North, South, East, and West
Blotting Techniques Expand the Utility of Electrophoresis
by Cheryl Scott

Electrophoresis is the basis of all blotting methods, and BPI Lab covered it last month (1). Electroblotting is a method for transferring electrophoretically separated proteins or nucleic acids onto a polyvinylidene fluoride (PVDF) or nitrocellulose membrane for permanence using electric current and a transfer buffer solution. This allows for analysts to further study them using probes, ligands, or stains. Capillary blotting is a variation designed to work with capillary electrophoresis.

After electrophoresis the following are stacked in cathode-to-anode order: a sponge, filter paper soaked in transfer buffer, the gel, a membrane, more soaked filter paper, and another sponge. When current is applied, it drives proteins or nucleic acids from the gel onto the membrane, where they are typically stained using a dye such as Coomassie Brilliant Blue to confirm that a sufficient quantity of material has been transferred. Immunoblotting involves the addition of specific antibodies with which proteins will interact. Affinity blotting involves other ligands in the same way.

A Brief History
The electroblotting technique was patented in 1989 by William J. Littlehales while he was working for American Bionetics (2). In 1991, BioGenex Laboratories acquired all the assets—including intellectual property—of that company when it went bankrupt. Before electroblotting, transferring gels to membranes was a more difficult and manual process.

Southern Blot: The names of all specific blotting methods come from the fact that the first was developed by Sir Edwin Southern, a British molecular biologist, when he was a professor at the University of Edinburgh in Scotland in the mid-1970s. Now a professor at Oxford, Southern is also chairman and chief scientific officer of Oxford Gene Technology. And he has been multiply awarded for his invention.

Northern Blot: Developers of the northern blot technique named it thus because of its similarity to the Southern technique, which came first. This variation came about in 1977 from the work of James Alwine, David Kemp, and George Stark at Stanford University.

Western Blot: The next method to appear originated almost simultaneously in the laboratory of Harry Towbin at the Friedrich Miescher Institute in Switzerland and the work of W. Neal Burnette, a postdoctoral researcher the Nowinski group at the Fred Hutchinson Cancer Center in Seattle. Burnette’s attempt at publication of his (slightly different) version of the technique was initially rejected—but his joking name for it caught on as colleagues and friends passed it around. The 1980s had arrived, and now the naming convention for blotting methods was set. In fact, when other scientists published a technique for DNA-binding proteins that combined Southern and western approaches in 1980, they named it the southwestern blot even though Burnette’s article hadn’t officially been published yet!

Eastern Blot: Further cementing the importance of Burnette’s work, multiple sets of authors have since dubbed a number of new methods eastern blotting. Among these is far-eastern blotting, which was developed (and named as such) in 1994 by scientists at the Tokyo Medical and Dental University in Japan.

Not surprisingly, with all the variations on the blotting theme, there are more suppliers providing services, membranes, reagents and probes, gels, instruments, and standards for this technology than can easily be counted. Among the most prominent are Abcam, Bio-Rad Laboratories, Cell Signaling Technology, Cyanagen, Li-Cor Inc., Life Technologies, Lonza, Molecular Devices, Molecular Probes, Perkin Elmer, Pierce Biotechnology, Protein Simple, Roche Applied Science, Rockland Immunochemicals, R&D Systems,
Methods and Applications

The applications of blotting techniques are as varied as the methods themselves.

Southern Blot:
A method of detecting specific DNA sequences in mixed DNA samples, Southern blotting involves transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection with probe hybridization. One use is homology-based cloning based on the amino acid sequence of a target gene’s protein product. Southern transfer also can be used to identify methylated sites in particular genes.

The northern blot is used in molecular biology research to study gene expression by detection of RNA (or isolated mRNA). Analysts can observe a particular gene’s expression pattern among tissues, organs, developmental stages, environmental stress levels, pathogen infection, and over the course of treatment. So this technique is used in diagnostics as well as cell-line analysis. Online databases help scientists compare their results to published examples. Although gene expression can be analyzed using many different methods (e.g., polymerase chain reaction, RNase protection assays, and microarrays), northern blotting can detect small changes in gene expression that other methods cannot.

References:
methods may not. However, it is limited in scope, usually involving one or a small number of genes at a time — but membranes can be stored and used for further analysis years later.

**Western Blot:** Sometimes called protein immunoblotting, the western technique is widely accepted for detecting specific proteins in complex samples. After sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (1), separated proteins are transferred to a membrane and stained with antibodies specific to a target protein. With applications in diagnostics and protein expression analysis (among others), this technique is very widely used in biopharmaceutical laboratories. Excellent western blotting guides can be found online from suppliers such as Abcam plc, Bio-Rad Laboratories, and GE Healthcare (4–6). And the *Western Blot Encyclopedia* from Sino Biological Inc. is another valuable online resource (7).

**Eastern Blot:** Although many definitions have been applied to the name, in general eastern blotting is used to analyze posttranslational modifications of proteins (often carbohydrate epitopes in glycosylation). This makes it useful for sponsors of complex protein products.

### References


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**Cyanagen Srl**

Via degli Stradelli Guelfi, 40/c 40138 Bologna-Italy

Tel/Fax +39 051 534063

**info@cyanagen.it**

www.cyanagen.com

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