VU MEDICAL ZONE

BIO 203 Current Final Term Fall 2020

Qno1: Difference between Blotting Technique on basis of detection.

SOUTHERN BLOTTING

The Southern blot is used to detect the presence of a particular piece of DNA in a sample by a molecular probe.

NORTHERN BLOTTING

Northern blot is a method used for detection of a specific RNA sequence in RNA samples.

WESTERN BLOTTING

A technique used to detect the presence of a specific protein in a complex protein mixture.

Qno2: Write down the limitations of Western blotting?

A minute imbalance at any level blotting of the procedure can skew the results of the entire process.
Incorrect labeling of the protein can happen due to the reaction of secondary antibody.
Cause erroneous in bands or no bands due to insufficient transfer.
Well trained technicians are required for this technique.
Primary antibody availability is crucial.
It is just a semi-quantitative at best. Only an approx. estimation & not a precise measurement of molecular weight of the protein are possible.

Qno3: Nomenclature of enzyme.

A suitable system was proposed by Smith and Nathans (1973)

The species name of host organisms is identified by the first letter of genus and first two letters of specific epithet

E. coli = Eco

H. influenzae = Hin

Strain identification is written as EcoK

- In case, host strain has several restriction and modification systems, these are identified by roman numerals, for example, in case of H. influenzae
- HindI, HindII, HindIII etc.

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All restriction enzymes have general name endonuclease R and modification-methylase M followed by the system name, for example, in case of H. influenzae

- R. HindIII or M. HindIII.

Qno4. Write properties of probes?

PROBES

- Labeled material to detect a target.
- For DNA: 20-30 nucleotides, complementary to a region in the gene or DNA.

Qno5: Write applications of PCR in clinical microbiology.

PCR-Medicine

- PCR has major impact on medicine especially in the field of clinical microbiology for diagnosis.
- Molecular tools have also allowed performing prenatal genetic diagnosis.

Qno6: Explain R-M system.

Types of restriction and modification (R-M) system

- At least four R-M systems are known
- Type I
- Type II
- Type III
- Type IIs

Type I

- Type I systems were the first to be characterized from E. coli K12
- The active enzyme consists of two restriction subunit, two modification subunit and one recognition subunit
- Type I systems are of little value for gene manipulation.

Type II

- Most of the useful R-M system is Type II.
- Type II enzymes recognize a defined sequence and cut within it.

Type III

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Type III enzymes have symmetrical recognition sequences but otherwise resemble type I systems and are of little value.

Type IIs

▪Type IIs systems have similar cofactors and structure to type II but restriction occurs at a distance from recognition site that limits their usefulness.

Qno7: Define SNP.

A **SNP** is defined as a single base change in a DNA sequence that occurs in a significant proportion (more than 1 percent) of a large population.

Qno8: Write down method of DNA Finger printing.

Types of DNA fingerprinting methods are…

- Electrophoresis.
- Polymerase chain reaction (PCR).
- Restriction fragment length polymorphism (RFLP).
- Random Amplified Polymorphic DNA (RAPD).
- Amplified fragment length polymorphism (AFLP).

Qno9: Explain Western Blotting.

WESTERN BLOTTING

A technique used to detect the presence of a specific protein in a complex protein mixture.

WESTERN BLOTTING

To determine the molecular weight of a protein.

To measure relative amounts (quantitation) of the protein present in complex mixtures of proteins that are not radiolabeled.

WESTERN BLOTTING

Western blots have become one of the most common analytical tools for the detection of viral proteins.

Characterization of monoclonal and polyclonal antibody preparations and in determining the specificity of the immune response to viral antigens.

Qno10: When does RFLP occur?

An RFLP occurs when the length of a detected fragment varies between individuals. Each fragment length is considered an allele, and can be used in genetic analysis.

Qno11: Write down benefits is secondary antibodies in western blotting.

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A species-specific, labeled secondary antibody directed against the constant region of the primary antibody is then used. The most common antibody label used in Western blots is HRP.

Qno12: Write down the Methods of joining DNA fragment.

Methods of joining DNA fragments:

▪Mainly three methods are used for joining DNA invitro
i)Joining covalently annealed cohesive ends by DNA ligase
ii)Joining blunt-ended fragments by DNA ligase from phage T4infected E.coli.

iii) The third utilizes the enzyme terminal deoxynucleotidyl transferase to synthesize homopolymeric 3**'single stranded tails at the** ends of fragments.

Qno13: DNA ligase Activity.

DNA ligase:

 E. coli and phage T4 encode enzyme, DNA ligase, which seals single stranded nicks between adjacent nucleotides in a duplex DNA chain.

DNA ligase to create covalent recombinant DNA

DNA ligase can repair the nicks produced after the association of cohesive ends of DNA strands.

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