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COMPARE STUDIES OF SOUTHERN –NORTHERN- WESTERN BLOTTING

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ABSTRACT
The extraction of biomolecules, DNA, RNA, and protein, is the most crucial method used in molecular biology.[1] These biomolecules can be isolated from any biological material for subsequent downstream processes, analytical, or preparative purposes.[2] Southern blotting, Northern blotting and Western blotting are the major blotting to find out DNA, RNA and protein respectively. In the world all life are standing in amino acid molecules. Because DNA means deoxyribonucleic acid and main ingredient of nucleic acid is amino acid. Amino acids are the building block of protein and proteins are the long chain of amino acids. There are some important factors of amino acids are charge, hydrophilicity or hydrophobicity, size and functional groups.[3]

KEYWORDS: Amino acids, protein, blotting, hydrophilicity, functional group.

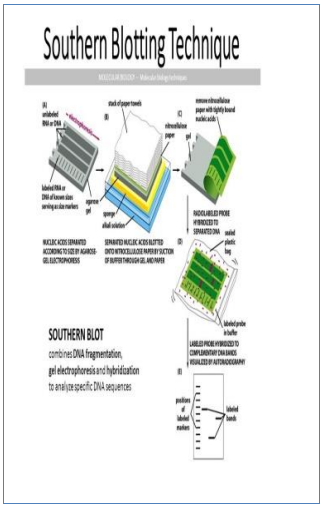
INTRODUCTION
The blot analysis technique is a powerful method to detect specific biomolecules in samples of complex composition. It can be applied to biomolecules that will adhere stably to a support material such as a nitrocellulose, nylon or paper membrane and are still able to bind their cognate ligand.[4] DNA, RNA and Protein blot analysis techniques are developed by Edwin M. Southern (in 1975 at Edinburg University), James Alwine David Kemp and George Stark (in 1977 at Stanford University) and Harry Towbin and colleagues (in 1979) respectively. For DNA purification required four important steps: effective disruption of cells or tissue; denaturation of nucleoprotein complexes; inactivation of nucleases, for example, RNase for RNA extraction and DNase for DNA extraction; away from contamination.[6] RNA is especially unstable due to the ubiquitous presence of RNases which are enzymes present in blood, all tissues, as well as most bacteria and fungi in the environment.[7,8] Strong denaturants has always been used in intact RNA isolation to inhibit endogenous RNases.[6] Protein purification is required to determine its unique characteristics, including size, charge, shape, and function.[9] Specific buffer conditions are recommended to be maintained because of the sensitivity of proteins toward environmental pH changes.[10] The hydrolyzation of protein and detergent are the main process for protein degradation.

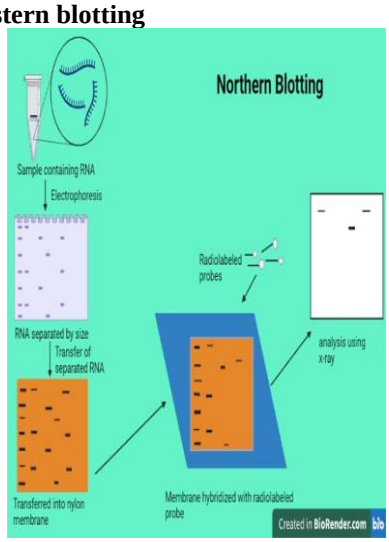
Principle of the Southern, Northern and Western blotting
The southern blotting: The DNA to be analyzed is digested with restriction enzymes and fractionated by size by the process of agarose gel electrophoresis followed by the identification by labeled probe hybridization. The DNA stands are denatured by alkaline treatment and are transferred to nylon or nitrocellulose membrane or filter in sandwiched between gel and stock paper towels which draws the transfer buffer from gel through capillary action by the blotting process. The desire DNA is detected by using a labeled probe (single strand DNA) complementary to the desire DNA.[5]

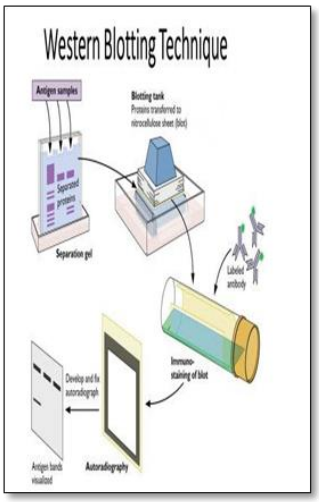
The northern blotting: Total RNA isolation is performed using the Tri-Reagent protocol. RNA samples (30µ g each) are run on 15% acrylamide denaturing (urea) Criterion precast gels (Bio-Rad), and then transferred onto Hybond-n + membrane.[5] Northern blotting starts with the formaldehyde contain agarose gel electrophoresis of separate RNA sample by size, based on the charge of nucleic acid sequence. RNA is single strands, not required for restriction enzymes. Since gel molecules are fragile in nature, the separated sequences are transferred to the nylon membranes. The selection of nylon membrane is contributed to the factor that nucleic acids are negatively charged in nature. Once the RNA molecules are transferred it is immobilized by covalent linkage. Aminobengoxy methyl filter paper is placed instead of nitrocellulose filter paper. The probe is then adde, the probe can be complementary an ssDNA sequence.[5]

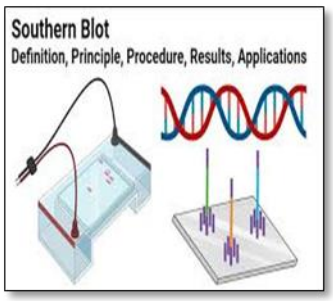
The western blotting: Protein has both positive and negative charge. For separation, need to any one charge of protein. Disulphide bond is found in between amino acid and protein. So at first to break disulphide bond by bêta marcapto ethanol which is the reducing agent. After getting primary structure of protein where different kind of amino acid present. SDS-PAGE(use generally coomassie dye blue) is a strong reducing agent and give negative charge, after binding of protein. Protein is separate according to the molecular weight in size by SDS-PAGE, to be transferred to the solid support (nitrocellulose or polyvinylidene difluoride) membrane. The solid support can absorb the protein and keep its biological activity unchanged. The transferred solid support membrane is called a blot and is treated with a protein solution to block the hydrophobic binding site on the membrane. The membrane is treated with the primary antibody of the target proteins. Only the proteins to be studied can specifically bind to the primary antibody to form an antigen-antibody complex. Primary and secondary anti body binds together and indicate their location.

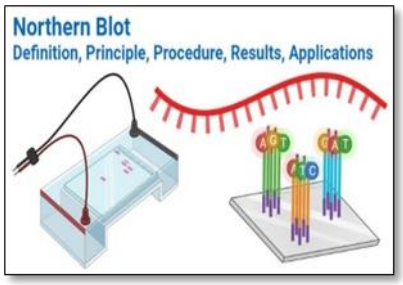
Methods of southern, northern and western blotting

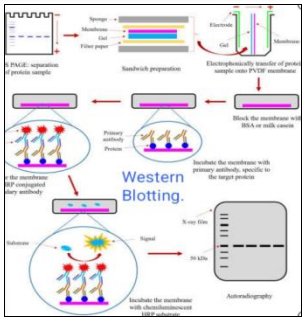












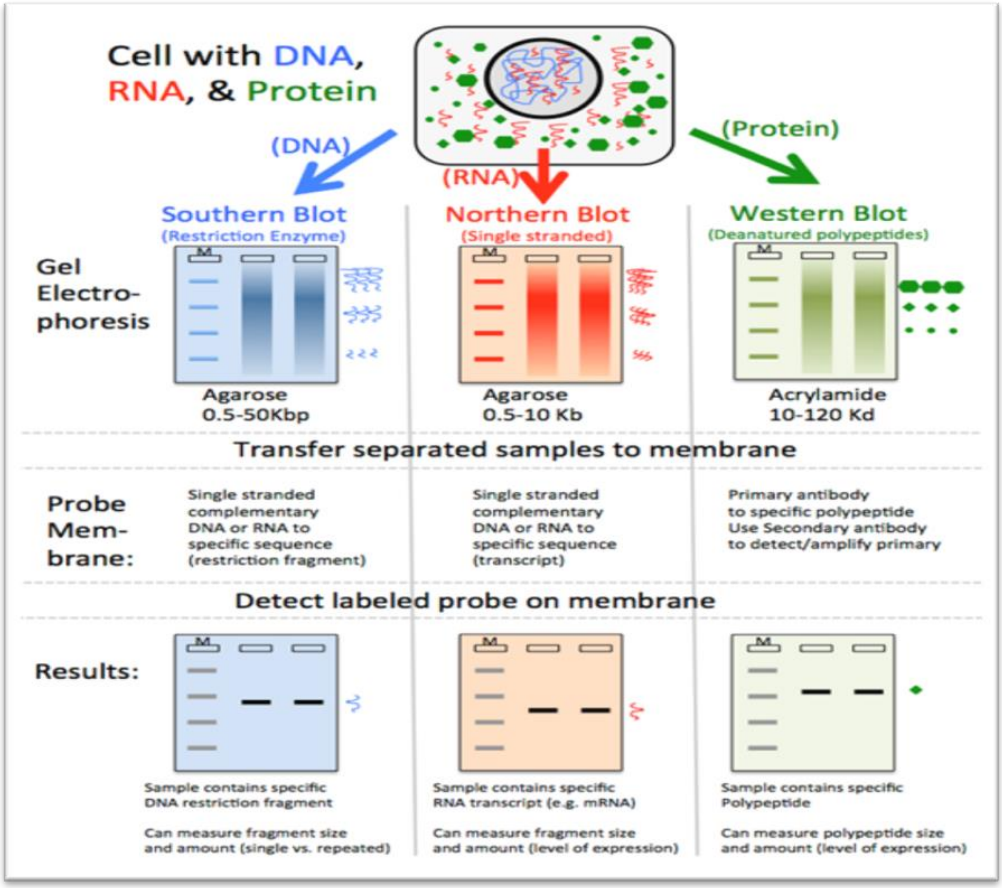
Procedure

Southern blotting	Northern blotting	Western blotting
Digestion of genomic DNA turns to DNA fragments. Size-separation of the fragments (standard agarose gel electrophoresis). In situ denaturation of the DNA fragments (by incubation in high temperature). Transfer of denatured DNA fragments into a solid support (nylon or nitrocellulose). Hybridization of the immobilized DNA to a labeled probe (DNA, RNA). Detection of the bands complementary to the probe (e.g. by autoradiography). Estimation of the size and number of the bands generated after digestion of the genomic DNA which is placing to the target DNA within a context o restriction sites.	RNA isolation(total or poly(A) RNA. Probe generation. Denaturing formaldehyde with agarose gel electrophoresis. Transfer of RNA from gel to the nylon membrane. Immobilization. Pre-hybridization and hybridization with probe. Washing. Detection. Stripping and re-probing. Under the radiography in the form of bands from the markers can be used to determine the length and semi quantification of the RNA fragments.	Proteins separated by SDS-PAGE are transferred from the polyacrylamide gel to a membrane, using a specialized apparatus. Nitrocellulose and PVDF membranes are commonly used. Stripping the membrane of antibodies after detection, and probing with another antibody. Different buffers are used for transfer, depending on the molecular weight and the nature of the target proteins. Blocking and probing with antibodies. Probing with antibodies. Determining an optimal concentration of the primary antibody. HRP or alkaline phosphatase labeled secondary antibodies. Detection of bands by Colormetric method.

RESULTS AND DISCUSSION

Blotting techniques mean the way of detecting of macro molecules that are present in the sample of cell as DNA, RNA and protein molecules. DNA is detecting by Southern blotting. RNA is detecting by Northern blotting and protein is detected by Western blotting. Among these three blotting Southern and Northern blotting are very similar where as Western blotting is different. Western blotting is most sensitive among them because it is detected up to neon gram. All kind of the blotting has generally four steps, these are (i) extraction of molecule, (ii) electrophoresis or separation of molecule, (iii) blotting process and (iv) probing of the molecule. Agarose gel electrophoresis is used for DNA, formaldehyde with agarose gel is used for RNA (because RNA is found in secondary structure format in linear) and where as SDS-PAGE is used in protein. In both Southern and Northern blotting time capillary force is working in blotting time but in Western blotting time electro elution technique is working. Single strand DNA probe with radioactive molecule is using to binding DNA-DNA hybridization. But Northern blotting DNA-RNA hybridization is occurred. Whereas in Western blotting primary and secondary antibodies are using as a probe. Radioactivity, chemilumn and colorimetric analysis are using in both Southern and Northern blotting as a detection system. But in Western blotting chemilumn and colorimetric analysis are using as a detection system.

Compare all three blotting



Comparison of Southern, Northern, and Western blots. Size and amount of DNA, RNA, and polypeptides can be determined using similar blotting methods. DNA is in blue, RNA in red, and polypeptides in green. A marker lane is shown in the left of each gel to determine size. A eukaryote cell is shown, but the same methods can be applied to prokaryotes.

APPLICATION AND CONCLUSIONS

Southern blotting is used in detection of DNA, DNA finger print, paternity testing, criminal identification, victim identification, isolate and identify desire gene of interest, restriction fragment length polymorphism, to identify mutation or rearrangement in the sequence of DNA, diagnosis of disease caused by genetic defects, use to identify infectious agents[11], etc.

Northern blotting is used in identification and separation of RNA fragments collected from different sources, transcription of DNA fragments that is used in Southern blotting as a probe, detection and quantification of specific mRNAs from different tissues and different living organisms, tool for gene expression studies such as cancer causing genes, gene expression during transplant rejects, molecular tool diagnosis of diseases like Crohn's

disease, detection of viral micro RNAs in viral infection.[12] etc.

Western blotting is using to determine the size and amount of protein in given sample, disease diagnosis, confirmatory test for HIV, to detect defective proteins, definitive test for Creutzfeldt-jacob disease, Lyme disease, Hepatitis B and Herpes[13], etc.

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