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# Depertment (Bs)MLT

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# Paper AML

# Q1. What is fluorescent in situ hybridization? Write down the principle and procedure of this technique.

Fluorescent in situ hybridization

It is a cytogenetic technique used for the detection of some duplication and deletion of nucleotides in a chromosome within a morphologically preserved tissue or cells by using fluorescent complementary DNA probes; it is used for the detection chromosomal abnormalities.

## Principle

 The complementary DNA probes will hybridize to the complementary regions of chromosome for visualizing specific location on chromosomes.

# Procedure of FISH

1. Denature the double stranded DNA strands into single stranded DNA molecules by heating.
2. The probes are denatured and then added to the sample.
3. If a small duplication is present that is complementary to the probes, it will hybridize and via the fluorescent dye it will be visualize them to be seen.

Q: 3 What are the application of the following technique?

 Dna sequencing.

* it is used to determine the sequencing of indiudual genome
* .used to determine the sequencing of entire the cell genome.
* Indentification of mutation .
* Detect different genes that are cause disease.

FISH

* It is used is used for find the specific feature in DNA for use the genetic conceling ,median and species.
* Fish it is technique used to visualing a specific cytogenic chromosome.
* Fish it is a genetic technique used to dignose congenital disease such as Edward syndrome down syndrome and also dignose infection disease.
* Detection of gene detation and gene amplication.

Chromotography

* Chromo mean color,graphy mean to write or draw graph.
* A labortary technique used for the separation of compound of mixture.
* Chromotograping technique is also be used in the separation of vitamin ,protein,lipids.
* Paper chromatography is used to determine some type of sugar, aminoacid body fluid which are associated heredetiry metabolic disorder.
* Radio immune assay
* it is primiraly used to analyse antigen (Notably) certain harmones or protein in the serum simple.
* It is used to analyse of vatimain,harmone,metabolic diagnostic marker
* For example. Fish T3,T4, AcTH, Testosteron and vitamin B 12 etc.
* It is technique infection hiv hepatitis B,A etc.
* An determine RBCs volume and whole blood volume.
* Northern Bloating
* Is the techniqueused for the gene expression detection of particular RNA in sample.
* Northern blotting are particularly useful for determine the specific gene are being expressed at MRna level.
* Northern blotting this technique also be used to show overexpression of uncogene and tumer suppressant gene in cancerous cell.compare to normal tissue
* Also used for study in MRna splicing
* Used CDNA prob

Q: 4.Define the following?

* Apharesis
* It is greek word which mean to take away.
* Apheresis a taking away, ia a medical technology in which the blood of a donar ot patient is passed through on apparatus that separate out one particular constituent and return the reminder to the circulation.

OR

* A procedure in which the blood is collected, part of the blood such as platelets or white blood cells is taken out,and the rest of the blood is returned to the donoris called apheresis.

Stationary Phase

* The solid or liquid phase of chamotheraphy system on which the material to be separated are selectively adsorbed.
* Adsorption or retention or partition or both or any other principal of a substance on the other stationary.

Radi activity

* A binding assay in which the binder is an antibody which uses radioactivity to measure the amount of bound and OR free antigen radioactivity labeled antigen is called ,tracer radioactive isotope are usually H (beta) or I (gamma) is called radioactivity.
* And radioisotopes are one of the main factor for immodiagnostic technology.

RAST

* Radio\_immuno sorbent test.
* A test used primarly for quantifiying total serum immunoglobin E (Ige) level in the blood serum .
* A radioallergosorbent test (RAST) is a blood test using radioimmunoassay test to detect specific IgE antibodies to determine the substance a subject is allergic to this is different form a skin allergy test.
* A person suffer from a severe skin condition such as widespread aczema.
* Leucapheresis
* The leukocyte are specifically the granulocyte can be heaviest from a donor to supplay granulocyte to help fight against infection in patient such as neanote.
* Leacocute in some cases of leukemia with very high blood cell (WBC) removal of increase WBC, may help that prevent complication of the thrombosis, severe neutropenia.

* Q:2 Differentiate between types of probes.
* Centromere.

 Centromere probes target centrameric region of a particulary chromosome this types of probes allow us to determine the quantative and qualatitive number of the centromerge probes binds to repatative alpha satellite DNA sequence.

* Eg.centromere it is used as a refence probes for HERZ/NEU
* Also use for determing the copies of a particular chromosome
* Telomere
* It is specific for a single chromosome arm they contain a locus estimated the end of the chromosome.
* Telomere are DNA structure at the end of eukaryatic chromosome that protect then from degradation od DNA repair activity.
* Whole chromosome paint
* It is also use for determination of composition of the marker chromosome and for centrimination of the preseuse of chromosome rearrangement.
* Cocus
* Use for the determination of the presence or absence or location of a particular gene .
* Cocus specific probes target a specific gene sequence of interest.these probes can determine wheather gene is amplific detect or present in a normal copy number.
* Q: 2 Different between centrifugation method.
* INTERMITENT FLOW CENTRIFUGATION.
* Performed in cycles and blood is collected from an individual to prevent clotting .
* Blood compare into centrifuge through inlet part and components are separated through specific gravity
* With 1 venipuncture blood is with drawn and reinfused through the same needle and with 2 venipuncture one for phelbotamy and 1 for reinfusing.
* CENTINOUS FLOW CENTRIFUGATION
* It this technique the blood is processed and return the blood to individual simaltanlous.
* It is contrast to iFc procedure which complete a cycle before beginning a new one.
* Always need 2 venipuncture

Q:5..compare and contrast between the different types of blotting? Also give application of each technique.

BLOTTING TECHNIQUE .

The visualization of specific DNA,RNA and protein among many thousand of contaminating molecules requires the convergene of number of technique which are collectively called blotting.

OR

It is technique which is used for transfusing DNA,RNA and protein.

There are three types of blotting

Southern blotting

Sir Edwin southern professor of the biochemistry was developed this method.

This method involves separation transfer and hybridization .this method is routinely used in the molecular biology for detection of the specific DNA sequence in DNA sample and the DNA can be detect single gene and can be a large part and piece of DNA such as viral genome.

Southern blotting is combines Agarose gel electrophoresis for the size separation of the DNA with the method of hybridization.

Application of Southern blotting:

Identification of the transformed gene in transgenic individual.

Southern blotting are used in gene discovery ,mapping,evolution and development studies, diagnostics and forensic.

Analyze the genetic patterns which appear in the person ,s DNA.

Western blotting:

It is immunoblotting technique which rely on the specificity of the binding between a protein of the interest and a probe (antibody) raised against that particular protein to allow detection if the protein of interest in the mixture of many similar molecules.it was discover in (1981)

Application:

The confirmatory HIV test

Western blotting is also used as the definitive test for bovine spongiform encephalopathy (BSE)

Some fprm lyme disease testing can be performed in western blotting.