



Blotting: A Smart Strategy for Enabling the Detection of Molecules of Interest

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Abstract

Blotting is a classical technique for detecting macro-biomolecules of interest from a mixture of molecules. The technique is generally composed of four steps: 1) gel electrophoresis-based separation of molecules, 2) blotting (transfer and immobilization) of separated molecules from the gel onto the membrane, 3) specific hybridization of probes to target molecules on the membrane, and 4) visualization of the probe/target molecule complex. The present article briefly introduces blotting techniques for readers, since the direction-named techniques are confusing for people beyond the biotechnology field. Particularly, the smartness of the blotting step in a blotting technique is emphasized. Specifically, the blotting step allows for fixation of size-separated molecules on the membrane, and permits subsequent hybridization of the target molecule to labeled probe, collectively allowing target molecules to be detected by imaging. In conclusion, not only is blotting a smart strategy for enabling the detection of molecules of interest but also the concept of blotting plays an important role in biotechnology.

Subject Areas

Biotechnology

Keywords

Blotting, Southern Blot, Northern Blot, Western Blot

1. Introduction

Blotting is a classical investigative technique used to detect macro-biomolecules (such as nucleic acids and proteins) of interest from samples (mixture of molecules). In general, a blotting technique is accomplished by: 1) size-based separation of molecule mixture from samples by gel electrophoresis; 2) transfer and

subsequent immobilization of size-fractionated molecules from the gel onto a membrane support, allowing the formation of a faithful and stable replica of the gel pattern on the membrane; 3) specific hybridization of labeled probes to the molecules of interest (target molecules) immobilized on the membrane; 4) visualization of the labeled probe/target molecule complex through imaging, allowing identification of the presence, size, and amount of the target molecule by the presence, location, and thickness of a specific band on the image, respectively (Figure 1).

The main three blotting techniques are Southern blot (developed by Edwin Southern, 1975) [1], northern blot (developed by James Alwine, David Kemp, and George Stark, 1977) [2], and western blot (developed by Harry Towin, Theophil Staehelin, and Julian Gordon, 1979) [3] [4], which are used for the detection of DNA, RNA, and protein, respectively. Other modified blotting techniques such as middle-eastern blot, eastern blot, far-eastern blot, northwestern blot, southwestern blot, and far-western blot, following the direction-oriented naming tradition, have been subsequently developed based on the classical three techniques (Figure 2). It is worth mentioning that, the directional word that precedes “blot” should be lowercase except for Southern blot, as only Southern blot is named after a person (Edwin Southern) [5].

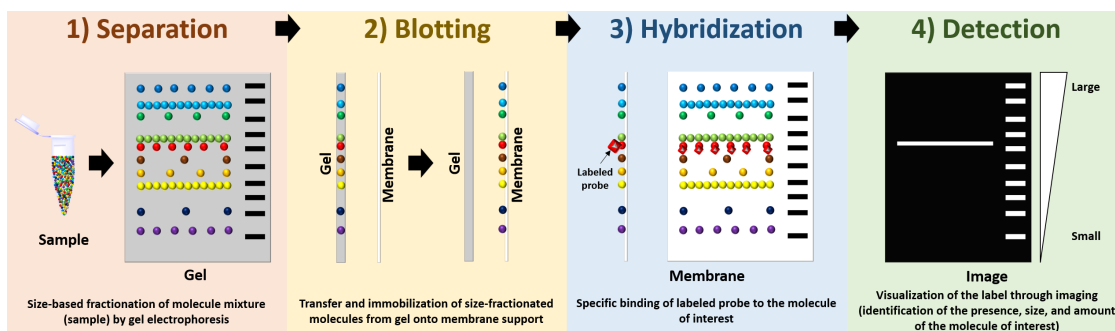


Figure 1. Simplified diagram of a blotting technique.

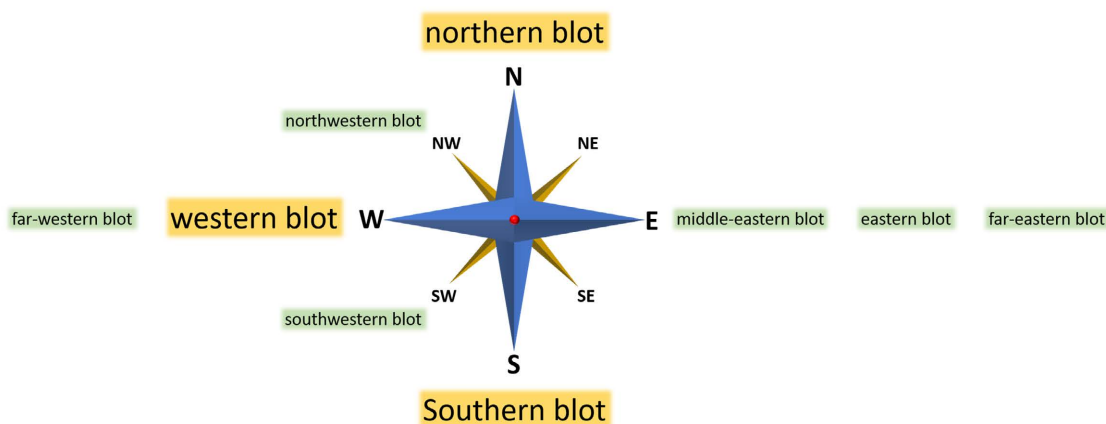


Figure 2. Blotting compass containing the main three techniques (Southern blot, northern blot, and western blot) and some other relative techniques using the direction-oriented naming system.

The present article is aimed to provide a brief introduction to the blotting technique process for readers since the direction-named techniques can be confusing, especially for readers from outside the biotechnology field. In particular, I would like to emphasize the smartness of the blotting step in a blotting technique (**Figure 1**), which truly enables the subsequent detection of target molecules in a convenient way.

2. Blotting Techniques

2.1. Southern Blot

Southern blot is used to detect specific DNA sequences in a complex DNA mixture (digested genomic DNA resulting from restriction endonuclease cleavage). In most applications, the mixture of restriction DNA fragments is size-separated by agarose gel electrophoresis [6]. The fragments are then transferred from the gel to a membrane (either nitrocellulose or nylon) by capillary action, followed by immobilization via baking (for nitrocellulose) or ultraviolet irradiation (for nylon) [7]. Notably, the post-transfer immobilization step is not required when the combination of positively charged nylon membrane and alkaline transfer buffer is applied, since the binding between DNA and positively charged membrane is irreversible under alkaline conditions [7]. After blotting (transfer and immobilization), the membrane carries a reproduction of the gel pattern, allowing target DNA sequences on the membrane to be detected selectively by hybridization with labeled RNA or DNA probes (complementary to the target sequence) and subsequent visualization according to the nature of labels [6] [7]. For instance, target DNA/radioactive-labeled probe hybrid can be detected by radioautography or fluorography [1]. Other labeling systems including fluorescent and chemiluminescent reagents have been applied as well [8].

2.2. Northern Blot

Northern blot is a further development of Southern blot for detecting specific RNA sequences. Whole cell RNA, *i.e.* “total RNA” (RNA mixture) is size-fractionated by either agarose gel (for large RNAs such as mRNA) or polyacrylamide gel (for small RNAs) electrophoresis [9]. The separated RNAs are usually transferred and covalently bound to a positively charged nylon membrane [9]. Capillary blotting and electroblotting are the two main approaches of the blotting step, which are used for transferring RNAs from agarose gel and polyacrylamide gel, respectively [9]. Subsequently, RNAs of interest are hybridized with specific labeled nucleic acid probes and detected.

2.3. Western Blot

Western blot (also referred to as immunoblotting or protein blotting) evolved from Southern blot and northern blot [10]. This is a routine technique for qualitative and semiquantitative identification of specific proteins from a complex mixture of proteins extracted from a biological sample [11] [12]. The separation

step of western blot is based on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, followed by transfer of the fractionated polypeptides (denatured proteins) to a membrane support, either a nitrocellulose or polyvinylidene fluoride (PVDF) membrane that binds the proteins nonspecifically. After subsequent blocking of nonspecific binding sites (usually by skim milk or bovine serum albumin), the membrane is incubated with antibodies for selective immunodetection of the immobilized protein of interest [13], which is depending on the specificity of antigen-antibody interaction [11], in contrast to the hybridization step of Southern blot and northern blot that depends on base pairing between nucleic acid target and its probe. Furthermore, the hybridization step of virtually all western blots is composed of two stages: Specific binding of target protein to an unlabeled primary antibody, and subsequent binding of the primary antibody to a reporter-labeled secondary antibody which is directed against the host species of primary antibody [11]. Therefore, identification of the protein of interest is achieved by detection of the labeled secondary antibody.

2.4. Other Blotting Techniques

The “middle-eastern blot” is a modified northern blot by coating the membrane support with polyuridylic acid (“poly-U”) for specific immobilization and detection of mRNAs, as they are “poly-A” containing molecules [14]. The “eastern blot” is an extension of western blot since it is used to detect post-translational modifications [15]. The “far-eastern blot” detects lipids by separating them on a high-performance thin layer chromatography (HPTLC) plate followed by blotting on a PVDF membrane [16]. The “northwestern blot” and “southwestern blot” are used for identification of RNA/protein interaction and DNA-binding proteins, respectively [17]. The “far-western blot” is a modified western blot by using a protein rather than an antibody for detecting target protein [18].

3. Blotting: A Super Smart Step in a Blotting Technique

Among the four basic steps in a blotting technique (Figure 1), the blotting step, as an adjunct to gel electrophoresis [8], is a super smart one in my view. Although gel electrophoresis allows separation of molecule mixture perfectly, a gel is not appropriate solid support for the purpose of probing due to three reasons: 1) The fractionation pattern of molecules on the gel is not permanent as molecules diffuse over time; 2) probes are difficult to access the molecules as they are located within the gel matrix rather than on its surface; 3) a gel is relatively fragile compared to membrane support. As stated by Edwin Southern in his groundbreaking work [1], without a blotting method, although target DNA detection can be done by gel slicing, DNA elution, and hybridizing the DNA to probe either in solution or after binding to filters, such a method is time-consuming, and inevitably causes some loss in the resolving power of the gel electrophoresis. The introduction of a blotting step retains the high resolving power of a gel by generating an exact replica of the banding pattern of the gel onto a membrane. Also, the blotting step in

western blot technique retains the high resolving power of SDS polyacrylamide gel electrophoresis by allowing easy access to proteins with their probes on membrane support to which the proteins are transferred [10]. In sum, a blotting step not only allows for fixation of size-fractionated molecules on a membrane (evading the diffusion of molecules in gel), but also permits easy hybridization of target molecule to labeled probe (evading the difficulty in target/probe binding in gel matrix), collectively allowing target molecules to be conveniently detected as a visible sharp band on an image.

4. Conclusion

At present, many of the applications of Southern blot and northern blot have been largely replaced by polymerase chain reaction (PCR) and Sanger sequencing, however, the western blot is still a standard methodology for protein analysis. Furthermore, the basic concept of blotting, as a smart strategy for enabling the detection of molecules of interest, continues to play a vital role in molecular biotechnology.

Conflicts of Interest

The author declares no conflicts of interest.

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