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 SC® indicates that this paper has been cited in over 1,235 publications since 1966.]

This week's Citation Classic

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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 \( \Phi X 174 \) replication in vivo and I wanted to develop a more biochemical approach. The model of \( \Phi X \) 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 DNA from \( E. coli \) DNA.

"About that time, a publication by Gillespie and Spiegelman\(^3\) 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.\(^4\)

"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 \( \Phi X \) DNA synthesis in vitro I thought it wise to demonstrate that it could be used to follow \( \Phi X \) 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 'minireprint'.

"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."