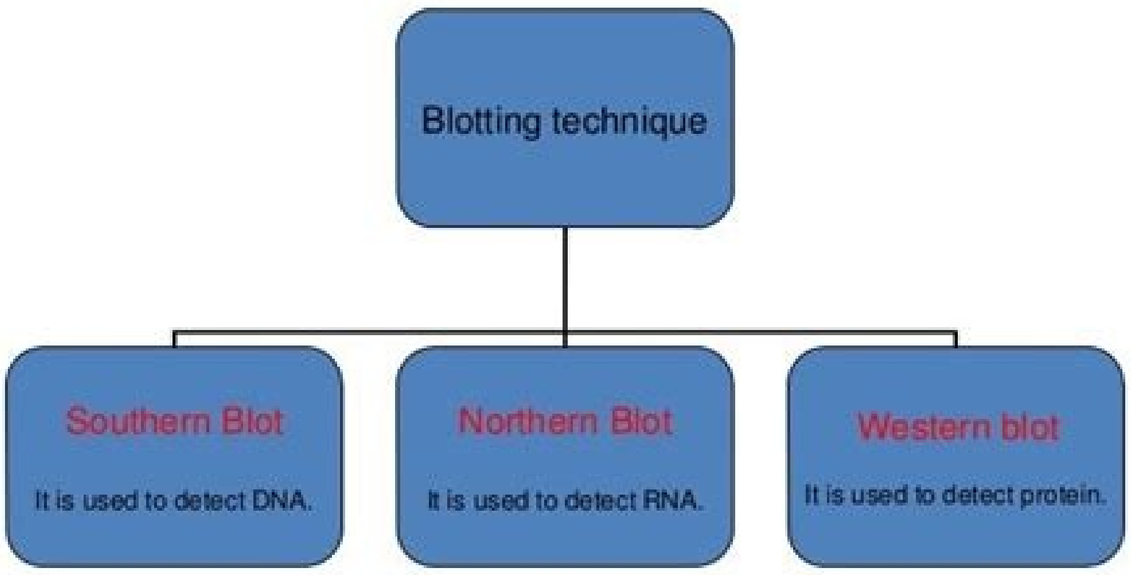
Southern northern and western blotting techniques pdf

TYPES OF BLOTTING TECHNIQUES



Umesh Goutam

Comparison Between Northern Blotting & Southern Blotting

Southern Blotting

- DNA molecules detected
- Agarose gel electrophoresis
- DNA/DNA hybridization
- Detecting systems are Colori, Radioactive, Chemical
- Probe is ssDNA
- Blotting method is capillary action
- Nitrocellulose membrane is

Northern Blotting

- RNA molecules detected
- Agarose denaturing gel electrophoresis with extra formaldehyde
- DNA/RNA hybridization
- Detecting systems are Colori, Radioactive, Chemical
- Probe is ssDNA
- Blotting method is capillary action
- Amino benzoxy methyl membrane is used

Step 1

Isolate RNA:

To detect rare mRNA, isolate the poly A^+ mRNA.

RNA is both biologically and chemically more labile than DNA. Thus eliminate RNases.

Step 2

Electrophoresis:

Performed in formaldehyde agarose gel to prevent RNA from folding on itself.

Stain with EtBr to visualize the RNA bands.

PAGE can be used with Urea.

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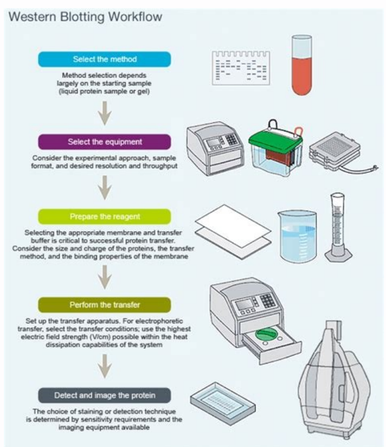
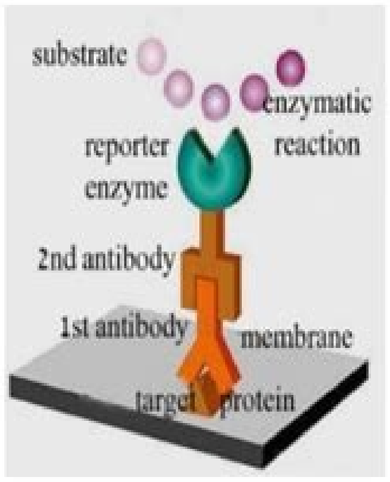


5. After washing for removal of non-specifically bound Ab1, second antibody (Ab2) is added.

- Ab2 specifically recognizes the primary antibody and binds.
- Ab2 is radioactively labeled, or is covalently linked to a reporter enzyme, which allows to visualize the protein-Ab1-Ab2 complex.



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1. Dr Ravi Kant Agrawal, MVSc, PhD Senior Scientist (Veterinary Microbiology) Food Microbiology Laboratory Division of Livestock Products Technology ICAR-Indian Veterinary Research Institute Izatnagar 243122 (UP) India 2. Hybridization is the process of establishing a non-covalent, sequence-specific interaction between two or more complementary strands of nucleic acids or proteins into a single hybrid. DNA,RNA or proteins will bind to their complement under normal conditions, so two perfectly complementary strands will bind to each other readily. Standard nucleic acid hybridization assays involve using a labeled nucleic acid probe to identify related DNA or RNA molecules (that is, ones with a significantly high degree of sequence similarity) within a complex mixture of unlabeled nucleic acid molecules, the target nucleic acid. Nucleic acid probes may be made as single-stranded or double- stranded molecules, but the working probe must be in the form of single strands. HYBRIDISATION 3. Blotting: History Southern Blotting is named after its inventor, the British biologist/Biochemist Professor Edwin Southern (1975). Other blotting methods (i.e. western blot, WB, northern blot, NB) that employ similar principles, but using protein or RNA, have later been named in reference to Edwin Southern's name. 4. 1. Southern Blotting This method involves seperation, transfer and hybridization. The Southern blotting is used to detect the presence of a particular piece of DNA in a mixture of sample. The DNA detected can be a single gene, or it can be part of larger piece of DNA such as viral genome. The key to this method is Hybridization. HYBRIDIZATION: Process of forming a double stranded DNA molecule between a single-stranded DNA probe and a single stranded target patient DNA. 5. Principle 1. The mixture of molecule is separated. 2. The molecules are immobilized on a matrix transferred to a solid support (blot). 3. The labeled probe is added to the matrix to bind to the molecule. 4. Any unbound probes are then removed. 5. The place where the probe is connected corresponds to the location of the immobilized target molecule. 6. Steps in Southern blotting 1. The DNA to be analyzed, such as the total DNA of an organism, is digested to completion with a restriction enzyme. 2. The complex mixture of fragments is subjected to gel electrophoresis to separate the fragment according to size. 7. Cont… 3.The restriction fragments present in the gel are denatured with heat or alkali and transferred onto a nitrocellulose or nylon membrane by blotting. This procedure preserves the distribution of the fragments in the gel, creating a replica of the gel on the filter. 8. Cont… 4.The filter is incubated under hybridization conditions with a specific radio labeled DNA probe. 5.The probe hybridizes to the complementary DNA restriction fragment. 6. Excess probe is washed away and the probe bound to the filter is detected by autoradiography, which reveals the DNA fragment to which the probe hybridized. 9. • Labeled material to detect a target. • For DNA: 20-30 nucleotides, complementary to a region in the gene • Methods of labeling: •Non-radioactive e.g. Biotin•Radioactive e.g. 32P Target DNA Probe Biotin Avidin* Target DNA Probe * Probes •Sensitive •Relatively cheap •Hazardous You should follow the radioactive waste disposal regulations. •Sensitive •Relatively expensive 10. Dr. Azhar Chishti The binding between ss labeled probe to a complementary nucleotide sequence on the target DNA. Detection of hybridization depends on method of probe labeling (radioactive or non-radioactive system e.g. biotin-avidin. Hybridization 11. Steps Digestion of genomic DNA (w/ ≥ one RE) DNA fragments Size-separation of the fragments (standard agarose gel electrophoresis) In situ denaturation of the DNA fragments (by incubation @ ↑temp/alkali treatment) Transfer of denatured DNA fragments into a solid support (nylon or nitrocellulose membrane). 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 & number of the bands generated after digestion of the genomic DNA w/ different RE placing the target DNA within a context of restriction sites) 12. METHODS OF TRANSFER Downward Capillary Transfer Upward Capillary Transfer Simultaneous Transfer to Two Membranes Electrophoretic Transfer Vacuum Transfer 13. Example of Transfer Upward Capillary Transfer Weight Glass Plate Whatman 3MM paper Gel Paper towels Membrane (nylon or nitrocellulose) Whatman 3MM paper Transfer buffer 14. Buffer drawn from a reservoir passes through the gel into a stack of paper towels DNA eluted from the gel by the moving stream of buffer is deposited onto a membrane weight tight connection 15. Southern blots are used in gene discovery, gene mapping, evolution and development studies, diagnostics and forensics. In regard to genetically modified organisms Southern blots is used for testing to ensure that a particular section of DNA of known genetic sequence has been successfully incorporated into the genome of host organism. Detection of Mutations A change in one nucleotide may alter the nucleotide sequence so that the restriction endonuclease fails to recognize and cleave at that site. The presence of a mutation affecting a restriction site causes the pattern of bands to differ from those seen with a normal gene (for example, in Figure, person 2 lacks a restriction site present in person 1). Applications 16. Dr. Azhar Chishti A ction A age A oph s d on 5- Hybridi tion e.g with 32P labeled probe 17. Example of Application of SB in diagnosis of mutation in globin gene 18. Dr. Azhar Chishti Example of Application of SB in diagnosis of mutation in globin gene 19. 2. Northern Blotting Northern blotting is a technique for detection of specific RNA sequences. Northern blotting was developed by Jamse Alwine and George Stark at Stanford University and was named such by analogy to Southern blotting. 20. Steps involved in Northern blotting 1. RNA is isolated from several biological samples (e.g. various developmental stages of same tissue etc.) * RNA is more susceptible to degradation than DNA 21. APPLICATIONS 1. A standard for direct study of gene expression at the level of mRNA (messenger RNA transcript). 2. Detection of mRNA transcript size . 3. Study RNA degradation. 4. Study RNA splicing – can detect alternatively spliced transripts. 5. Study RNA half-life. 6. Often used to confirm and check transgenic/ knockout mice (animals). 22. SOUTH WESTERN HYBRIDISATION Based along the lines of Southern blotting (which was created by Edwin Southern) and first described by B. Bowen and colleagues in 1980) It is a lab technique which involves identifying and characterizing DNA-binding proteins (proteins that bind to DNA) by their ability to bind to specific oligonucleotide probes. The proteins are separated by gel electrophoresis and are subsequently transferred to nitrocellulose membranes similar to other types of blotting. The name southwestern blotting is based on the fact that this technique detects DNA-binding proteins, since DNA detection is done by Southern blotting and protein detection is by western blotting. 23. DOT BLOT HYBRIDIZATION METHOD DNA/RNA samples are spotted onto a membrane and hybridized with a labeled probe that hybridizes to a specific DNA/RNA molecule., a dot represents the hybrid formation. Protocol : Tissue samples are collected and stored in a manner so that they don't decompose. Tissue sample is ground in a buffer and the debris removed by centrifugation Small amount of the liquid, e.g. 5 µl, is spotted on the nylon membrane Air dry the samples for 1 hour. 24. Membranes are treated with a denaturing solution to form ssDNA, which binds to the nylon membrane The labeled probe is prepared Membrane and labeled probe are placed in a hybridization solution. Membranes are washed to remove any probe that was not hybridized to the test DNA Membranes dried and placed adjacent to X-ray film. The intensity of dot in the autoradiograph corresponds with the extent to which DNA or RNA is represented in the sample. 25. Dot blots are faster to set up than southern or northern blots because gels don’t have to be run and therefore, the transfer of nucleic acids to the filter is far quicker. However, dot blots give no information on the size and number of different sequences contributing to the hybridization signal. Dot blots are well-suited for analysis of many samples at once. Advantages 26. COLONY HYBRIDIZATION Colony Hybridization is the screening of a library with a labeled probe (radioactive, bioluminescent, etc.) to identify a specific sequence of DNA, RNA, enzyme, protein, or antibody. 27. In situ hybridization In situ hybridization (ISH) is based on the complementary pairing of labelled DNA or RNA probes with normal or abnormal nucleic acid sequences in intact chromosomes, cells or tissue sections. Application of nucleic acid hybridization technology used to detect the presence of nucleic acids in cells can be used to identify either DNA or RNA molecules in cells. DNA: Cytogenetic analysis; location of genes on chromosomes, chromosomal rearrangements RNA: detect populations of cells expressing a particular mRNA transcript 28. Cells are treated with ribonuclease (RNase) and sodium hydroxide (NaOH) to degrade RNA and to denature their DNA without affecting their structural integrity. The treated cells unpack, exposing their DNA segments that become available for DNA:DNA/DNA:RNA pairing. A sample of gene is radioactively labeled and applied to chromosome preparation. After appropriate interval, the cell preparation is washed to remove any unbound radioactive DNA (or RNA) probe. Hybridization occurs between the gene and its chromosomal copy, resulting in dark spot on autoradiograph Probe can be detected with a fluorescent antibody in a technique called fluorescence in situ hybridization (FISH). 29. ISH Using Digoxigenin-labelled DNA Probe 30. APPLICATIONS It is used in the diagnosis of diseases, particularly for the detection of viruses in cells and tissues. It has been applied for the detection of cytomegalovirus, human papilloma virus, human immunodeficiency virus, JC virus, B 19 parvovirus, HSV- 1, Epstein-Barr virus, hepatitis B virus, hepatitis delta virus, measles virus, Chlamydia trachomatis, Salmonella and Mycoplasma. It has also been applied to karyotype preparations to study chromosomal changes in tumours and viral infections. ISH has been applied using cDNA probes for detection of mRNAs coding for regulatory peptides in the neuroendocrine system. 31. Microarray A technique which allows for the rapid and simultaneous screening of many thousands of genes. Hybridisation is the basis behind the technique of DNA microarrays. Oligonucleotides or PCR probes are immobilized on a solid support (the matrix) and due to their specificity to a target gene they will detect complementary sequences present in a mixture which is to be analyzed. 32. Protocol 1.Preparation of probe: The DNA arrays are basically of 2 types: (a) Spotted DNA array: The source of DNA is typically purified cDNAs that have been amplified by PCR in individual wells of a 96-well microtitre plate. Samples of about 0.05 µl are transferred by a computer-controlled print head to predetermined positions on a series of microscope slides. These have been coated with positively charged poly-L-lysine to enhance adhesion. (b) Oligonucleotide chips: Oligonucleotides of any sequence can be used. Oligonucleotides of specific sequence can either be synthesized in situ or prepared separately and attached post-synthetically. The maximum length attainable for oligonucleotides in an array is about 25 meters. A density of oligonucleotides of at least 400,000/1.6 cm2 can be achieved at present. 33. Sample preparation: RNA is isolated from the sample and quickly degraded. It is converted into complementary DNA (cDNA) by using reverse transcriptase. The cDNA is then labeled with suitable fluorescent probe. The DNA microarray is used for hybridization with cDNA probes. The unbound probes are removed by washing. Each labeled cDNA molecule will hybridize with a DNA molecule of that dot of microarray, to which it is complementary. These spots will fluoresce and will be detected as coloured spot. The last step is the data analysis, where in accordance to the intensity of colour produced, the inference is taken. 34. Microarrays detect expressed genes by hybridization Each spot has a different synthetic oligonucleotide complementary to a specific gene. 35. APPLICATIONS In genomics, In Diagnostics, In Gene expression analysis and DNA sequencing. 36. Thanks Acknowledgement: All the material/presentations available online on the subject are duly acknowledged. Disclaimer: The author bear no responsibility with regard to the source and authenticity of the content. Questions???