



## BLOTTING METHODS FOR THE INVESTIGATION OF DNA, RNA, AND PROTEINS

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### ABSTRACT

Blotting is a typical procedure which are southern, western, northern and eastern are relevant for various kinds of macromolecules like lipids, RNA, DNA and proteins.. Western blotting (or immunoblotting) is a generally utilized method to distinguish proteins just as posttranslational changes on proteins, utilizing counter acting agent based tests to get clear data about objective proteins from multifaceted examples. Southern blotting is the first smearing strategy, which began the naming framework. It was intended by Edwin Southern. The Southern smear is utilized to recognize the measure of DNA in a blend. The term 'northern smudge' really alludes explicitly to the hair like exchange of RNA from the electrophoresis gel to the smearing layer. The eastern smear, or eastern blotting, is a biochemical method used to look at protein post-translational modifications (PTM) including the development of lipids, phosphates, and glycoconjugates. It is normally used to recognize sugar epitopes.

**Keywords:** Southern, Northern, Western Blotting, Probe, Hybridization, Antibody, Membrane.

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### INTRODUCTION:

Blotting is method in which nucleic acids i.e., RNA and DNA or proteins are transferred onto a precise membrane [1,2]. This membrane is perhaps nitrocellulose PVDF or nylon membrane. This method can be done just after the gel electrophoresis, by transferring the molecules from the gel onto the surface of blotting membrane. But occasionally it can be done by directly transferring the molecules onto the membrane. And then we can envisage these transferring molecules by using staining [3-4]. Examples: Ethidium bromide, Crystal violet, Safranin and Osmium tetroxide etc. [5-7].

Blotting is utilized in molecular biology for the identity of proteins and nucleic acids and is extensively used for diagnostic functions.

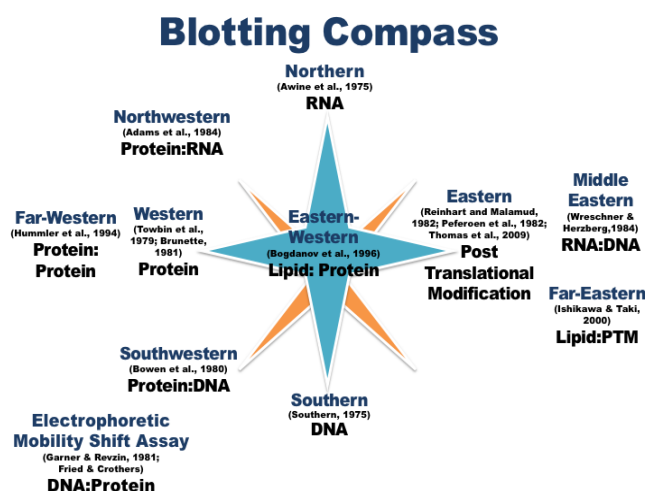
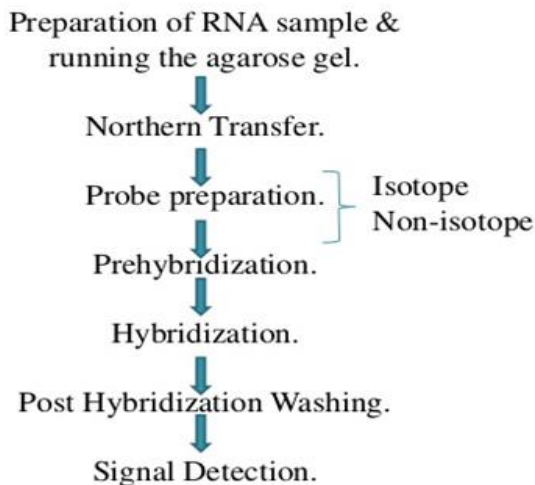


Figure 1: FIG 1-Blotting compass [8]

**FLOW CHART:-**



The Oxford College of Science

**Chart 1: Blotting processes [9]**

**Molecular treasure**

**Table 1: Molecular treasure [10]**

The type of molecular treasure you're hoping to find dictates the type of probe you use to bind!

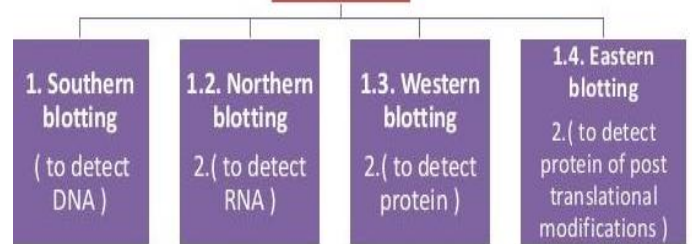
blot "direction"	Looks for specific	Using
northern	RNA	DNA
eastern	protein modifications	antibodies
Southern	DNA	DNA
Southwestern	DNA-binding proteins	DNA
western	protein	antibodies
far western	protein-binding proteins	other proteins
northwestern	RNA-binding proteins	RNA
reverse northern	DNA	RNA

**Types of Blotting:**

**Definition:**

A **blot**, in molecular biology and genetics, is a method of transferring proteins, DNA or RNA onto a carrier [11].

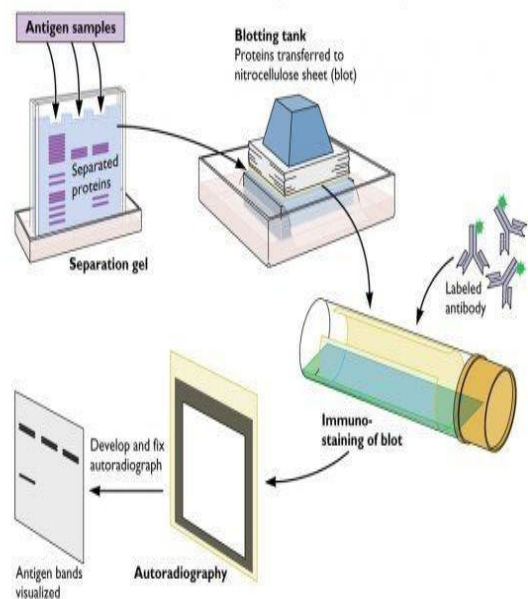
**Types:**



**Chart 2: Types of Blotting [12]**

**Western Blotting:**

**Western Blotting Technique**



**Figure 2: Western blotting technique [13]**

**Principle:**

Western Blotting isolates proteins by size and names protein of enthusiasm with a counter acting agent. Western smearing (additionally called Protein Immunoblotting on the grounds that a counter acting agent is utilized to explicitly identify its antigen) is a broadly acknowledged logical method used to recognize explicit proteins in the given example. [14]

**Procedure of Western Blotting:**

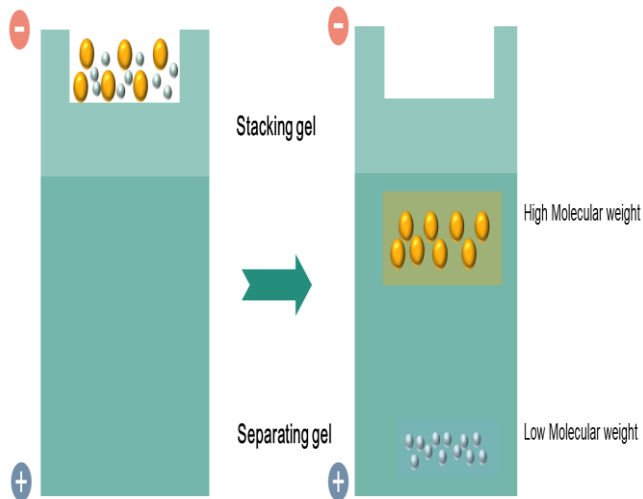
There are six steps implicated in western blot, including sample preparation, gel electrophoresis, proteins transfer, blocking, antibody incubation, and proteins detection and visualization.

**1. Sample preparation.**

Proteins can be extracted from unusual samples, such as tissues or cells. While tissue samples exhibit a higher degree of structure, the tissues are primary broken down by the mechanical innovation, such as homogenizer or sonication. Protease and phosphatase inhibitors are

usually used to prevent the digestion of the sample at cold temperatures. Behind protein extraction, it is imperative to detect the concentration of proteins, which permits the mass of proteins loaded into each well. And a spectrophotometer is often used for proteins concentration.

## 2. Gel electrophoresis.

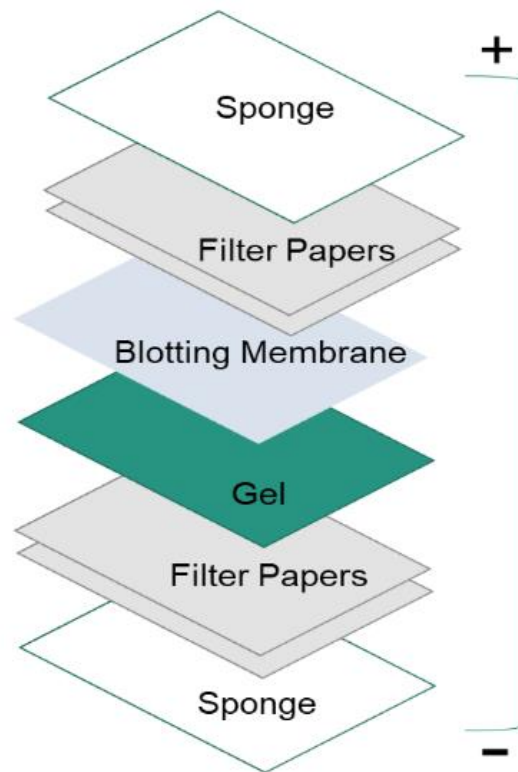


**Figure 3: Western blotting gel electrophoresis**

The most frequently used gel is polyacrylamide gels (PAG) and buffers loaded with sodium dodecyl sulfate (SDS). Western blot uses two types of agarose gel: stacking gel that is used for concentrate all proteins in one band and separating gel that allows for separating proteins according to their molecular weight. Smaller proteins drift faster in SDS-PAGE when a voltage is applied. PAGE can separate proteins ranging from 5 to 2,000 kDa according to the uniform pore size which is illicit by the Different concentration of PAG. Typically separating gels are prepared in 5%, 8%, 10%, 12% or 15%. When we prefer the appropriate percentage of the separating gel, we should consider the size of the target proteins. The minor the known weight of proteins is the higher percentage of gels should be use.

## 3. Proteins transfer

Behind separating proteins by gel electrophoresis, proteins are moved from within the gel onto a solid hold up membrane to make the proteins available to antibody detection. The most important method for transferring proteins is called Proteins transfer. , which uses an electric field oriented perpendicular to the surface of the gel, to pull proteins out of the gel and shift into the membrane. It can be done semi-dry or wet conditions, whereas wet conditions are generally more dependable as it is less probable dry out the gel. As shown in the left figure, the membrane is placed among the gel surface and filter. The transfer sandwich is formed as follows: a fiber pad (sponge), filter papers, the gel, a membrane, filter papers, a fiber pad (sponge).



**Figure 4: Proteins Transfer**

## 4. Blocking

Blocking is a basic advance in the western smear to keep antibodies from restricting to the film vaguely. The most habitually utilized commonplace blockers are BSA and non-fat dry milk. At the point when the layer is put in the weakened arrangement of proteins, the proteins append to all spots in the film where the objective proteins have not joined. Along these lines, the "commotion" in the eventual outcome of the western smear can be diminished and result in more clear outcomes.

## 5. Antibody incubation

Subsequent to obstructing, the essential counter acting agent ties to target protein when the essential immunizer is hatched with the layer. Washing the membrane with the antibody-buffer solution is supportive for minimizing background and removes boundless antibodies. Subsequent to rinsing the membrane, the membrane is exposed to the precise enzyme conjugated secondary antibody. When performing optional immune response brooding, the named auxiliary neutralizer can tie to the essential immunizer which has responded with target proteins. Based on the class of the primary antibody, we can select the appropriate secondary antibody.

## 6. Protein detection and visualization.

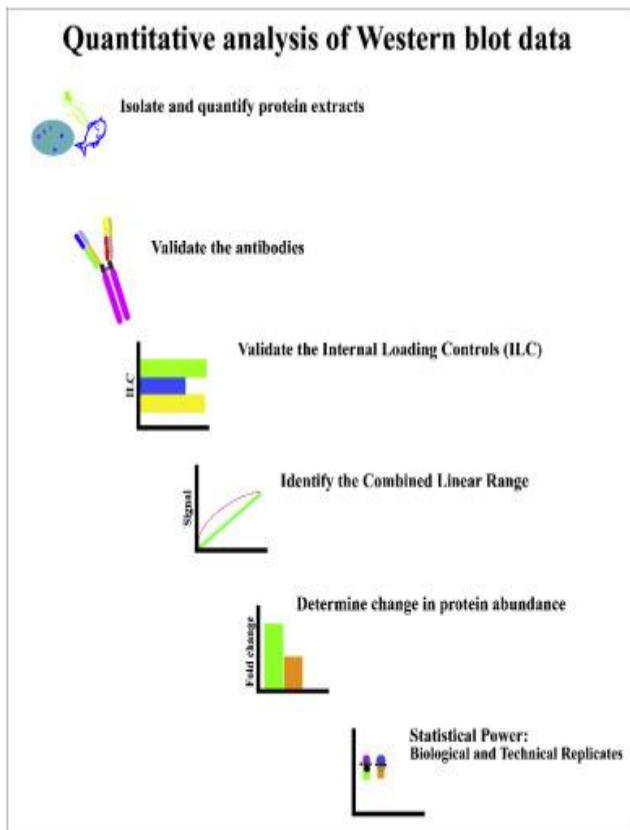
A substrate reacts with the enzyme that is bound to the secondary antibody to create colored substance. It enables us to know the densitometry and place of the targets protein. And the size approximations are taken by comparing the proteins bands to the marker.

There are numerous detection systems accessible for protein visualization, such as colorimetric detection, chemiluminescent detection, radioactive detection, and fluorescent detection. The electrochemiluminescence (ECL) system is the most common detection method. [13]

**Application:**

The western blot is usually used for qualitative detection of proteins and post-translational modifications (e.g. phosphorylation). Furthermore, it likewise can be utilized in clinical diagnostics, for example, the HIV-test or BSE-test. [13]

**Quantative Analysis:**

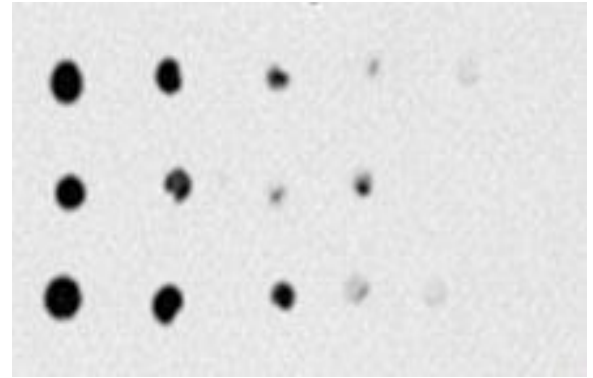


**Figure 5: Quantitative analysis of western blot data [14]**

**Dot Blot:**

A dab blotch (or space smear) is a strategy in atomic science used to identify proteins. It speaks to rearrangements of the western smear strategy, with the special case that the proteins to be recognized are not first isolated by electrophoresis. Rather, the example is applied legitimately on a film in a solitary spot, and the smudging system is performed.

The strategy offers noteworthy reserve funds in time, as chromatography or gel electrophoresis, and the complex smearing strategies for the gel are not required. Notwithstanding, it offers no data on the size of the objective protein. [15]



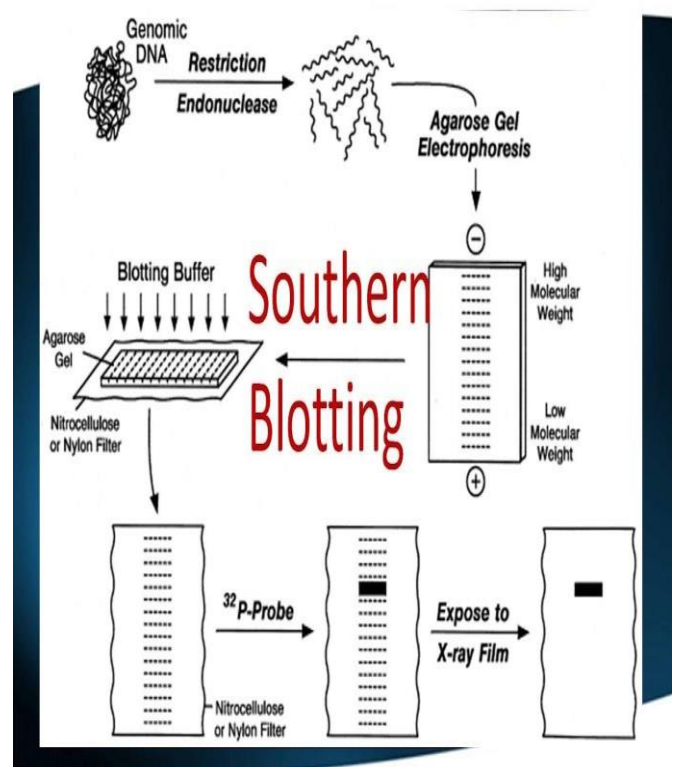
**Figure 6: Dot-blot blotting**

Typical dot blot membrane. Darker dots indicate more protein. [16].

**Applications:**

1. Performing a dab smear is analogous in thought on stage out a western blotch, with the upside of quicker speed and lower cost.
2. Dot blotches are moreover performed to screen the coupling capacities of an antibody.[17]

**Southern Blotting:**



**Figure 7: Southern blotting technique [18]**

**Principle:**

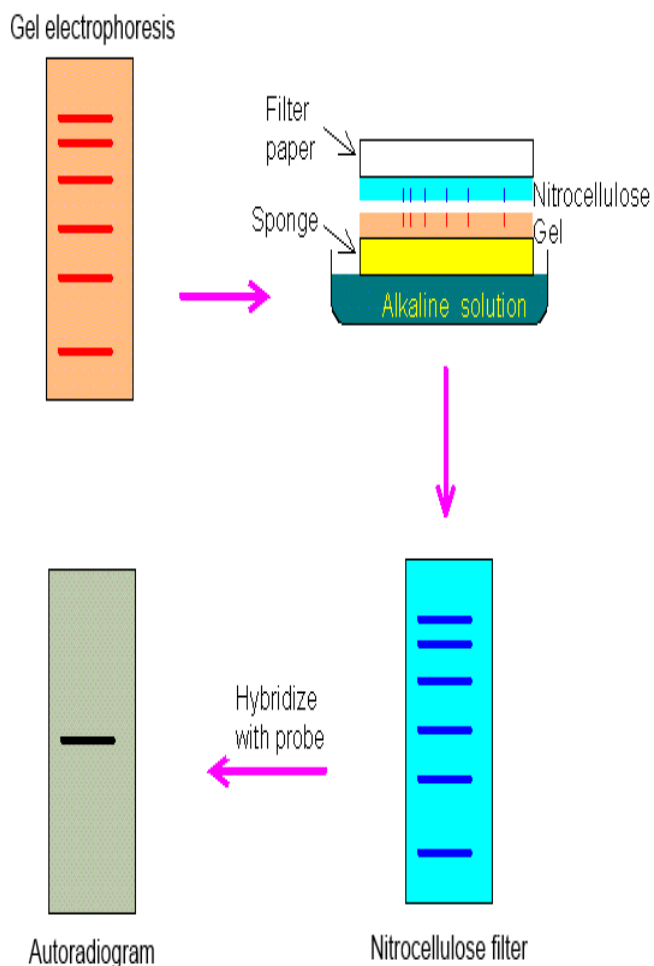
The method includes the exchange of electrophoresis-isolated DNA sections to a transporter film which is usually nitrocellulose and the resulting discovery of the objective DNA part by test hybridization. Hybridization alludes to the way toward shaping a twofold abandoned DNA atom between a solitary abandoned DNA test and a solitary abandoned objective DNA.



While the test and target DNA are corresponding to one another, the response is explicit which helps in the recognition of the particular DNA section. [19] Southern blotting is a method for detecting specific DNA fragments in a multifaceted mixture. The procedure was imagined in mid-1970s by Edward Southern. It has been applied to recognize Restriction Fragment Length Polymorphism (RFLP) and Variable Number of Tandem Repeat (VNTR) Polymorphism. The later is the premise of DNA fingerprinting.

**Procedure of Southern Blotting:**

- a) The DNA to be analyzed is digested with restriction enzymes and then alienated by agarose gel electrophoresis.
- b) The DNA fragments in the gel are denatured with alkaline solution and transferred onto a nitrocellulose sort out or nylon membrane by blotting, preserving the allotment of the DNA fragments in the gel.
- c) The nitrocellulose filter is incubated with a precise probe. The location of the DNA fragment that hybridizes with the probe can be displayed by autoradiography.



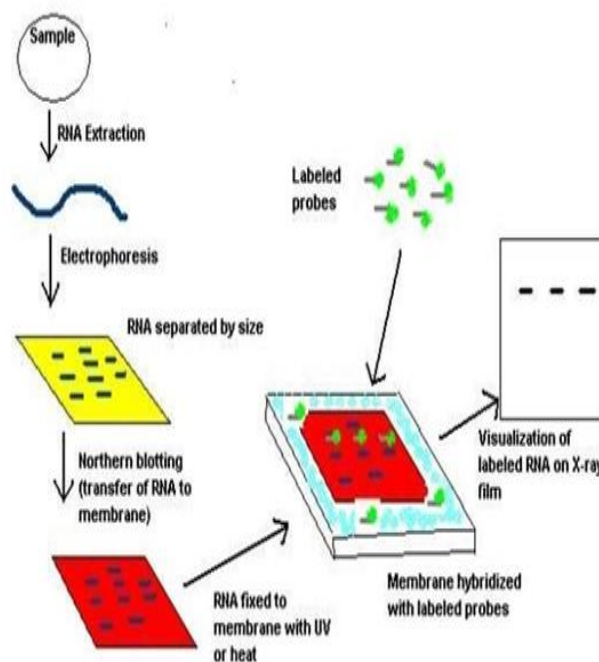
**Figure 8: Gel electrophoresis [20]**

**Applications:**

- i) It is used in the technique called RFLP (Restriction fragment length polymorphism) mapping [21].
- ii) Additionally utilized in phylogenetic examination [11, 22].

iii) To identify the gene rearrangements [23].

**Northern Blotting:**



**Figure 9: Northern blotting technique [24]**

**Principle:**

Northern smearing begins with the electrophoresis to isolate RNA tests by size. Electrophoresis isolates the RNA atoms dependent on the charge of the nucleic acids. The charge in the nucleic acids is relative to the size of the nucleic corrosive arrangement. [22] This technique was developed in 1977 by James Alwine, David Kemp and George Stark at Stanford University. Northern blotting takes its name from its similarity to the first blotting technique, the Southern blot. The major difference is that RNA, rather than DNA, is analyzed in the northern blot. [17]

**Procedure of Northern Blotting:**

The blotting method starts with extraction of total RNA from a homogenized tissue sample. The mRNA can then be isolated throughout the use of oligo (dT) cellulose chromatography to sustain only those RNAs with a poly (A) tail. RNA samples are then alienated by gel electrophoresis. A nylon film with a positive charge is the majority effectual for use in northern smearing while the contrarily charged nucleic acids have a high partiality for them. The transfer buffer used for the blotting typically contains formamide because it lowers the annealing temperature of the probe-RNA interaction preventing RNA ruin by high temperatures. one time the RNA has been transferred to the membrane it is immobilized throughout covalent association to the membrane by UV light or heat. Behind a test has been named, it is hybridized to the RNA on the layer. The layer is washed to guarantee that the test has bound only. The half and half signals are then distinguished by X-beam film and can be evaluated by densitometry [17].

**Applications:**

1. Northern Blotting is a procedure utilized for the investigation of quality articulation.
2. This blotching method can likewise be utilized for the development of a tissue or life form.
3. It additionally helps in the distinguishing proof of strange, ailing or contaminated condition at the atomic level [25]

**Eastern Blotting:**

**Principle:**

The eastern smear, or eastern smirching, is a biochemical method used to dismember protein post-translational changes (PTM) together with the development of lipids, phosphates, and glycoconjugates. It is much of the time used to recognize sugar

epitopes. Along these lines, eastern smear can be seen as a development of the biochemical strategy for western smudge. A variety of methodologies have been depicted by the articulation "eastern blot(ting)", most use of protein spread from SDS-PAGE gel on to a PVDF or nitrocellulose layer. Moved proteins are examined for post-translational modifications using tests that may recognize lipids, starch, phosphorylation or some other protein change. Eastern spreading should be used to propose procedures that perceive their goals through express alliance of the PTM and the test, remembering them from a norm far-western smudge. On a central level, eastern blotching resembles lectin spreading (for instance disclosure of sugar epitopes on proteins or lipids) [21].

**Procedure of Eastern Blotting:**

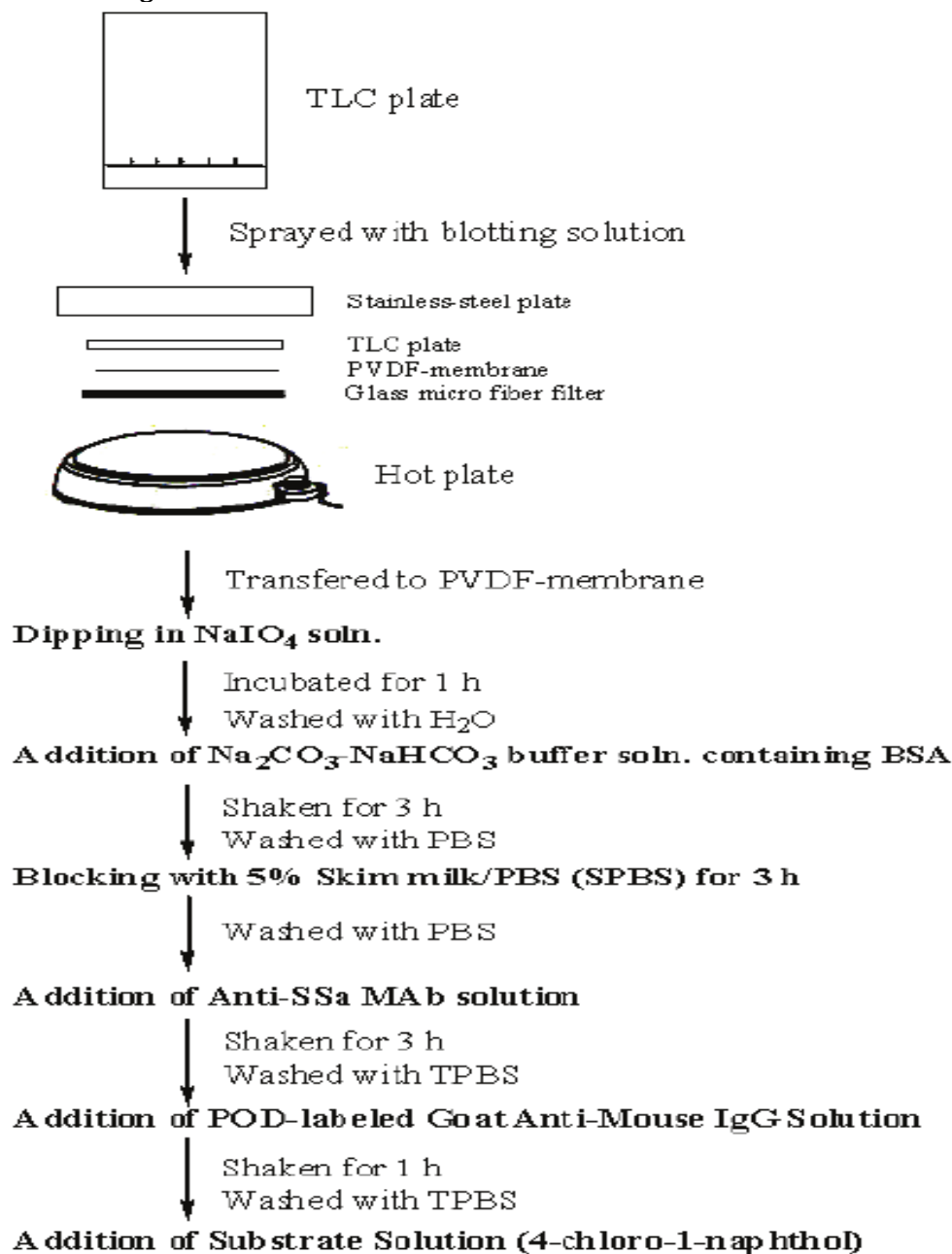


Chart 3: Procedure of eastern blotting [25]

**APPLICATIONS:**

- Utilization of this process remembers identification of protein adjustments for two bacterial species Ehrlichia-E. muris and IOE. Cholera poison B subunit (which ties to gangliosides), concanavalin A (which identifies mannose-containing glycans) and nitrophenylphosphate-methyl green (which distinguishes phosphoproteins) were utilized to identify protein adjustments.
- The strategy indicated that the antigenic proteins of the non-destructive E. muris is more post-translationally changed than the profoundly harmful IOE [26,27]

**SIGNIFICANCE:**

- Most proteins that are decoded from mRNA experience changes before getting utilitarian in cells. These progressions are all things considered known as post-translational changes (PTMs).
- The early or crumbled proteins, which are consistent under physiological conditions, are then presented to a battery of express compound catalyzed changes as a bit of hindsight chains or spines.
- Post-translational difference in proteins can include: acetylation, acylation (myristoylation, palmitoylation), alkylation, arginylation, ADP-ribosylation, biotinylation, formylation, geranylgeranylation, glutamylation, glycosylation, glycylation, hydroxylation, isoprenylation, lipoylation, methylation, nitroalkylation, phosphopantetheinylation, phosphorylation, prenylation, selenation, S-nitrosylation, succinylation, sulfation, transglutamination and ubiquitination (sumoylation, neddylation).
- Post-translational changes occurring at the N-end of the amino destructive chain accept a noteworthy activity in development across normal layers. These recall secretory proteins for prokaryotes and eukaryotes and moreover proteins that are intended to be combined in various cell and organelle layers, for instance, lysosomes, chloroplast, mitochondria and plasma film. Enunciation of posttranslated proteins is noteworthy in a couple of diseases. [28,29]

**Difference:**

**Table 2: Comparison between Southern blotting Northern blotting and western blotting [30]**

	Southern blotting	Northern blotting	Western Blotting
<b>Molecule detected</b>	DNA(ds)	mRNA	Protein
<b>Gel electrophoresis</b>	Agarose gel	Formaldehyde agarose gel	Polyacrylamide gel
<b>Gel pretreatment</b>	Depurination denaturation and neutralization		
<b>Blotting method</b>	Capillary transfer	Capillary transfer	Electric transfer
<b>Probes</b>	DNA radioactive or non radioactive	RNA radioactive or non radioactive	Primary antibody
<b>Detection system</b>	Autoradiography chemiluminescent Colormetric	Autoradiography chemiluminescent Colormetric	chemiluminescent Colormetric

**FUTURE ADVANCEMENTS:**

The applications of smearing strategies will undoubtedly addition in the following decade. Approximately indeed, instead of being utilized in detention they will complement other regions of mechanical proceed laid out in this agreement. An model of this is the polymerase chain response, in which exclusive heat safe DNA polymerase chemicals are utilized to duplicate very little sections of DNA that would otherwise go undetected. Once improved such DNA sections are known by Southern smearing. This technique has been utilized to identify antagin with HIV numerous months formerly antibodies can be notable in the patient's serum.<sup>34</sup> Smearing methods, which authorize detection of genomic material or on the other hand proteins sensibly basically also, reasonably with high individuality, are broadly utilized in clinical research. In any case, the consequences depend on the immaculateness of the materials utilized also, the specialized capability of those utilizing the [28]

**CONCLUSION:**

Southern, western, northern and eastern are material for unusual sorts of macromolecules like lipids, RNA, DNA and proteins. Western blotting has points of interest over other protein discovery procedures. Northern smudging takes its name from its proximity to the first blotting method, the Southern smear, named for scholar Edwin Southern. Various methods have been portrayed by the expression "eastern blot (ting)".

**REFERENCES:**

- Tani Y, et al. Evaluation of a Novel Automated Machine, the Auto2D, for Two-Dimensional Gel Electrophoresis. J Proteomics Bioinform. 2014;7:108-111.
- Marcel Binnebösel, et al. Gentamicin supplemented polyvinylidene fluoride mesh materials enhance tissue integration due to a transcriptionally reduced MMP-2 protein expression BMC Surgery. 2012;121-122
- Li A. Encoding Genetic Information and Catalyzing Chemical Reactions: The Multifunctional Roles of Nucleic Acids. Hereditary Genet. 2015;40-45
- Polly P, et al. Evaluation of an adaptive virtual laboratory environment using Western Blotting for diagnosis of disease. BMC Medical Education. 2014;14:222..
- About O and Griffin W. Silver Staining of Alzheimer's Disease. J NeurolDisord. 2014;2:i103.
- Itodo SE, et al. Phytochemical Properties and Staining Ability of Red Onion (Allium cepa) Extract on Histological Sections. J CytolHistol. 2014;5:275
- Manu Tomar, "Types of Blotting", Research & Reviews: Journal of Pharmaceutics and Nanotechnology, Vol . 4 , 30-09-2016 , Page No. 147
- [https://en.wikipedia.org/wiki/File:Blotting\\_Compass\\_for\\_Molecular\\_Probes.png](https://en.wikipedia.org/wiki/File:Blotting_Compass_for_Molecular_Probes.png).
- <https://images.app.goo.gl/USWXejqkqTzocbJA>.

10. <http://thebumblingbiochemist.com/365-days-of-science/blotting-compass-northern-eastern-southern-western-blotting-more/>.
11. [https://en.wikipedia.org/wiki/Blot\\_\(biology\)](https://en.wikipedia.org/wiki/Blot_(biology))
12. <https://images.app.goo.gl/oTmca1uFrRjxWw7V9>
13. <https://www.mybiosource.com/learn/westernblotting>
14. <https://www.bosterbio.com/protocol-and-troubleshooting/western-blot-principle>.
15. <https://www.creative-proteomics.com/blog/index.php/the-principle-and-procedure-of-western-blotting>
16. <https://www.sciencedirect.com/science/article/pii/S000326971930750X>
17. "Test Blots, Slot Blots & Dot Blots - Immunodetection | Bio-Rad". Bio-Rad
18. [https://en.wikipedia.org/wiki/Dot\\_blot](https://en.wikipedia.org/wiki/Dot_blot)
19. Rupprecht, Kevin R.; Nair, Rad K.; Harwick, Larissa C.; Grote, Jonathan; Beligere, Gangamani S.; Rege, Sushil D.; Chen, Yon-Yih; Lin, Zhen; Fishpaugh, Jeffrey R. (15 December 2010). "Development of a dot-blot assay for screening monoclonal antibodies to low-molecular-mass drugs". *Analytical Biochemistry*: 160–164. doi:10.1016/j.ab.2010.08.003. PMID 20696124
20. <https://www.mybiosource.com/learn/southern-blotting/>
21. Freeze, HH (1993). "Preparation and analysis of glycoconjugates". *Current Protocols in Molecular Biology*. Chapter 17: 17.7.1–17.7.8. doi:10.1002/0471142727.mb1707s23. PMID 18265163
22. <https://www.web-books.com/MoBio/Free/Ch9D.htm>
23. Levéziel H. Identification of the two common alleles of the bovine  $\kappa$ -casein locus by the RFLP technique, using the enzyme Hind III *Génétique, sélection, evolution*. 1988;20:247.
24. May ST, et al. Using genomic DNA-based probe-selection to improve the sensitivity of high-density oligonucleotide arrays when applied to heterologous species *Plant Methods*. 2005;1:10.)
25. Nynke HD. Essential validation methods for E. coli strains created by chromosome engineering *Journal of Biological Engineering*. 2015;9:11.
26. <https://www.mybiosource.com/learn/testing-procedures/northern-blotting/>
27. [https://www.researchgate.net/figure/Eastern-blotting-protocol\\_fig1\\_221913278](https://www.researchgate.net/figure/Eastern-blotting-protocol_fig1_221913278)
28. <http://eagri.org/eagri50/GPBR311/lec25.pdf>
29. Thomas; Thirumalapura, N; Crossley, EC; Ismail, N; Walker, DH; et al. (2009). "Antigenic protein modifications in Ehrlichia". *Parasite Immunology*. **31** (6): 296–303. doi:10.1111/j.1365-3024.2009.01099.x. PMC 2731653. PMID 19493209
30. <https://images.app.goo.gl/sXVAii8dNbxw1XqZ7>