

This Week's Citation Classic

Sanger F, Brownlee G G & Barrell B G. A two-dimensional fractionation procedure for radioactive nucleotides. *J. Mol. Biol.* **13**:373-98, 1965.
[Medical Research Council Laboratory of Molecular Biology, Cambridge, England]

A two-dimensional procedure for fractionating ³²P-labelled oligonucleotides is described. The first dimension uses high-voltage electrophoresis on cellulose acetate strips and the second electrophoresis on DEAE-cellulose paper. The method is used to fractionate ribonuclease digests of RNA, and micro techniques are described for analysing and sequencing the purified oligonucleotides. [The SC[®] indicates that this paper has been cited over 870 times since 1965.]

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"This paper represents my first venture into the field of nucleic acids. My previous work had been concerned with amino acid sequence determination in proteins and I was particularly interested in the development of methods. By 1965, techniques for protein sequencing were well developed and already somewhat standardised, whereas little had been done on the determination of sequences in the other biologically important polymeric molecules—the nucleic acids. This seemed a worthwhile challenge and, together with a bright and enthusiastic PhD student, G.G. Brownlee, and B G Barrell, who was a technical assistant at that time, I set to work on the problem.

"Prior to this work the only RNA sequence that had been determined was that of the alanine tRNA, which was done by Holley and his collaborators.¹ The methods used by them were largely developed for protein chemistry and involved rather laborious procedures

such as counter-current distribution and ion-exchange chromatography. It seemed to us that in order to be able to sequence the many large nucleic acids present in living matter more rapid and simple methods were needed that could be applied to small amounts of material. In particular we needed a method of fractionating the complex mixture of oligonucleotides obtained by partial digestion of RNA. One important development described in this paper was the use of ³²P-labelled RNA of high specific activity. This made it possible to work on a small scale and to use two-dimensional 'paper' fractionation techniques, which had high resolving power as well as being rapid and simple to carry out. As ³²P can be detected at very low concentrations by radioautography and assayed by counting techniques, ³²P-labelled nucleic acids have been used in most subsequent studies on sequences of RNA and DNA.

"In general, nucleotides do not fractionate well by 'paper' methods and we spent a good deal of time trying out different systems of chromatography and electrophoresis on paper and various modified papers, but usually the products were not well resolved and the radioautographs produced contained only streaks and blotches rather than well-defined bands or spots. The turning point in this work came one morning when Barrell showed me a film he had developed that contained a large number of clear, well-defined spots. This was what we had been looking for and the two-dimensional fractionation we had used formed the basis of the method described in this paper. It was possible to elute the purified nucleotides from the paper and we developed micro methods for analysing and sequencing them. The various methods described in this paper have formed the basis for many subsequent studies on RNA sequences."^{2,3}

1. Holley R W, Apgar H, Everett G A, Madison J T, Marquisee M, Merrill S H, Penswick J R & Zamfr A. Structure of a ribonucleic acid. *Science* **147**:1462-5, 1965.
2. Adams J M, Jeppein P G N, Sanger F & Barrell B G. Nucleotide sequence from the coat protein cistron of R17 bacteriophage RNA. *Nature* **223**:1009-14, 1969.
3. Fiers W, Contreras R, Duerinck F, Haegeman G, Iserentant D, Merregaert J, Min Jou W, Molemans F, Raeymaekers A, Van den Berghe A, Volckaert G & Ysehaert M. Complete nucleotide sequence of bacteriophage MS2 RNA: primary and secondary structure of the replicase gene. *Nature* **260**:500-7, 1976