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Types of Blotting

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Review Article

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ABSTRACT

Blotting is a common technique which is widely used in the field of molecular biology. These methods such as southern, western, northern and eastern are applicable for different types of macromolecules like lipids, RNA, DNA and proteins. Each technique depends upon the following factors such as the size of molecule and their binding ability to the solid support. Finally, by using probe we have to detect the molecule of interest.

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

INTRODUCTION

Blotting is technique in which nucleic acids i.e., RNA and DNA or proteins are transferred onto a specific membrane [1,2]. This membrane may be nitrocellulose PVDF or nylon membrane. This process can be done just after the gel electrophoresis, by transferring the molecules from the gel onto the surface of blotting membrane. But sometimes it can be done by directly transferring the molecules onto the membrane. And then we can visualize these transferring molecules by using staining [3-6]. Examples: Ethidium bromide, Crystal violet, Safranine and Osmium tetroxide etc [7,8].

TYPES OF BLOTTING

There are basically 4 types of blotting:

1) Southern blotting
2) Western blotting
3) Northern blotting
4) Eastern blotting

1) Southern blotting

Southern blotting is named after Edward M. Southern. This method is used for analysis of DNA sequences [9]. It involves the following steps:

- Firstly, large weighted DNA is cut into small fragments by using Restriction endonucleases [10].
- Then, these fragments are electrophoresed on separating gel so that they can separate according to their size [11].
- If DNA fragments are much larger in size so firstly the gel should be treated with HCl, causes depurination of DNA fragments [12-14].
- After separating these fragments, placed a nitrocellulose sheet over the separating gel. Apply pressure over the membrane so that proper interaction can occur between these two [15-17].

- After that the membrane is exposed to ultraviolet radiation so that the fragments are permanently attached to the membrane [18].
- Then the membrane is exposed to hybridization probe. But the DNA probe is labeled so that it can easily detect, when the molecule is tagged with a chromogenic dye [19-23].
- After hybridization process, excess probe is washed away by using SSC buffer and it can be visualized on X-ray film with the help of autoradiography [24-29].

  Applications:

  i) It is used in the technique called RFLP (Restriction fragment length polymorphism) mapping [30].

  ii) Also used in phylogenetic analysis [30,31].

  iii) To identify the gene rearrangements [32-36].

2) Western blotting

Western blotting is named after W. Neal Burnette. This method is used for detection and analysis of protein in a given sample [37,38]. It involves the following steps:

- Firstly, isolating the protein from particular sample.
- After that beta- mercaptoethanol (BME) and Sodium dodecyl Sulfate (SDS) is added into the protein suspension [39-41].
- Then, protein- SDS complex is placed on top of the gel in the well. A molecular weight marker is also loaded in one of the well in order to determine the molecular weight of other proteins. After that the samples are added in the remaining wells [42].
- Once the samples and markers are loaded then current is passed across the gel. Protein is pulled down to the positive pole of the well because it is tightly bound to SDS which is negatively charged. Movement of protein is inversely proportional to its size [43-45].
- After this step, gel is placed against a membrane and current is passed across the gel so that all the proteins are transferred onto the membrane [46].
- Then Immunoblotting has to be done. In this method, firstly block the membrane with non-specific protein in order to prevent antibody from binding to the membrane where the protein is not present [47].
- After that primary antibody is added to the solution. These antibodies are responsible for recognizing a specific amino-acid sequence. Then wash it to remove unbound primary antibody and add secondary antibody [48-50].
- Now these antibodies are conjugated with an enzyme and recognize the primary antibody. Lastly, another wash is done to remove unbound secondary antibody [51-55].
- Here, chemiluminescent substrate is used for detection. The light is being emitted once the substrate has been added and can be detected with film imager [56-58].

  Applications:

  i) Used in clinical purposes.

  ii) Used to detect specific protein in low quantity.

  iii) Used to quantifying a gene product [59-61].

3) Northern blotting

Northern blotting is given by Alwine. This method is used to analyse and detection of RNA in a sample [62,63].

- Firstly, extraxt and purify mRNA from the cells [64,65].
- Separate these RNA on agarose gels containing formaldehyde as a denaturing agent for the RNA [66,67].
- This gel is immersed in depurination buffer for 5-10 minutes and washed with water [68-71].
- Then transfer these RNA fragments onto the carrier membrane i.e aminobenzyloxymethyl filter paper [72].
- After transferring the RNA,it is fixed to the membrane by using UV or heat.
- Add DNA labellled probe for hybridization [73-75].

  Wash off the unbound probe and at the end mRNA-DNA hybrid are then detected by X-ray film [76,77].

  Applications:

  i) Used in screening [78].

ii) For studying the gene expression [79,80].
iii) In disease diagnosis.

4) Eastern blotting

Eastern blotting is given by Bogdanov. This method is used to identify carbohydrate epitopes including glycoconjugates and lipids [81-84]. Mostly blotted proteins after transferring onto the membrane are analyzed for PTMs by using a probe and hence identify carbohydrates and lipids [85-87]. It involves the following steps:

- Firstly, targeted molecules are vertically separated by using gel electrophoresis [88-90].
- Then, these separated molecules are transferred horizontally on the nitrocellulosic membrane [91-94].
- After that primary antibody is added to the solution. These antibodies are responsible for recognizing a specific amino-acid sequence. Then wash it to remove unbound primary antibody and add labelled secondary antibody [95-98].
- These labelled probes confirm the molecule of interest.
  i) Detection of protein modification.
  ii) Used for binding studies by using various ligands [99-100].
  iii) Used to purify various phospholipids.

CONCLUSION

Different blotting is used to detect different type of macromolecules such as southern blotting is used for DNA analysis, western blotting is for protein analysis, northern blotting is for RNA analysis and eastern for carbohydrate detection. The remaining of this article is focus on different techniques and applications used in particular blotting.

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