

Advances in MLT

BS MLT

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

Fluorescent in situ hybridization

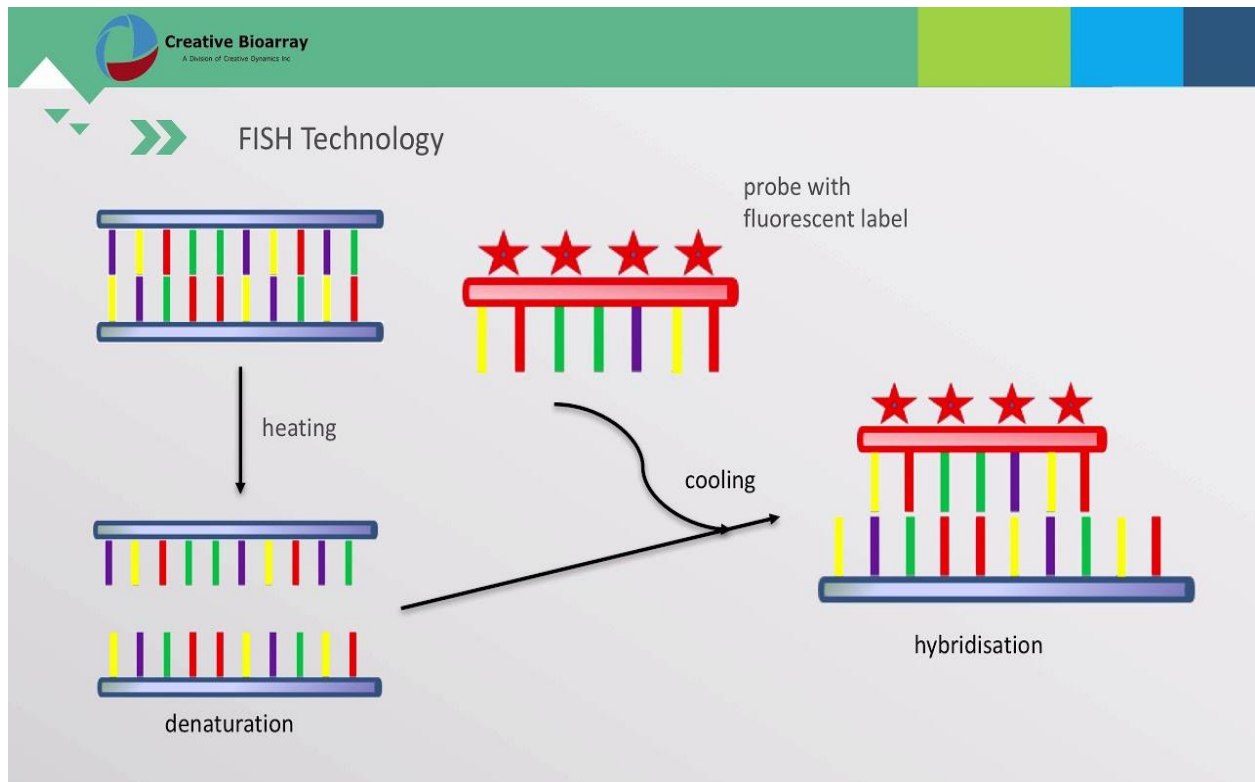
It is a cytogenetic technique used for the detection of some duplication and deletion of nucleotides in a chromosome within a morphologically preserved tissue or cells by using fluorescent complementary DNA probes; it is used for the detection chromosomal abnormalities.

Principle of FISH

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

Procedure of FISH

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.



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

Centromere Probes

Centromere probes target centromeric region of a particular 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 HER2/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	CONTINUOUS FLOW CONFIGURATION
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.

Q3. What are the applications of the following?

DNA Sequencing

1. Used to identify child's paternity and endangered & protected species.
2. Gene mapping of microbes.
3. Detection of genes that causes disease.
4. By knowing the DNA sequencing the human genome project also completed.

FISH (Fluorescent in situ hybridization)

1. FISH can be used in formalin fixed, paraffin embedded sections or fresh frozen tissues
2. It can be used either in large tumors or tumor where the malignant component contribute to a small proportion of the overall cellular population.
3. It allows simultaneous interrogation of multiple cytogenetic signatures.

Northern Blotting

1. This technique is used for over expression of oncogenes and TNF.
2. Also used for mRNA splicing.
3. Used for regulation of tumor suppressor genes in cancerous cells.
4. Used for detection of specific mRNA in sample and for screening recombinant which are successfully transformed with transfer.

Chromatography

1. The technique is used for the purification of proteins, in Pharmaceuticals (drugs are purified) fine chemicals and also provides high purity and high recovery in short time.
2. Also for used for the analysis of hormones and vitamins.
3. Helpful in the analysis of complex mixture.

Immunoassay

1. This technique is used the analysis of hormones (ACTH, FSH, T3 T4, Glucagon, Insulin, Testosterone etc..) vitamins, and other metabolites.
2. Also used for the diagnostic purposes such as detection of Antibodies in conditions like HBS, HCS, and HIV etc....

Q4. Definitions of the followings.

Apheresis

It is Greek word which means to take away, It is a technique used for the separation of blood cells from whole blood via the Apheresis machine.

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 Immune sorbent test)

A test used primarily for quantifying total serum immunoglobulin E (IgE) level in the blood serum.

A radio immune sorbent 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 from a skin allergy test.

A person suffers from a severe skin condition such as widespread eczema.

Leucopheresis

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. Compare and contrast b/w different types of blotting? Give application of each technique.

Southern Blotting	Northern Blotting	Western Blotting
This technique was developed by M. Southern in 1975	This technique was developed by Alwine and his colleagues in 1979.	This technique was developed by George shark's group in 1979.
Technique used to identify specific sequence of DNA	This procedure is used for detection of specific RNA sequence by hybridization with cDNA.	This technique is used for identification of specific amino acids sequence in protein.
It involves agarose gel electrophoresis.	This technique involves denaturing formaldehyde agarose gel	Involves SDS PAGE.
It involves capillary transfer	It involves capillary transfer	It involves electric transfer
DNA probes are used.	DNA probes are used.	Primary and secondary antibodies are used.
Applications	Applications	Applications
Uses DNA fingerprinting.	Used in gene expression during analysis.	Used for disease diagnosis.
Used in phylogenetic analysis	Used for over expression oncogenes and suppression TNF in cancerous cells	Used for the analysis of viral genome to diagnose HIV, HBS, HCV and bacterial infection like Lyme disease.