North, South, or East? Blotting Techniques

M.W. Nicholas[1] and Kelly Nelson[2]

Journal of Investigative Dermatology (2013) **133**, e10; doi:10.1038/jid.2013.216

One of the cornerstones of modern molecular biology, blotting is a powerful and sensitive technique for identifying the presence of specific biomolecules within a sample. Subtypes of blotting are differentiated by the target molecule that is being sought. The first of these techniques developed was the Southern blot, named for Dr. Edwin Southern, who developed it to detect specific DNA sequences (Southern, 1975). Subsequently, the method was modified to detect other targets. The nomenclature of these techniques was built around Dr. Southern's name, resulting in the terms northern blot (for detection of RNA), western blot (for detection of protein), eastern blot (for detection of posttranslationally modified proteins), and southwestern blot (for detection of DNA binding proteins). Most researchers consider the eastern blot and the southwestern blot variations of western blots rather than distinct entities.

WHAT BLOTTING DOES

- Blotting allows specific and sensitive detection of a protein (western) or specific DNA or RNA sequence (Southern, northern) within a large sample isolate.
- Targets are first separated by size/charge via gel electrophoresis and then identified using a very sensitive probe.
- Variations of these techniques can detect post-translational modifications and DNA-bound proteins.
- Western blotting may also be used to detect a circulating antibody in a patient sample or confirm an antibody's specificity.

LIMITATIONS

- Blotting is more time- and labor-intensive than newer techniques and probably not as sensitive.
- Specificity and sensitivity can be altered by the technique and reagents chosen.
- Determination of the quantity of molecules present is not as accurate as with newer techniques.
- In the case of western blotting, the tertiary structure is destroyed and therefore the relevant epitope recognized by the primary antibody may not be recognized.

THE BLOTTING PROCESS

The basic principles underlying these techniques are virtually identical. First, the target molecules (DNA, RNA, or protein) are separated by a combination of their size and charge using the appropriate method of gel electrophoresis; second, separated molecules are transferred to a membrane; and third, the membrane is queried with a probe directed against the specific molecule of interest (Figure 1). In the first step, separation of target molecules by gel electrophoresis, large target molecules may first be modified to facilitate movement through the gel. Very large sequences of DNA are processed with restriction endonucleases to cut them into smaller pieces. RNAs often require no additional manipulation, and proteins may be denatured with specific detergents. Denaturing a protein means "unfolding" it from its naturally occurring three-dimensional (or tertiary) structure to a partially or completely linear structure. The denaturing step also disrupts most protein–protein interactions. Most commonly, sodium dodecyl sulfate, a detergent, is used to denature proteins in this setting. It also provides a stronger negative charge, which facilitates gel electrophoresis. The sample is then loaded onto a gel where, in response to application of an electrical charge, molecules vertically separate based on size and charge, with smaller and more charged molecules running through the gel more rapidly and thus traveling farther than large molecules in the same period of time. A "ladder" consisting of molecules of standardized and known sizes is run parallel with the samples, which allows the technician to estimate the size of each unknown molecule.

In the second step, the molecules are horizontally transferred to a membrane, again using an electrical gradient, which maintains the vertical separation established by gel electrophoresis. This step is akin to making a carbon copy, except the molecules themselves are transferred, rather than an image.

[1]*Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA and* [2]*Department of Dermatology, Duke University, Durham, North Carolina, USA*

Correspondence: M.W. Nicholas, Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA. E-mail: mnichola@unch.unc.edu

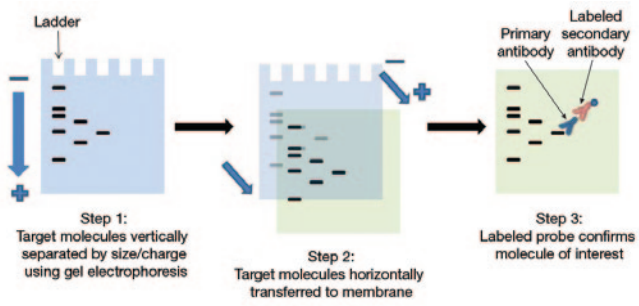

Figure 1. Overview of blotting steps.

Finally, the membrane is treated with probes that bind only the specific molecule being sought (Figure 1); the type of probe varies based upon the target molecule. For nucleic acids (DNA and RNA), the probe is a labeled complementary (antisense) strand that hybridizes to the target sequence. For proteins, a monoclonal antibody (the primary antibody) binds to the protein of interest; this is followed by a second antibody (the secondary antibody), which recognizes the Fc portion (the "fragment crystallizable" portion, or the "stem" of the Y shape) of the primary antibody and is itself labeled, allowing specific detection. This "two-step" method is used for cost control because labeling each specific monoclonal antibody would be quite expensive. Because of the use of antibodies, the western blot technique is sometimes referred to as immunoblotting. Regardless of which form of probe is used, the label may be a radioactive isotope or a fluorescent or chromogenic dye, all of which allow detection of the presence of the probe under specific conditions.

APPLICATIONS OF BLOTTING

These techniques, particularly the western blot, may also be used to detect the presence of biological probes (circulating antibodies) to a single protein or group of proteins. For example, if a patient showed clinical evidence of circulating antibodies to a skin protein (e.g., collagen VII in epidermolysis bullosa acquisita), the target protein could be separated via gel electrophoresis and transferred to a membrane, which would then be treated with a serum sample from the patient; this would act as the primary antibody or probe. If antibodies recognizing collagen VII were present, they would bind to their target protein on the membrane and could be detected with labeled antihuman Fc antibodies. One could imagine how this technique might also enable the determination of targets for unknown circulating autoantibodies by providing a "slurry" of known proteins for the patient's serum to react against. One notable example of a disease illuminated by this technique is paraneoplastic pemphigus; patients' serum was found, via western blot, to contain antibodies recognizing multiple proteins expressed in the epidermis. However, a significant limitation of the western blot process arises in the protein denaturing required to allow passage through the gel: this commonly disrupts the tertiary structure of the protein, which may compromise the three-dimensional epitope structure required for autoantibody recognition.

CURRENT USE AND NEW TECHNOLOGY

Blotting techniques have been widely employed for more than 30 years and have provided the foundation of our understanding of molecular biology. However, these techniques have been largely—and in some cases completely—usurped by new technologies (Table 1). Southern blots have been replaced by multiple techniques. Real-time PCR boasts incredible sensitivity; theoretically, this method is able to detect even a single copy of the target sequence and compare relative copy numbers across samples rapidly and reliably, with little technical expertise required. Fluorescent *in situ* hybridization (FISH) allows detection of specific sequences within a tissue sample with high sensitivity and precise localization. Northern blots have given way to reverse-transcription real-time PCR, again a more sensitive and more user-friendly technique. Both PCR and FISH also boast more accurate determination of the quantity of the target present, another major advantage.

Western blots are still considered by many to be a gold standard for examining protein expression, and they are still used routinely in research. In a single recent issue of the *Journal of Investigative Dermatology*, three studies employed western blotting (Gordon *et al.*, 2013; Kidwai *et al.*, 2013; Wang *et al.*, 2013). Gordon *et al.* used it to confirm the expression of particular proteins in culture. In this case, the label was a radioisotope, and the images shown are X-ray films that were incubated layered on the membrane. It is important to note that western blotting was used even though the proteins' mRNA was detected in the samples; this is because the presence of mRNA does not ensure the presence of the protein itself (Figure 2).

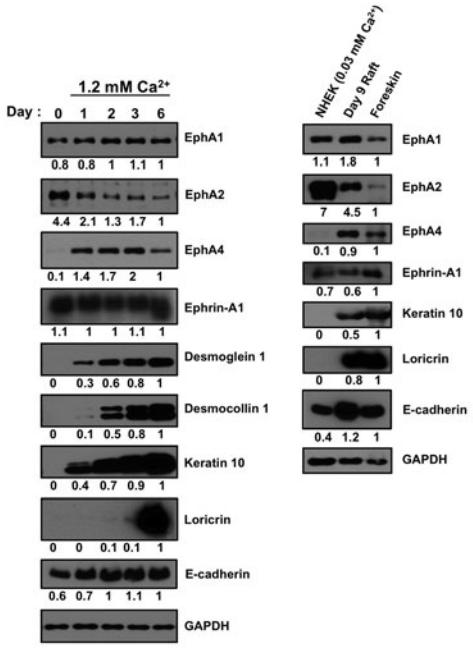

Figure 2. Western blot analysis for expression of various proteins in culture. Radioactive labeled probes were used and blots were incubated overlying X-ray film. Reprinted with permission from Gordon *et al.* (2013).

Table 1. Blotting techniques

Name	Target	Probes used	Newer alternative techniques
Southern	DNA	Complementary (antisense) sequence of DNA or RNA	PCR, real-time PCR, FISH
Northern	RNA	Complementary sequence of DNA or RNA	RT-PCR, real-time RT-PCR
Western	Protein	Monoclonal antibody	ELISA, immunohistochemistry, immunofluorescence, flow cytometry

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FISH, fluorescent *in situ* hybridization; RT-PCR, real-time reverse transcription PCR.

Although the western blot continues to enjoy popularity, newer techniques including immunohistochemistry, immunofluorescence, and flow cytometry are more sensitive and precise, allowing examination of protein expression within whole preserved tissues or specific cell types and not requiring a homogenized sample. ELISA is another technique that has replaced the western blot in many settings; it may be mass produced to quickly and efficiently detect a known target and is frequently cheaper and less technologically complex than a western blot. Determination of the quantity of protein present is also more accurate and straightforward when compared with western blot. Another key advantage of ELISA (enzyme-linked immunosorbent assay) is that it does not typically require disruption of the three-dimensional structure of a target protein, allowing preservation of immunologic epitopes. However, in many settings, western blot can be rapidly and relatively inexpensively employed and may often provide the answer sought, thus explaining its continued popularity.

USE IN CLINICAL PRACTICE

Although these techniques are not frequently used in current clinical practice, they do have their place. Western blots are used to diagnose infectious diseases—most commonly and famously, HIV. Some clinical immunofluorescence laboratories employ western blotting techniques as confirmatory tests as well (such as detecting circulating anti–collagen VII antibody in epidermolysis bullosa acquisita or multiple anti–self proteins in paraneoplastic pemphigus, as above). Often, however, these clinical applications are supplanted by mass-produced ELISA with reduced cost and expedited throughput.

CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Answers and a PowerPoint slide presentation appropriate for journal club or other teaching exercises are available at http://dx.doi.org/10.1038/jid.2013.216.

REFERENCES

Gordon K, Kochkodan JJ, Blatt H *et al.* (2013) Alteration of the EphA2/Ephrin-A signaling axis in psoriatic epidermis. *J Invest Dermatol* 133:712–22

Kidwai FK, Liu H, Toh WS *et al.* (2013) Differentiation of human embryonic stem cells into clinically amenable keratinocytes in an autogenic environment. *J Invest Dermatol* 133:618–28

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98:503–17

Wang Z, Kirkwood JS, Taylor AW *et al.* (2013) Transcription factor Ctip2 controls epidermal lipid metabolism and regulates expression of genes involved in sphingolipid biosynthesis during skin development. *J Invest Dermatol* 133:668–76

QUESTIONS

Answers are available as supplementary material online and at http://www.scilogs.com/jid/.

1. The target molecule type for Southern blotting is:

A. RNA.

B. DNA.

C. Protein.

D. Lipids.

2. Limitations of the western blot technique include all of the following *except*:

A. Low specificity.

B. Loss of antibody epitope with denaturing.

C. Less accurate determination of quantity.

D. Higher cost as compared with ELISA.

3. Which of the following techniques is most commonly employed in modern research?

A. Southern blot.

B. Northern blot.

C. Western blot.

D. Eastern blot.

4. Place the following blotting steps in order:

A. Transfer to membrane.

B. Detect probe.

C. Separate via gel electrophoresis.

D. Treat with probe.