

③ Dead S-strain injected into mice

- Mice survived
- Bacteria was dead

④ Live R-strain + Dead S-strain

- Mice died
- Because DNA from dead S-strain bacteria entered the live R-strain bacteria & changed them into virulent S-strain bacteria.



IMPORTANCE

- Causes genetic variation
- Helps in transfer of antibiotic resistant genes.
- Used in biotechnology & genetic engineering.

TRANSDUCTION

- Transduction is a method of genetic transfer in bacteria in which bacterial DNA is transferred from one bacterium to another by a virus called a Bacteriophage.
- The bacteriophage acts as a carrier of genetic material b/w bacteria.

COMPONENTS OF TRANSDUCTION

- Donor bacterium
- Recepient bacterium
- Bacteriophage

TYPES OF TRANSDUCTION

It is of two types:

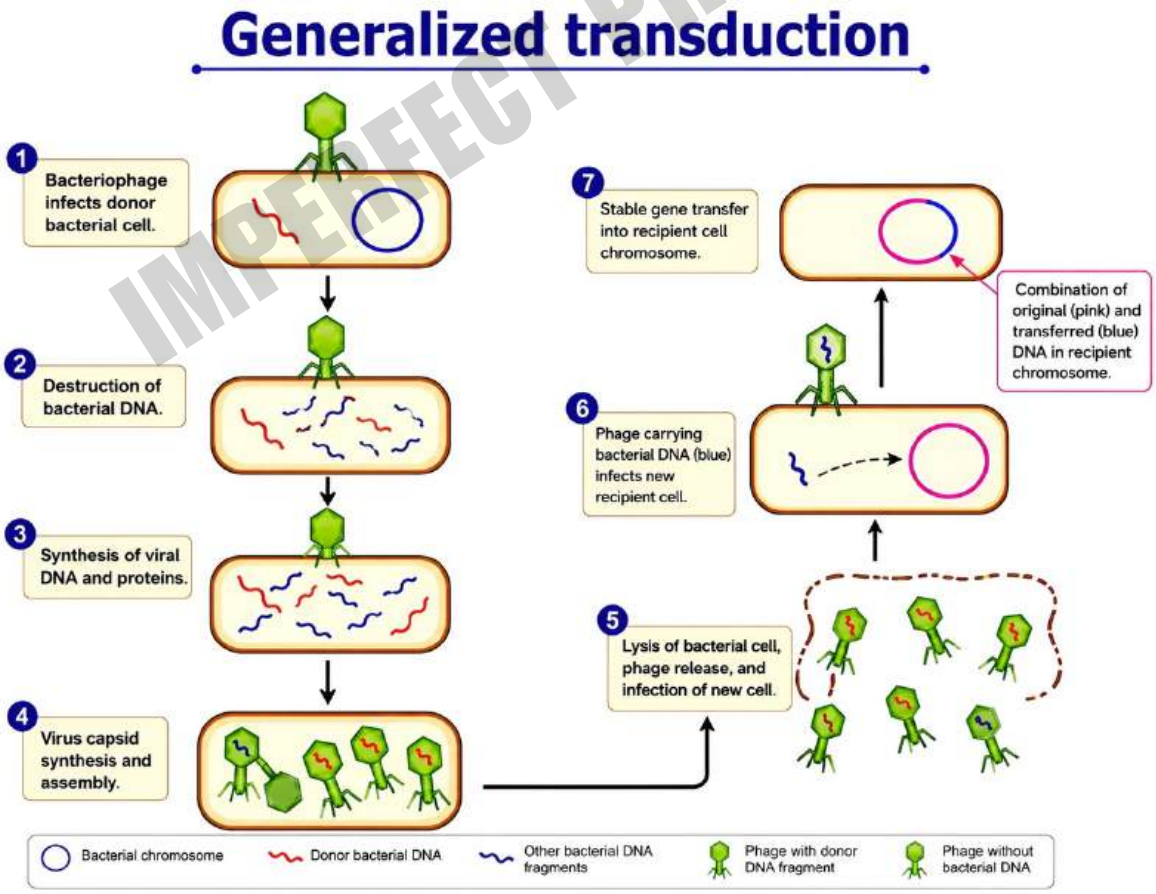
1. Generalized Rransduction
2. Specialized Transduction

1. GENERALIZED TRANSDUCTION

- It is a type of transduction in which any bacterial gene can be transfered from one bacterium to another by a bacteriophage.
- It usually occurs during the lytic cycle when bacterial DNA fragments are accidently packed into phage particles.

Steps Of Generalized Transduction

- A bacteriophage infects a donor bacterial cell.
- The phage multiplies inside the bacterium & breaks the bacterial chromosomes into fragments.
- By mistake, some bacterial DNA fragments get packed inside new phage particles.
- The donor bacterial cell bursts & releases the phages.
- The phage carrying bacterial DNA infects another recipient bacterium.
- The transferred bacterial DNA enters the recipient cell & combines with its chromosome.
- The recepient bacterium shows new genetic characters.



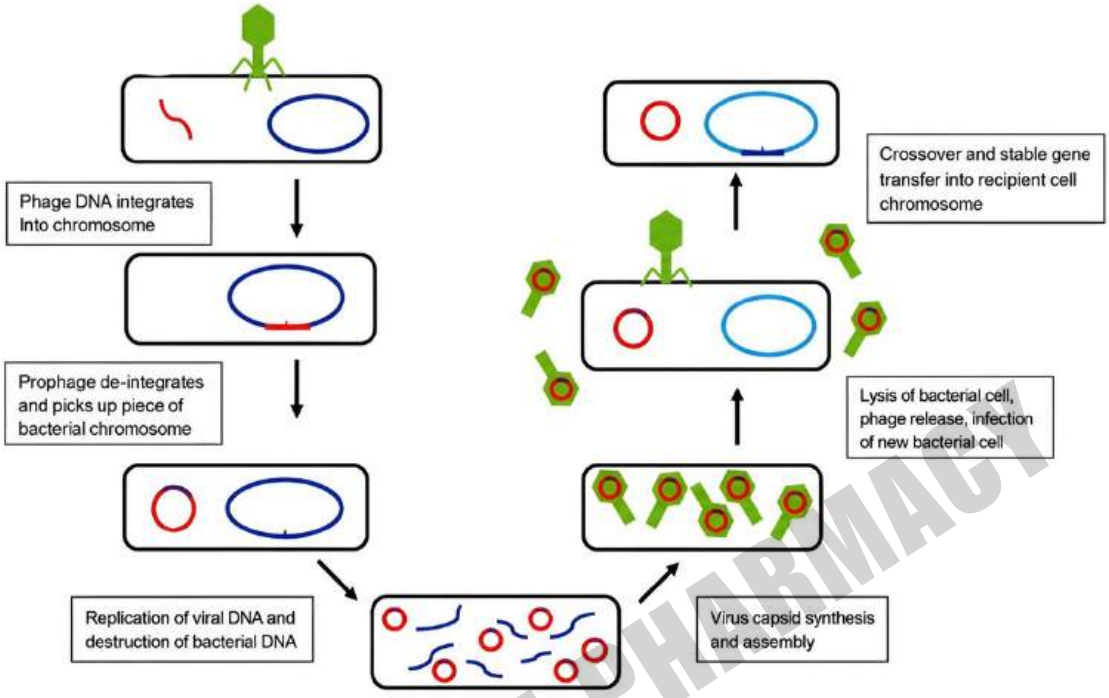
② Specialized Transduction

- It is a type of transduction in which only specific bacterial genes are transferred by a bacteriophage.
- It occurs during the lysogenic cycle when a prophage removes itself incorrectly from the bacterial chromosome & carries nearby bacterial genes along with it.

Steps Of Specialized Transduction

- A temperature bacteriophage infects a bacterial cell.
- The phage DNA becomes integerated into the bacterial chromosome & forms a Prophage.
- During induction, the prophage separates from the bacterial chromosome.
- Sometimes, the prophage carries nearby bacterial genes along with its own DNA.
- New phages containing both phage DNA & bacterial DNA are formed.
- These phages infect another recipient bacterium.
- The transferred bacterial genes become incorporated to the recipient chromosome, producing new characters.

Specialized transduction



IMPORTANCE

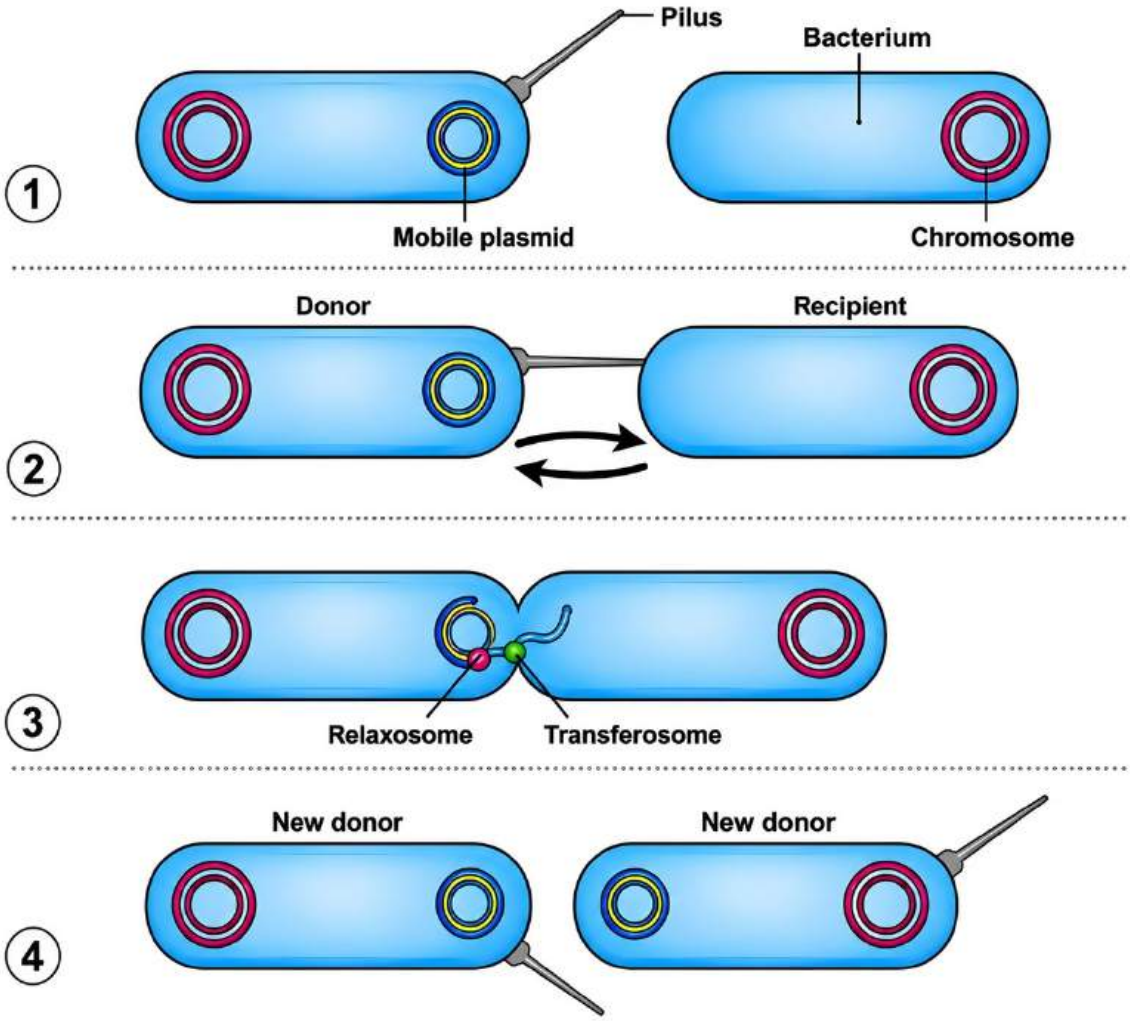
- Causes genetic variation in bacteria.
- Helps in transfer of antibiotic resistance genes.
- Used in microbial genetics research.
- Important in genetic engineering & biotechnology.

CONJUGATION

- Conjugation is a method of genetic transfer in bacteria in which genetic materials is transfered from one bacterial cell to another through direct cell-to-cell contact.
- This process usually occurs through a thin-tube like structure called the sex pilus or conjugation pilus.
- The bacteriam that donates DNA is called the donor cell (F^+ cell) & the bacteriam that recieves DNA is called the recepient cell (F^- cell).

STEPS OF CONJUGATION

- The donor (F^+) bacterial cell forms a sex pilus.
- The sex pilus attaches the donor cell to the recipient (F^-) cell.
- A conjugation bridges is formed b/w the two cells.
- The Plasmid DNA from the donor cell is copied.
- One strand of plasmid DNA is transfered into the recipient cell.
- Both cell synthesize the complementary DNA strand.
- The recipient cell becomes F^+ & can also transfer DNA to other bacteria.



Types Of Conjugation

① $F^+ \times F^-$ Conjugation

Transfers of F plasmid from donor to recipient cell

② Hfr Conjugation

Transfer of chromosomal genes along with F factor.

③ F' conjugation

Transfer of F plasmid carrying some bacterial genes.

Importance Of Conjugation

- Produces genetic variation in bacteria
- Transfers antibiotic resistance genes
- Important in recombinant DNA technology
- Used in microbial genetics & biotechnology.

PLASMIDS

- Plasmids are small, circular, double-stranded DNA molecules found mainly in bacteria.
- They're separate from the main chromosomal DNA & replicate independently.

FEATURES OF PLASMIDS

- Circular DNA structure
- Double-stranded DNA
- Self-replicating (have their own origin of replication)
- Usually small in size (few kb)
- Provides advantages such as antibiotic resistance to bacteria.

TYPES OF PLASMIDS

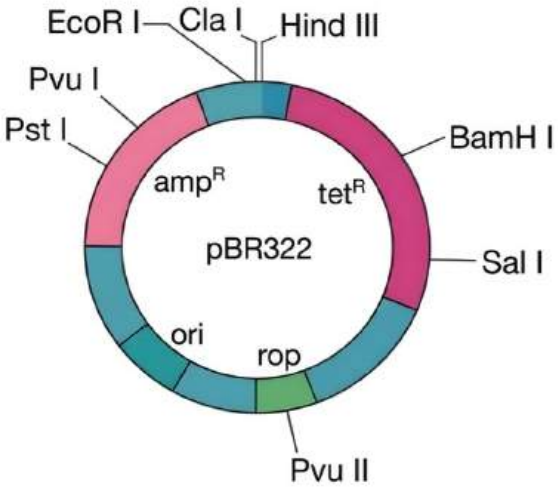
① Fertility (F) Plasmids: Helps in bacterial conjugation

② Resistance (R) Plasmids: Provide antibiotic resistance

③ Col Plasmids: Produce bacteriocins (Toxins to kill other bacteria)

④ Degradative Plasmids: Helps in metabolism of unusual substance

⑤ Virulence Plasmids: Increase pathogenecity



IMPORTANCE

- Transfer antibiotic resistance
- Used as vectors in genetic engineering
- Important in recombinant DNA technology
- Help bacteria survive under harmful conditions.

TRANSPOSONS

- Transposons, also called jumping genes, are movable DNA sequences that can change their position from one place to another within the genome.
- They're fist discovered by Barbara McClintock.
- Transposons may move from:
  - One part of chromosome to another
  - Chromosome to plasmid
  - Plasmid to chromosome
  - The movement is called Transposition.

CHARACTERISTICS OF TRANSPOSONS

- Mobile genetic elements
- Present in bacteria & eukaryotes
- Can replicate & insert into new sites
- May carry antibiotic resistance genes
- Cause mutations & genetic variations

STRUCTURE OF TRANSPOSONS

① Transposase gene

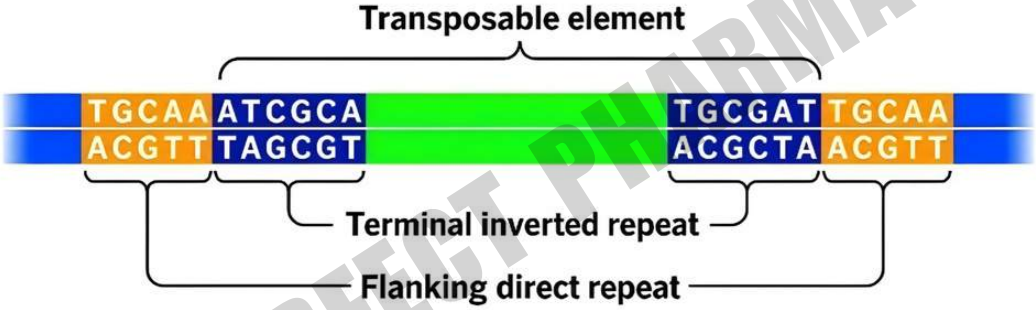
- Codes for the enzyme transposase.
- Helps in cutting & inserting the transposon into another DNA site.

② Inverted Repeat (IR) Sequence

- Short repeated sequences present at both ends.
- Recognized by transposase enzyme.

③ Additional genes

- Present in composite & non composite transposons.
- Example: Antibiotic resistance gene.



Types Of Transposons

- Transposons can be classified on following basis:

① On the basis of structure
② On the basis of movement
③ On the basis of ability to move
④ On the basis of mechanism

① On The Basis Of Structure

ⓐ Insertion Sequences

- IS elements are the simplest type of transposons.
- They contain transposase gene & inverted repeat sequences
- They do not carry extra genes
- Example: IS1, IS2

ⓑ Composite Transposons

- Composite transposons contain two IS elements with extra genes
- such as antibiotic resistance gene present b/w them.
- Example: Tn5, Tn10

ⓒ Non - composite Transposons

- These transposons don't contain complete IS elements at both ends.
- They carry additional functional genes.
- Example: Tn3

IS element

Composite transposon

Non-composite transposon

② Based On Movement

ⓐ DNA Transposons

- These transposons move directly in the form of DNA.
- They usually follow cut & pase transposition.

ⓑ Retrotransposons

- Retrotransposons move through an RNA intermediate.
- DNA is first covered into RNA
- Reverse transcriptase again converts RNA into DNA.
- They usually follow copy-&-paste mechanism
- Increase genome size

③ Based On Ability To Move

ⓐ Autonomous Transposons

- These transposons can move by themselves.
- They contain transposase gene.

ⓑ Non-autonomous Transposons

- These transposons can't move independently.
- They lack transporase gene
- They depend on enzymes produce by autonomous transposons for movement.

Mechanism Of Transposition

- The movement of transposons is called transposition.
- It occurs mainly in two ways:

① Cut & Paste Mechanism

- Transposon is removed from one site.
- Inserted into another site
- Original copy is lost.

② Copy & Paste Mechanism

- A new copy of transposon is formed.
- One copy remains at original site.
- Another copy inserts into new site.

Importance

- Causes genetic variation
- Transfer antibiotic resistance
- Used in genetic engineering & biotechnology
- Help in evolution and mutation studies.

Microbial Biotransformation

- Microbial biotransformation is the chemical modification of substances by microorganisms such as bacteria, fungi & yeast using thier enzymes.
- In simple words, microbes convert one compound into another compound through biochemical reactions.
- It is widely used in Pharmaceutical industry for pharmaceutical preparations.

Importance Of Microbial Biotransformation

- Produces useful drugs
- Eco-Friendly process
- Highly specific reactions
- Works under mild temperature & pH
- Used in steroid & antibiotic production

Types Of Biotransformation Reactions

① Oxidation Reaction

- Oxidation involves addition of oxygen or removal of hydrogen
- It is mainly used in vinegar production by alcohol oxidation.
- Example: Ethanol ⟶ Acetaldehyde by Acetobacter.

② Reduction Reaction

- Reduction involves addition of Hydrogen or removal of Oxygen.
- It is mainly used in fermentation industry.
  Example: Acetaldehyde → Ethanol using Saccharomyces cerevisiae (Yeast)

③ Hydrolysis Reduction

- Hydrolysis involves breakdown of complex molecules by the addition of water.
- It is mainly used in food industry & industrial enzymes production.
  Example: Starch → Glucose by Bacilus Subtilis.

④ Hydroxylation Reaction

- Hydroxylation involves introduction of a hydroxyl (-OH) group into a compound.
- It is mainly used in steroid drug production.
  Example: Progesterone → 11α - Hydroxyprogesterone by Rhizopus nigricans.

⑤ Dehydrogenation Reaction

- Dehydrogenation involves removal of Hydrogen atoms.
  Example: Succinate → Fumerate by Pseudomonas bacteria.

⑥ METHYLATION REACTION

- Methylation involves addition of Methyl group ($-CH_3$)
  Example: Catechol → Guaiacol by Aspergillus species.

⑦ DEMETHYLATION REACTION

- Demethylation involves removal of Methyl group.
  Example: Caffiene → Theophylline using Pseudomonas putida.

⑧ ISOMERIZATION REACTION

- Isomerisation is the conversion of one isomer into another.
  Example: Glucose → Fructose by Streptomyces Fructose

⑨ ACETYLATION REACTION

- In Acetylation: Acetyl group is added.
  Example: Sulfonamide → Acetyl sulfonamide

⑩ ESTERIFICATION REACTION

- In esterification Acid & alcohol combine to form ester.
  Example: Organic acid + Alcohol → Ester using Saccharomyces cerevisiae.

Sources Of Biocatalyst

① Growing Cells

- Growing cells are actively dividing microbial cells that carry out biotransformation during their growth phase.
- Enzymes are continuously produced.

Example: Production of antibiotics using Penicillium species.

② Non - Growing Cells

- Non - growing cells are resting microbial cells that do not divide but still possess active enzymes for biotransformation.

Example: Steroid transformation using resting bacterial cells.

③ Immobilized Cells

- Immobillized cells are microbial cells trapped or attached to a solid support while retaining catalytic activity.
- In this cells can be reused.

Example: Ethanol production using immobillized yeast cells.

④ Immobilized Enzymes

- Immobilized enzymes are enzymes fixed onto or within an insoluble support material while maintaining their activity.

Example: Immobilized glucose isomerase used in high fructose corn syrup production.

APPLICATIONS

① Pharmaceutical Industry

- Steroid conversion
- Antibiotic modification
- Vitamin synthesis

② Environmental Applications

- Biodegradation of pollutants
- Wastewater treatment
- Detoxification of toxic chemicals

③ Food Industry

- Fermentation
- Flavor production
- Organic acid synthesis

④ Agriculture

- Pesticide degradation
- Biofertilizer production

MUTATION

- A mutation is a sudden, permanent & heritable change in the genetic material (DNA or RNA) of an organism.
- It changes the sequence of nucleotides in genes & may alter the structure or function of proteins.
- Mutations is the main source of genetic variation & plays an important role in:
  - Evolution
  - Adaptation
  - Drug resistance
  - Genetic disorders
  - Cancer development

TYPES OF MUTATION

GENE MUTATION

- Point Mutation
- Frameshift Mutation

CHROMOSOMAL MUTATION

- Deletion
- Duplication
- Inversion
- Translocation

GENE MUTATION

- Gene mutation are changes that occur in the small nucleotide sequence of DNA.
- They're of two types:

① Point Mutation
② Frameshift Mutation

① POINT MUTATION

A Point mutation is a type of gene mutation in which a single nucleotide base pair in DNA is changed or replaced.
It can be either spontaneous or induced.
It is further divided into two categories on following basis:

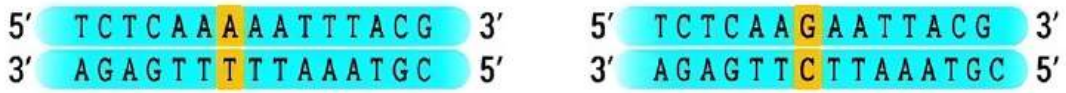
ⓐ On the basis of Base Substitution
ⓑ On the basis of change in function

ⓐ ON THE BASIS OF BASE SUBSTITUTION

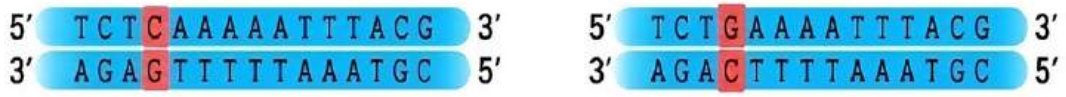
① Transition: • In this substitution occurs b/w same types of bases:
- Purine ⟷ Purine
- Pyrimidine ⟷ Pyrimidine

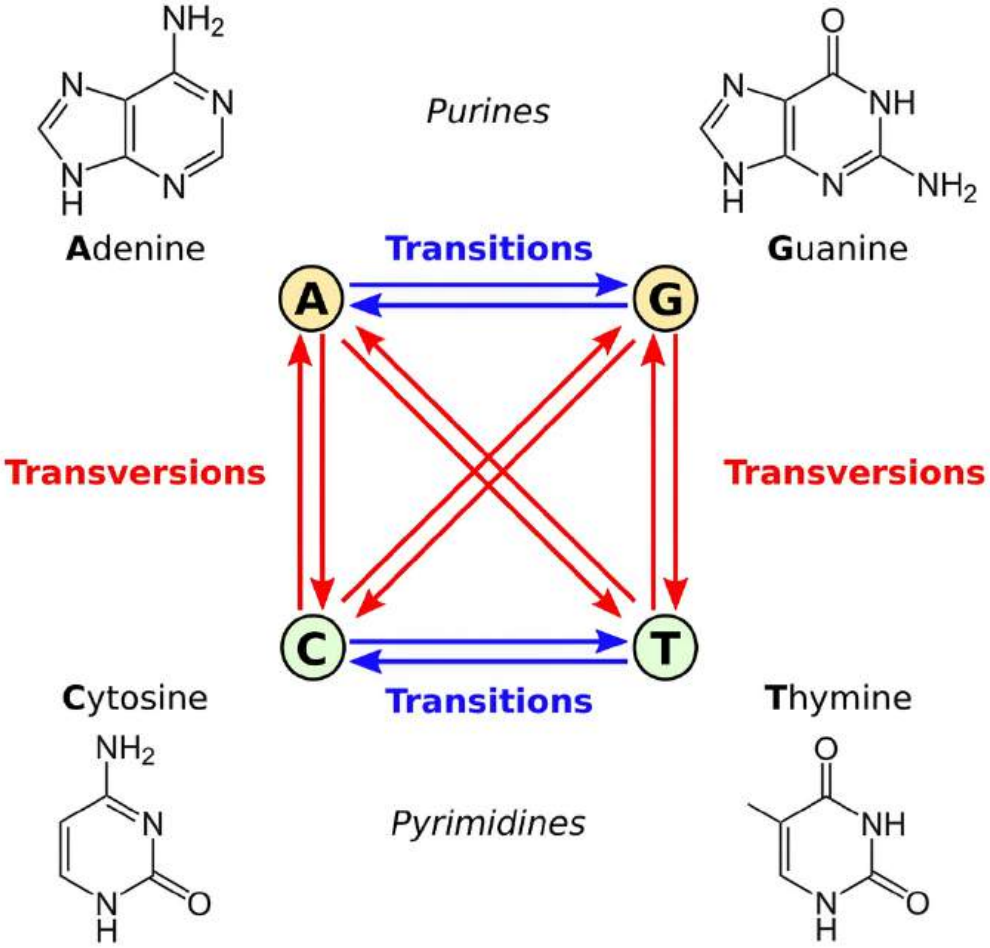
② Transversion: • In this substitution occurs b/w different types of bases:
- Purine ⟷ Pyrimidine

a) Transition mutation



b) Transversion mutation





② On The Basis Of Change In Function

① **Silent Mutation:**
- In this, the change in DNA square doesn't alter the resulting amino acid.
- Example: UUU → UUC
- Both code for Phenylalanine

② **Missence Mutation:**
- In this, change in DNA sequence results in formation of different amino acids.
- Example: Normal coden → GAG (Glutamic Acid)
  Mutated coden → GTG (valine)

③ **Nonsence Mutation:**
- In this, change in DNA sequence creates a STOP Codon
- This result in the formation of short, non-functional protein
- Example: UAU → UAA
- UAA is a stop codon.

② Frameshift Mutation

- A Frameshift Mutation is a type of mutation caused by the insertion or deletion of one or more nucleotides in DNA, which changes the normal reading frame of codons during protein synthesis.
- Since codons are read in groups of three bases, adding or removing bases shifts the entire reading frame.

Types Of Frameshift Mutation

@ Insertion Mutation

- In this Mutation 1 or more nucleotides are added into the DNA sequence.
- Example: Normal : AUG - AAA - GGC
  Mutated : AUG - UAA - AGG - C

ⓑ Deletion Mutation

- In this mutation 1 or more nucleotides are removed from DNA
- Example: Normal : AUG - AAA - GGC
  Mutated : AUG - AAG - GC

CHROMOSOMAL MUTATION

- Chromosomal mutation refers to changes in the structure of chromosomes.
- These mutations affect large segments of DNA & may involve several genes.
- The major types of chromosomal mutations are:

① DELETION

- In deletion, a segment of the chromosome is lost or removed.

② DUPLICATION

- In duplication, a segment of the chromosome is repeated one or more times.

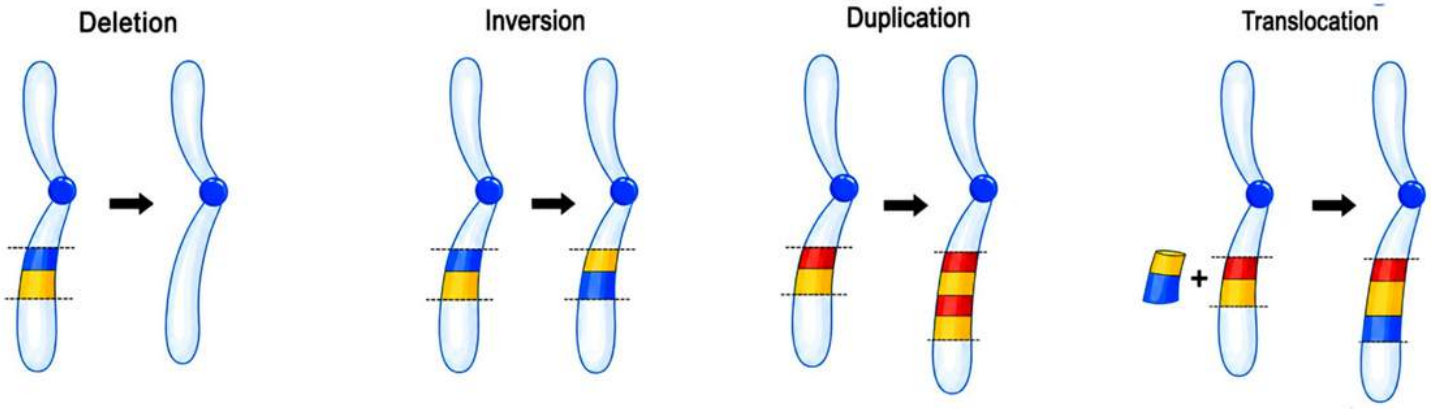
③ INVERSION

- In inversion, a chromosome segment breaks off, rotates 180°, and reattaches in the reverse direction.

④ TRANSLOCATION

- In translocation, a chromosome segment breaks & attaches to another non-homologous chromosome.

Chromosomal mutation



MUTANTS

- Mutants are organisms (Bacteria, Viruses, Fungi) or cells that undergo a mutation, resulting in a permanent change in their genetic material (DNA).
- These changes may alter the organism's structure, metabolism, growth, or resistance properties.

TYPES OF MUTANTS

① MORPHOLOGICAL MUTANTS

- These mutants show changes in shape, size, color or colony appearance.
- Examples: Change in bacterial color, shape & size.

② LETHAL MUTANTS

- These mutations are so harmful that they cause death of the organism or cell.
- Example: Mutation affecting vital metabolic enzymes.

③ Condition Lethal Mutants

- These mutants survive only under certain environmental conditions but die under others.
- Example:
  - Temperature-sensitive Mutants: survive at one temperature but not at another.
  - Nutritional Mutants: require special nutrients for growth

④ Biochemical Mutants

- These mutants have defects in metabolic pathways due to enzyme changes.
- They became unable to synthesize certain nutrients.
- Example: Auxotrophic mutants that can't produce amino acids or vitamins.

MUTAGENS

- A Mutagen is any physical, chemical, or biological agent that causes changes (mutations) in the genetic material (DNA) of an organism.

TYPES OF MUTAGEN

① PHYSICAL MUTAGENS

- These include radiations that damage DNA.
- They cause DNA breaking or abnormal bonding b/w bases.
- Examples: - X-Rays
  - Gamma Rays
  - Ultravoilet (UV) Rays

② CHEMICAL MUTAGENS

- They're chemicals that react with DNA & alter nucleotide sequences.
- They cause base substitution, deletion, or insertion mutations.
- Examples: - Nitrous Acid
  - Mustard Gas
  - Ethidium Bromide

③ Biological Mutagens

- They're living organisms or biological elements that induce mutations.
- Examples: - Viruses
  - Transposons

THANK YOU

FOR CHOOSING IMPERFECT PHARMACY AS YOUR STUDY PARTNER

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