This Week's Citation Classic

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Denhardt D T. A membrane-filter technique for the detection of complementary DNA. *Biochem. Biophys. Res. Commun.* 23:641-6, 1966.
[Biological Laboratories, Harvard University, Cambridge, MA]

This paper describes a technique for detecting specific DNA sequences in solution by annealing them to nitrocellulose filters carrying complementary DNA sequences. Prior to the hybridization the filters are incubated with a solution of Ficoll, polyvinylpyrrolidone, and bovine serum albumin. [The SCI® indicates that this paper has been cited in over 1,235 publications since 1966.]

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"I went to Harvard University in late-1964 fresh from my PhD at the California Institute of Technology with the goal of developing an *in vitro* DNA replicating system. For the preceding four years I had worked in Bob Sinsheimer's laboratory on aspects of ΦX174 replication *in vivo* and I wanted to develop a more biochemical approach. The model of ΦX replication that had evolved from our studies on the intact cell had led me to believe that DNA replication was occurring on the cell membrane. Now we needed a method to detect DNA replication in cell extracts. The incorporation of label from radioactive triphosphates into DNA would be ideal if we could develop a simple quantitative procedure to distinguish ΦX DNA from *E. coli* DNA.

"About that time, a publication by Gillespie and Spiegelman[1] appeared. They had extended earlier studies of Nygaard and Hall[2] by first binding single-stranded (SS) DNA to nitrocellulose filters and then using them to quantify complementary RNA sequences in solution. I realized I could use a similar procedure if I could block the nonspecific sticking of the SS DNA to the nitrocellulose without interfering with the annealing reaction. At high ionic strengths denatured DNA and poly[rA] adhere to nitrocellulose (which is also acetylated and fairly hydrophobic) because of the open, unstacked character of the hydrophobic bases; the bases in RNA are more stacked and less available for hydrophobic interactions.[3]

"To prevent the nonspecific binding of the denatured DNA I cast about for suitable compounds. Among the many I tried were Ficoll (a polymer of sucrose), polyvinylpyrrolidone (PVP) (I thought it might resemble an array of bases), and bovine serum albumin (BSA). BSA alone had a profound effect on the nonspecific sticking and together with Ficoll and PVP reduced the background to less than one percent. Before using this procedure to detect ΦX DNA synthesis *in vitro* I thought it wise to demonstrate that it could be used to follow ΦX DNA synthesis *in vivo*. It worked well and the results were published together with the technique in *Biochemical and Biophysical Research Communications*; it was my third independent publication. I was so overwhelmed with reprint requests that the only way I could afford to honor them all was to reduce the six pages to one photographically and send out one-page Xerox copies—perhaps the first 'miniprint' reprint.

"Despite the reprint requests, I saw very few applications of the technique until recombinant DNA technology came into use. Examples of recent applications of DNA-DNA hybridization include the analysis of Southern Blots and the detection of specific cloned sequences in plaques or colonies.[4] Some improvement in the signal-to-noise ratio has been obtained by increasing the concentrations of the several components and including dodecyl sulfate and nonhomologous DNA or poly[rA] in the hybridization reaction. Dextran sulfate also helps to reduce the background and to accelerate the rate of hybridization.[5] This publication has been widely cited because it describes a simple and inexpensive, yet effective, procedure to detect specific DNA sequences."

1. **Gillespie D & Spiegelman S.** A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. *J. Mol. Biol.* 12:829-42, 1965.
2. **Nygaard A P & Hall B D.** A method for the detection of RNA-DNA complexes. *Biochem. Biophys. Res. Commun.* 12:98-104, 1963.
3. **Cashion P, Sathe G, Javed A & Kuster J.** Hydrophobic affinity chromatography of nucleic acids and proteins. *Nucl. Acid. Res.* 8:1167-85, 1980.
4. **Wu R, ed.** Recombinant DNA. (Whole issue.) *Methods Enzymol.* 68, 1979. 555 p.
5. **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. US* 76:3683-7, 1979.