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This Week's Citation Classic®

Wahl G M, Stern M & Stark G R. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. *Proc. Nat. Acad. Sci. USA* 76:3683-7, 1979.
[Department of Biochemistry, Stanford University School of Medicine, CA]

This paper described two techniques to increase the speed and reproducibility of nucleic acid hybridization to DNA immobilized on solid supports. The first involved a single chemical method to fragment DNA *in situ* in agarose gels to facilitate transfer to a solid support. The second described a means for accelerating hybridization rates 100-1,000-fold. [The *SCI*® indicates that this paper has been cited in over 2,930 publications.]

Simple Methods Facilitate Nucleic Acid Detection

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This paper describes two procedures to increase the speed at which DNA blotting and hybridization can be performed. As is often the case, modifications of techniques or new techniques are often developed out of necessity. The motivation for developing the methods described in this paper was that I and many other investigators experienced difficulty obtaining reproducible, high-quality results from the original Southern blotting procedure,[1] and the hybridization and film development steps required substantial amounts of time. In 1978, when the technique reported here was developed, I can remember commiserating with colleagues attempting to perform Southern blot hybridizations to detect unique sequence genes in mammalian DNA, about incomplete transfer of DNA fragments, multiday hybridizations, and two-week film exposures. The frustration encountered as films put up for multiweek exposures slowly emerged from the developer to reveal spots, blotches, and smears obscuring crucial bands, quickly made me decide to develop a procedure that would yield results much faster.

I embarked on this work with two goals in mind: (1) to develop a method to transfer DNA fragments from the gel to a solid support independent of their initial size, and (2) to develop a procedure that would dramatically reduce the time required for the hybridization reactions to proceed to completion and that would produce more intense signals while maintaining low backgrounds. Luckily, I was in the Department of Biochemistry at Stanford and had access to people like George Stark, Ron Davis, and Tom St. John who had substantial knowledge of DNA hybridization and nucleic acid chemistry. The conversations with these people led me to develop a simple procedure that involved treating agarose gels, subsequent to electrophoresis of DNA, with dilute HCl followed by concentrated NaOH to break the DNA backbones. Single-stranded DNA fragments with optimal sizes for rapid transfer and retention on solid supports were generated. Davis told me about a paper by J.G. Wetmur[2] that described the use of anionic dextran polymers to accelerate hybridization reactions in solution, and he warned me that many investigators had failed to obtain satisfactory results with hybridization of probes to DNA affixed to solid supports in the presence of dextran sulfate. Luckily, the first experiments performed by Mike Stein, then a Stanford undergraduate, worked beautifully. Later experiments revealed variable background. The variability of the results led me to investigate the parameters involved in probe preparation to obtain reproducibly successful DNA blots. The information I accumulated on this subject was ultimately disseminated in a review article published in *Analytical Biochemistry*.[3]

The methods published in this paper have continued to find new applications in molecular biology. For example, acid depurination to fragment DNA to enable efficient transfer has turned out to be important for the analysis of very large DNA. Indeed, implementation of methods for fragmenting DNA is crucial to enable transfer and subsequent detection of single copy sequences embedded within the large DNA molecules fractionated by pulsed field gel electrophoresis. Dextran sulfate has been employed for some time with *in situ* hybridization techniques designed to detect the positions of single copy genes in the chromosomes of many species.[4-6]

The frequency of Southern blots and other techniques that employ the methods described in this paper have increased steadily each year. Thus, it is curious that the number of citations to this work has leveled off or decreased. I interpret this observation in two ways. First, people are using the "cloning manuals" (e.g., see reference 7) with ever increasing frequency and citing them for methods instead of the original literature. Second, the information contained in this article is now generally regarded to be common knowledge. Indeed, perhaps the true mark of a *Citation Classic* is when the information a paper contains is so well known that citations to it are deemed superfluous.

1. **Southern E M.** Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503-17, 1975. (Cited 16,380 times.)
2. **Wetmur J G.** Acceleration of DNA renaturation rates. *Biopolymers* 14:2517-24, 1975. (Cited 55 times.)
3. **Meinkoth J & Wahl G M.** Hybridization of nucleic acids immobilized on solid supports. *Anal. Biochem.* 138:267-84, 1984. (Cited 595 times.)
4. **Harper M E & Saunders G F.** Localization of single-copy DNA sequences on G-banded human chromosomes by in situ hybridization. *Chromosoma* 83:431-9, 1981. (Cited 590 times.)
5. **Wahl G M, Vitto L, Padgett R A & Stark G R.** Single-copy and amplified CAD genes in Syrian hamster chromosomes localized by a highly sensitive method for in situ hybridization. *Mol. Cell. Biol.* 2:308-19, 1982. (Cited 70 times.)
6. **Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J & Gray J.** Fluorescence in situ hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. *Proc. Nat. Acad. Sci. USA* 85:9138-42, 1988. (Cited 20 times.)
7. **Sambrook J, Fritsch E F & Maniatis T,** eds. *Molecular cloning: a laboratory manual.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989. 7 vols.

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