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Citation Classic

Western Blotting

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Featured Article: Burnette WN. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate–polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal Biochem 1981;112:195–203.[2]

Three decades have flown by, and it is now difficult to imagine a world of biological science in which graphic representations of antigen reactions with specific antibodies did not exist. Nevertheless, the need for facilitated screening of antibody-producing hybridomas in the late 1970s gave rise to Western blotting, again validating the Swiftian dictum of necessity leading to invention. As a new member of Bob Nowinski's laboratory at the Fred Hutchinson Cancer Research Center, I was interested in RNA tumor virus (now called "retrovirus") protein–RNA interactions. The main thrust of the laboratory, however, was using the then-recent innovation of monoclonal antibodies to assess antigenic epitopes of retrovirus structural proteins. Contributing to this effort, I volunteered to find a means to simplify the tedious RIAs being used, which were very low–throughput methods of screening.

That the invention of Western blotting was not blindingly intuitive to someone like me, who was performing Southern and Northern blots *(1, 2)* on a regular basis, perhaps seems incomprehensible now, but struggle I did. In defense of my rather amusing early efforts to develop something useful, I did quickly focus on antibody reactivity with proteins resolved by SDS-PAGE. The problem inherent in melding RIAs with SDS-PAGE was that no suitable means existed to bind antibodies to antigens "fixed" (to avoid diffusion) in a porous gel matrix; elution of individual proteins from the gel still meant performing RIAs, rendering SDS-PAGE nothing more than another laborious antigen-purification method.

There were several "Eureka moments" that occurred in rapid succession. The first—in the category of "the solution is right in front of you, stupid"—was the realization that, just like Southern and Northern blots, producing a replica of the gel-resolved proteins would facilitate handling and antibody binding. Some quick experiments suggested that conditions favoring passive capillary transfer of proteins from a gel to a nitrocellulose paper replica were slow, were inefficient, and produced unacceptable band spreading. Like Kekulé's apocryphal dream of the tail-biting serpent and the benzene ring *(3)*, I was overcome, so to speak, by a flash of clarity that I might electromotively transport proteins out of an unfixed gel directly onto nitrocellulose sheets in a protein gel–destaining chamber I used almost every day.

Unaware of the work of Renart et al. *(4)*, I initially used diazobenzyloxymethyl-treated nitrocellulose sheets as the replica medium, as with nucleic acid blots, but soon found that unmodified sheets were more satisfactory and, moreover, required no time-consuming derivatization. Various buffer compositions, electrophoretic transfer conditions, nitrocellulose pore sizes, "blocking" agents for protein-adsorptive sites on the replica paper, antibody incubation and replica washing conditions, and the substitution of radioiodinated *Staphylococcus aureus* protein A for second-antibody detection of antigen–antibody complexes by radiography all were assessed in a couple of weeks. Gratifyingly, the new technique worked exactly as hoped for the specificity screening of hybridomas. It dawned on me that this methodology had potential for much greater applicability, both as a broadly applicable laboratory technique for detection of protein antigens in complex (e.g., cellular) mixtures and as a general immunodiagnostic tool.

About this time, there appeared the report of Towbin et al. *(5)*, describing an immunoblotting technique very similar to mine. There were, however, substantial enabling differences between the 2 methods, and I believed that Western blotting greatly simplified antigen transfer, antibody detection, and complex visualization. Undaunted, I therefore submitted my manuscript to *Analytical Biochemistry*, only to have it dismissed—not on the basis of the Towbin publication but because the technique was transparently obvious, made no fundamental contribution to the scientific literature, and, most damning, had been given the flippant name of "Western blotting" in honor, of course, of Edwin Southern and of the West Coast location of its invention. Word of the method continued to spread widely as a consequence of multiplicative expansion of the preprints sent to numerous colleagues. Thus, within a year of countenancing nonattributable references to my work, *Analyti-*

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[2] This paper has been cited more than 7300 times since publication.
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cal Biochemistry invited me to resubmit, and the article was published straightaway. Although a "citation classic," it is perhaps better recognized as one of the most highly cited scientific journal articles ever to have been initially rejected for publication.

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References

1. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975;98:503–17.
2. Alwine JC, Kemp DJ, Stark GR. Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes. Proc Natl Acad Sci U S A 1977;74:5350–4.
3. Kekulé A. Benzolfest: Rede. Ber Dtsch Chem Ges 1890;23:1302–11.
4. Renart J, Reiser J, Stark GR. Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera: a method for studying antibody specificity and antigen structure. Proc Natl Acad Sci U S A 1979;76:3116–20.
5. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 1979;76:4350–4.