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Sanger F, Nicklen S & Coulson A R. DNA sequencing with chain-terminating inhibitors. *Proc. Nat. Acad. Sci. USA* 74:5463-7, 1977.
[Medical Research Council Laboratory of Molecular Biology, Cambridge, England]

A rapid and simple method for sequencing DNA is described. It employs 2',3'-dideoxyribonucleotides (ddNTP) and arabinonucleotides (araNTP), which are analogues of the normal triphosphates and act as chain-terminating inhibitors of DNA polymerase. Under appropriate conditions, including the incorporation of a ^{32}P-labelled deoxynucleotide, a mixture of radiolabelled oligonucleotides all ending in the same nucleotide is produced. After electrophoresis on acrylamide gel, the DNA sequence can be read off from the distribution of the bands on an autoradiograph of the gel. [The *SCI*® indicates that this paper has been cited in over 2,600 publications.]

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Earlier methods for sequencing proteins and nucleic acids depended on the principle of partial hydrolysis; these were very time-consuming and not easily applicable to DNA. In 1975 at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England, we developed a new method,[1] which we called the "plus and minus" method. This depended on an entirely different principle involving the copying of the DNA with DNA polymerase rather than degradation. Although much more satisfactory than any of the previous systems, it was still not as good in practice as the principle suggested it could be, and so we continued to test different techniques using this new approach.

At a meeting in Germany, I was fortunate to meet K. Geider, who told me that he had some ddTTP. He let me have a sample, and on trying it out we got a startlingly good result, with sharp bands of equal intensity going up to the top of the electrophoresis gel, whereas with the plus and minus method the bands could vary greatly in intensity and were only spread over a small region of the gel. This was clearly the method we should use. However, the problem was that we needed the other three dideoxynucleotide triphosphates, and these had never been made. Alan R. Coulson and I had not done any nucleotide chemistry before and were unable to persuade anyone else to make them, so we had to settle down to making them ourselves. Eventually, we were able to obtain small amounts of all three, of uncertain purity, but when we tried them out it was clear that this was a great improvement on the plus and minus method, which it completely replaced, and it is still the most widely used technique.

Further development in experimental conditions has led to considerable improvements, and gels produced today are more impressive than those shown in the paper. From the scientific point of view, the 1975 paper was probably more important than this one since it described an entirely new approach and represented a turning point in DNA sequencing that led to the vast amount of data that is being obtained today. The present paper is more widely cited because it describes the actual method that is being used.

Another rapid DNA sequencing technique was developed at about the same time by A.M. Maxam and W. Gilbert,[2] and the two methods led to a surge of interest and activity in DNA sequencing. The scope and use of the "dideoxy" method was greatly increased by the introduction of a cloning procedure by J. Messing and his colleagues.[3] This made it possible, at least in theory, to sequence any DNA, however large, and most of the papers published today on DNA sequences use this system.

While Coulson and I were working on the dideoxy derivatives, Steve Nicklen was studying the *ara* [arabinosyl] compounds, some of which were commercially available. Although they were less satisfactory than the dideoxys and are not used now, the two systems were sometimes employed to resolve possible amibiguities in early experiments, and so were included in this paper. Steve was a very bright PhD student, but he decided to give up science and had left the lab by the time the paper was written.

This paper is referred to in most sequencing work, though a more recent reference with later modifications and improvements is usually included to give the exact experimental conditions. Frequently, this is from instructions provided with the commercially available sequencing kits.

For my work on DNA sequencing, I was awarded the University of Chicago G.W. Wheland Award (1978); the Gairdner Foundation Annual Award, the Louisa Gross Horwitz Prize, and the Albert Lasker Basic Medical Research Award (1979); and the Biochemical Analysis Prize of the German Society for Clinical Chemistry and the Nobel Prize in chemistry (1980).[4]

1. **Sanger F & Coulson A R.** A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J. Mol. Biol.* 94:441-8, 1975. (Cited 375 times.)
2. **Maxam A M & Gilbert W.** A new method for sequencing DNA. *Proc. Nat. Acad. Sci. USA* 74:560-4, 1977. (Cited 4,770 times.)
3. **Messing J & Vieira J.** A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. *Gene* 19:269-76, 1982. (Cited 1,750 times.)
4. **Sanger F.** Sequences, sequences, and sequences. *Annu. Rev. Biochem.* 57:1-28, 1988.

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