

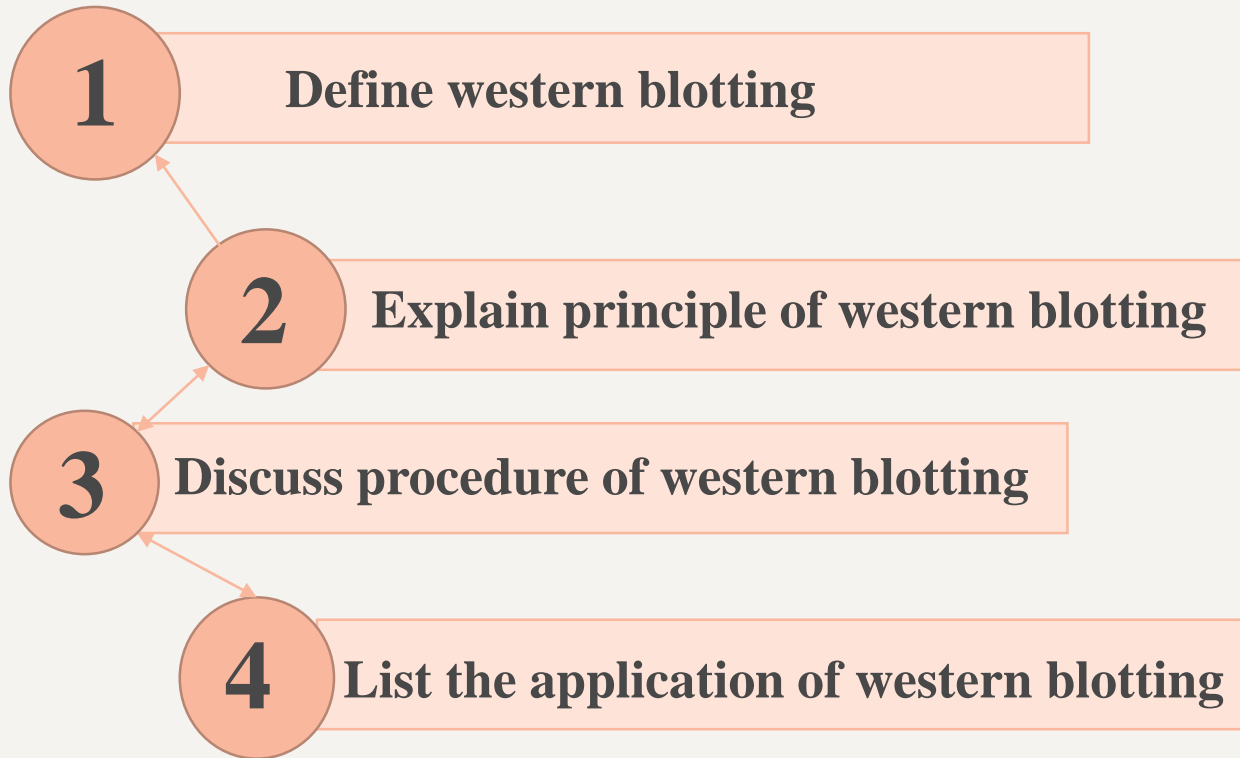
Western Blotting

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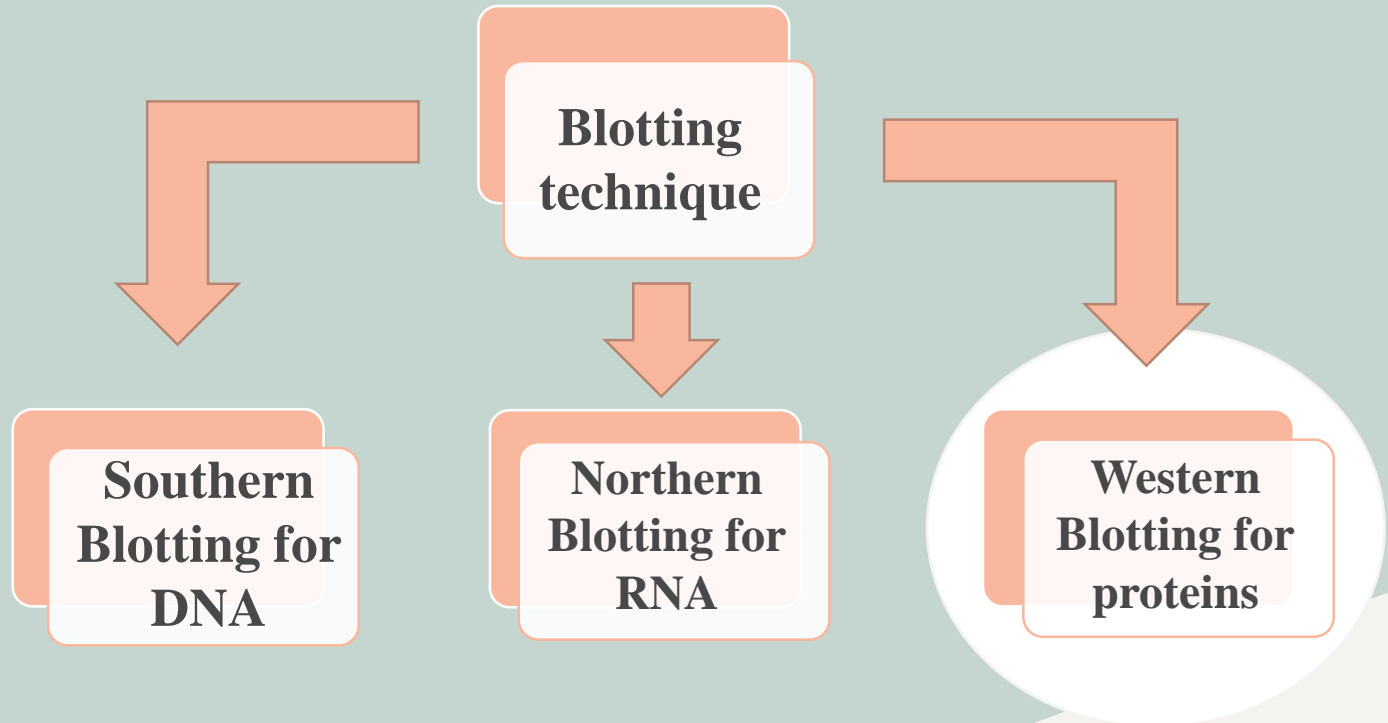
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□ Introduction:

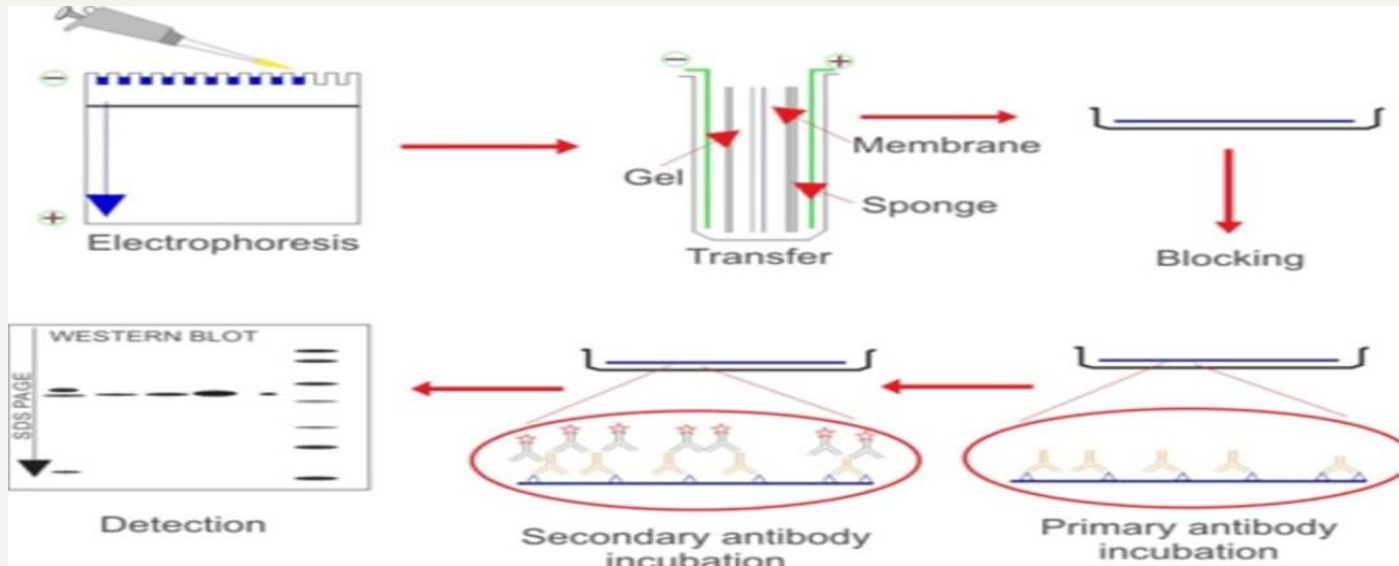
➤ What is blotting?

Blots are techniques for transferring **DNA**, **RNA** and proteins onto a carrier so they can be separated, and often follows the use of a gel electrophoresis.



□ Define Western Blotting:

- **Western blotting (or protein immunoblot):** is a laboratory technique used to detect a specific protein in a blood or tissue sample.
- Is a rapid and sensitive assay for detection and characterization of proteins.
- It is based on the principle of immuno chromatography where proteins are separated into polyacrylamide gel according to their molecular weight.



❑ Principle of Western Blotting:

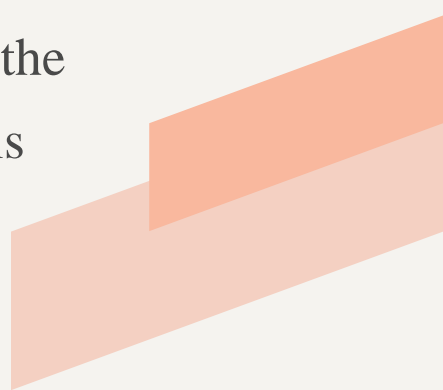
Protein extraction:

- Protein gel electrophoresis is used to separate and resolve proteins prior to blotting.
- The prepared protein mixture is run on a polyacrylamide gel to sort proteins by molecular weight and charge.



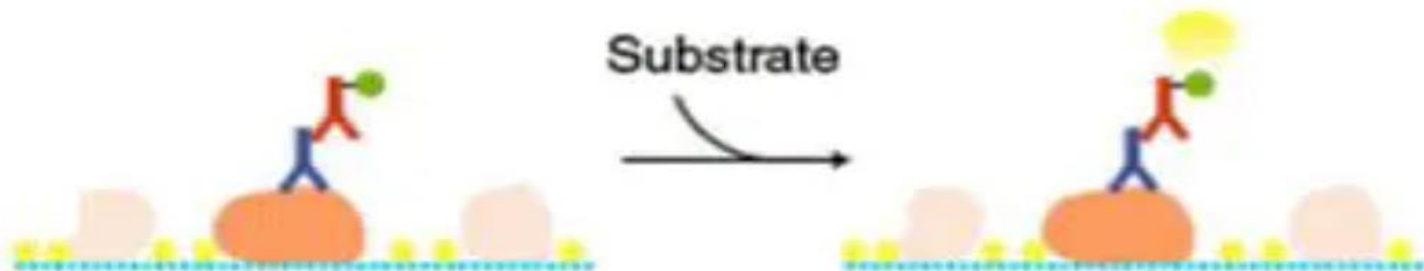
□ Cont. Principle of Western Blotting:

Gel electrophoresis:

- The standard technique used to separate proteins prior to immunoblotting is called discontinuous polyacrylamide gel electrophoresis (PAGE).
 - Electrophoresis refers to the movement of charged particles in an electric field, and requires two electrodes of opposite charge (anode and cathode) separated by a conducting medium (the buffer). When charged molecules, such as proteins and DNA, are exposed to an electric field under these conditions, they migrate towards the electrode with their opposite charge .
 - The mobility of molecules in gel electrophoresis is dictated by the molecules charge, size, and shape, as well as the electrophoresis conditions.
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□ Cont. Principle of Western Blotting:

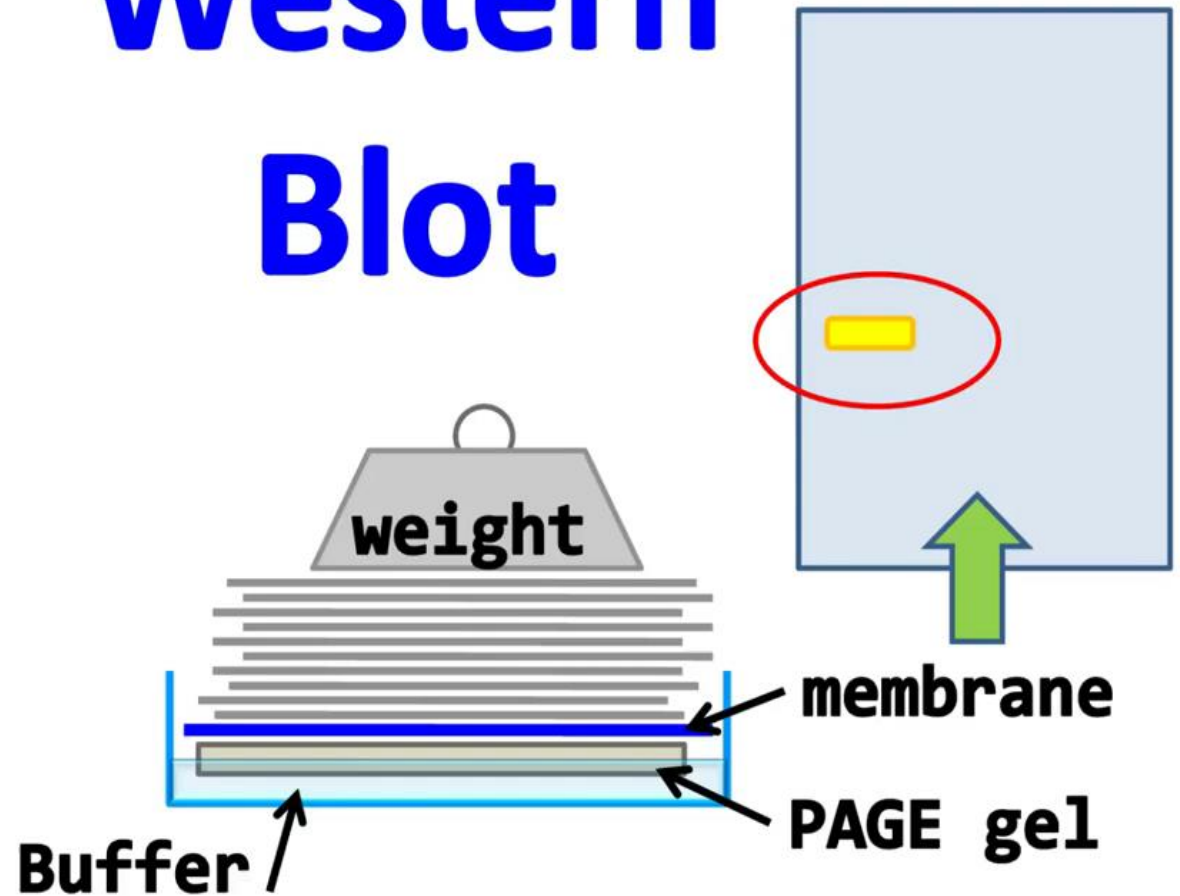
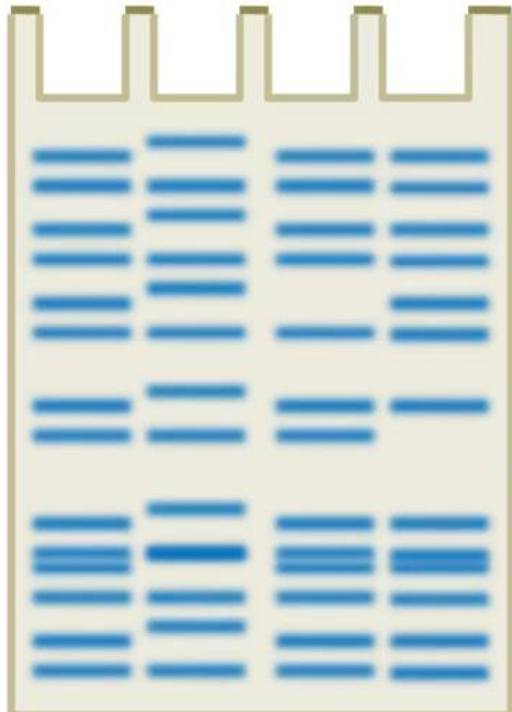
- **Blotting:** after electrotransfer of protein to a membrane, we will now block the blot applying a primary antibody specific for our protein of interest and then a secondary antibody which will recognize the primary antibody.
- **Detection of bands** a reaction mixture containing a substrate is added to the membrane. The enzyme attached to the antibody catalyzes a reaction that emits light, which is detected by X-ray film or a cooled CCD camera.



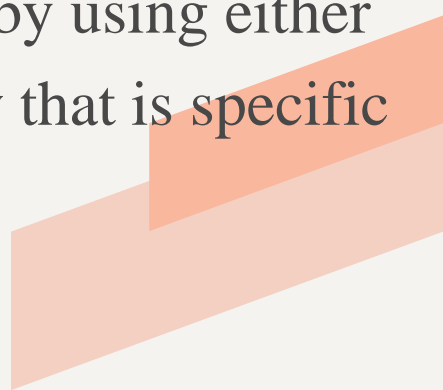
□ Procedure of Western Blotting:

Quickly
Understand

Western Blot



❑ Application of Western Blotting:

- Identification of a specific protein in a complex mixture of proteins. In this method, known antigens of well-defined molecular weight are separated by SDS-PAGE and blotted onto nitrocellulose.
 - The separated bands of known antigens are then probed with the sample suspected of containing antibodies specific to one or more of these antigens.
 - The reaction of an antibody with a band is detected by using either a radiolabeled or enzyme-linked secondary antibody that is specific for the species of the antibodies in the test sample.
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❑ Cont. Application of Western Blotting:

- Estimation of the size of the protein as well as the amount of protein present in the mixture.
- It is most widely used as a confirmatory test for the diagnosis of HIV, where this procedure is used to determine whether the patient has antibodies that react with one or more viral proteins or not.
- Demonstration of specific antibodies in the serum for diagnosis of neurocysticercosis and tubercular meningitis.



□ Summary:

- Blots are techniques for transferring DNA , RNA and proteins onto a carrier so they can be separated.
- Western blotting is a laboratory technique used to detect a specific protein in a blood or tissue sample.
- Western Blotting is based on the principle of immuno chromatography.
- Western Blotting is applied to identify a specific protein in a complex mixture of proteins and to estimate the size of the protein.



☐ References:

- ✓ <https://www.antibodies.com/es/western-blotting>
- ✓ https://www.sigmaaldrich.com/LY/en/applications/protein-biology/western-blotting?gclid=CjwKCAjwoduRBhA4EiwACL5RP1Q42LiW25oGlkEtxiF2MVtexjuQ2w88tTX_3PC_FjWsVU7qfaCeMRoC21gQAvD_BwE
- ✓ [\(479\) Western blot – YouTube](#)
- ✓ <https://microbenotes.com/western-blotting-introduction-principle-and-applications/>
- ✓ <https://microbenotes.com/western-blotting-introduction-principle-and-applications/>

Thank You

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