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Helling R B, Goodman H M & Boyer H W. Analysis of endonuclease R·*Eco*RI fragments of DNA from lambdoid bacteriophages and other viruses by agarose-gel electrophoresis. *J. Virology* 14:1235-44, 1974.
[Depts. Microbiology and Biochemistry and Biophysics, Univ. California, San Francisco, CA and Div. Biological Sciences, Univ. Michigan, Ann Arbor, MI]

A procedure for low voltage agarose-gel electrophoresis of DNA molecules generated by site-specific endonucleases was developed. Its utility in making DNA size estimations, separating and purifying specific DNA molecules, and mapping and analyzing chromosomes was demonstrated. [The *SCI*® indicates that this paper has been cited in over 605 publications, making it the most-cited paper for this journal.]

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I planned to attempt *in vitro* recombination during my first sabbatical leave from the University of Michigan. It seemed clear that restriction endonucleases would be important in generating DNA segments and analyzing products. I knew that Herb Boyer, who had been a fellow graduate student, was studying restriction and modification of DNA. When I called him he said that he was very interested in such a project, and that his student R. Yoshimori had identified two restriction enzymes. We arranged to work together at the University of California in San Francisco from 1972 to 1973 while Boyer took his sabbatical in residence. Unexpectedly, and before I arrived, the EcoRI enzyme was found to produce staggered cuts in DNA, thus making a direct procedure for cloning genes obvious.

Nevertheless, the necessary purification and analysis of specific DNA molecules was far from simple. Assaying restriction enzyme activity and resolving DNA mixtures required sucrose-gradient centrifugation, which was tedious and of poor resolving power. Boyer suggested that I try polyacrylamide gel electrophoresis, which I did but without success. I explored agarose-acrylamide mixed gels, gradient gels, and continuous flow electrophoresis. Ultimately, agarose alone gave promising results. After testing the effects of agarose concentration, field strength, buffer composition, and temperature, I achieved excellent separations. Initially I analyzed the gels by using radioactive DNA and counting gel slices after electrophoresis—an extremely laborious and costly procedure. Fortunately, Joe Sambrook and Phil Sharp suggested staining with ethidium bromide,[1] and the entire procedure became simple.

The cloning experiments worked marvelously well.[2,3] After initial attempts to use lambda DNA as the cloning vector, we invited Stan Cohen (and his associate Annie Chang) to collaborate with us in using plasmids. The history of these experiments has been documented at the request of the US Patent Office and can be released with the permission of Stanford University.[4]

When I returned to Michigan, I completed experiments using the gel procedure to analyze DNA from viruses, plasmids, bacteria, and higher organisms. The choice of a journal in which to publish was not obvious and was finally dictated by Boyer's interest in publishing in a virology journal so that he would have proper credentials if he wished to attend a forthcoming virology congress. The clumsy title of the paper as it appeared resulted from the refusal of a reviewer to accept my original "Agarose-gel electrophoresis of DNA" and is the concoction of that reviewer.

The first published demonstration of the use of the gel procedure was in the basic cloning paper.[2] As a result I received requests for the details from scientists around the world. The excellence of the procedure depended on the use of low voltage, agarose as the support matrix, and a simple and reversible staining procedure. At the time I cast the gels in tubes. Subsequently, a simple horizontal gel apparatus was developed[5] and is now in widespread use.

Recently, the development of pulsed-field gel electrophoresis and variant procedures[6,7] has extended the theory of gel electrophoresis and allows the separation of molecules of very large size. I believe our paper is still read because it demonstrates how gel electrophoresis can be applied in simple but powerful ways to analyze genomes and because its use was pioneered in the first paper in which the successful cloning of genes was described.

1. **Aaij C & Borst P.** The gel electrophoresis of DNA. *Biochim. Biophys. Acta* 269:192-200, 1972. (Cited 140 times.)
2. **Cohen S N, Chang A C Y, Boyer H W & Helling R B.** Construction of biologically functional bacterial plasmids *in vitro*. *Proc. Nat. Acad. Sci. USA* 70:3240-4, 1973. (Cited 525 times.)
3. **Morrow J F, Cohen S N, Chang A C Y, Boyer H W, Goodman H M & Helling R B.** Replication and transcription of eukaryotic DNA in *E. coli*. *Proc. Nat. Acad. Sci. USA* 71:1743-7, 1974. (Cited 335 times.)
4. **Board of Trustees, Stanford University and University of California.** *Process for producing biologically functional molecular chimeras.* US patent 4,237,224. 2 December 1980.
5. **McDonell M W, Simon M N & Studier F W.** Analysis of restriction fragments of T7 DNA and determination of molecular weights by electrophoresis in neutral and alkaline gels. *J. Mol. Biol.* 110:119-46, 1977. (Cited 1,185 times.)
6. **Schwartz D C & Cantor C R.** Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* 37:67-75, 1984. (Cited 230 times.)
7. **Chu G, Vollrath D & Davis R W.** Separation of large DNA molecules by contour-clamped homogeneous electric fields. *Science* 234:1582-5, 1986.

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