Subject /Course	Pharmaceutical Biotechnology
Subject/Course ID	BP605T
Module Title	Immuno-Blotting Techniques, Mutation
Course coordinator	ChamanpreetKaur
Mobile No.	7087592588
Email id	Chamanpreetmann495@gmail.com

Learning Outcome of module-4

LO	Learning Outcome (LO)	Course Outcome Code
LO1	Upon completion of topic, students will be able to understand about various immune-blotting techniques	BP605.4
LO2	They will be familiar with genetic organization of eukaryotes and prokaryotes	BP605.4
LO3	They will have knowledge about microbial biotransformation and its applications	BP605.4
LO4	They shall be able to understand the concept of mutation and its type	BP605.4

MODULE CONTENT TABLE

Topic
• Immuno-Blotting Techniques-ELISA • Immuno-Blotting Techniques- Western blotting • Immuno-Blotting Techniques- Southern blotting • Genetic organization of Eukaryotes and Prokaryotes • Microbial genetics including transformation, transduction, conjugation, plasmids and transposons • Introduction to Microbial biotransformation and applications • Types of mutation/mutants.

IMMUNOBLOTTINGTECHNIQUES

ELISA

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying peptides, proteins, antibodies and hormones. In an ELISA, an antigen must be immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

ELISAs are typically performed in 96-well (or 384-well) polystyrene plates, which will passively bind antibodies and proteins. It is this binding and immobilization of reagents that makes ELISAs so easy to design and perform. Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay. This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.

Principle

Enzyme-linked immunosorbent assays (ELISA) principles are very similar to other immunoassay technologies. ELISA's rely on specific antibodies to bind the target antigen, and a detection system to indicate the presence and quantity of antigen binding. In order to maximize the sensitivity and precision of the assay, the plate must be carefully coated with high-affinity antibodies – a process that Boster Bio has mastered.

Procedure

An ELISA begins with a coating step, in which the first layer, consisting of a target antigen or antibody, is adsorbed onto a 96-well polystyrene plate. This is followed by a blocking step in which all unbound sites are coated with a blocking agent. Following a series of washes, the plate is incubated with enzyme- conjugated antibody. Another series of washes removes all unbound antibody. A substrate is then added, producing a calorimetric signal. Finally, the plate is read.

Because the assay uses surface binding for separation, several washes are repeated in each ELISA step to remove unbound material. During this process, it is essential that excess liquid is removed in order to

prevent the dilution of the solutions added in the next assay step. To ensure uniformity, specialized plate washers are often used.

ELISAs can be quite complex and include multiple intervening steps, especially when measuring protein concentration in heterogeneous samples such as blood. The most complex and varying step in the overall process is detection, where multiple layers of antibodies can be used to amplify signal.

ELISA Types

ELISAs can be performed with a number of modifications to the basic procedure: direct, indirect, sandwich or competitive. The key step, immobilization of the antigen of interest, can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected either directly (enzyme-labeled primary antibody) or indirectly (enzyme-labeled secondary antibody). The detection antibodies are usually labeled with alkaline phosphatase (AP) or horseradish peroxidase (HRP). A large selection of substrates is available for performing the ELISA with an HRP or AP conjugate. The choice of substrate depends upon the required assay sensitivity and the instrumentation available for signal-detection (spectrophotometer, fluorometer or luminometer).

Among the standard assay formats, where differences in both capture and detection were the concern, it is important to differentiate between the particular strategies that exist specifically for the detection step. However an antigen is captured to the plate (by direct adsorption to the surface or through a pre-coated "capture" antibody, as in a sandwich ELISA), it is the detection step (as either direct or indirect detection) that largely determines the sensitivity of an ELISA.

Blotting Techniques

Introduction:

Blotting is used in molecular biology for the identification of proteins and nucleic acids and is widely used for diagnostic purposes. This technique immobilizes the molecule of interest on a support, which is a nitrocellulosic membrane or nylon. It uses hybridization techniques for the identification of the specific nucleic acids and genes. The blotting technique is a tool used in the identification of biomolecules such ad DNA, mRNA and protein during different stages of gene expression. Protein synthesis involves expression of a DNA segment which gets converted to mRNA to produce the respective protein. Molecules such as DNA, RNA and proteins are subjected to biochemistry analysis which are separated using blotting techniques. In the case of a cell, these molecules are present

altogether and hence with the help of blotting scientists are able to recognise a specific molecule out of all others. Blotting is performed by allowing a mixture of molecules of interest pass through a block of gel which separates the molecules based on their molecular sizes. The hence processed molecules are required to be hard- pressed against a suitable membrane which will in turn transfer the molecules from the gel onto a suitable membrane (nylon, nitrocellulose or PVDF) via capillary action. After the molecules are transferred to the membrane their position does not change.

Southern blotting was introduced by Edwin Southern in 1975 as a method to detect specific sequences of DNA in DNA samples. The other blotting techniques emerged from this method have been termed as Northern (for RNA), Western (for proteins), Eastern (for post-translational protein modifications) and South-western (for DNA-protein interactions) blotting.

Subtypes of blotting such as northern, western & southern depend upon the target molecule that is being sought. When a DNA sequence is the foundation or code for a protein molecule, the particular DNA molecule of interest can be blotted using Southern Blotting technique. During gene expression, when the DNA is expressed as mRNA for a protein production, this process can be identified by Northern blotting. Finally, the coded mRNA produces the concerned protein, this protein identification can be done by Western Blotting.

Western blot

A western blot is a laboratory method used to detect specific protein molecules from among a mixture of proteins. This mixture can include all of the proteins associated with a particular tissue or cell type. Western blots can also be used to evaluate the size of a protein of interest, and to measure the amount of protein expression. This procedure was named for its similarity to the previously invented method known as the Southern blot.

The first step in a western blot is to prepare the protein sample by mixing it with a detergent called sodium dodecyl sulfate, which makes the proteins unfold into linear chains and coats then with a negative charge. Next, the protein molecules are separated according to their sizes using a method called gel electrophoresis. Following separation, the proteins are transferred from the gel onto a blotting membrane. Although this step is what gives the technique the name "western blotting," the term is typically used to describe the entire procedure.

Once the transfer is complete, the membrane carries all of the protein bands originally on the gel. Next, the membrane goes through a treatment called blocking, which prevents any nonspecific

reactions from occurring. The membrane is then incubated with an antibody called the primary antibody, which specifically binds to the protein of interest. Following incubation, any unbound primary antibody is washed away, and the membrane is incubated yet again, but this time with a secondary antibody that specifically recognizes and binds to the primary antibody. The secondary antibody is linked to a reporter enzyme that produces color or light, which allows it to be easily detected and imaged. These steps permit a specific protein to be detected from among a mixture of proteins.

Southern Blotting

The first of these techniques developed was the Southern blot, named after Dr. Edwin Southern who developed it to identify specific DNA sequences. Southern blotting is a detection technique used to find the target DNA sequences in the DNA sample in the field of molecular biology. The process starts from electrophoresis of DNA molecules which are hybridized in a blotting membrane followed by a transfer step where DNA from gel is transferred onto the blotting membrane.

Principle

Restriction endonucleases, which is an enzyme, is used to break the DNA into small fragments. These fragments are then separated using electrophoresis. The fragments achieved is then classified according to their size (kDa). Thus, DNA fragments are transferred to the blotting paper where it is incubated with probes. Probes used in the Southern blotting can be highly selective. They can selectively bind with a resolution of 1 in a million and the characteristics to bind to the intended target fragments.

Organization of Genetic Material in Prokaryotes and Eukaryotes

The vast majority of an organism's genome is organized into the cell's **chromosomes**, which are discrete DNA structures within cells that control cellular activity. Recall that while eukaryotic chromosomes are housed in the membrane-bound nucleus, most prokaryotes contain a single, circular Chromosome that is found in an area of the cytoplasm called the **nucleoid**. A chromosome may contain several thousand genes.

Organization of Eukaryotic Chromosome

Chromosome structure differs somewhat between eukaryotic and prokaryotic cells. Eukaryotic chromosomes are typically linear, and eukaryotic cells contain multiple distinct chromosomes. Many eukaryotic cells contain two copies of each chromosome and, therefore, are **diploid**.

The length of a chromosome greatly exceeds the length of the cell, so a chromosome needs to be packaged into a very small space to fit within the cell. For example, the combined length of all of the 3 billion base pairs[1] of DNA of the human genome would measure approximately 2 meters if completely stretched out, and some eukaryotic genomes are many times larger than the human genome. DNA **supercoiling** refers to the process by which DNA is twisted to fit inside the cell. Supercoiling may result in DNA that is either underwound (less than one turn of the helix per 10 base pairs) or overwound (more than one turn per 10 base pairs) from its normal relaxed state. Proteins known to be involved in supercoiling include **topoisomerases**; these enzymes help maintain the structure of supercoiled chromosomes, preventing overwinding of DNA during certain cellular processes like DNA replication.

During **DNA packaging**, DNA-binding proteins called **histones** perform various levels of DNA wrapping and attachment to scaffolding proteins. The combination of DNA with these attached proteins is referred to as **chromatin**. In eukaryotes, the packaging of DNA by histones may be influenced by environmental factors that affect the presence of methyl groups on certain cytosine nucleotides of DNA. The influence of environmental factors on DNA packaging is called **epigenetics**. Epigenetics is another mechanism for regulating gene expression without altering the sequence of nucleotides. Epigenetic changes can be maintained through multiple rounds of cell division and, therefore, can be heritable.

Organization of Prokaryotic Chromosomes

Chromosomes in bacteria and archaea are usually circular, and a prokaryotic cell typically contains only a single chromosome within the nucleoid. Because the chromosome contains only one copy of each gene, prokaryotes are haploid. As in eukaryotic cells, DNA supercoiling is necessary for the genome to fit within the prokaryotic cell. The DNA in the bacterial chromosome is arranged in several supercoiled domains. As with eukaryotes, topoisomerases are involved in supercoiling DNA. DNA gyrase is a type of topoisomerase, found in bacteria and some archaea, that helps prevent the overwinding of DNA. (Some antibiotics kill bacteria by targeting DNA gyrase.) In addition, histone-like proteins bind DNA and aid in DNA packaging. Other proteins bind to the origin of replication, the location in the chromosome where DNA replication initiates. Because different regions of DNA are packaged differently, some regions of chromosomal DNA are more accessible to enzymes and thus may be used more readily as templates for gene expression. Interestingly, several bacteria, including

Helicobacter pylori and *Shigella flexneri*, have been shown to induce epigenetic changes in their hosts upon infection, leading to chromatin remodeling that may cause long-term effects on host immunity.

MICROBIAL GENETICS

Horizontal gene transfer (HGT) is the movement of genetic material between organisms. It plays a key role in bacterial evolution and is the primary mechanism by which bacteria have gained antibiotic resistance and virulence. Scientists have studied how HGT occurs in nature and have learned how to introduce genetic materials into cells in the lab.

The introduction of foreign DNA or RNA into bacteria or eukaryotic cells is a common technique in molecular biology and scientific research. There are multiple ways foreign DNA can be introduced into cells including transformation, transduction, conjugation, and transfection. Transformation, transduction, and conjugation occur in nature as forms of HGT, but transfection is unique to the lab. Let's take a look at these different methods of DNA insertion.

Transformation

Transformation is the uptake of genetic material from the environment by bacterial cells. In nature, this genetic material often comes from adjacent lysed bacteria and can include plasmid DNA or fragmented DNA released into the environment. Various factors promote natural transformation in different bacteria such as growth phase of the cells (Baltrus and Guillemin, 2006) or the presence of specific substances.

Though not all bacteria are naturally competent to take up DNA, they can be made competent through chemical manipulation in the lab. This is commonly done using calcium chloride which permeabilizes the cell membrane so the bacteria can easily uptake your plasmid of interest. Scientists can also use electroporation, the application of an electrical charge to cells, to increase cell membrane permeability and thus transformation efficiency. Check out Addgene's blog to learn about making your own competent cells and our protocols page to learn about bacterial transformation in the lab.

Transduction

Transduction occurs when foreign DNA or RNA is introduced into bacterial or eukaryotic cells via a virus or viral vector.

One example are bacteriophages that attach to bacterial membranes and inject their genetic material into the cell. Once inside, phages can follow one of two different life cycles: lytic or lysogenic. Lytic phages hijack the bacterial hosts machinery to make more viral particles. Eventually the cell lyses releasing the newly formed viral particles that can infect other bacteria. In the lysogenic cycle, the phage's genetic material is incorporated into the host's genome at a particular integration site. The integrated phage remains dormant until it is triggered to enter the lytic cycle.

During both of these life cycles bacterial DNA can be accidentally packaged into the newly created phages. Transfer of this DNA to another cell is referred to as transduction. Transferred DNA once inside the infected bacterium can either exist as transient extrachromosomal DNA, like a plasmid, or it can integrate into the host bacterium's genome through homologous or site directed recombination.

Transduction is a common tool used by scientists to introduce different DNA sequences of interest into a bacterial cell or a host's genome. To do this scientists commonly use phagemids, a DNA cloning vector that contains both bacteriophage and plasmid properties. The phagemids are packaged into replication- incompetent phage particles with assistance from a 'helper' phage prior to transduction.

Scientists also use transduction to introduce foreign DNA into eukaryotic cells, like mammalian cell lines. This can be done with lentiviral and Adeno Associated Viruses (AAV). Lentiviral and AAV can be used to create both transient cell lines, where a gene of interest is expressed but not integrated into the genome and stable cell lines, where foreign DNA is incorporated into the cell's genome and is thus passed down through cell division.

Bacterial conjugation

Conjugation was the first extensively studied method of gene transfer and was discovered in 1946 by Joshua Lederberg and Edward Tatum when they observed genetic recombination between two nutritional deficient E. coli strains that resulted in a wild type E. coli.

During conjugation, genetic material is transferred from a donor bacterium to a recipient bacterium through **direct contact**. The donor bacterium contains a DNA sequence called the Fertility factor (F-factor). The F-factor is found on an episome, a piece of DNA that can replicate on its own or be integrated within a bacterial chromosome and allows the donor bacterium to make a small "bridge" or sex pilus that attaches to the recipient cell drawing it close. Once in contact the donor can transfer genetic material to the recipient bacterium. The genetic material transferred is commonly a plasmid and can infer genetic advantages such as antibiotic resistance.

Plasmids

In addition to the bacterial chromosome, many bacteria often contain small nonchromosomal DNA molecules called plasmids. Plasmids usually contain between 5 and 100 genes. Plasmids are not essential for normal bacterial growth and bacteria may lose or gain them without harm. They can, however, provide an advantage under certain environmental conditions. For example, under normal environmental growth conditions, bacteria are not usually exposed to antibiotics and having a plasmid coding for an enzyme capable of denaturing a particular antibiotic is of no value. However, if that bacterium finds itself in the body when the particular antibiotic that the plasmid-coded enzyme is able to degrade is being given to treat an infection, the bacterium containing the plasmid is able to survive and grow.

Structure and Composition

Plasmids are small molecules of double stranded, helical, non-chromosomal DNA. In most plasmids the two ends of the double-stranded DNA molecule that make up plasmids covalently bond together forming a physical circle. Some plasmids, however, have linear DNA. Plasmids replicate independently of the host chromosome, but some plasmids, called episomes, are able to insert or integrate into the host cell's chromosome where their replication is then regulated by the chromosome.

Although some plasmids can be transmitted from one bacterium to another by transformation and by generalized transduction, the most common mechanism of plasmid transfer is conjugation. Plasmids that can be transmitted by cell-to-cell contact are called conjugative plasmids. They contain genes coding for proteins involved in both DNA transfer and and the formation of mating pairs.

Functions

Plasmids code for synthesis of a few proteins not coded for by the bacterial chromosome. For example, R-plasmids, found in some Gram-negative bacteria, often have genes coding for both production of a conjugation pilus (discussed later in this unit) and multiple antibiotic resistance. Through a process called conjugation, the conjugation pilus enables the bacterium to transfer a copy of the R-plasmids to other bacteria, making them also multiple antibiotic resistant and able to produce a conjugation pilus. In addition, some exotoxins, such as the tetanus exotoxin, *Escherichia coli* enterotoxin, and *E. coli* shiga toxin discussed later in Unit 2 under Bacterial Pathogenicity, are also coded for by plasmids. Thousands of different plasmids are known to exist.

Transposons

Transposons (transposable elements or "jumping genes") are small pieces of DNA that encode enzymes that transpose the transposon, that is, move it from one DNA location to another, either on the same molecule of DNA or on a different molecule. Transposons may be found as part of a bacterium's nucleoid (conjugative transposons) or in plasmids and are usually between one and twelve genes long. A transposon contains a number of genes, coding for antibiotic resistance or other traits, flanked at both ends by insertion sequences coding for an enzyme called transpoase. Transpoase is the enzyme that catalyzes the cutting and resealing of the DNA during transposition. Thus, such transposons are able to cut themselves out of a bacterial nucleoid or a plasmid and insert themselves into another nucleoid or plasmid and contribute in the transmission of antibiotic resistance among a population of bacteria.

Plasmids and conjugative transposons are very important in horizontal gene transfer in bacteria. Horizontal gene transfer , also known as lateral gene transfer, is a process in which an organism transfers genetic material to another organism that is not its offspring. The ability of *Bacteria* and *Archaea* to adapt to new environments as a part of bacterial evolution most frequently results from the acquisition of new genes through horizontal gene transfer rather than by

the alteration of gene functions through mutations. (It is estimated that as much as 20% of the genome of *Escherichia coli* originated from horizontal gene transfer.)

MICROBIAL BIOTRANSFORMATION

Biotransformation is the process by which an organism or its enzyme bring out chemical changes on compounds that are not part of their metabolism and they result in the formation of novel or useful products that are often difficult or impossible to obtain by conventional chemical means. The total chemical transformation of one steroid to another not only requires many stages but an expensive process although provide only low yield.

The biotransformation is used for the preparation of products of defined chemical structure that are related to the substrate or starting material for the reaction by only a small number of chemical changes and in many cases the changes are brought about by the action of only a single enzyme. Biotransformation reactions reported in the chemical literature of nineteenth century was developed as part of the synthetic routes for the production of L-ascorbic acid (vitamin C) and ephedrine.

Oxidation of alcohol to acetic acid by bacterium *Acetobacer xylinum*; oxidation of glucose to gluconic acid by *Acetobacter acetii*, sorbitol to sorbose by Acetobacter but were not fully utilized. Biotransformation is realised beyond doubt in early part of 20th century when the conversion of D-sorbitol to L-sorbose by *Acetobacter suboxidans* and benzaldehyde to phenyl (lactyl carbinol) by yeast. Mamoli and Vercellone (1937) were the first to demonstrate the oxidation of nuclear hydroxyl group of steroid and reduction of nuclear double bond of steroid by yeast.

Welsch and Hongshem (1948) have not only confirmed but also enlarged the above result by employing a Streptomces sp. Kramli and Horvath (1949) could oxidize cholesterol to hydroxyl cholesterol by Pencillium roseum and Azatobacter sp. Hench and his associates (1949) demonstrated the curative effect of cortisone on rheumatoid arthritis was possible only by introducing O2 at its 11th carbon atom with the help of Rhizopus arrhizus which chemically was very difficult.Subsequently several people could accomplish this task by using different Fungi, Actinomycetes and Bacteria. Presently varieties of biocatalysts are in use for carrying biotransformation reaction.

Types of Biotransformation Reactions

i. **Oxidation:** Hydroxylation, epoxidation, dehydrogenation of C-C bonds, oxidation of alcohol and aldehydes, oxidative degradation of alkyl, carboxyalkyl or ketoalkyl chains, oxidative

removal of substituents, oxidative deamination, oxidation of heterofunctions and oxidative ring fission.

ii. Reductions:

Reduction of organic acids, aldehydes, ketones and hydrogenation of C-C bonds, reduction of heterofunctions, dehydroxylations and reductive elimination of substituents.

iii. Hydrolysis:

Hydrolysis of esters, amines, amides, lactones, ethers, lactams etc.

iv. Condensation:

Dehydration, O- and N-acylation, glycosidation, esterification, lactomization and amination.

v. Isomerization:

Migration of double bonds or oxygen functions, racemization, rearrangements, formation of C-C bonds or hetero-atom bonds.

vi. Mixed Reactions:

Hydroxylation with reduction; Hydroxylation with oxidation; hydroxylation with side chain degradation; rupture of C-C linkages with oxidation of side chain.

Methods of Biotransformation:

Transformation of organic compounds may be accomplished by use of microorganism, isolated enzyme, immobilization techniques and solvent selection. The submerged fermentation is carried out in a stainless steel tank with minimal nutritional quantities to allow maximum transformation and use of easy extraction and purification of transformation product.

The microorganisms are grown in a suitable medium for 12-72 hrs depending on bacterium and fungus at optimum temperature, pH, aeration and agitation.

The fermentation is carried out in two phases:

1. Growth phase
2. Product formation phase

At the end of suitable incubation period (growth phase), measured quantity of organic compound to be transformed is added to the growing culture. The enzyme produced by the microorganism act upon

the organic compound and does the desired function (product formation phase). At the end of suitable incubation period, the microbial biomass is separated from the fermentation broth. The broth is subjected to separation of both added substratum and product formed by the transformation.

For analysis, if the product samples are obtained at regular intervals upto end of incubation period (1-5 days), which are analysed by using TLC, paper chromatography, gas chromatography or HPLC technique. The extraction of product is done by appropriate organic solvents such as methylenechloride, chloroform, ethylacetate and methyl isobutylketone. Product obtained from cell and substratum should be extracted separately. Different factors like pH, temperature, addition of steroid and mineral content are reported to influence biotransformations. Biotransformations in a large scale are carried out under sterile conditions in aerated and stirred fermenter. The conversion process is being monitored chromatographically or spectroscopically. The process is terminated when a maximal titer is reached. Sterility is required because contamination can suppress the desired reaction, induce the formation of faulty conversion products or cause total substrate break down.

Advantages of microbial transformation

Many benefits can be obtained through microbial transformations studies. The process required in microbial transformation may most probably have the ability to operate at near neutral pH, ambient temperatures and atmospheric pressures . In contrast, chemistry often requires extremes of these conditions which are not exactly environmentally friendly and industrially undesired. Furthermore, extreme pH, temperature and pressure may provide harmful effects toward personnel operating the harsh procedures and may also affect community surrounding the areas.

More importantly biocatalysts are highly reaction specific, enantiomer-specific and regio-specific [6]. This is mainly and directly referring to the chemical structure of a compound one may want to obtain specifically. Many versatile microorganisms can be utilized to carry out extremely specific conversions using substrates of low cost.

MUTATION

A mutation is a change in a genetic sequence. Mutations include changes as small as the substitution of a single DNA building block, or nucleotide base, with another nucleotide base. Meanwhile, larger mutations can affect many genes on a chromosome. Along with substitutions, mutations can also be caused by insertions, deletions, or duplications of DNA sequences.

Some mutations are hereditary because they are passed down to an offspring from a parent carrying a mutation through the germ line, meaning through an egg or sperm cell carrying the mutation. There are also nonhereditary mutations that occur in cells outside of the germ line, which are called somatic mutations. Mutations can be introduced due to mistakes made during DNA replication or due to exposure to mutagens, which are chemical and environmental agents that can introduce mutations in the DNA sequence, such as ultraviolet light. Some mutations do not result in changes in the amino acid sequence of the encoded protein and can be described as silent mutations. Other mutations result in abnormal protein products. Mutations can introduce new alleles into a population of organisms and increase the population's genetic variation.

Types of Mutations

There are three types of DNA Mutations: base substitutions, deletions and insertions.

Base Substitutions

Single base substitutions are called point mutations, recall the point mutation Glu > Val which causes sickle-cell disease. Point mutations are the most common type of mutation and there are two types.

Transition: this occurs when a purine is substituted with another purine or when a pyrimidine is substituted with another pyrimidine.

Transversion: when a purine is substituted for a pyrimidine or a pyrimidine replaces a purine.

Mutant

A Mutant is an organism or a new genetic character arising or resulting from an instance of mutation, which is generally an alteration of the DNA sequence of the genome or chromosome of an organism.

IMPORTANT QUESTION

2 Marks Questions

1. Define Immuno Blotting.
2. What is ELISA?
3. Diffrentiate between eukaryotes and prokaryotes.
4. What are Transposons?
5. Define Microbial Biotransformation.
6. What is Mutation?
7. Define Mutants.
8. Define Transformation, Transduction , Conjugation and Plasmids.

5 Marks Question

1. Explain Northeren Blotting.
2. Write a detail note Western Blotting.
3. Explain applications of Western and Northeren Blotting.
4. Explain Enzyme Linked Immuno Sorbent Assay (ELISA).
5. Explain Transposons.

10 Marks Questions

1. Explain the application of Microbial transformation.
2. What are the factors affecting rate on mutation?
3. Write a note on methods used for isolation of mutants.
4. Explain mutation with its types.
5. Describe in brief the applications of microbes in industry.
6. Explain procedure for transformation, transduction and conjugation.