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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 of FISH

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

# Procedure of FISH

* Denature the double stranded DNA strands into single stranded DNA molecules by heating.
* The probes are denatured and then added to the sample.
* 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: 2 What are the application of the following technique?
  + ***DNA sequencing***.
* it is used to determine the sequencing of individual genome
* .used to determine the sequencing of entire the cell genome.
* Identification 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 visualize a specific cryptogenic chromosome.
* Fish it is a genetic technique used to diagnose congenital disease such as Edward syndrome Down syndrome and also diagnose infection disease.
* Detection of gene dilation and gene implication.
  + Chromatography
* Chromo mean color,graphy mean to write or draw graph.
* A laboratory 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, amino acid body fluid which are associated hereditary metabolic disorder.
* Radio immune assay
* it is primarily used to analyze antigen (Notably) certain harmones or protein in the serum simple.
* It is used to analyze of vatimain,harmone,metabolic diagnostic marker
* For example. Fish T3,T4, AcTH, Testosterone and vitamin B 12 etc.
* It is technique infection hiv hepatitis B,A etc.
* And determine RBCs volume and whole blood volume.
* Northern Bloating
* Is the technique used 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 over expression of uncongen and tumor suppressant gene in cancerous cell. Compare to normal tissue
* Also used for study in MRna splicing
* Used CDNA prob
* Q:4 Define the following?
* **Apheresis**
* It is Greek word which means to take away.
* Apheresis a taking away, ia a medical technology in which the blood of a donor 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 donors called apheresis.
  + Stationary Phase
* The solid or liquid phase of chemotherapy 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.
  + Radio 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 primarily for quantifying 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 suffers from a severe skin condition such as widespread aczema.
* ***Leucapheresis***
* The leukocyte are specifically the granulocyte can be heaviest from a donor to supple granulocyte to help fight against infection in patient such as neonate.
* Leucocytes 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.
  + Q5:-
  + There are three type of bloating technique
  + The bloating technique in the way of detecting the macromolecules that are present in synthesis cell
  + ***Characteristics:- southern bloating northern bloating western bloating***
  + Molecular detected. Detect DNA . detect of RNA detect proteins.
  + Electrophoresis; used Agarose gel. Used Agarose gel

+ formaldehude used SDS

Acrylamide

* Bloting methyodes:- capillary force methods same electroelution

Technique

* Prob used :- used DNA probs
  + - * + SsDNA are used DNa/SsDNA/RNa target

With hybridization used antibody primary and secondry.

* Detection system :- radioactivity , same radioactivity are
  + - * + Colorimetric analysis not used

Colorimetric analysssis

Used.

* 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 form lyme disease testing can be performed in western blotting.

**Q2. (1) Differentiate between different types of probes.**

# Centro mere Probes

Centro mere probes target centromeric region of a particularly chromosome. This type of probes allow us to determine the quantitative and qualitative number of the centromere probes binds to repetitive alpha satellite DNA sequence.

**E.g.**

* Centromere it is used as a reference probes for HERZ/NEU.
* And to determine the copies of a particular chromosome

# Telomere Probes

* It is specific for a single chromosome arm that contains a locus estimated the end of the chromosome.
* Telomeres are DNA structure at the end of eukaryotic chromosome that protect them from degradation and DNA repair activity.

# Whole chromosome painting Probes

* It is also use for determination of composition of the marker chromosome and for confirmation of the presence of chromosome rearrangement.

# Locus

* Used for the determination of the presence or absence or location of a particular gene.
* Locus specific probes target a specific gene sequence of interest, these probes can be determine whether gene is amplified detect or present in a normal copy number.

**Q2. (2) Different between centrifugation methods.**

|  |  |
| --- | --- |
| INTERMITTENT FLOW CENTRIFUGATION | CONTINOUS FLOW CONFIGERATION |
| Performed in cycles and blood is collected from an individual to prevent clotting. | **In this technique the blood is processed and returns the blood to individual simultaneously.** |
| Blood compare into centrifuge through inlet part and components are separated through specific gravity. | **It is totally opposite or contrast to Intermittent Flow Configuration procedure which complete a cycle before begging a new one.** |
| With 1 veinipuncture blood is withdrawn and rein fused through the same needle and with 2 veinipuncture one for phlebotomy and one for rein fusing | **And in this technique there is always need of two veinipuncture.** |