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Q: 1

What are the applications of the following technique?

* DNA Sequencing
* FISH
* Northern blotting
* Radio immune assay
* Chromatography
* **DNA Sequencing**

We understand the function of the DNA sequencing and square responsible for any disease

With the help of comparative DNA, we can have deducted any mutation of genes.

Wide use in DNA fingerprinting

By knowledge the whole DNA sequencing.

* **FISH**

Also used in germ cell of diagnosis of some abnormal techqunie like aneuploidies

Use to deducted the missing of specific DNA sequence

To find the specific sequence of the DNA which are used for genes counselling.

It can have used to localized specific mRNA

Compare the Genome of two biological species.

* **Northern blotting**

Southern blots are used in genes discovery.

Delectation and insertion.

Structural rearrangement

Polymorphism

Presence of particular bite of DNA in the sample.

* **Chromatography**

The chromatography technique is used for the separation of protein, amino acid and carbohydrate.

It is also used for the finding of hormones vitamins and drugs.

On this technique we determined the molecular width of the protein.

This technique also helps in the qualitative and quantities measurement of the molecule.

* **Radio immune assay**

Finding of hormones, vitamins and also diagnostic marker

Example- FSH, T3, T4, ATCH

Therapeutic drugs monerting

Example-morphine and digoxin

Q: 2

Compare and Contrast between the different types of blotting? Also give Application of each technique.

The problem can be solved by three type of blotting method

1. Southern blotting
2. Northern blotting
3. Western blotting

**Southern blotting:**

Southern blotting is technique for detecting specific DNA fragments in a complex mixture. This technique was invented in mid-1970 by Edward southern

It has been applied to detect Restriction fragments length polymorphisms RfLP and variable number of tandem repeat polymorphisms VNTR the letter is the basis of DNA fragments

Restriction fragments length polymorphisms

Polymorphisms refer to DNA sequence variation between individuals of a species

The most well-known example is the RFLP due to beta globulin Gene mutation.

**Application of Southern blotting:**

* Southern blotting technique is used to detect DNA in given sample.
* DNA finger printing is an example of southern blotting
* Used in diagnosis of disease caused by genetic defects
* Used to identify infectious agents
* Used for paternity testing, criminal identification, victim identification
* To identify mutation or gene rearrangement in the sequence of DNA

**Northern Blotting.**

Northern Blotting is used to detecting RNA fragments instead of DNA fragments. This technique was invented by person named Northern

In the southern blotting DNA fragments are denatured with alkaline solution

In The northern Blotting RNA fragments are treated with formaldehyde to insure linear confirmation.

**Application of northern blotting:**

* Southern blots are use in gene discovery, mapping evolution and development studies etc.
* It is an invaluable method in gene analysis.
* Important for the conformation of DNA cloning.
* Highly useful for the determination of RELP.

**Western blotting.**

* Western blotting is used to detect particular protein in a mixture. The probe used is therefore not DNA or RNA but antibodies. This technique also called immune blotting.

**Application of western blotting:**

* Identification of a specific protein in a complex mixture of proteins. In this method, known antigens of well-defined molecular weight are separated by SDS-PAGE and blotted onto nitrocellulose. The separated bands of known antigens are then probed with the sample suspected of containing antibody specific for one or more of these antigens. Reaction of an antibody with a band is detected by using either radiolabeled or enzyme-linked secondary antibody that is specific for the species of the antibodies in the test sample.
* Estimation of the size of the protein as well as the amount of protein present in the mixture.
* It is most widely used as a confirmatory test for diagnosis of HIV, where this procedure is used to determine whether the patient has antibodies that react with one or more viral proteins or not.
* Demonstration of specific antibodies in the serum for diagnosis of neurocysticercosis and tubercular meningitis.

**Q: 3**

Define the following terms.

* APHAERESIS
* Stationary phase
* Radioactivity
* RASTs
* Leukapheresis

Ans:

**Aphaeresis:**

* Aphaeresis is a Greek word meaning to take away.
* The processes of apheresis involved removal of whole blood from donor through instrument which are basically design just like a centrifuge.
* The component of whole blood is separate.one of the separate portion is withdraw and then remaining component are transfused to the donor.
* This apheresis machine was invented by American medical technologist Hereb cullis 1972.

**Radioactivity:**

* Radioisotopes are one of the main factors for immunodiagnostic technology.
* Usually, Iodine isotope 125-I labels are used. Although both carbon isotopes such as C14 and H3 have been used nowadays.
* Usually, for the pure antigen, by iodination, high specific activity for radio-labeled (125-I) antigen is prepared on its tyrosine residue(s) using method such as chloramine-T or peroxidase methods and then the radio-labeled antigen from the free-isotope using gel-filtration or HPLC.
* The pure antigen is used as the standard or calibrator along with the specific antibody against the antigen.

**RASTs:**

* RASTs are often used to test for allergies when:
* a physician advises against the discontinuation of medications that can interfere with test results or cause [medical complications](https://en.wikipedia.org/wiki/Complication_(medicine));
* a patient suffers from severe skin conditions such as widespread [eczema](https://en.wikipedia.org/wiki/Eczema) or
* a patient has such a high sensitivity level to suspected allergens that any administration of those allergens might result in potentially serious side effects.
* IgE is the [antibody](https://en.wikipedia.org/wiki/Antibody) associated with [Type I allergic response](https://en.wikipedia.org/wiki/Type_I_hypersensitivity): for example, if a person exhibits a high level of IgE directed against [pollen](https://en.wikipedia.org/wiki/Pollen), the test may indicate the person is allergic to pollen (or pollen-like) proteins.
* A person who has outgrown an allergy may still have a positive IgE years after exposure

**Stationary phase:**

The [phase](https://www.britannica.com/science/phase-state-of-matter) over which the mobile phase passes in the technique of [chromatography](https://www.britannica.com/science/chromatography).

Chromatography is a separation process involving two phases, one stationary and the other mobile. Typically, the stationary phase is a porous [solid](https://www.britannica.com/science/solid-state-of-matter) (e.g., [glass](https://www.britannica.com/technology/glass), [silica](https://www.britannica.com/science/silica), or [alumina](https://www.britannica.com/science/alumina)).

**Leukapheresis:**

Leukapheresis is a laboratory procedure in which white blood cells are separated from a sample of blood. It is a specific type of apheresis, the more general term for separating out one particular constituent of blood and returning the remainder to the circulation

Q: 4

1. Diffrentiate between diffrent types of probes.
2. Diffrentiate between diffrent type of centrifugation.

Ans:

**Differentiate between different types of probes:**

There are four different types of probes.

1. Centromere
2. Telomere
3. Whole chromosome paint.
4. Locus

**Centromere:**

* This probe is designed to hybridize centromere, fluorescent brightly due to large number of repeats in centromere.
* Useful for determining the number of copies of a particular chromosome

**Telomere:**

* It has specificity for a single human chromosome arm.
* They contain a locus estimated the end of the chromosome.

**Whole chromosome paint:**

* used to determine composition of marker chromosomes, confirm the presence of chromosome rearrangements.

**Locus:**

* used to detect the presence absence or location of a particular gene**.**
* Diffrentiate between diffrent type of centrifugation methods:

**Centrifugation methods:**

There are two main types of centrifugation method which are:

1. Intermittent flow centrifugation.
2. Continuous flow centrifugation.

**Intermittent flow centrifugation:(IFC)**

They are stepwise:

* It is performed in various cycles (known as passes)
* Blood is collected from an individual.
* To prevent clotting, anticoagulant added to tubing blood pumped into centrifuge bowled through inlet port
* Bowl rotates and components separated according to specific gravity
* RBCs packed against outer rim of bowl (greatest density)
* Followed by WBCs, platelets and plasma
* Separated components flow from bowl through outlet port into separate collection bags
* Undesired components are diverted into reinfusion bag and returned to the individual
* Reinfusion completes one cycle
* Cycles are repeated until the desired quantity of product obtained (e.g.: platelets pheresis usually takes six to eight cycles to collect a therapeutic dose)
* IFC can be done:

1. With one venepuncture (one arm procedure)-blood is withdrawn and reinfused through the same needle
2. With two venepuncture (two arm procedure) ­-one for phlebotomy and one for reinfusion.

**continuous flow centrifugation: (CFC)**

* It withdraws, process and returned the blood to individual simultaneously.
* This is in contrast to IFC procedure, which completes a cycle before beginning a new one.
* Always done with two when puncture sites.
* ADV- low extracorporeal blood volume is used- so useful in elderly in children.

Q: 5

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

Ans: **fluorescence in situ hybridization:**

Fluorescence in situ hybridization (FISH) is widely used for the localization of genes and specific genomic regions on target chromosomes, both in metaphase and interphase cells.

It is cytogenic technique which is used for the localization of the presence or absence of specific DNA sequence on chromosome.

A specific technique used to determine the visualize various cytogenic abnormalities.

FISH results must be correlate with pathological, clinical and molecular information.

It used fluorescent probes which can bind to those part of chromosome which have the high degree of sequence similarity.

It also used for the finding of other features of DNA.

These features also used in genetic counseling, medicine and species identification.

**Principal of FISH:**

1. DNA probes and targeted sequence
2. DNA probes are labelled.
3. The labeled probes and targeted DNA are denatured.
4. Annealing of complementary DNA sequence.
5. This is followed by hybridization of the probe & DNA.
6. Counter staining is performed to observe the cells or chromosomes.
7. Stained slides are observed using required filters.
8. Florescence have the characteristic color of emission and absorption.
9. Probes refers to a sequence of nucleotides.

**Procedures of FISH:**

1. Slides preparation for cells morphology and fixation using standard cytogenetic procedure.
2. Denature the DNA or chromosomes at 5°C for 5 min)
3. The probe Labelling.
4. Hybridization

Then, the labeled probe and the target DNA are denatured.

hybridization done at (37°C for 16 hrs.)

Counter staining (DAPI)

1. Fluorescence staining.
2. Post Washing of Excess Probes.
3. Examine slides or store in the dark