Course	**: PGPathshala-Biophysics**
Paper 11	**: Cellular and Molecular Biophysics**
Module 20	**: Theory and Practicals of Bloting Techniques in Molecular biology**
Content Writer	**: Dr. Subhradip Karmakar, AIIMS, New Delhi**

Abstract :

Blotting is a method in which a macromolecule is immobilized on a solid matrix and subsequently probed with a detectable ligand to determine whether the macromolecule binds specifically to its ligand. Depending on whether the immobilized macromolecule is DNA, RNA or protein, one generates DNA blots (Southern blots), RNA blots (Northern blots) (1), or protein blots (Western blots). The macromolecule can be applied to the blotting matrix directly (dot blot), or it can be derived and eluted from an electrophoretic gel (gel blot).

Blotting techniques are used to separate DN, RNA and protein types of molecules. In cells, they exist as a mixture. Blotting allows researchers to find one protein among many, like a needle in a haystack. Blotting is generally done by letting a mixture of DNA, RNA or protein flow through a slab of gel. This gel allows small molecules to move faster than bigger ones. The separated molecules are then pressed against a membrane, which helps move the molecules from the gel onto the membrane. The molecules stick to the membrane, but stay in the same location, apart from each other, as if they were still in the gel.

Introduction :

To isolate a gene, genomic DNA is extracted from a selected tissue. For a better handling the relatively large DNA molecules are cut into a mixture of fragments by restriction endonucleases. The fragments are then separated from each other according to their size by gel electrophoresis. A procedure called Southern blotting is used to verify the presence of the desired gene in one of the DNA fragments separated on an agarose gel. The DNA fragments are transferred from the gel to a filter whereby the original fragment pattern is maintained. Then, a single-stranded DNA or RNA probe specific for the gene to be isolated is hybridized to its target fragments fixed to the filter. A radioactive or fluorescent tag is attached to the probe for subsequent identification. In cases where only transcribed sequences are to be isolated cytoplasmic messenger RNA (mRNA) is prepared instead of DNA. Analysis of RNA by a technique similar to Southern blotting is termed Northern blotting. Preservation of DNA sequences is usually achieved by DNA cloning. DNA cloning involves the insertion of a DNA fragment into a DNA vector and the stable incorporation of the recombinant DNA into a suitable host.

Blotting is a common laboratory procedure in which biological molecules in a gel
matrix are transferred onto nitrocellulose or nylon membrane for further scientific analysis. The biological molecules transferred in this process are DNA, RNA or proteins. The blotting procedure is named differently depending on the type of the molecules being transferred. When DNA fragments are transferred the procedure is called a Southern bl, named after Edward Southern that first developed it. The Northern blotting procedure, which transfers RNA molecules, was developed shortly thereafter and humorous named Northern blotting.

Western blotting involves the transfer of proteins. All blotting procedures begin with a standard process called gel electrophoresis when DNA, RNA, or proteins are loaded on to an agarose or acrylamide gel and separated on the gel through an electric field. Two types of gels are commonly used: agarose gels and acrylamide gels. Transfer is initiated when nitrocellulose or nylon membrane is laid on top of the gel and biological molecules are transfer from the gel to the membrane. Hybridization / blotting is a technique in which biological molecular (DNA, RNA or protein) are immobilized onto a nylon or nitrocellulose membrane. A probe (a piece of nucleic acid with identical and specific sequence to the organism or gene of interest) can then hybridize (join) to the biological molecules (DNA, RNA or protein) with identical sequence on the membrane.

The hybridization between the blotted DNA and probe is visualized by labeling the probe in some way.Short fragments of DNA that have a nucleotide sequence complementary to the molecule being analyzed are normally used as probes in Southern and Northern blots. Antibodies that react with the protein being analyzed are used as probes in a Western blot

Blotting technique is an extremely powerful tool for analyzing gene structure and used to study gene expression, once cloned cDNA is isolated. There are three important types of blotting techniques are: 1. Southern Blotting 2. Northern Blotting 3. Western Blotting.

Objectives :

1. **Southern Blot**
2. **Northern Blot**
3. **Western Blot**
4. **SouthWestern Blot**
5. **NorthWestern Blot**
6. **Far Western Blot**

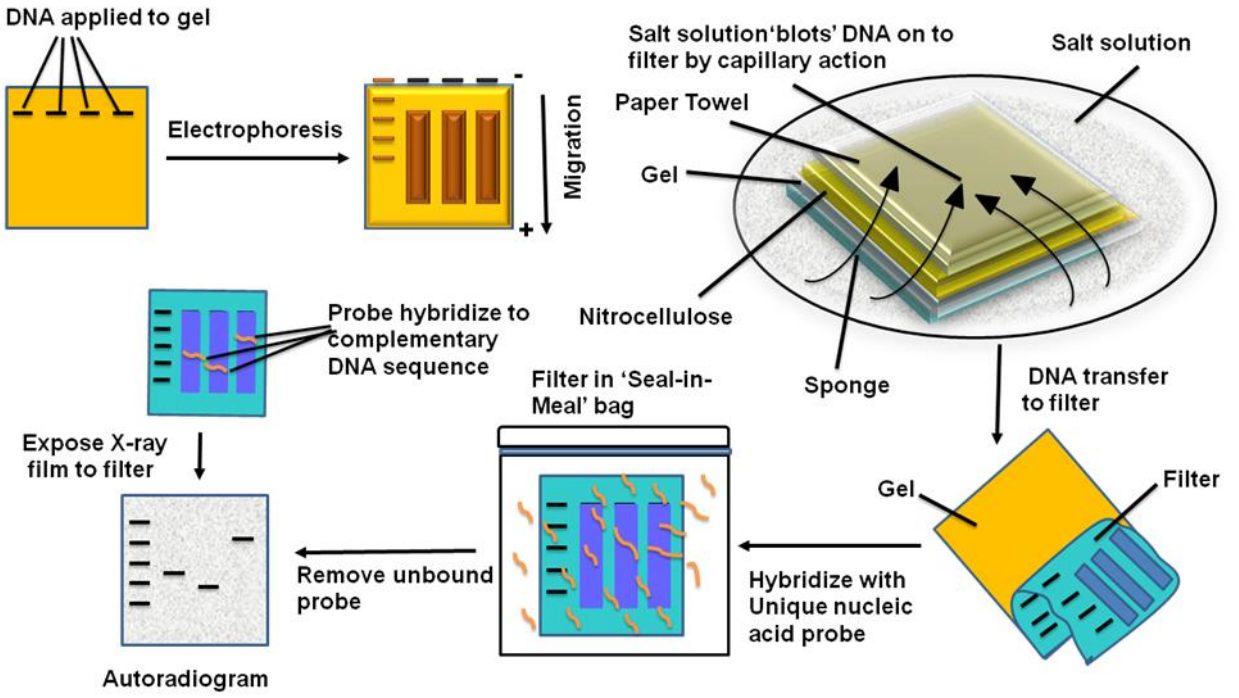
Technique # 1. Southern Blotting:

Developed by E.M. Southern, the technique of Southern blotting is one of the most important methods used in molecular biology. In Southern blotting, DNA is transferred from a gel to a membrane for hybridization analysis. In this technique, the DNA is cut with suitable restriction enzymes and run on a gel. Treatment with NaOH denatures the DNA to form single strand.

- Southern blotting is an example of RFLP (restriction fragment length polymorphism).. Southern blotting is a hybridization technique for identification of particular size of **DNA** from the mixture of other similar molecules**.** This technique is based on the principle of separation of DNA fragments by gel electrophoresis and identified by labelled probe hybridization.

- Basically, the DNA fragments are separated on the basis of size and charge during electrophoresis. Separated DNA fragments after transferring on nylon membrane, the desired DNA is detected using specific DNA probe that is complementary to the desired DNA.
- A hybridization probe is a short (100-500bp), single stranded DNA. The probes are labeled with a marker so that they can be detected after hybridization.

The transfer of DNA from agarose gel to the membrane is performed by capillary action. The gel is placed above the buffer saturated filter paper. The nitrocellulose membrane is placed above the gel and covered by 2-3 layers of dry filter paper towel. A flow of buffer occurs through the gel and membrane to the top papers.



Work flow for Southern Blot

Step 1: DNA digestion

Obtaining complete fragmentation of your DNA at the intended restriction enzyme sites is a critical step in Southern blot analysis.

Step 2: Gel electrophoresis

Fragmented DNA is typically electrophoresed on an agarose gel to separate the fragments according to their molecular weights. Acrylamide gels can alternatively be used for good resolution of smaller DNA fragments (<800 bp).

Step 3: Blotting

After electrophoresis, DNA is transferred to a positively charged nylon membrane. Traditional transfer of DNA is done overnight using an upward-transfer method. For reliable and consistent transfer with minimal background, Nylon Membranes are highly recommended. The membranes are ideal for use with radiolabeled and nonisotopic probes to achieve maximum hybridization signal.

Step 4: Probe labeling

A nucleic acid probe with sequence homologous to the target sequence under study is labeled with radioactivity, fluorescent dye, or an enzyme that can generate a chemiluminescent signal when incubated with the appropriate substrate. The choice of the label depends on several factors such as the nature of your probe or probe template, sensitivity needed, quantification requirements, ease of use, and experimental time.

Step 5: Hybridization & washing

During hybridization, the labeled probe is incubated with the DNA fragments that are immobilized on the blot under conditions that promote hybridization of complementary sequences. When used for both prehybridization and hybridization, can increase sensitivity up to 100 times compared to other hybridization solutions by pushing hybridization to completion without increasing background. As few as 10,000 target molecules can be detected. Because ULTRAhyb® buffer maximizes blot sensitivity, for many targets hybridization can typically be performed in just 2 hours.

After hybridization, the unhybridized probe is removed by washing in several changes of buffer. Low stringency washes (e.g., with 2X SSC or SSPE) remove the hybridization solution and unhybridized probe. High-stringency washes (e.g., with 0.1X SSC or SSPE) remove partially hybridized probe molecules. The result is that only fully hybridized labeled probe molecules, with complementary sequence to the region of interest, remain bound.

Step 6: Detection

In the detection step, the bound, labeled probe is detected using the method required for the particular label used. For example, radiolabeled probes may be detected using X-ray film or a phosphorimaging instrument, and enzymatically labeled probes are typicallly detected by incubating with a chemiluminescent substrate and exposing the blot to X-ray film.

Applications of Southern blotting:

1. Southern blotting technique is used to detect DNA in given sample.
2. DNA finger printing is an example of southern blotting.
3. Used for paternity testing, criminal identification, victim identification
4. To isolate and identify desire gene of interest.
5. Used in restriction fragment length polymorphism
6. To identify mutation or gene rearrangement in the sequence of DNA
7. Used in diagnosis of disease caused by genetic defects
8. Used to identify infectious agents

Applications in research :

A relatively new method for the diagnostic characterization of malignant lymphomas was established with the introduction of gene probes usinfg Souther Blot for the immunoglobulin and T cell receptor gene segments . Though the method is not absolutely specific for the determination of lineage and clonality of a given lymphoid neoplasm, it provides a lot of additional information for the pathologist. It is the first method that gives proof to the clonality of T cell lymphomas; reactive lymph node processes can be distinguished from true neoplasms; within lymph nodes of mixed lymphoid populations the clonally proliferated can be detected and the lineage can be determined. Therefore the Southern Blot Analysis is a method that should be applied for the diagnosis of malignant lymphomas together with histology and immunohistochemistry. Using the combination of all these methods an extensive characterization of lymphoid neoplasms can be made.

Reference : Pathol Res Pract. 1989 Apr;184(4):455-63.

Technique # 2. Northern Blotting:

RNA Isolation

A northern blot is a laboratory method used to detect specific RNA molecules among a mixture of other population of RNA. Northern blotting can be used to analyze a sample of RNA from a particular tissue or cell type in order to measure the RNA expression of particular genes. This method was named for its similarity to the technique known as a Southern blot.

The first step in a northern blot is to denature, or separate, the RNA within the sample into single strands, which ensures that the strands are unfolded and that there is no bonding between strands. The RNA molecules are then separated according to their sizes using a method called gel

electrophoresis. Following separation, the RNA is transferred from the gel onto a blotting membrane. (Although this step is what gives the technique the name "northern blotting," the term is typically used to describe the entire procedure.) Once the transfer is complete, the blotting membrane carries all of the RNA bands originally on the gel. Next, the membrane is treated with a small piece of DNA or RNA called a probe, which has been designed to have a sequence that is complementary to a particular RNA sequence in the sample; this allows the probe to hybridize, or bind, to a specific RNA fragment on the membrane. In addition, the probe has a label, which is typically a radioactive atom or a fluorescent dye. Thus, following hybridization, the probe permits the RNA molecule of interest to be detected from among the many different RNA molecules on the membrane.

Obtaining high-quality, intact RNA is a critical step in performing northern blot analysis. All protocols, techniques, and commercially available kits used to isolate RNA share these common attributes:

- Cellular lysis and membrane disruption
- Inhibition of ribonuclease activity
- Deproteinization
- Recovery of intact RNA

The detail steps involved are :

The steps involved in Northern analysis include:

- RNA isolation (total or poly(A) RNA)
- Probe generation
- Denaturing agarose gel electrophoresis
- Transfer to solid support and immobilization
- Prehybridization and hybridization with probe
- Washing
- Detection
- Stripping and reprobing (optional)

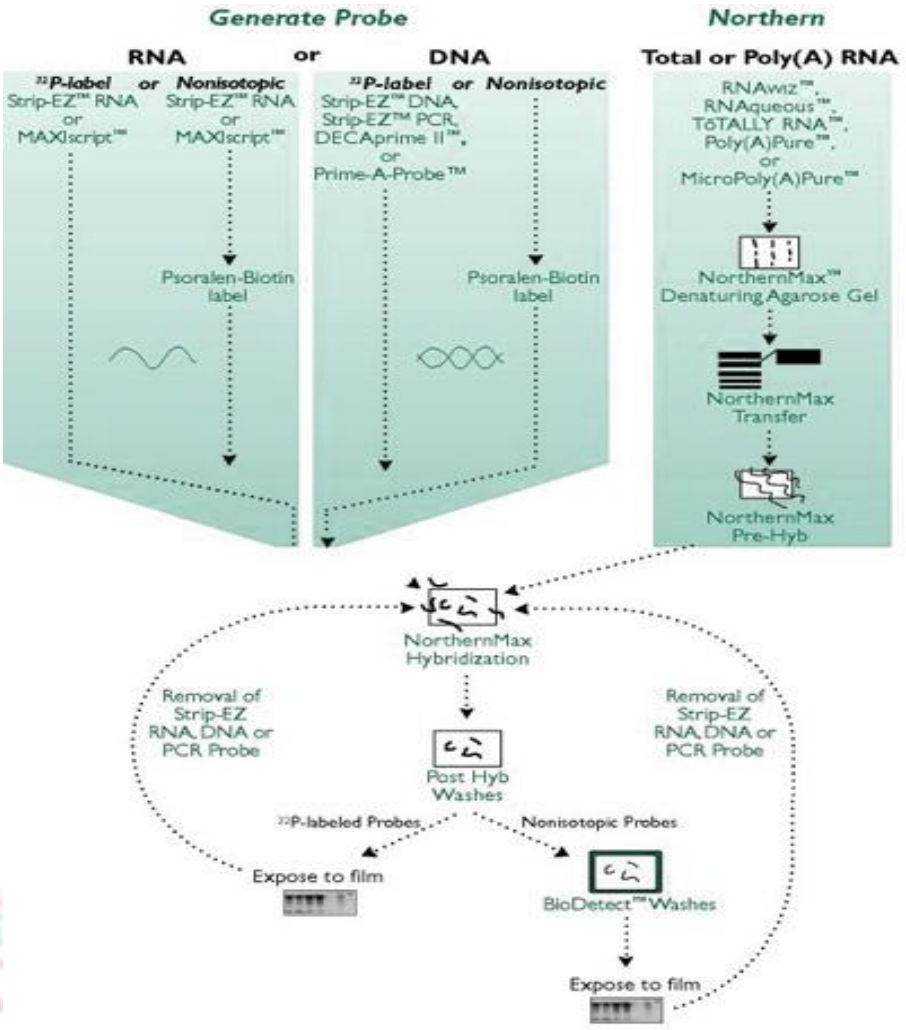
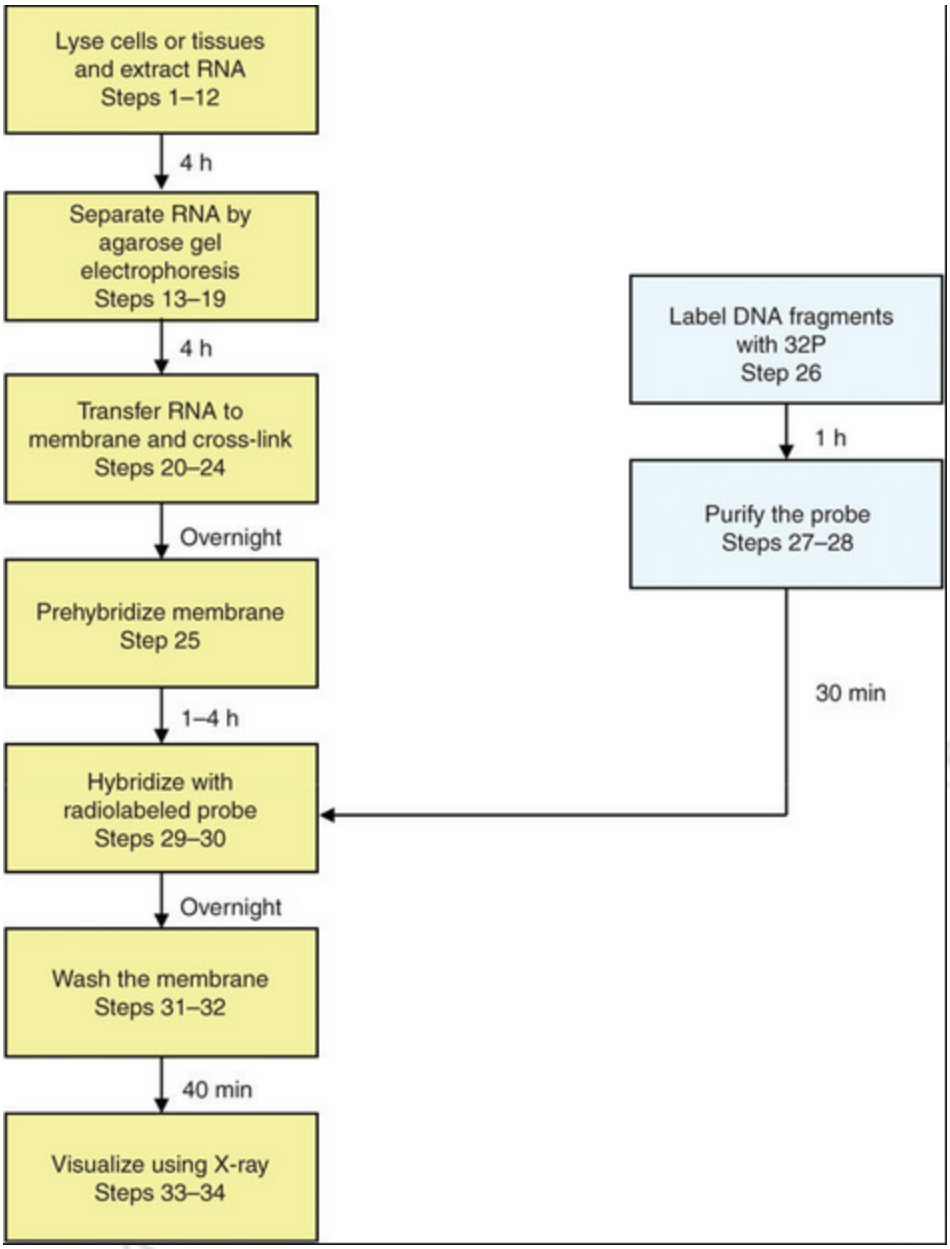


Image adapted from : https://www.thermofisher.com

Northern blots can be probed with radioactively or nonisotopically labeled RNA, DNA or oligodeoxynucleotide probes. Research at Ambion has revealed startling differences in the signal sensitivities on Northern blots achieved by three methods of probe synthesis when using standard formamide or aqueous hybridization buffers — random-priming of DNA, asymmetric PCR-generated DNA and in vitro transcription of RNA. While probes for Northerns and Southerns have been historically synthesized by random-primed labeling, our results indicate that probes synthesized by asymmetric PCR are 3-5 fold more sensitive than random-primed probes, and that RNA probes provide an additional 10-fold increase in sensitivity. RNA probes have the added advantage that they can be hybridized and washed under more stringent conditions, which results in lower background and fewer problems with cross-hybridization.



Workflow for Northern Blot

Applications for Northern Blot :

APPLICATIONS

- Southern blots are used in gene discovery , mapping, evolution and development studies, diagnostics and forensics (It is used for DNA fingerprinting, preparation of RFLP maps)
- identification of the transferred genes in transgenic individuals, etc.
- It is an invaluable method in gene analysis.
- Important for the conformation of DNA cloning results.
- Highly useful for the determination of restriction fragment length polymorphism (RFLP) associated with pathological conditions.

Difference between Norther and Southern Blots :

Southern blotting	Northern blotting
Southern name of inventor	Northern a misnomer
Separation of DNA	Separation of RNA
Denaturation needed	Denaturation not needed
Nitrocellulose filter membrane	Amino benzyloxymethyl filter paper Membrane
DNA-DNA Hybridization	RNA-DNA Hybridization

Technique # 3. Western Blotting:

The first step in a western blotting procedure is to separate the macromolecules in a sample using gel electrophoresis. Subsequently, the separated molecules are transferred or blotted onto a second matrix, generally a nitrocellulose or polyvinylidene difluoride (PVDF) membrane. Next, the membrane is blocked to prevent any nonspecific binding of antibodies to the surface of the membrane. Most commonly, the transferred protein is then probed with a combination of antibodies: one antibody specific to the protein of interest (primary antibody) and another antibody specific to the host species of the primary antibody (secondary antibody). Often the secondary antibody is complexed with an enzyme, which when combined with an appropriate substrate, will produce a detectable signal. Chromogenic substrates produce a precipitate on the membrane resulting in colorimetric changes visible to the eye. The most sensitive detection methods use a chemiluminescent substrate that produces light as a byproduct of the reaction with the enzyme conjugated to the antibody. The light output can be captured using film. However, digital imaging instruments based on charge-coupled device (CCD) cameras are becoming popular alternatives to film for capturing chemiluminescent signal. Alternatively, fluorescently-tagged antibodies can be used, which require detection using an instrument capable of capturing the fluorescent signal. Fluorescent blotting is a newer technique and is growing in popularity as it affords the potential to multiplex (detect multiple proteins on a single blot). Whatever system is used, the intensity of the signal should correlate with the abundance of the antigen on the membrane.

Procedures vary widely for the detection step of a western blot experiment. One common variation involves direct versus indirect detection. With the direct detection method, an enzyme- or fluorophore-conjugated primary antibody is used to detect the antigen of interest on the blot. This detection method is not widely used as most researchers prefer the indirect detection method for a variety of reasons. In the indirect detection method, an unlabeled primary antibody is first used to bind to the antigen. Subsequently, the primary antibody is detected using an enzyme- or flourophore-conjugated secondary antibody. Labels (or conjugated molecules) may include biotin, fluorescent probes such as Invitrogen AlexaFlour or DyLight flourophores, and enzyme conjugates such as horseradish peroxidase (HRP) or alkaline phosphatase (AP). The indirect method offers many advantages over the direct method, which are described below.

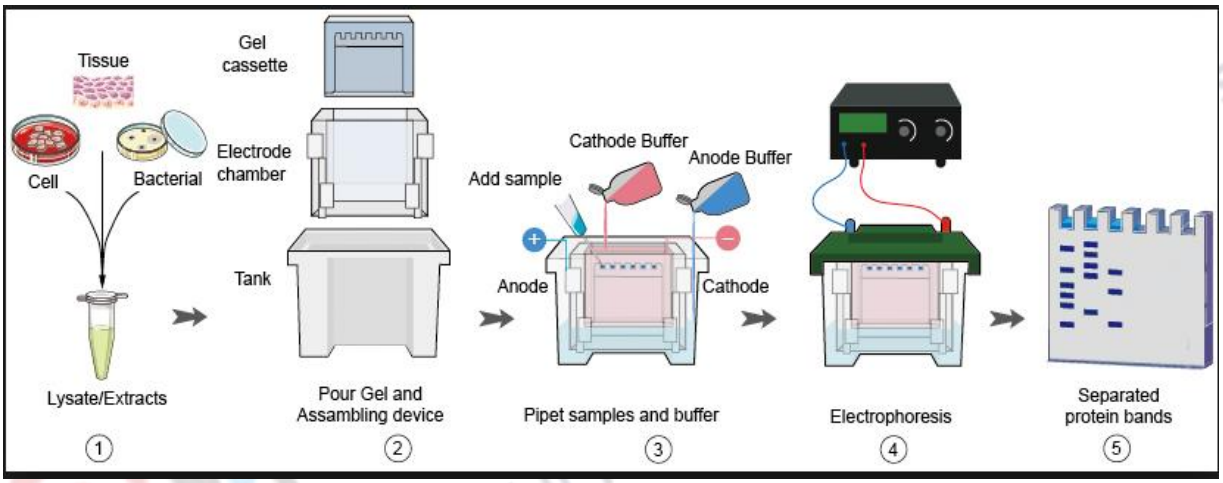
The Biomedical Application of Western Blot:

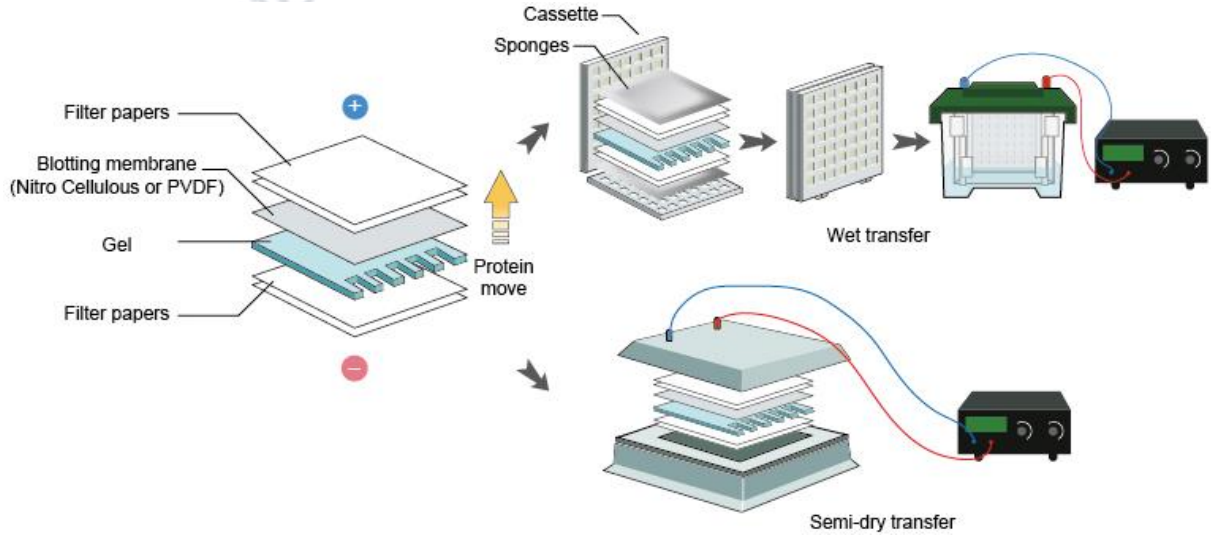
1. Western blot is applied in a confirmatory HIV-test to detect anti-HIV antibody in a human serum sample. Proteins like gp41, gp120, from known HIV-infected cells are separated and blotted on a membrane. Then, in the primary antibody incubation step, the serum to be tested is applied; free antibody is washed away, and a secondary anti-human antibody conjugated with an enzyme signal is added. Then the stained bands will indicate whether the patient's serum contains anti-HIV antibody. This is the main principle of western blot medical diagnosis assay for HIV infection.

2. Under appropriate conditions, the western immunoblotting technique is quantitative. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separated viral proteins .Viral proteins are transferred quantitatively to nitrocellulose by electroblotting in SDS-containing

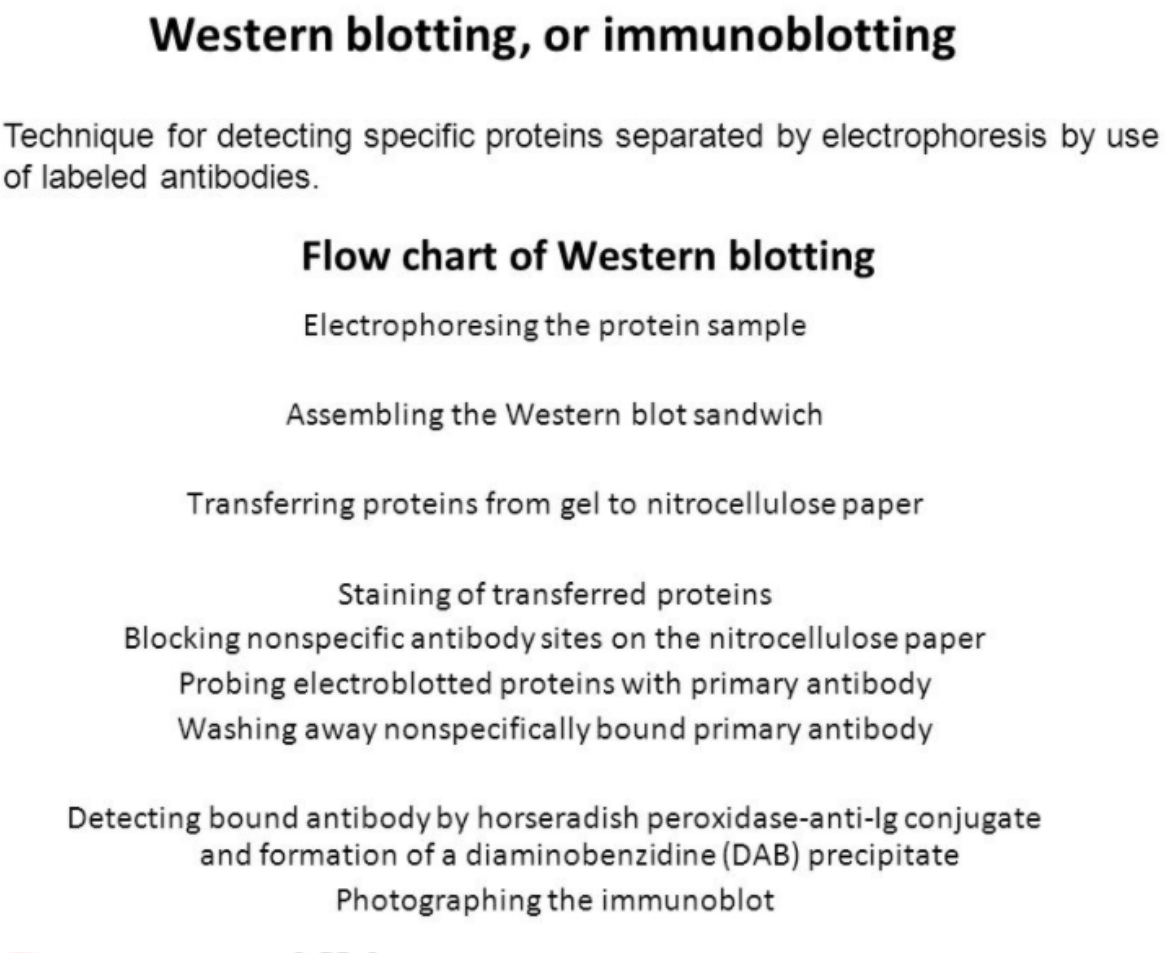
buffer. Monoclonal antibodies directed against previously defined epitopes on the viral proteins were used as probes to detect viral protein synthesis and expression, as well as processing in animal tissues. Because of their polypeptide specificities, circulating polyclonal antibodies were also probed and characterized. Finally, a highly sensitive dot immunoblotting assay can analyze the sensitivity and denaturation of various epitopes on the viral proteins. Picogram quantities of viral antigens and antibodies were detected by this assay.

Setup for Western Blot:





Working Protocol :



<u>Applications of Western Blot :</u>

Application1: It can identify the nature of the protein or epitope effectively. Also, it can be applicated as a tool of quantitative analysis of the micromolecule antigen in cooperation with immunoprecipitation.

Application2: Epitope mapping

Epitope mapping can identify the process of the binding sites, or 'epitope', of antibodies on their target antigens (which are proteins). The identification and characterization of the binding sites of can help us to discover and develop new therapeutics, diagnostics and vaccines. Epitopes (the binding sites on the protein) can be divided into conformational and linear. Conformational epitopes are made up of amino acids that are discontinuous in the protein sequence but are

assembled on the basis of three-dimensional protein folding, while Linear epitopes are formed by a continuous sequence of amino acids in a protein. Most of antigen-antibody interactions have conformational epitopes.Because of the difficulty in expressing and purifying these types of antigens, epitope mapping of complex target antigens, such as integral membrane proteins or multi-subunit proteins, is often challenging.

Western blot applicaiton in epitope mapping

Application3: Amino acid composition and sequence analysis, extremely trace protein (10 pmol) transferd to PVDF membrane .Amino acid composition or sequence analysis of transferred protein or peptide bands after dyed by coomassie brilliant blue.

Application4: Spots imprinting analysis, available chromatography components analysis, sucrose gradient analysis or pulse tracking experimental analysis.

Application5: Test the endogenous or exogenous expression phosphoprotein so as to detect the phosphorylation signal.

Application6: Protein resilience in the function experiment.

Application7: Structure domain analysis.

Application8: Analysis of the protein expression level.

Application9: Analysis of some protein content in the serum.

Application10: Eliminate the albumin and IgG in the serum.

Application11: Analysis of regulation protein expressed in the cell cycle

Troubleshooting :

Even though the procedure for western blot is simple, many problems can arise, leading to unexpected results. The problem can be grouped into five categories: (1) unusual or unexpected bands, (2) no bands, (3) faint bands or weak signal, (4) high background on the blot, and (5) patchy or uneven spots on the blot.

Unusual or unexpected bands can be due to protease degradation, which produces bands at unexpected positions. In this case it is advisable to use a fresh sample which had been kept on ice or alter the antibody. If the protein seems to be in too high of a position, then reheating the sample can help to break the quaternary protein structure. Similarly, blurry bands are often caused by high voltage or air bubbles present during transfer. In this case, it should be ensured that the gel is run at a lower voltage, and that the transfer sandwich is prepared properly. In addition, changing the running buffer can also help the problem. Nonflat bands can be the result of too fast of a travel through the gel, due to low resistance. To fix this the gel should be

optimized to fit the sample. Finally, white (negative) bands on the film are due to too much protein or antibody.

Conclusion :

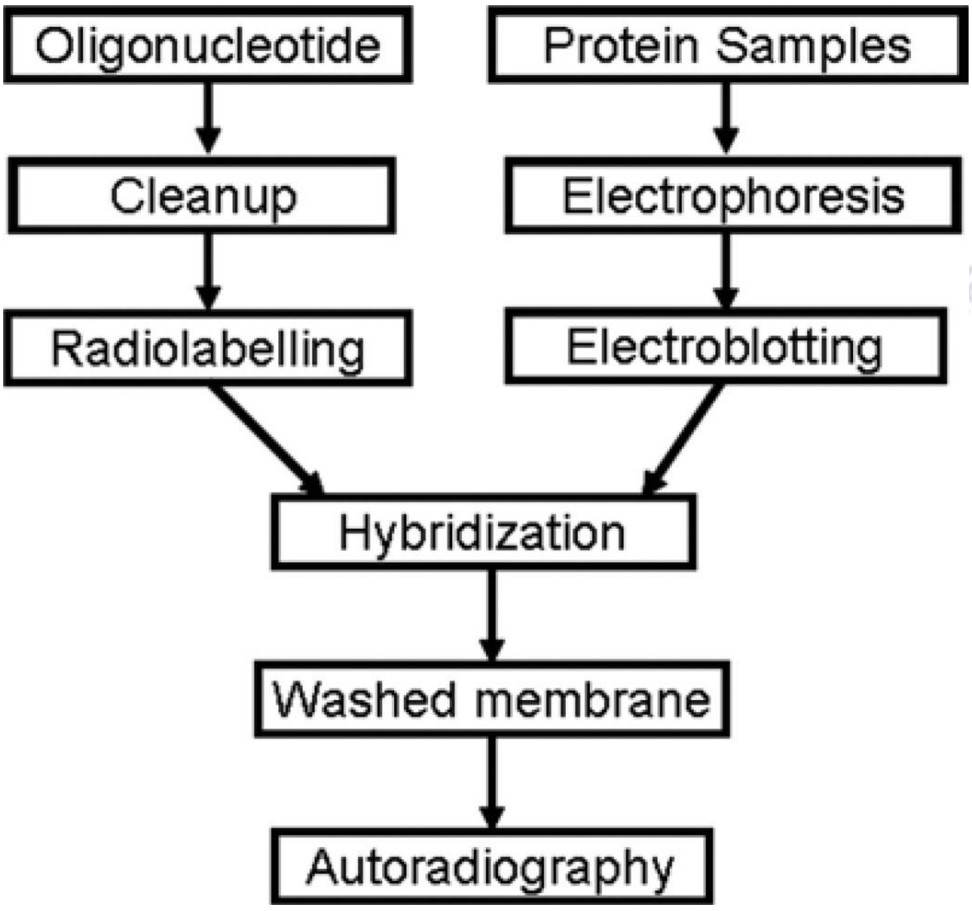
Western blot is a technique that is very useful for protein detection as it allows the user to quantify the protein expression as well. Though not as specific like PCR, western blot can be seen as an intricate balance, as the researcher attempts to get a nonspecific, yet strong signal.

Technique # 4. Southwestern Blotting:

Southwestern blotting is used to investigate DNA-protein interactions. The advantage of this technique over other related methods such as electrophoretic mobility shift assay (EMSA) and DNA footprinting is that it provides information regarding the molecular weight of unknown protein factor. This method combines the features of Southern and Western blotting techniques; a denaturing SDS-PAGE is first employed to separate proteins electrophoretically based on size, and after transferring the proteins to a membrane support, the membrane-bound proteins are renatured and incubated with a (32)P-labeled double-stranded oligonucleotide probe of specific DNA sequence. The interaction of the probe with the protein(s) is later visualized by autoradiography. This technique could be combined with database searching (TransFac, http://www.gene-regulation.com/pub/databases.html#transfac), prediction of potential protein factors binding onto a target motif (e.g., Patch search), in vitro supershift EMSA and in vivo chromatin immunoprecipitation (ChIP) assays for effective identification of protein factors. The whole Southwestern blotting procedure takes approximately 4 d to complete. South Western blot is mainly for rapid characterization of both DNA binding proteins and their specific sites on genomic DNA is described. Proteins are separated on a sodium dodecyl sulfate (SDS) polyacrylamide gel, renatured by removing SDS in the presence of urea, and blotted onto nitrocellulose by diffusion. The genomic DNA region of interest is digested by restriction enzymes selected to produce fragments of appropriate but different sizes, which are subsequently end-labeled and allowed to bind to the separated proteins. The specifically bound DNA is eluted from each individual protein-DNA complex and analyzed by acrylamide gel electrophoresis. Evidence that tissue-specific DNA binding proteins may be detected by this technique is presented. Moreover, their sequence-specific binding allows the purification of the corresponding selectively bound DNA fragments and may improve protein-mediated cloning of DNA regulatory sequences

This method detects specific DNA-binding proteins by incubating radiolabeled DNA with a gel blot, washing, and visualizing through autoradiography. A blot resulting from 1-dimensional SDS-PAGE reveals the molecular weight of the binding proteins. To increase separation and determine isoelectric point a 2-dimensional gel can be blotted. Additional dimensions of electrophoresis, such as a gel shift (EMSA), can precede isoelectric focusing and SDS-PAGE to further improve separation. Combined with other techniques, such as mass spectrometry, the DNA-binding protein can be identified.

Two-dimensional Southwestern blotting (2D-SW) described here combines several steps. Proteins are separated by two-dimensional gel electrophoresis and transferred to nitrocellulose (NC) or polyvinylidene fluoride (PVDF) membrane. The blotted proteins are then partially renatured and probed with a specific radiolabeled oligonucleotide for Southwestern blotting (SW) analysis. The detected proteins are then processed by on-blot digestion and identified by LC-MS/MS analysis. A transcription factor, bound by a specific radiolabeled element, is thus characterized without aligning with protein spots on a gel. In this study, we systematically optimize conditions for 2D-SW and on-blot digestion.

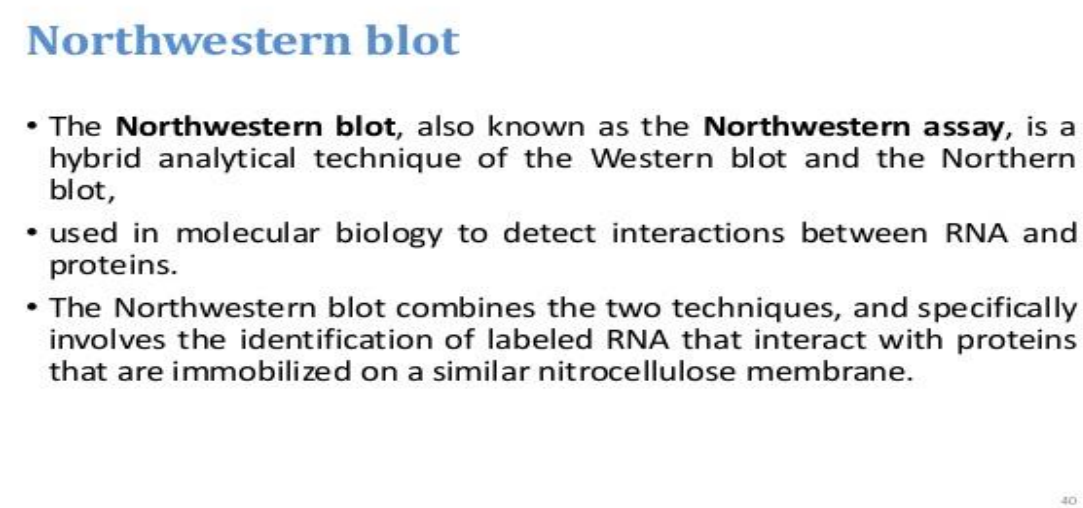


Workflow for Southwestern Blot :

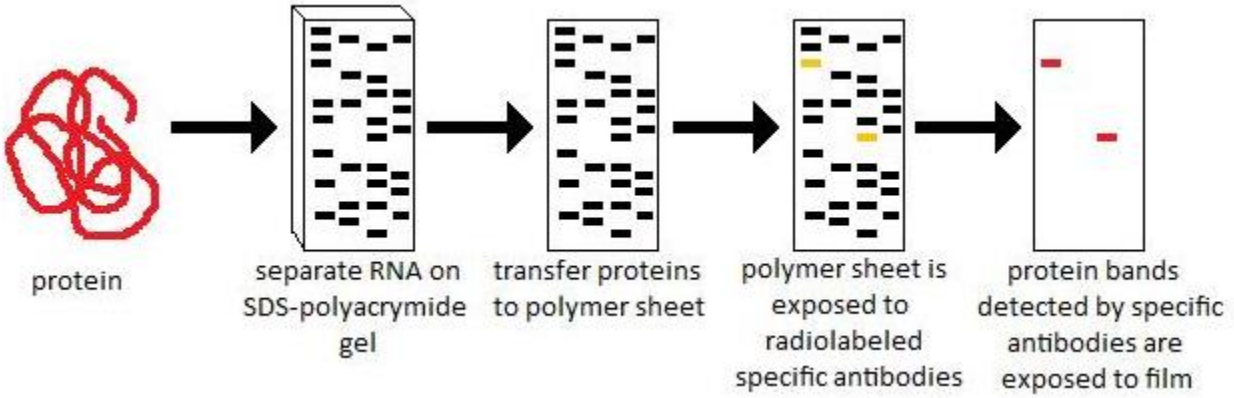
Technique #6. North Western Blotting:

Northwestern assays detect a direct binding of a given RNA molecule to a protein immobilized on a nitrocellulose membrane. Here, we describe protocols to prepare (32)P-labeled RNA probes and to use them to assay for RNA-protein interactions after partially purified protein preparations are resolved on denaturing SDS-polyacrylamide gels. The method can unambiguously determine whether the protein of interest can directly and independently bind RNA even in the presence of contaminating bacterial proteins or degradation products that at times may hinder interpretation of results obtained from gel mobility shift or RNP immunoprecipitation assays.

The northwestern assay is employed to study the interaction between protein and RNA. The RNA binding proteins tend to bind to different kinds of RNA through either known domains or unknown sequences of proteins. Northwestern assays detect a direct binding of a given RNA molecule to a protein immobilized on a nitrocellulose membrane. Here, we describe protocols to prepare ^{32}P-labeled RNA probes and to use them to assay for RNA–protein interactions after partially purified protein preparations are resolved on denaturing SDS-polyacrylamide gels. The method can unambiguously determine whether the protein of interest can directly and independently bind RNA even in the presence of contaminating bacterial proteins or degradation products that at times may hinder interpretation of results obtained from gel mobility shift or RNP immunoprecipitation assays.



Work Flow :



Southwestern and Northwestern blotting

- South western blotting : **This technique combine the principles of southern and western blots and has been efficiently used for screening and isolation of clones expressing sequence specific DNA-binding proteins by using ss DNA probe**
- North western blotting : **This technique is devised to isolate sequence specific RNA-binding proteins by using ss RNA probe**

- Both of these techniques are most efficient when the oligonucleotides contain the binding sequence in multimeric form

Technique #6. FarWestern Blotting:

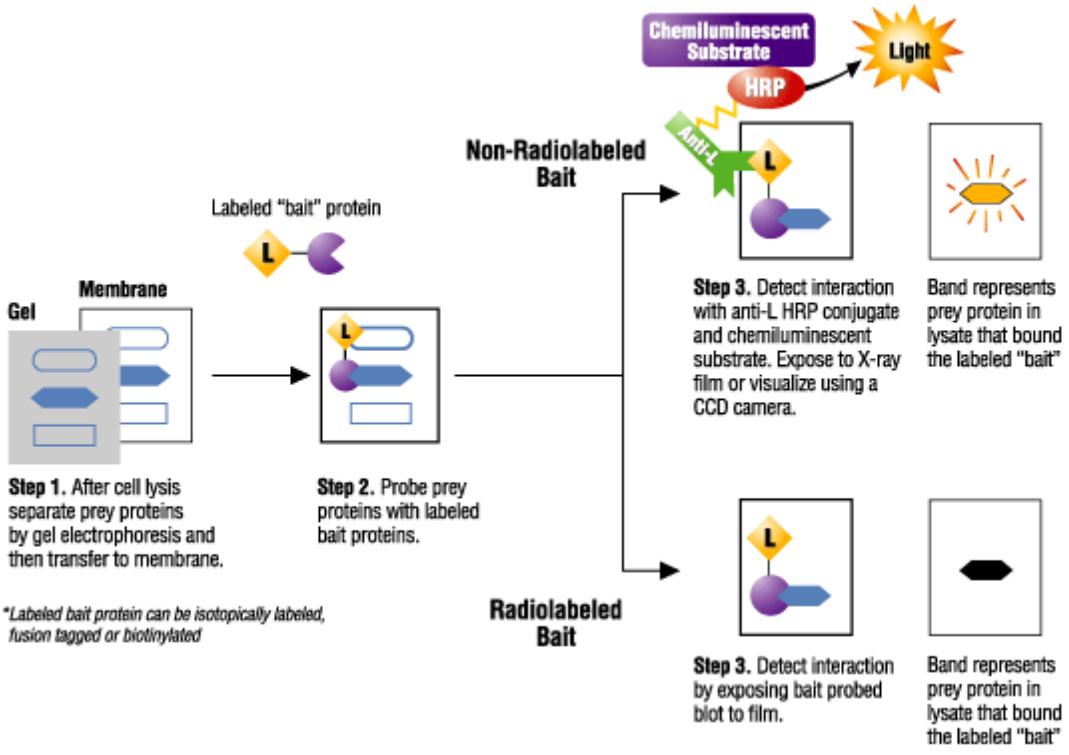
Far-Western blotting was originally developed to screen protein expression libraries with ^{32}P-labeled glutathione S-transferase (GST)-fusion protein. Far-Western blotting is now used to identify protein:protein interactions. In recent years, far-Western blotting has been used to determine receptor:ligand interactions and to screen libraries for interacting proteins. With this method of analysis it is possible to study the effect of post-translational modifications on protein:protein interactions, examine interaction sequences using synthetic peptides as probes, and identify protein:protein interactions without using antigen-specific antibodies.

Far-Western Blotting vs. Western Blotting

The far-Western blotting technique is quite similar to Western blotting. In a Western blot, an antibody is used to detect the corresponding antigen on a membrane. In a classical far-Western analysis, a labeled or antibody-detectable "bait" protein is used to probe and detect the target "prey" protein on the membrane. The sample (usually a lysate) containing the unknown prey protein is separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) or native PAGE and then transferred to a membrane. When attached to the surface of the membrane, the prey protein becomes accessible to probing. After transfer, the membrane is blocked and then probed with a known bait protein, which usually is applied in pure form. Following reaction of the bait protein with the prey protein, a detection system specific for the bait protein is used to identify the corresponding band

Step	Western Blotting	Far-Western Analysis
Gel Electrophoresis	Native or Denaturing (usually)	Native (usually) or Denaturing
Transfer System	Optimal membrane and transfer system determined empirically	Optimal membrane and transfer system determined empirically
Blocking Buffer	Optimal blocking system determined empirically	Optimal blocking system determined empirically
Detection (several possible strategies)*	Unlabeled primary antibody.–> Enzyme-labeled secondary antibody.–> Substrate Reagent	Unlabeled bait protein.–> Enzyme-labeled bait-specific antibody.–> Substrate Reagent
	Enzyme-labeled primary antibody.–> Substrate Reagent	Radiolabeled bait protein.–> Exposure to film
[Arrows designate sequence of steps of detection strategy]	Biotinylated antibody.–> Enzyme-labeled streptavidin.–> Substrate Reagent	Biotinylated bait protein.–> Enzyme-labeled streptavidin.–> Substrate Reagent
		Fusion-tagged bait protein.–> Tag-specific antibody.–> Enzyme-labeled secondary antibody.–> Substrate Reagent

Workflow for far Western Blot



Conclusions :

Blotting Techniques

- Analytical tools for the specific identification of desired DNA or RNA fragments from thousands of molecules.
- Blotting is a process of immobilization of sample nucleic acids on solid support (nitrocellulose or nylon membranes).
- Blotted nucleic acids are used as targets in the hybridization experiments.

Blotting Compass