1 mark Questions

1. ***What is recon?***

The unit of recombination is called recon.

2. ***What is cistron?***

Cistron is the smallest unit of genetic material which coded for a single polypeptide for the transmission of genetic information. Cistron is present in the DNA of prokaryotic and eukaryotic cells.

3. ***What is terminator codon? Give any one example.***

"Terminator codons are the codons responsible for bringing about the termination of the protein chain.(terminator codons are also called nonsense codon). Nonsense codons are a set of 3 nitrogenous bases which do not code for any amino acid and bring about chain termination (end or stop) during protein synthesis. Ex: UAA, UAG & UGA

4. ***Define initiator codon. Give example.***

"Initiator codon is the codon which is located at the 5^1end of m-RNA and responsible for the initiation (starts) of protein synthesis". EX: AUG

5. ***What do you mean by splicing?***

The removal of introns (non-coding portions) from the Hn RNA to make it homogeneous is callled RNA splicing.

6. ***What do you mean by capping?***

The first nucleotide at the 5' end of the Hn RNA is covered by cap of a molecule-7 methyl guanosine triphosphate (or 7mGTP). It is a modified guanosine triphosphate molecule. This event is called capping.

7. ***Define glycosylation.***

Glycosylation is the attachment of carbohydrates to the backbone of a protein through an enzymatic reaction. A protein that is glycosylated is known as a glycoprotein. The two most common types of protein glycosylation are known as N-glycosylation and O-glycosylation.

8. ***Define autophagy.***

A process by which a cell breaks down and destroys old, damaged, or abnormal proteins and other substances in its cytoplasm (the fluid inside a cell). The breakdown products are then recycled for important cell functions, especially during periods of stress or starvation.

(Autophagy, or cellular self-digestion, is **a cellular pathway involved in protein and organelle degradation**, with an astonishing number of connections to human disease and physiology. For

example, autophagic dysfunction is associated with cancer, neurodegeneration, microbial infection and ageing).

9. ***What is dark field microscopy?***

"A microscope in which objects are light at a very low angle from the side so that the background appears dark and the objects shows up against this dark background".

10. What is phase contrast microscopy?

"Phase contrast microscopy is an optical microscopy technique that converts phase shifts in the light passing through a transparent specimen to brightness changes in the image".

11. ***Expand TLC and HPLC.***

TLC: Thin Layer Chromatography. **HPLC:** High Performance Liquid Chromatography.

12. Expand HPLC and GC.

HPLC: High Performance Liquid Chromatography and GC: Gas chromatography

13. Expand CGRP.

CGRP: Calcitonin gene related peptide

14. Expand GTP and UTR.

GTP: Guanosine Tri Phosphate and **UTR:** Untranslated region

15. Expand NC and PVDF.

NC: Neurocellulose and **PVDF:**Polyvinylidene difluoride

16. What is Spectrophotometer?

The spectrophotometer is an optical instrument for measuring the intensity of light relative to wavelength.

17. ***What is Autoradiogram?***

Autoradiography records the distribution of radioactive materials in botanical and histological specimens placed in contact with a photographic emulsion. This technique has been applied to the study of metabolism of plants and animals; it records the activity of organic compounds of radioactive isotopes introduced.

18. Mention the two electrodes of pH meter.

There are two electrodes in pH meter namely, Glass electrode and reference electrode.

19. ***Expand PCR and ELISA.***

PCR: Polymerase Chain Reaction. **ELISA:** Enzyme Linked Immuno Sorbant Assay

20. ***What is translation?***

"Synthesis of a protein (or a polypeptide) molecule by ribosome under the guidance of m-RNA and t-RNA called translation".

21. What is transcription?

"Synthesis of m- RNA using the DNA template is called transcription". (Transcription is the process by which an m-RNA is synthesized from the DNA template).

22. ***Name the fields in which gel-elecrophoresis used.***

Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA, RNA or proteins in a matrix of agarose.

23. What is Agarose gel - Electrophoresis?

Agarose gel electrophoresis is a technique of choice for separating nucleic acids. (Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA, RNA or proteins in a matrix of agarose)

24. Which blotting technique is used to identify the RNA?

Northern blotting is used to identify the RNA.

25***. Which reagent is used to break disulfide bonds in polyacrylamide gel electrophoresis?***

B-mercaptoethenol is used to break disulfide bonds in polyacrylamide gel electrophoresis.

Short Notes (5mark Questions)

1. Explain the translation mechanism in eukaryotes.

Translation involves translating the sequence of a messenger RNA (mRNA) molecule to a sequence of amino acids during protein synthesis. It is the process in which ribosomes in the cytoplasm or ER synthesize proteins after the process of transcription of DNA to RNA.

Translation is a cyclical process, and ribosomal subunits that participate in initiation are derived by recycling of post-termination ribosomal complexes (post-TCs), which comprise and 80S ribosome still bound to mRNA, P-site deacylated tRNA and at least one release factor, eukaryotic release factor 1 (eRF1).

This process involves the transport of amino acids to the ribosome, where they are assembled in the polypeptide chain. After which they will assemble into proteins somewhere in the cytoplasm. This process is accomplished by t RNA and occurs in several stages.

The major steps involved in this processes is as follows.

1. **Activation of amino acids:** Here, when an amino acid(AA) and adenosine triphosphate(ATP), is mediated by aminoacyl synthetases enzyme, the enzyme complex AMP is formed.
2. **Transfer of amino acid to transfer RNA:** The enzyme complex above is reacted with the specific t RNA. As a result, an amino acid is transferred into t RNA and the enzyme and AMP are liberated.
3. **Initiation of polypeptide chain:** Now the charged transfer RNA shifts to the ribosome. In the presence of an initiation factor, it begins in the 5'-end of m RNA. The large and small

are the two subunits of the ribosome. The m RNA binds to the small subunit and the large subunit bind to the small subunit to complete the initiation complex. The aminoacyl t RNA binding site(A site) and peptidyl site(P site) are the two sites of binding in large sub-units.

4. **Elongation of polypeptide chain:** The first codon m RNA binds with the anticodon of the methionyl t RNA complex in the P site. The other aminoacyl t RNA complex with the appropriate amino acids thus enters the ribosome and attaches to A site.
   The peptide bond is formed between the first and second amino acids when the anticodon binds to the second codon in the m RNA in the presence of an enzyme, peptidyl transferase. then, the translocation takes place i.e. when the first amino acids and the t RNA are broken, this t RNA is removed from the P site and the second t RNA from the A-side is pulled to the P site along with the m RNA.
5. **Termination:** The termination is signalled by one of the three-terminal codons-UAG, UGA, UAA. The polypeptide chain, t RNA, and m RNA are released after signalling and the ribosome subunits will be dissociated.

Note: Two classes of polyribosomes have been identified during protein translocation, free polyribosomes, and membrane-bound polyribosomes.

2. Explain the translation mechanism in prokaryotes.

It is the process of synthesis of proteins from messenger RNA transcripts (mRNA) after the process of transcription of DNA to RNA. It takes place in the cytoplasm by specialized organelle known as ribosomes. There are no endoplasmic reticulum in the prokaryotes and ribosomes are suspended in the cytoplasm, whereas endoplasmic reticulum are present in eukaryotes which harbors ribosomes – translation takes place on rough endoplasmic reticulum (RER) in eukaryotes, whereas translation occurs freely in cytoplasm in the prokaryotes. The codons on the mRNA are translated into amino acid sequence which leads to the synthesis of protein. Translation requires a variety of cellular components, such as proteins, RNAs and different small molecules. It has also three main steps:

1. **Initiation** : Formation of mRNA-ribosome complex,
2. **Elongation** : Formation of polypeptide chain complimentary to the mRNA
3. **Termination**: Termination of polypeptide chain 2 The “Central Dogma

Translation process refers to the process of polymerization of amino acids to form a polypeptide. The order and sequence of amino acids are defined by the sequence of bases in the mRNA. The amino acids are joined by a bond which is known as a peptide bond. Formation of a peptide bond requires energy. Therefore, in the first phase itself amino acids are activated in the presence of ATP and linked to their cognate tRNA – a process commonly called as

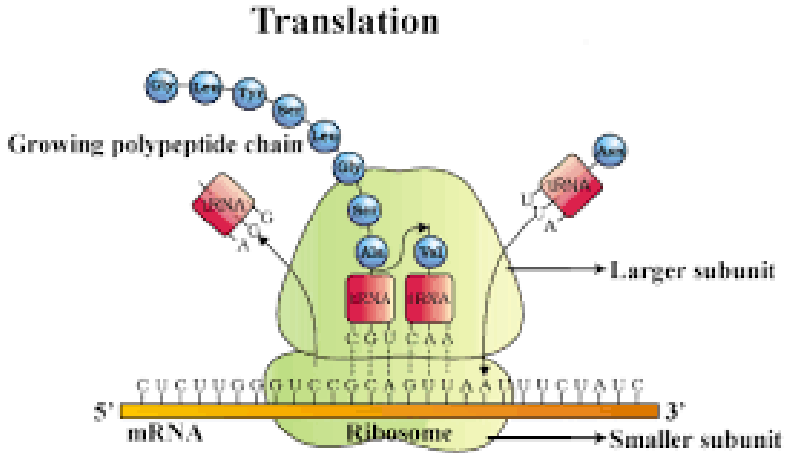
charging of tRNA or aminoacylation of tRNA to be more specific. If two such charged tRNAs are brought close enough, the formation of peptide bond between them. tRNA - the adapter molecule would be favored energetically. The presence of a catalyst would enhance the rate of peptide bond formation. The cellular factory responsible for synthesizing proteins is the ribosome. The ribosome consists of structural RNAs and about 80 different proteins. In its inactive state, it exists as two subunits; a large subunit and a small subunit. When the small subunit encounters an mRNA, the process of translation of the mRNA to protein begins. There are two sites in the large subunit, for subsequent amino acids to bind to and thus, be close enough to each other for the formation of a peptide bond. The ribosome also acts as a catalyst (23S rRNA in bacteria is the enzyme- ribozyme) for the formation of peptide bond A translational unit in mRNA is the sequence of RNA that is flanked by the start codon (AUG) and the stop codon and codes for a polypeptide An mRNA also has some additional sequences that are not translated and are referred as untranslated regions (UTR). The UTRs are present at both 5' -end (before start codon) and at 3' - end (after stop codon). They are required for efficient translation process

Translation has 3 steps

1. Initiation: For initiation, the ribosome binds to the mRNA at the start codon (AUG) that is recognised only by the initiator tRNA

2. Elongation: The ribosome proceeds to the elongation phase of protein synthesis. During this stage, complexes composed of an amino acid linked to tRNA, sequentially bind to the appropriate codon in mRNA by forming complementary base pairs with the tRNA anticodon. The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one, translated into Polypeptide sequences dictated by DNA and represented by mRNA

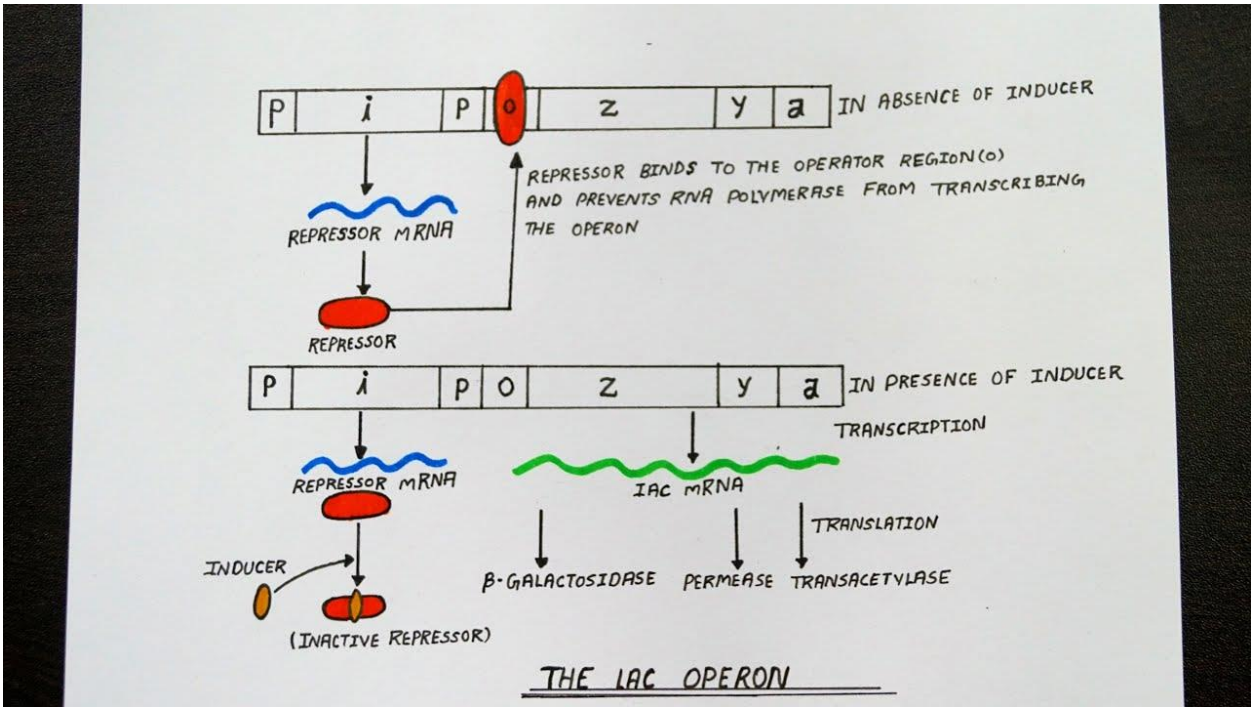
3. Termination: At the end, a release factor binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome.



3. Write a short note on Lac-Operon model.

"Lac operon is an operon or a group of genes with a single promoter that encode genes for the transport and metabolism of lactose in E.coli and other bacteria."

The lac operon is a cluster of genes through which Escherichia coli catabolizes lactose. It was first proposed by **F. Jacob and J. Monod**. The lactose operon (also known as the lac operon) is a set of genes that are specific for uptake and metabolism of lactose and is found in E. coli and other bacteria.
The mechanisms of regulation of expression of genes responsible for coding the involved enzymes for synthesis of β-galactosidase enzyme in E. coli. According to the model, an operon consists of operator gene and structural genes.



Lac operon structure and function:

It is comprised of three structural genes namely, a promotor, an operator and a repressor , and a terminator. The structural genes make proteins called enzymes that the bacteria need to metabolize (digest) lactose sugar.

Lac Operon Concept:

Gene regulation in prokaryotes can be explained with the help of the Lac Operon model. Here the alteration in physiological and environmental conditions can be observed leading to an alteration in expression in prokaryotes. It was observed by Jacob and Monod. The lac operon consists of:

- Regulatory gene *i* – It codes for the repressor protein.
- *z* gene – It codes for beta-galactosidase which catalyzes the hydrolysis of lactose into glucose and galactose.
- *y* gene – It codes for permease which regulates the lactose permeability in the cell.
- *a* gene – It codes for transacetylase which assists the enzyme beta-galactosidase.

Hence, all these genes help in lactose metabolism. In lac operon, lactose acts as an inducer. If lactose is provided in the medium for the bacteria, the regulatory gene is activated. The inducer will bind to the repressor protein and render it inactive which allows transcription of the operon. Thus, the lac operon is negatively regulated in this case.

3 types of genes in lac operon:

The lac operon consists of three structural genes: lac Z, which codes for β-galactosidase, which acts to cleave lactose into galactose and glucose; lac Y, which codes for lac permease, which is a transmembrane protein necessary for lactose uptake and lac A, which codes for a transacetylase that transfers an acetyl group .
The lac operon is **essential for the metabolism and transfer of lactose in Escherichia coli and other bacteria**. As glucose is the mandatory source of energy, but when it is completely absorbed, this operon enables lactose digestion using the beta-galactosidase enzyme to provide energy to the cell.
Lac operon **contains genes involved in metabolism**. The genes are expressed only when lactose is present and glucose is absent. The operon is **turned on** and **off** in response to the glucose and lactose levels, catabolite activator protein and lac repressor. The lac repressor blocks the transcription of the operon.
Mechanism of lac operon model:

In the absence of inducer lactose, the regulator gene R produces a repressor protein which binds to the operator site and prevents transcription of structural genes. When inducer lactose is introduced in the medium, it binds to the repressor and prevents it from binding to the operator.

The activity of the promoter that controls the expression of the lac operon is regulated by two different proteins. **One of the proteins prevents the RNA polymerase from transcribing (negative control), the other enhances the binding of RNA polymerase to the promoter (positive control)**.
Operons can be of two types:

Inducible : This type of operon is switched on in the presence of an inducer, e.g. Lac operon.

Repressible : It is usually present in anabolic pathways. The operon is active and the functional product or enzyme is present normally in the cell.

- Lac operon contains genes involved in metabolism.
- The genes are expressed only when lactose is present and glucose is absent.

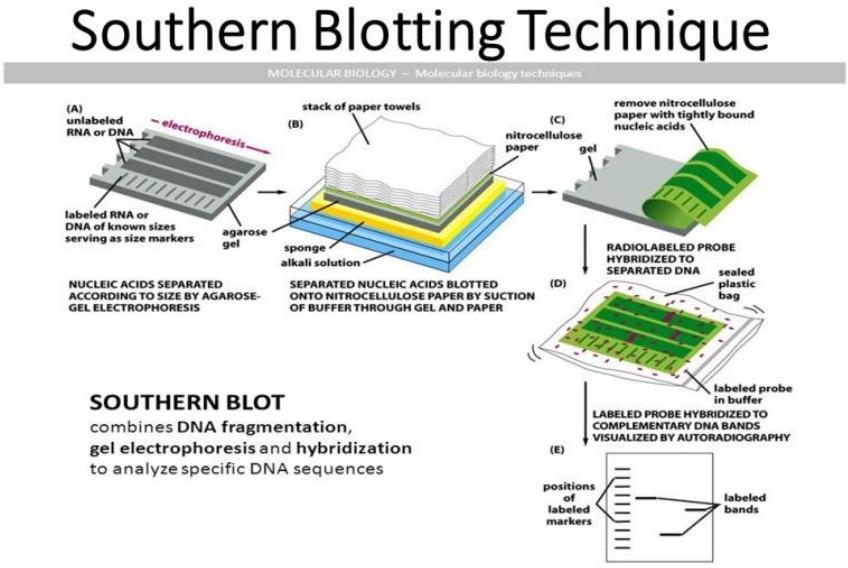
- The operon is turned on and off in response to the glucose and lactose levels: catabolite activator protein and lac repressor.
- The lac repressor blocks the transcription of the operon. In the presence of lactose, it stops acting as a repressor.
- catabolite activator protein activates the transcription of the operon, only when glucose levels are low.

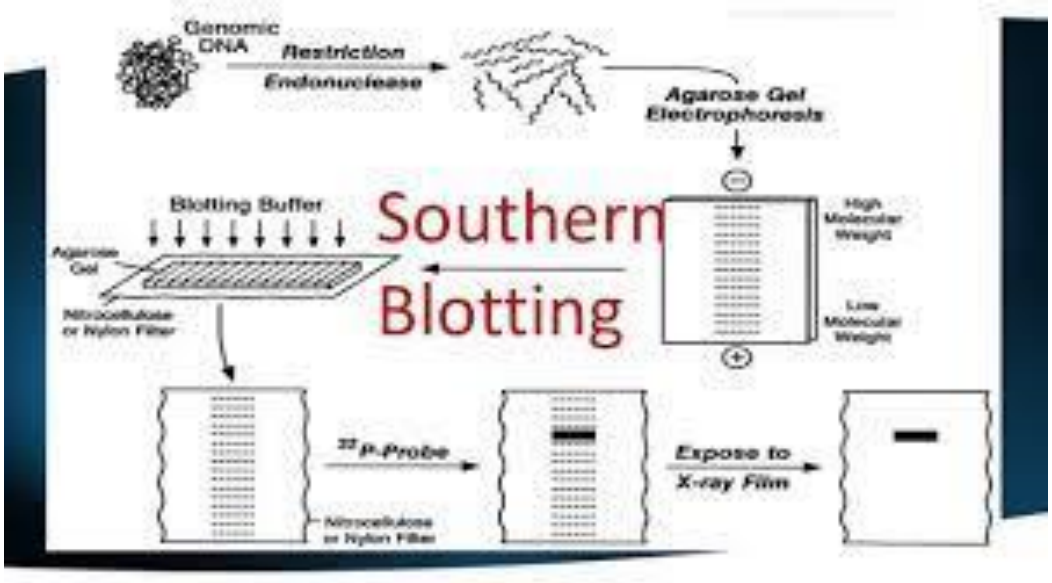
4. Describe the principles and applications of Southern blotting.

Principle: Southern blotting is an example of RFLP (restriction fragment length polymorphism). It was developed by Edward M. Southern (1975). Southern blotting is a hybridization technique for identification of particular size of **DNA** from the mixture of other similar molecules**.** Southern blotting is based on the principle of separation of DNA fragments by gel electrophoresis followed by the identification by labeled probe hybridization. The DNA fragments are separated based on their size and charge during electrophoresis.

Separated DNA fragments after transferring on nylon membrane, the desired DNA is detected using specific DNA probe that is complementary to the desired DNA. A hybridization probe is a short (100-500bp), single stranded DNA. The probes are labeled with a marker so that they can be detected after hybridization.

Southern blotting technique is used **to detect DNA in given sample**. DNA finger printing is an example of southern blotting. Used for paternity testing, criminal identification, victim identification. To isolate and identify desire gene of interest.





Application of Southern blotting:

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

Procedure/ Steps of southern blotting:

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

Step I: Restriction digest

- The DNA is fragmentized by using suitable restriction enzyme. RE cuts the DNA at specific site generating fragments

- The number of fragments of DNA obtained by restriction digest is amplified by PCR

Step II: Gel electrophoresis

- The desired DNA fragments is separated by gel electrophoresis

Step III: Denaturation

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

Step IV: Blotting

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

Step V: Baking and blocking

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

Step VI: Hybridization with labelled probes

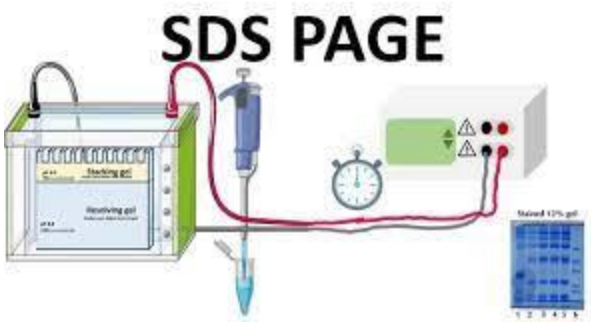
- The DNA bound to membrane is then treated with labelled probe
- The labelled probe contains the complementary sequences to the gene of interest
- The probe bind with complementary DNA on the membrane since all other non-specific binding site on the membrane has been blocked by BSA or casein.

Step VII: Visualization by Autoradiogram

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

5. List out the applications of SDS-PAGE gel-electrophoresis.

SDS PAGE or Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis is a technique used for the separation of proteins based on their molecular weight. It is a technique widely used in **forensics, genetics, biotechnology and molecular biology to separate the protein molecules based on their electrophoretic mobility**.



Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used to obtain high resolution separation of complex mixtures of proteins. The method initially denatures the proteins that will undergo electrophoresis.

Applications of SDS-PAGE:

1. It is used to measure the molecular weight of the molecules.
2. It is used to estimate the size of the protein. Used in peptide mapping
3. It is used to compare the polypeptide composition of different structures.
4. Agricultural testing.
5. Medical research.
6. DNA sequencing.
7. Protein purification and research.
8. Food industry.
9. Analysis of antibiotics, terpenoids and steroids.
10. Testing the purity of thyroid hormones.
11. Separation of free insulin from plasma proteins.
12. It is a technique widely used in **forensics, genetics, biotechnology and molecular biology to separate the protein molecules based on their electrophoretic mobility**.
13. In the separation of DNA fragments for DNA finger printing to investigate crime scenes.
14. To analyze results of polymerase chain reaction.
15. To analyze genes associated with a particular illness.

6. Briefly explain the properties of genetic code.

Properties of genetic code:

- **Genetic code is universal:** the genetic code is biologically universal. One particular codon codes for the same amino acid in all organism irrespective of plants or animals, prokaryotes (ex:- bacteria) or eukaryotes (ex:- man)

  For example: AUG –codes for aminoacid methionine

  UUU - codes for aminoacid phenylalanine

UUG – codes for aminoacid leucine

- **Codons are degenerate (degeneracy or redundancy) :**

a single amino acid is coded by more than one triplet code. The first two bases are the same but the third base is different called wobbly base.

Leucine is coded by UUG but it is also coded by CUU, CUC, CUA, CUG & UUA. Aminoacid arginine is coded by CGU.

- **Codons are non- overlapping:** the bases on m-RNA read continuously in groups of three from the 5^1 end to the 3^1 end without any demarcating signals in between.
- **Initiator codon:** protein synthesis is initiated only with a particular codon designated as initiatior codon. In most of the organisms AUG is the initiatior codon. If AUG is not present, GUG acts as an initiatior codon. When AUG occurs in the middle of an m-RNA, it codes for methionine. As an initiatior codon AUG binds to f- met-t-RNA complex.
- **Terminator codon (non- sense codon):** out of 64 triplet codons, only 61code for 20 aminoacids. The remaining 3 acts as terminator or nonsense codons. Terminotor codons at 3^1 end of m-RNA bring about termination of protein synthesis.
  Ex: UAG, UAA & UGA.

- **Codons are commaless:** triplet codons read continuously on and m-RNA without marks like comma, 'full stop' when the protein synthesis is going on.
- **Codons are sensible:** most of the triplet codons code for specific amino acids & therefore they are said to be sensible. But rarely some codons may code wrong amino acids when they are said to be ambiguous.

7. Explain the principle and applications of phase contrast microscopy.

Phase contrast microscopy:

"Phase contrast microscopy is an optical microscopy technique that converts phase shifts in the light passing through a transparent specimen to brightness changes in the image". It was first described by Dutch Scientist Frits Zernike in 1934.

This is a type of optical microscope whereby small light deviations known as phase shifts occurs during light penetration into unstained specimen. The phase contrast microscope produce high contrast images when using a transparent specimen more so those of microbial culture

Principles of Phase contrast microscopy:

- When light passes through the cells, small phase shifts take place, which are invisible to the human eye.
- It based on the wavelength of light rays and the fact that light rays can be in phase or out of phase.

- Different shade of grey are distinguished to our eyes due to differences in amplitude of light rays.
- Living cells can be observed in their natural state without previous fixation.
- No need of fixation or staining. It saves lot of time.
- Examining intracellular components of living cells at relatively high resolution. Ex: The dynamic movement of mitochondria, mitotic chromosomes and vacuoles in the cytoplasm.
- In a phase contrast microscope, one set of light rays comes directly from the light sources.
- The other set comes from light that is reflected or diffracted from a particular structure in the specimen.

Applications of Phase contrast microscopy:

- It is most commonly used to provide contrast of transparent specimens such as living cells or small organisms.
- Useful in observing cells cultured in vitro during mitosis.
- Phase contrast enables visualization of internal cellular components.
- It is used in the examination of growth, dynamics and behavior of a wide variety of living cell culture.
- It applied for equipment from the study of the living biological specimens, medical application, study of live blood cells, and other biological and science application.
- It is used in diagnosis of tumour cells.
- The possible applications of Zernike's phase contrast microscope in microscopy are evident in the fields of molecular and cellular biology, microbiology and medical research.
- Specimens that can be observed and studied include live microorganisms such as protozoa, erythrocytes, bacteria, molds and sperm, thin tissue slices, lithographic patterns, fibers, glass fragments and sub-cellular particles such as nuclei and organelles.

8. Briefly explain the principle and applications of Dark field microscope.

Dark field microscopy:

"A microscope in which objects are light at a very low angle from the side so that the background appears dark and the objects shows up against this dark background"

Dark field microscope was invented by J.J.Lister in 1830. In this type of the microscope the condenser system is modified so that the specimen is not illuminated directly. The condenser directs the light obliquely so that the light is deflected or scattered from the specimen, which then appears bright against a bark background. Living specimens may be observed more readily with dark field than with bright field microscopy. In this microscope, the standard bright field condenser is replaced with a single or double reflecting dark field condenser.

Principles of Dark field microscopy:

- A hollow cone of light is focused on the specimen in such a way that unreflected and unrefracted rays do not enter the objective.
- The only light that has been reflected by the specimen appears black or dark while the object itself is brightly illuminated.
- A dark field microscope is arranged so that the light source is blocked off, causing light to scatter as it hits the specimen.
- This is ideal for making objects with refractive values similar to the back ground appear bright against a dark ground.
- The result is a "cone of light" where rays are diffracted, reflected and refracted off the object, ultimately, allowing the individual to view a specimen in dark field.

Applications of Dark field microscopy:

- The dark field microscope is used to identify very thin bacteria not visible under ordinary illumination.
- It is also useful for the demonstration of the motility of flagellated bacteria and protozoa
- DFM used to observe the blood cells.
- Considerable internal structure in microorganisms can be revealed by the DEM.
- It is used to study the diamonds and other Persian stones.
- It helps to characterize the embedded nonmaterial in cells by combined with hyper spectral imaging.
- This is a frequently used method for rapid demonstration of *Treponema pallidum* in clinical specimens.
- DEM is used to study marine organisms such as algae, planktons, diatoms, insects, fibers, hairs, yeast and protozoans.
- DEM is also used to study for some minerals, crystals, thin polymers and some ceramics.
- Dark field microscope is used to study mounted cells and tissues.
- Here, No need to stain the specimen.
- It is useful for the specimens that are transparent and absorb little light.
- It is more useful for the study of external structures such as outlines, edges, surface boundaries etc.

9. Give an account of thin layer chromatography.

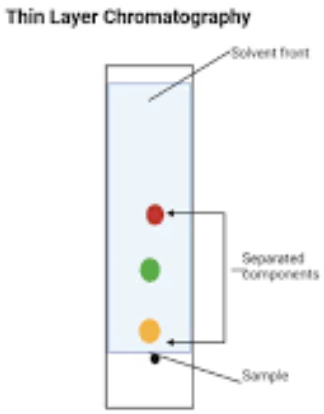
Thin layer chromatography:

Definition: "Thin-layer chromatography is a separation technique where the stationary phase is applied as a thin layer on a solid support plate with a liquid mobile phase".

Principle of Thin-layer chromatography (TLC):

Thin layer chromatography is a separation technique where the stationary phase is applied as a thin layer on a solid support plate with a liquid mobile phase.

- This method is based on the principle that components of a mixture are separated when the component having an affinity towards the stationary phase. In contract , other components are eluted with the mobile phase
- The substrate is bonded to the stationary phase so that the reactive sites for the binding of components are exposed.
- Now, the mixture is passed through the mobile phase where the components with binding site to the substrate on the stationary phase while the rest of the comments are elucted out with the mobile phase.
- After the separation , the molecules are seen as spots at a different location through out the stationary phase .
- The detection of molecules is performed by various techniques.



Steps of Thin-layer chromatography (TLC):

- The stationary phase is uniformly applied on the solid support (glass, thin plate or aluminum foil) and dried.
- The sample is injected as spots on the stationary phase about 1 cm above the edge of the plate.
- The sample loaded plate is then carefully dipped into the mobile phase not more than the height of 1 cm. After the mobile phase reaches near the edge of the plate, the plate is taken out.
- The retention factor is calculated as in paper chromatography, and the separated components are detected by different techniques.

Uses of Thin-layer chromatography (TLC):

- Thin-layer chromatography is routinely performed in laboratories to identify different substances present in a mixture.
- This technique helps in the analysis of fibers in forensics.
- TLC also allows the assay of various pharmaceutical products.
- It aids in the identification of medicinal plants and their composition.

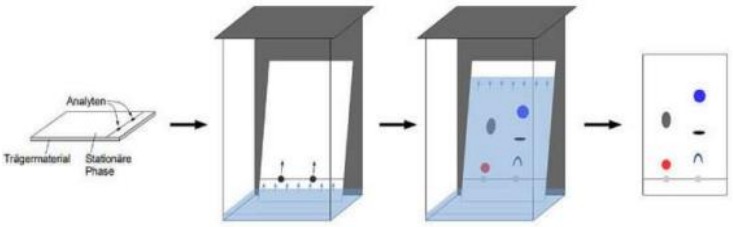


Figure: Thin-layer chromatography (TLC)

Applications:

25. TLC is routinely is used in laboratory to identify different substance present in the mixture.
26. This technique helps in the analysis of fibers in forensics.
27. TLC also allows the assay of various pharmaceutical products.
28. It aids to the identification of medical plants and their composition.

10. List out the applications of centrifugation.

Centrifugation: "Centrifugation is a method of separating molecules having different densities by spinning them in solution around an axis (in a centrifuge rotor) at high speed."

Applications of centrifugation

1. To separate two miscible substance.
2. To analyze the hydrodynamic properties of macromolecules.
3. Purification of mammalian cell.
4. Fractionation of subcellular organelles (including membrane).
5. Separating chalk power from water.
6. Removing fat from milk to produce skimmed milk.
7. Separating particles from an air – flow using.
8. The clarification and stabilization of wine.
9. Separation of urine components and blood components in forensic and research labs.
10. Aids in the separation of proteins using purification techniques ex: ammonium sulfate precipitation.

Centrifugation of Blood:

Collect whole blood in a micro centrifuge tube. After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15-30 minutes. Remove the clot by centrifuging at 1,000-2,000 x g for 10 minutes in a refrigerated centrifuge.

Uses of centrifuge:

- Centrifuges are used in various laboratories **to separate fluids, gases, or liquids based on density**.
- In research and clinical laboratories, centrifuges are often used for cell, organelle, virus, protein, and nucleic acid purification.

10 marks questions

11 Explain the steps involved in the process of transcription mechanism.

Definition: Transcription is the process in which a DNA sequence is transcribed into an RNA molecule with the help of enzyme RNA polymerase. One of the DNA strands acts as a template to make a complementary RNA strand. (Transcription is defined as a process in which the information is copied from a DNA strand to a messenger mRNA).

Transcription is the first step of gene expression. During this process, the DNA sequence of a gene is copied into RNA.

Before transcription can take place, the DNA double helix must unwind near the gene that is getting transcribed. The region of opened-up DNA is called a **transcription bubble**.

In transcription, a region of DNA opens up. One strand, the template strand, serves as a template for synthesis of a complementary RNA transcript. The other strand, the coding strand, is identical to the RNA transcript in sequence, except that it has uracil (U) bases in place of thymine (T) bases.

Example: Coding strand: 5'-ATGATCTCGTAA-3' Template strand: 3'-TACTAGAGCATT-5' RNA transcript: 5'-AUGAUCUCGUAA-3'

In translation, the RNA transcript is read to produce a polypeptide.

Example: RNA transcript: 5'-AUG AUC UCG UAA-3' Polypeptide: (N-terminus) Met - Ile - Ser - [STOP] (C-terminus)

Transcription uses one of the two exposed DNA strands as a template; this strand is called the **template strand**. The RNA product is complementary to the template strand and is almost identical to the other DNA strand, called the **non template** (or **coding**) **strand**. However, there is one important difference: in the newly made RNA, all of the T nucleotides are replaced with U nucleotides.

The site on the DNA from which the first RNA nucleotide is transcribed is called the +1+1plus, 1 site, or the **initiation site**. Nucleotides that come before the initiation site are given negative numbers and said to be **upstream**. Nucleotides that come after the initiation site are marked with positive numbers and said to be **downstream**.

If the gene that's transcribed encodes a protein (which many genes do), the RNA molecule will be read to make a protein in a process called translation.RNA polymerase

RNA polymerases are enzymes that transcribe DNA into RNA. Using a DNA template, RNA polymerase builds a new RNA molecule through base pairing. For instance, if there is a G in the DNA template, RNA polymerase will add a C to the new, growing RNA strand.

RNA polymerase synthesizes an RNA strand complementary to a template DNA strand. It synthesizes the RNA strand in the 5' to 3' direction, while reading the template DNA strand in the 3' to 5' direction. The template DNA strand and RNA strand are antiparallel.

RNA transcript: 5'-UGGUAGU...-3' (dots indicate where nucleotides are still being added at 3' end) DNA template: 3'-ACCATCAGTC-5'

RNA polymerase always builds a new RNA strand in the **5' to 3'** direction. That is, it can only add RNA nucleotides (A, U, C, or G) to the 3' end of the strand.

RNA polymerases are large enzymes with multiple subunits, even in simple organisms like bacteria. Humans and other eukaryotes have three different kinds of RNA polymerase: I, II, and III. Each one specializes in transcribing certain classes of genes. Plants have an additional two kinds of RNA polymerase, IV and V, which are involved in the synthesis of certain small RNAs.

Stages involved in transcription mechanism:

It involves three steps namely, initiation, elongation and termination.

Messenger mRNA is formed by the enzyme RNA polymerase.

For eg. In case of E.coli the polymerase has five polypeptide subunits. These are called holoenzymes. Once the transcription completes the enzyme disassembles.

Transcription is a process that requires a double stranded DNA. This helps to partially unwind in the region of mRNA synthesis. This is known as the transcription bubble. The proteins and enzymes bind to the DNA sequence. This process is known as promoter. The sequencing of the promoter is essential to determine the process of transcription.

1**. Initiation:** In the initial stage of transcription, RNA polymerase assembled holoenzyme assembles over the promoter. The factor allows it to proceed over the DNA template to synthesize the mRNA molecules by adding variable nucleotide pairs.

The strand undergoing transcription is called a template strand. The mRNA formed is similar to

the other single DNA strand. This other DNA strand is called a non template strand. This synthesis takes place in 5'to3'direction.
To begin transcribing a gene, RNA polymerase binds to the DNA of the gene at a region called the **promoter**. Basically, the promoter tells the polymerase where to "sit down" on the DNA and begin transcribing.

The promoter region comes before (and slightly overlaps with) the transcribed region whose transcription it specifies. It contains recognition sites for RNA polymerase or its helper proteins to bind to. The DNA opens up in the promoter region so that RNA polymerase can begin transcription. Each gene (or, in bacteria, each group of genes transcribed together) has its own promoter. A promoter contains DNA sequences that let RNA polymerase or its helper proteins attach to the DNA. Once the transcription bubble has formed, the polymerase can start transcribing.
2. Elongation: In case of elongation the DNA double stranded helix is broken and new hydrogen bonds are formed. The RNA polymerase acts as a template and stabilizes the DNA strand and the newly formed RNA strand.

Once RNA polymerase is in position at the promoter, the next step of transcription—elongation—can begin. Basically, e**long**ation is the stage when the RNA strand gets **long**er, thanks to the addition of new nucleotides.

During elongation, RNA polymerase "walks" along one strand of DNA, known as the **template strand**, in the 3' to 5' direction. For each nucleotide in the template, RNA polymerase adds a matching (complementary) RNA nucleotide to the 3' end of the RNA strand.

RNA polymerase synthesizes an RNA transcript complementary to the DNA template strand in the 5' to 3' direction. It moves forward along the template strand in the 3' to 5' direction, opening the DNA double helix as it goes. The synthesized RNA only remains bound to the template strand for a short while, then exits the polymerase as a dangling string, allowing the DNA to close back up and form a double helix.

In this example, the sequences of the coding strand, template strand, and RNA transcript are:

Coding strand: 5' - ATGATCTCGTAA-3'

Template strand: 3'-TACTAGAGCATT-5'

RNA: 5'-AUGAUC...-3' (the dots indicate where nucleotides are still being added to the RNA strand at its 3' end)

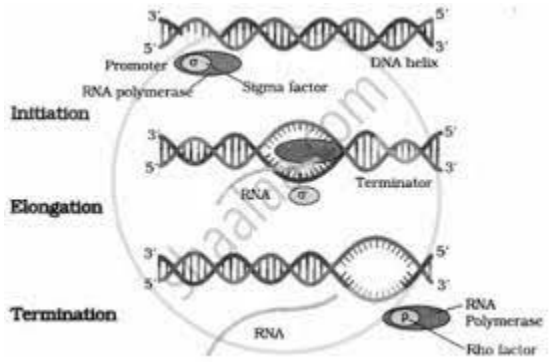
The RNA transcript is nearly identical to the **non-template**, or **coding**, strand of DNA. However, RNA strands have the base uracil (U) in place of thymine (T), as well as a slightly different sugar

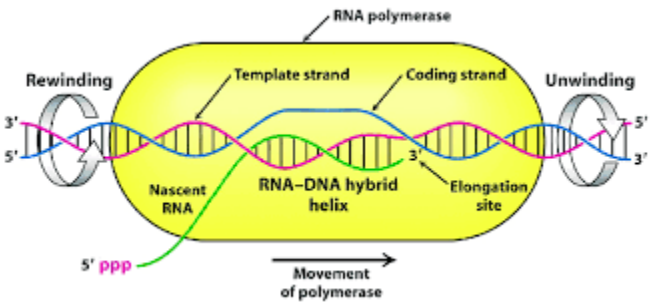
in the nucleotide. So, as we can see in the diagram above, each T of the coding strand is replaced with a U in the RNA transcript.

The picture below shows DNA being transcribed by many RNA polymerases at the same time, each with an RNA "tail" trailing behind it. The polymerases near the start of the gene have short RNA tails, which get longer and longer as the polymerase transcribes more of the gene.

In the microscope image shown here, a gene is being transcribed by many RNA polymerases at once. The RNA chains are shortest near the beginning of the gene, and they become longer as the polymerases move towards the end of the gene. This pattern creates a kind of wedge-shaped structure made by the RNA transcripts fanning out from the DNA of the gene.

3.Termination: Once the elongation completes termination comes into action. RNA polymerase dissociates from the DNA template. This leads to formation of mRNA.
The major difference between a prokaryote and eukaryote is — the nucleus in eukaryotes is membrane bound and in case of prokaryotes the nucleus lacks membrane.
In case of prokaryotes transcription occurs in cytoplasm and in case of eukaryotes the transcription occurs in the nucleus.
RNA polymerase will keep transcribing until it gets signals to stop. The process of ending transcription is called **termination**, and it happens once the polymerase transcribes a sequence of DNA known as a **terminator**.



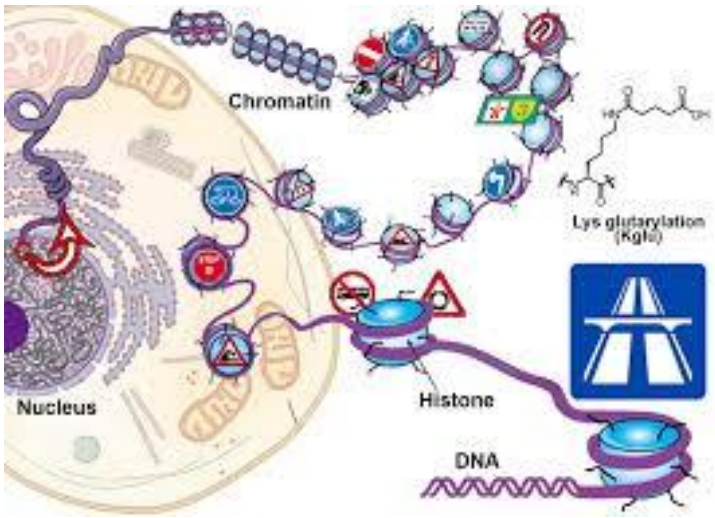


Note: Transcription is a process in both eukaryotes and prokaryotes. In case of eukaryotes it occurs in the nucleus. In case of prokaryotes it occurs in cytoplasm.

1. ***Explain the role of chromatin in gene expression.***

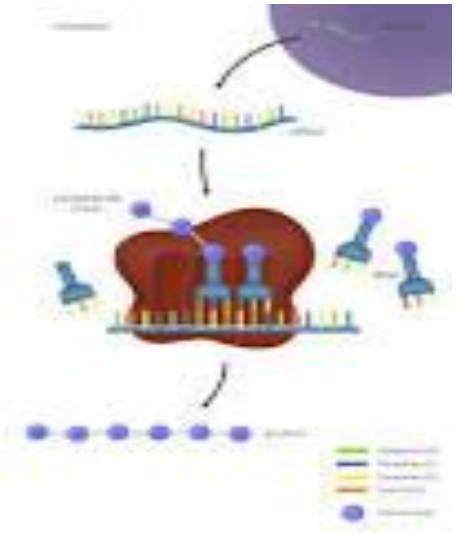
Chromosomes have two structurally and functionally distinguishable territories: euchromatin and heterochromatin. Heterochromatin is highly condensed, gene-poor, and transcriptionally silent, whereas euchromatin is less condensed, gene-rich, and more easily transcribed

Definition: Heterochromatin and euchromatin are two major categories of chromatin higher order structure. **Heterochromatin has condensed chromatin structure and is inactive for transcription, while euchromatin has loose chromatin structure and active for transcription**.
Chromatin **serves as a platform for numerous cellular signals to influence gene expression**. Post-translational modifications (PTMs) of histone proteins or covalent modifications of nucleotides influence a cell's transcriptional program, which ultimately impacts cellular behavior and cell fate.



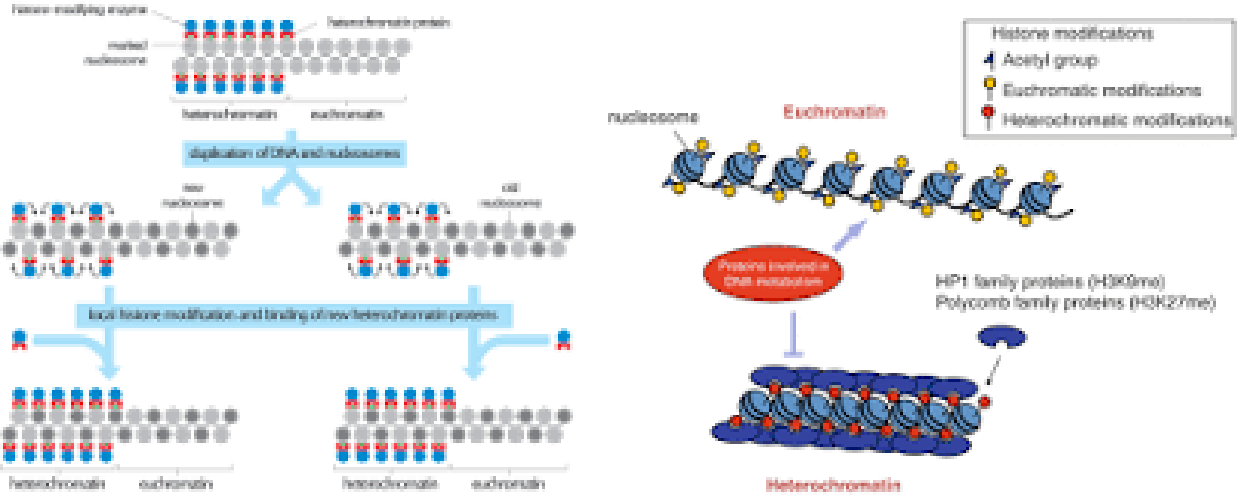
The primary functions of chromatin are 1) to package DNA into a more compact, denser shape, 2) to reinforce the DNA macromolecule to allow mitosis, 3) to prevent DNA damage, and 4) **to control gene expression and DNA replication**. The primary protein components of chromatin are histones that compact the DNA.

Role of gene expression:



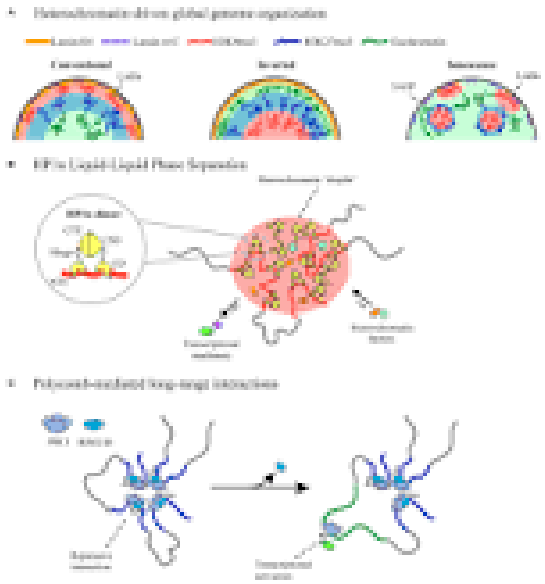
Gene expression is a tightly regulated process that **allows a cell to respond to its changing environment**. It acts as both an on/off switch to control when proteins are made and also a volume control that increases or decreases the amount of proteins made.

Euchromatin is **the part of the chromatin involved in the active transcription of DNA into mRNA**. As euchromatin is more open in order to allow the recruitment of RNA polymerase complexes and gene regulatory proteins, so transcription can be initiated.

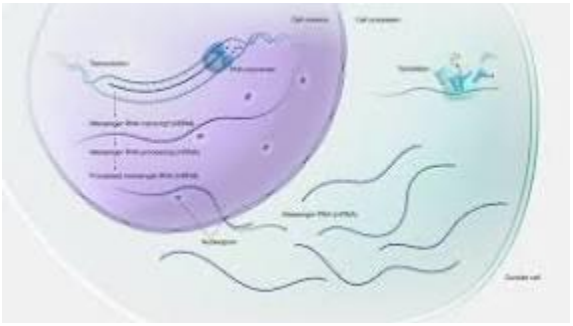


Role of heterochromatin and euchromatin:

Constitutive heterochromatin can affect the genes near itself (e.g. position-effect variegation). It is usually repetitive and forms structural functions such as centromeres or telomeres, in addition to **acting as an attractor for other gene-expression or repression signals**.



Heterochromatin is **a constituent of eukaryotic genomes with functions spanning from gene expression silencing to constraining DNA replication and repair**. Inside the nucleus, heterochromatin segregates spatially from euchromatin and is localized preferentially toward the nuclear periphery and surrounding the nucleolus.31-Au



Gene expression is the process by which the information encoded in a gene is turned into a function. This mostly occurs via the **transcription of RNA molecules that code for proteins or non-coding RNA molecules that serve other functions**.

2. Describe the applications of Fluorescence and Confocal microscopy.

Fluorescene microscopy:

"A fluorescence microscope is an optical microscope that uses fluorescence and phosphorescence instead or in addition to reflection and absorption to study the properties of organic and inorganic substances"

First working fluorescent microscope was developed by **Oscar Heimstaedt** in 1911.

Applications of Fluorescence microscopy:

- Fluorescence microscopy is a critical tool for academic and pharmaceutical research, pathology and clinical medicine.
- It is used to identify the structure in fixed and live biological samples.
- Fluorescence microscopy is a common tool for today's life science research.
- It allows the use of multicellular staining, labeling of the structure within the cells and measurements of the physiological state of the cell.
- It is even used for the demonstration of naturally occurring fluorescent material and other non–fluorescent substance or microorganism after staining with some fluorescent dyes. Eg : mycobacterium, tuberculosis, lipids, elastic fibers.
- It is also used for imaging the genetic material within a cell (DNA &RNA).
- Fluorescent microscopy is often used to image specific features of small specimens such as microbes.
- It is also used to visually enhance 3-D features at small scales.

Confocal microscopy:

Confocal microscopy is the most frequently confocal scanning microscopy or laser confocal scanning optical microscopy. It is an optical imaging technique for increasing optical resolution and contrast of a micrograph by means of using a spatial pinhole to block out of focus light in image formation.

A confocal microscopy was invented by **Marvin Minsky** in 1951.

Applications of Confocal microscopy:

- It is used for the examination of various eye diseases (corneal infections).
- It is used for the qualitative analysis and identification of endothelial cells of the cornea.
- It is used for optical scanning and recovery of damaged tissue.
- It also used to identify the fungal elements in the corneal stroma, during keratomycosis infection, or rapid diagnosis and quick therapeutic response
- Confocal microscopy is widely used for **fluorescence imaging in the life sciences**.
- Applications of confocal microscopy in the biomedical sciences.

3. Explain the steps involved in DNA finger printing.

Definition: "DNA fingerprinting is a procedure that shows the hereditary cosmetics of living things. It is a strategy for finding the distinction between the satellite DNA areas in the genome." **This technique was invented by** Alec Jeffreys **in 1984.**

DNA fingerprinting is a method used to identify an individual from a sample of DNA by looking at unique patterns in their DNA.

DNA fingerprinting is also known as DNA profiling. This is the method which is used to identify the criminals and suspects. There are many steps involved in DNA fingerprinting.

The next step is denaturing the DNA fragments. The next step is blotting the DNA The gel with the size-fractionated DNA is applied to a sheet of nitrocellulose paper or nylon membrane. In order to analyze a Southern Blot, a radioactive genetic probe is used in a hybridization reaction with the DNA. The X-ray film helps to analyse the fragments.

The Process of DNA Fingerprinting

Stage 1: Sample Collection: Collection of organic example blood, spit, buccal swab, semen, amniotic liquid, chorionic villi, skin, hair, body liquid, and different tissues are significant kinds of samples utilized.

Stage 2: DNA Extraction(Isolation of DNA): Isolating the desired DNA which can be performed chemically, mechanically or enzymatically.

Stage 3: Restriction Absorption, Enhancement or DNA Sequencing: Digestion of DNA by restriction endonucleases. The cutting the DNA into several pieces by restriction enzymes. Digestion by restriction endonucleases: Using a specific restriction enzyme, the DNA fragment is cut at a specific site. This is done to obtain RFLP (Restricted Fragment Length Polymorphism).

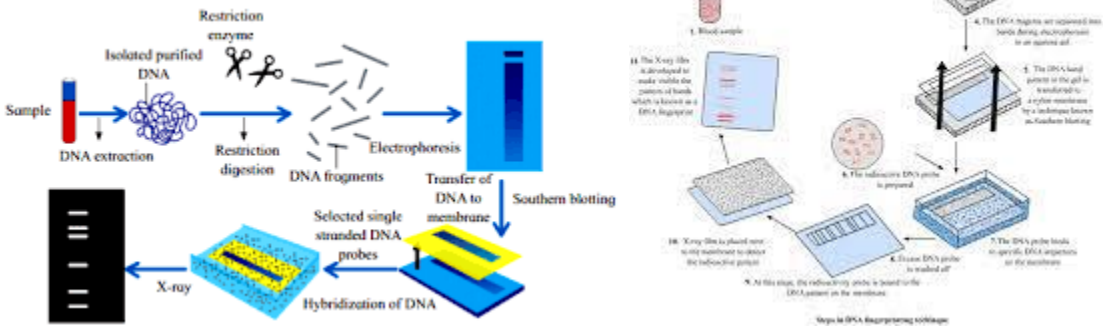
Stage 4: Analysis of Results Separation of DNA fragments by electrophoresis. Using gel electrophoresis, the DNA fragment of various sizes are obtained. Denaturing DNA fragment: The gel containing DNA fragments are then immersed in NaOH solution. This will denature DNA into single-stranded DNA.

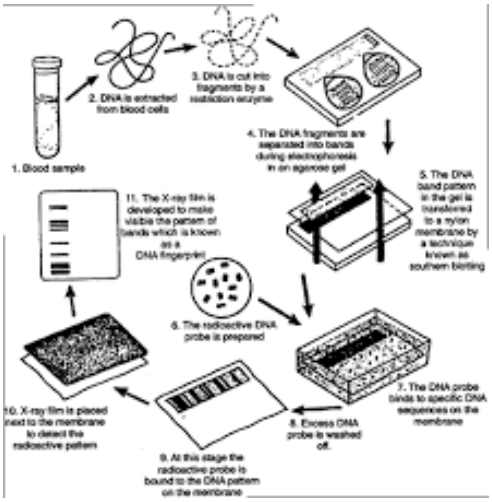
Southern blotting technique: This is performed to transfer single-stranded DNA from the gel onto the nitrocellulose membrane.

Hybridization: This DNA fragment is subjected to hybridization with a suitable DNA probe tagged with a radioactive substance.

Stage 5: Interpreting Results (Interpreting outcomes):

By looking at DNA profiles of different examples, varieties and likenesses between people can be distinguished. Comparing the sample of the suspect with the evidence: Using X-ray films, the DNA samples showing radioactivity are compared.



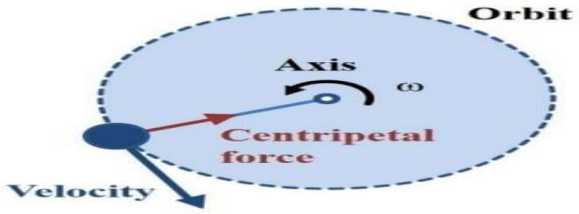


4. *Write the detail note on the Principle and Applications Of Centrifugation.*

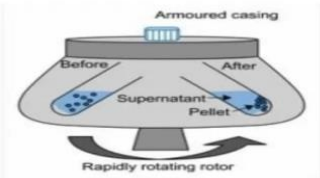
Principle of centrifugation:

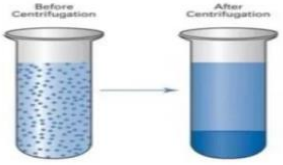
a centrifuge works by using the principle of sedimentation: **Under the influence of gravitational force (g-force), substances separate according to their density**. Different types of separation are known, including isopycnic, ultrafiltration, density gradient, phase separation, and pelleting.

- In a solution, particles whose density is higher than the solvent sink (sediment) , and particles that are lighter than it floats to the top .
- In greater the difference in density, the faster they move. If there is no difference in density, the particles stay steady.
- To take advantage of even tiny difference in density to separate various particles in a solution, gravity can be replaced either the much more powerful "centrifugal force'' provided by a centrifuge.
- A centrifuge is a piece of equipment that puts an object in rotation around the fixed axis , applying a potentially strong force perpendicular to the axis of spin.



- The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substance and particles to move outward in the redial direction.
- At the same time, object that is less dense are displaced and move to center.
- The redial acceleration causes denser particles to settle to the bottom of the tube, while low – density substance rise to the top.





Principle of Centrifugation

In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it floats to the top. The greater the difference in density, the faster they move. If there is no difference in density (isopycnic conditions), the particles stay steady. To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful "centrifugal force" provided by a centrifuge. A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward). The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction. At the same time, objects that are less dense are displaced and move to the center. In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low- density substances rise to the top.

Applications of centrifugation

- To separate two miscible substance.
- To analyze the hydrodynamic properties of macromolecules.
- Purification of mammalian cell.
- Fractionation of subcellular organelles (including membrane).
- Separating chalk power from water.
- Removing fat from milk to produce skimmed milk.
- Separating particles from an air – flow using.
- The clarification and stabilization of wine.
- Separation of urine components and blood components in forensic and research labs.
- Aids in the separation of proteins using purification techniques ex: ammonium sulfate precipitation.

Centrifugation of Blood:

Collect whole blood in a micro centrifuge tube. After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15-30 minutes. Remove the clot by centrifuging at 1,000-2,000 x g for 10 minutes in a refrigerated centrifuge.

Uses of centrifuge:

- Centrifuges are used in various laboratories **to separate fluids, gases, or liquids based on density**.

- In research and clinical laboratories, centrifuges are often used for cell, organelle, virus, protein, and nucleic acid purification.

5. Give the detail account of the types and functions of RNA polymerases.

Definition: RNA polymerase is a multi-unit enzyme that synthesizes RNA molecules from a template of DNA through a process called transcription. The transcription of genetic information into RNA is the first step in gene expression that precedes translation, the process of decoding RNA into proteins. The RNA molecules produced by RNA polymerase fulfill a variety of roles in the cell.

RNA polymerase or ribonucleic acid polymerase is an enzyme that synthesizes RNA from a DNA template. It is a multi-subunit enzyme that catalyzes the process of transcription from eukaryotes. The prokaryotic RNA polymerase initiates the process of transcription and has a sigma factor that dissociates the enzyme after the transcription initiation. In prokaryotes, the same RNA is used to catalyze the polymerization of coding and non-coding RNA. Whereas in eukaryotes, different RNA catalyzes the polymerization of coding and non-coding RNA.

RNA polymerase:

1. RNA polymerase enzyme synthesizes RNA from a template of DNA.
2. RNA polymerase catalyzes the transcription process.
3. RNA polymerase is responsible for the formation of ribosomal RNA, micro RNA, nuclear RNA etc.,
4. There are different types of RNA polymerase: RNA polymerase I, RNA polymerase II, RNA polymerase III, RNA polymerase IV and RNA polymerase V.

T**ypes of RNA** polymerases in eukaryotes are as follows.

All eukaryotes have three different RNA polymerases (RNAPs) which transcribe different types of genes.

1**. RNA polymerase I**:

- It helps in the polymerization of ribosomal RNA (which transcribe different types of genes).
- It is involved in more than 50% of RNA transcription.

6. **RNA polymerase II:**
   - It helps in the transcription of mRNA precursors (It transcribes mRNA, miRNA, snRNA, and snoRNA genes).
   - It forms micro RNA and small nuclear RNA.

3. **RNA polymerase III:**

- It is mainly involved in the transcription of t-RNA(It transcribes tRNA and 5S rRNA genes)..
- It is involved in the normal functioning of a cell such as transfer RNA and ribosomal RNA.

7. **RNA polymerase IV and RNA polymerase V:**
   - They are involved in the formation of small interfering RNA.
   - They help in the formation of heterochromatin in the nucleus.
   - They are exclusively found in plants.

   Various RNA polymerases in eukaryotes catalyze the process of transcription and are responsible for the formation of different types of RNA such as ribosomal RNA, micro RNA, nuclear RNA, etc.

RNA polymerase structure and function (in transcription):

The RNA polymerase enzyme is a large complex made up of multiple subunits[1]. The prokaryotic form of RNA polymerase has four subunits capable of transcribing all types of RNA. In eukaryotes, these enzymes have eight or more subunits that facilitate the attachment and processing of DNA throughout transcription.

The three stages of transcription involve various functions of RNA polymerase that result in the synthesis of RNA:

1. Initiation begins when RNA polymerase wraps around the promoter region of DNA. The promoter is a DNA sequence that guides RNA polymerase on where to bind upstream of a gene. While prokaryotic RNA polymerase can directly bind to DNA promoter sequences, eukaryotic forms require the assistance of transcription factors for initial binding. Once RNA polymerase successfully binds DNA at the targeted promoter region, the enzyme can continue with the second stage of transcription.

2. Elongation commences when RNA polymerase unwinds double-stranded DNA into two single strands. These DNA strands are used as genetic templates for RNA synthesis. As the DNA template strand moves through the RNA polymerase it builds an RNA strand that is complimentary to the transcribed DNA strand.

3. Termination is the final step of transcription. Once RNA polymerase encounters a terminator sequence or signal, it stops adding complementary nucleotides to the RNA strand. This is followed by the release of the RNA transcript, which marks the end of transcription for that template of DNA.

RNA polymerase vs DNA polymerase

DNA polymerase synthesizes double-stranded DNA molecules from unwound DNA strands during replication. Even though the end products of replication and transcription are different, they both work upon DNA by adding nucleotides in the same 5' to 3' direction. In contrast with RNA polymerase, DNA Polymerase is semi-conserved process that utilizes both strands of a double-stranded DNA molecule as a template for replication.

Comparison	**RNA Polymerase**	**DNA Polymerase**
Function	Transcription of DNA	Replication of DNA
Purpose	To make RNA copies of genes	To copy the entire genome
Time of occurrence	Used in transcription during G phase(s)	Used in replication during S phase
Primer	Not required for transcription	Required for initiation of replication
Base pairs used to synthesize product	Adenine, Guanine, Cytosine and Uracil	Adenine, Guanine, Cytosine and Thymine
Resulting product	Single-stranded RNAs (e.g. mRNA)	Double-stranded DNAs

7. Explain the role of euchromatin and heterochromatin in gene expression.

Chromosomes have two structurally and functionally distinguishable territories: euchromatin and heterochromatin. Heterochromatin is highly condensed, gene-poor, and transcriptionally silent, whereas euchromatin is less condensed, gene-rich, and more easily transcribed

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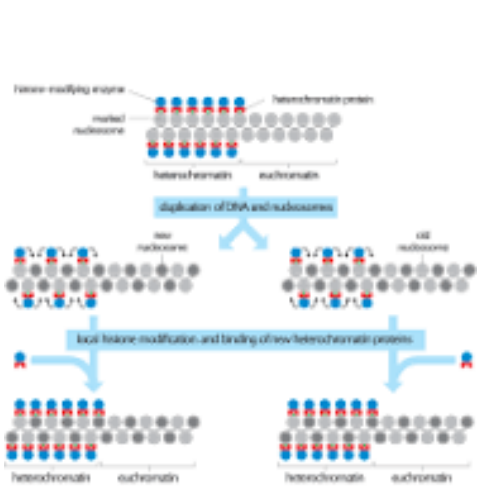
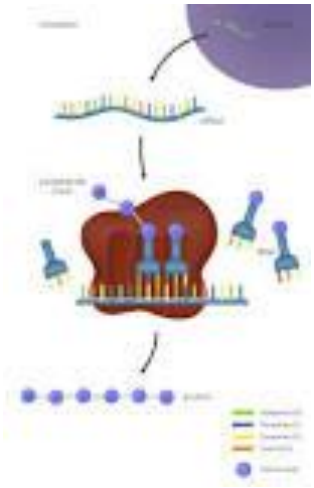
Role of gene expression:

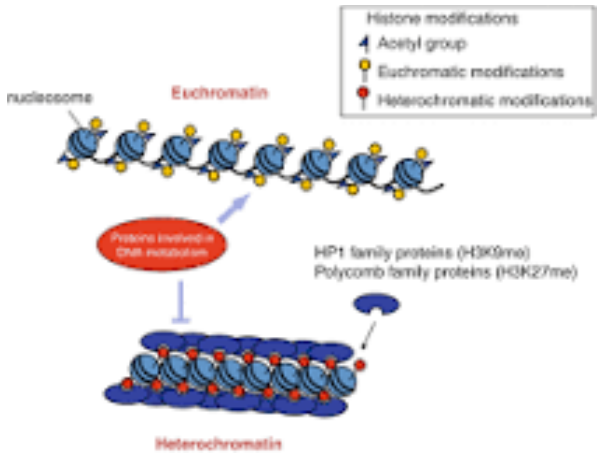
Gene expression is a tightly regulated process that **allows a cell to respond to its changing environment**. It acts as both an on/off switch to control when proteins are made and also a volume control that increases or decreases the amount of proteins made.

Euchromatin is **the part of the chromatin involved in the active transcription of DNA into mRNA**. As euchromatin is more open in order to allow the recruitment of RNA polymerase complexes and gene regulatory proteins, so transcription can be initiated.

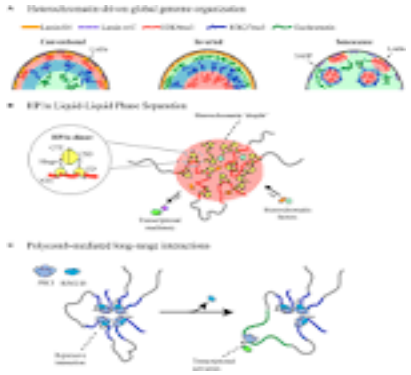


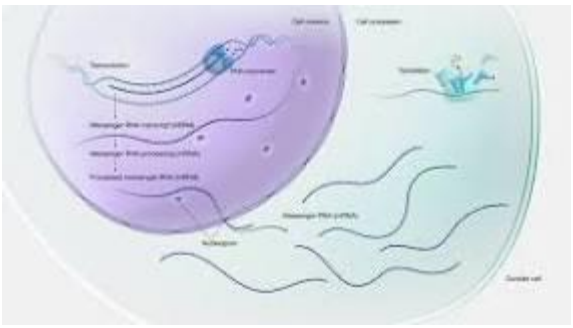


Role of heterochromatin and euchromatin:



Constitutive heterochromatin can affect the genes near itself (e.g. position-effect variegation). It is usually repetitive and forms structural functions such as centromeres or telomeres, in addition to **acting as an attractor for other gene-expression or repression signals**.





Heterochromatin is **a constituent of eukaryotic genomes with functions spanning from gene expression silencing to constraining DNA replication and repair**. Inside the nucleus, heterochromatin segregates spatially from euchromatin and is localized preferentially toward the nuclear periphery and surrounding the nucleolus.

Gene expression is the process by which the information encoded in a gene is turned into a function. This mostly occurs via the **transcription of RNA molecules that code for proteins or non-coding RNA molecules that serve other functions**.

8. *Describe the applications of Fluorescence and Electron microscopy.*

Fluorescene microscopy:

"A fluorescence microscope is an optical microscope that uses fluorescence and phosphorescence instead or in addition to reflection and absorption to study the properties of organic and inorganic substances"

First working fluorescent microscope was developed by **Oscar Heimstaedt** in 1911.

Applications of Fluorescence microscopy:

- Fluorescence microscopy is a critical tool for academic and pharmaceutical research, pathology and clinical medicine.
- It is used to identify the structure in fixed and live biological samples.
- Fluorescence microscopy is a common tool for today's life science research.
- It allows the use of multicellular staining, labeling of the structure within the cells and measurements of the physiological state of the cell.
- It is even used for the demonstration of naturally occurring fluorescent material and other non–fluorescent substance or microorganism after staining with some fluorescent dyes. Eg : mycobacterium, tuberculosis, lipids, elastic fibers.
- It is also used for imaging the genetic material within a cell (DNA &RNA).
- Fluorescent microscopy is often used to image specific features of small specimens such as microbes.
- It is also used to visually enhance 3-D features at small scales.

Electron microscopy (SEM and TEM):

Definition: An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination. **"An electron microscope is defined as the type of microscope in which the source of illumination is the beam of accelerated electrons".** It is a special type of microscope with a high resolution of images able to magnify objects in nanometers, which are formed by controlled use of electrons in a vacuum captured on a phosphorescent screen. **Ernst Ruska** (1906-1988), a German engineer and academic professor built the first electron microscope in 1931 and the same principles behind his prototype still given modern EMS.

Applications:

- Electron microscope is used to investigate the ultra structure of wide range of biological and inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals and crystals.
- Industrially, it is often used for fertility control and failure analysis.
- Modern EM produces electron micrographs using specialized digital cameras and frame grubbers to captures the images.
- The science of microbiology owes its development to the EM. The study of microorganisms like bacteria, virus and other pathogen has made the treatment of diseases very effective.
- Electron microscopy is often **used for industrial purposes to assist in developing new products and throughout the manufacturing process**. For example, electronics industries use electron microscopes for high-resolution imaging in the development and manufacturing processes of semiconductors and other electronics.

Advantages:

- Very high magnification
- Incredibly high resolution
- Material rarely deserted by preparation.
- It is possible to investigate a greater depth of field.

There are two types of electron microscope , namely, Transmission electron microscope (TEM) And Scanning electron microscope(SEM):

Transmission electron microscope (TEM):

Definition: "TEM is a powerful EM that uses a beam of electrons to focus on a specimen producing a highly magnified and detailed image of the specimen".

Electron microscope was developed by **Ernst Ruska** and **Max Knoll** in 1931 and was modified into TEM by Ernst Ruska along with the Siemens in 1939.

Applications of TEM:

TEM is used in wide variety of fields like Biology, microbiology, Forensic studies, nanotechnology etc.

- TEM is used to visualize and study the cell structure of bacteria, viruses and fungi.
- It is used to view a bacteria flagella and plasmids.
- It is used to study the shapes and sizes of microbial cell organelles.
- It is used to study and differentiate between plants and animal cells.
- It is also used nanotechnology to study nanoparticles such as ZnQ nano particles.
- TEM is used to detect and identify fractions, damaged micro particles which further enable repair mechanisms of the particles.
- It can be used in semiconductor analysis and production and the manufacturing of computer and silicon chips.
- It is used for spot chemical analysis in energy – dispersive X-rays spectroscopy.
- TEM is used in the analysis of cosmetic components which are very tiny in size.
- It is also used to study the filaments structures of microorganisms.
- It is used to study the topography of element used in industries.
- Quality control and failure analysis in industries are done with the help of an electron microscope.
- The images obtained in an electron microscope can be captured as electron micrographs with the help of specialized cameras.
- The study of metals and crystals became easy with the introduction of an electron microscope.

Advantages of TEM:

- It has a very powerful magnification o about 2 million times that of the light microscope.
- It can be used for a variety of applications ranging from basic biology to nanotechnology, to education and industrial uses.
- It can be used to acquire vast information on compounds and their structure.
- It produces very efficient high quality image with high clarity.
- It can produce permanent images. It is very easy to train and use the TEM.

Scanning electron microscope (SEM):

Definition: “A scanning microspore was focused beams of electrons to create an image of a specimen by scanning the surface area”. SEM was invented by German scientist **Max Knoll** in 1935.

Applications of SEM:

- It is used in life science, biology, gemology and medicine for research purposes.
- It is also used in forensic laboratories.
- In industrial technology, it is used for semiconductor inspection and assembly of microchips for computer.
- It is used to examine surface contaminants.
- SEM reveals spatial variations in chemical compositions.
- It provides qualitative chemical analysis and identifies crystalline structure.
- It provides information in microstructures.
- It can detect and analyze surface fractions.
- In modern materials science, investigations into nanotubes and nanofibres, high temperature superconductors, architectures and alloy strength, all are relatively on the use of SEMs for research and investigation.

Advantages of SEM:

- It provides a 30 and topographical image of the specimen with great detail.
- It require less time.
- It requires minimal preparation action of the sample.
- Modern SEM produces portable digital data.
- SEM is easy to operate with proper training.

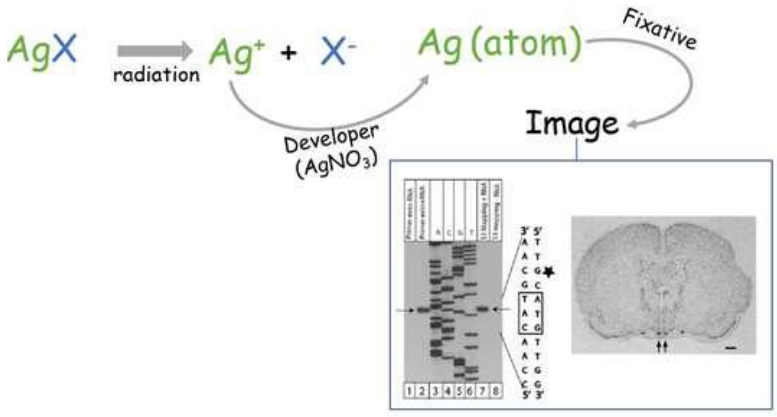
8. Write a detailed note on the principle and safety measures of Autoradiography.

Definition: "Autoradiography is an imaging technique that uses radioactive sources contained within the exposed sample". In vitro autoradiography methods involve the isolation of cellular components such as DNA, RNA, proteins or lipids, followed by labeling with suitable radioisotopes. In 1924 first biological experiment involving autoradiography traced the distribution of polonium in biological specimen. The first autoradiography was obtained accidently around 1867 when Niepce de St. victor was working on silver chloride and iodide by uranium salt.

Principles of autoradiography

- Autoradiography is based upon the ability of radioactive to expose the photographic film by ionizing it .
- In this technique a radioactive substance is put in direct contact with a thick layer of a photographic emulsion (thickness of 5-50 micrometer) having gelatin substance and sliver halide crystal .
- This emulsion differs from the standard photographic film in terms of having higher ratio of silver halide to gelatin and small size of grain .

- It is the left in dark for several days for proper exposure .
- The silver halide crystal are exposed to the radiation which chemically converts silver halide into metallic silver (reduced) giving a dark color band .
- The resulting radiography is viewed brelectron microscope & digital scanners
-



Application of autoradiography

- To find and investigate the various properties of DNA .
- To find the location and amount of particular substance within a cell including cell organelle , metabolites .
- Tissue localization of radioactive substance .
- To find the site and performance of targeted drug .
- To locate the metabolic activity site in the cell .
- Quantitativeanalysisofmacroautographs.
- AnalyzethelengthandnumberofDNA

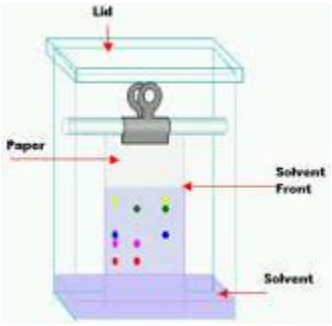
Safety measures using radiography

- Use spill trays and absorbent covering.
- Use fume hoods for handling potentially volatile material.
- Use glove box for handling large quantities of volatile material.
- Wear laboratory coat, disposable gloves, and laboratory safety glasses.
- Use gloves appropriate for the chemicals to be handled.

9. Give an account of the principle and applications of chromatography.

Chromatography: "Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of the mixture for qualitative and quantitative analysis".

The Russian botanist **Mikhail Tswett** coined the term chromatography in 1906.



Chromatography is **the general name given to the methods by which two or more compounds in a mixture are physically separated by distributing between two phases**: a stationary phase which can be a solid or liquid supported on a solid and a mobile phase, either a gas or a liquid which flows continuously around the ...

Principle:

- Chromatography is based on the principles where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase is separating from each other while moving with the aid of a mobile phase.
- The factors effective on this separation process include molecular characteristics related to adsorption, partition, and affinity or difference among their molecular weights.
- Because of these differences, some components of the mixture stay longer in the stationary phase and they move slowly in the chromatography system, while others pass rapidly into the mobile phase and leave the system faster.
- **Chromatography technique that uses paper sheets or strips as the adsorbent being the stationary phase through which a solution is made to pass** is called paper chromatography.

  It is an inexpensive method of separating dissolved chemical substances by their different migration rates across the sheets of paper.

Applications:

1. It is used in the study of metabolism and proteins along with nucleic acids for the research purpose.

2. HPLC is used in protein separation like Insulin Purification, Plasma Fractionation, enzyme Purification, fuel Industry, biotechnology and biochemical procedures.
3. In forensic pathology and crime scenes testing like analyzing blood and hair samples of Crime place.
4. Determining the nutritional quality of food.
5. In various life science application.
6. In testing water samples and also checks air quality.
7. It is used in Pharmaceutical sector (In drugs development).
8. Detects the unknown compounds and purity of mixture.
9. Separation of compound based on their molecular weight and elements composition.
10. It is used to identify and analyze samples for the presence of trace elements or chemicals .
11. HPLC and GC are very much used for detecting various contaminants such as polychlorinated biphenyl (PCBs) in pesticides and oils.
12. It is used in detecting the food spoilage and additive detection.
13. It is also used in the study of Molecular Biology.

Principle of Paper chromatography:

- Paper chromatography is of two types based on two different principles.
- The first is the paper adsorption chromatography that is based on the varying degree of interaction between the molecules and the stationary phase.
- The molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column.
- However, the molecules with lower affinity move with a faster movement, thus allowing the molecules to be separated in different fractions.
- The second type of paper chromatography is the paper partition chromatography. It is based on the principle that the moisture on the cellulose paper acts as a stationary phase for the molecules moving with the mobile phase.
- The separation of the molecules is thus based on how strongly they adsorb onto the stationary phase.
- An additional concept of ‘retention factor’ is applied during the separation of molecules in the paper chromatography.
- The retention value for a molecule is determined as a ratio of distance traveled by the molecule to the distance traveled by the mobile phase.
- The retention value of different molecules can be used to differentiate those molecules.