

This Week's Citation Classic®

Burnette W N. "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.* 112:195-203, 1981.
 [Fred Hutchinson Cancer Research Center, Seattle, WA]

A method is described whereby specific antigens can be distinguished in a complex protein mixture by fractionation in polyacrylamide gels followed by electrophoretic transfer of the protein pattern to nitrocellulose sheets, detection on the solid phase with antibody, and visualization by autoradiography. [The SCI® indicates that this paper has been cited in more than 3,810 publications.]

Western Blotting

W. Neal Burnette
 Amgen Inc.
 Amgen Center
 Thousand Oaks, CA 91320

In late 1977, I left my postdoc at Albert Einstein College of Medicine, to join Bob Nowinski's group at the Hutchinson Cancer Center in Seattle. Although my work involved alternative messenger splicing in retroviruses, I spent some of my research time contributing to the laboratory's efforts to map murine leukemia virus structural proteins with monoclonal antibodies. In seeking ways to screen hybridomas for their epitope specificity, it occurred to me that it should be possible to link principles of the radioimmunoassay with the resolving power of SDS-polyacrylamide gel electrophoresis in order to pinpoint specific antigens in a complex protein mixture (such as a cell extract) with antibodies. But it was unclear how I might physically visualize the in situ interaction of the gel-separated proteins with antibody; some of my initial attempts to do this were, in retrospect, laughingly naive.

To distinguish retrovirus mRNA species, I had been using a solid-phase hybridization procedure in which gel-fractionated RNA is passively transferred ("blotted") to activated nitrocellulose paper and detected with specific radiolabeled DNA probes. J.C. Alwine, et al.¹ had humorously nicknamed this technique "Northern" blotting in homage to the original developer of DNA blotting, E.M. Southern.² It dawned on me

that I might likewise make a solid-phase replica of a protein gel. After some experimentation, I found that electrophoresis facilitated the transfer of proteins from the SDS-gels, that unmodified nitrocellulose worked better than chemically modified paper, and that [¹²⁵I]protein A could bind most types of antibody-antigen complexes³ on blots and was more convenient to prepare than radiolabeling various second antibodies. By blocking nonspecific binding of antibody and protein A to the nitrocellulose replica of the gel, startlingly clear radiographic images of antibody-specific antigens could be obtained.

The term "Western" blotting was coined in a distantly remembered discussion with Bob Nowinski, an allusion to both the geographic location of our laboratory and to Southern and Northern blotting. About this same time, the protein blotting paper of H. Towbin and his colleagues⁴ appeared. The general principles of their technique and of Western blotting were similar; nevertheless, the differences in specific methodology encouraged me to submit a manuscript to *Analytical Biochemistry*. The reviewers were not impressed, objecting especially to the name Western blotting, and the paper was rejected.

My move to the Salk Institute obscured my disappointment over failure to publish the technique. However, the few preprints I had sent to colleagues seemed to have undergone logarithmic Xerox multiplication. I began receiving phone calls from researchers unable to read the umpteenth photocopied generation of the preprint, a sort of technical samizdat that I had to endlessly interpret. With a little prodding, *Analytical Biochemistry* eventually agreed to publish the paper, which finally appeared in 1981. Since then, Western blotting has become one of the most widely employed immunochemical techniques, probably a consequence of its relative simplicity, interdisciplinary applicability, and the visual clarity of the results. Perhaps its most important clinical implementation has been as a confirmatory diagnostic test for AIDS.⁵

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2. Southern E.M. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503-17, 1975. (Cited 20,370 times.)
3. Langone J J. [¹²⁵I]protein A: a tracer for general use in immunoassay. *J. Immunol. Methods*, 24:269-85, 1978. (Cited 143 times.)
4. Towbin H, Staehelin T & Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Nat. Acad. Sci. USA* 76:4350-4, 1979. (Cited 17,295 times.) [See also: Towbin H. Citation Classic, *Current Contents/Life Sciences* 3(1111):19, 14 March 1988.]
5. Esteban J I, Tai C-C, Kay J W-D, Shih J W-K, Bodner A J & Alter H J. Importance of Western blot analysis in predicting infectivity of anti-HTLV-III/LAV positive blood. *Lancet* 2:1083-6, 1985. (Cited 100 times.)

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