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J. A technique for radiolabeling DNA restriction endonuclease activity. *Anal. Biochem.* 132:6-13, 1983; and **Feinberg A P & Vogelstein B.** *Anal. Biochem.* 137:266-7, 1984.
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development of the ... for labeling DNA, by us... ...ide primers and single-stranded ... ...fied directly from agarose gels. [The ... ...dicates that these papers have been cited in more than 10,615 and 3,785 publications, respectively. The 1983 paper is the most-cited paper published after 1980 in the entire *SCI*®.]

DNA Labeling by Random Priming

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I had been in Bert Vogelstein's lab for only two months when Bert told me to question everything from first principles when learning a new method, and then immediately left for vacation. Thus, when I needed to radioactively label a DNA probe by the standard method of the time, nick-translation, I wondered what translation had to do with it, since there was no protein, and I also wondered whether DNase might be bad to have around during DNA synthesis. With Bert gone, I directed my questions directly to the vendor's booklet that came with the nick-translation kit, which only deepened my confusion. DNase was present in order to make short stretches of single-stranded template available for DNA polymerase. However, use of the enzyme required an almost cosmic sense of how long to permit a labeling reaction to go, since by the time one finished measuring incorporation, probe mass and length had changed considerably.

On his return, Bert directed me to the classical literature on DNA polymerases and to a recent paper by G.A. Ricca et al.,[1] who generated high specific activity probes using a specific primer, although the template first had to be subcloned into M13. On the other hand, 15 years earlier, M. Goulian et al.[2] had primed a single-stranded template using random oligonucleotides. An additional advantage of using a single-stranded template was that one could label a gel fragment directly, and Bert had earlier invented the glass milk technique for purifying them.

In order to use random priming to generate probes from single-stranded templates, the first problem was to maximize radionucleotide incorporation while minimizing probe degradation. DNA polymerase contains two exonuclease activities. I eliminated the 5′-3′ exonuclease by using the Klenow fragment, since the small fragment of Pol I contains this nuclease. However, I also found that at pH 6.6 there is negligible 3′-5′ exonuclease activity, and yet the enzyme retains approximately half the polymerization activity. Incidentally, some of the current commercial kits do not work well because they use neutral pH. The second technical problem was the stable and reproducible use of random oligonucleotides. Goulian had found that 8-12mers were optimal, but in those days I had to make my own oligonucleotides, and the quality varied greatly among sources and preparations. One day, while ordering isotope, Bert happened to notice that one vendor sold random hexamers, which saved me a good deal of trouble (and later made the vendor some money since they could claim an exclusive reagent). In order to work out the details of the method, I performed many thousands of labeling reactions on my bench, but learned an important lesson from Bert, that if you ask why it is that we do things the way we do them, the answer is often for no good reason at all.

After succeeding, Bert and I thought the technique might have some commercial applications. We brought it to the attention of the institutional patent office whose approach to patenting, at least in this instance, was to distribute the technique to most vendors in molecular biology, telling us that if they expressed interest in the method, then a patent would be filed and the vendors charged a licensing fee. As Bert would say, "They're no dummies": The companies expressed no interest and then did quite well after no patent was filed.

1. **Ricca G A, Taylor J M & Kalinyak J E.** Simple rapid method for the synthesis of radioactively labeled cDNA hybridization probes utilizing bacteriophage M13mp7. *Proc. Nat. Acad. Sci. USA* 79:724-8, 1982.
2. **Goulian M.** Initiation of the replication of single-stranded DNA by *Escherichia coli* DNA polymerase. *Cold Spring Harbor Symp.* 33:11-20, 1968.

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