CC/NUMBER 3
JANUARY 18, 1982

This Week's Citation Classic

Sharp P A, Sugden B & Sambrook J. Detection of two restriction endonuclease activities in *Haemophilus parainfluenzae* using analytical agarose-ethidium bromide electrophoresis. *Biochemistry* **12**:3055-63, 1973.
[Cold Spring Harbor Laboratory, Cold Spring Harbor, NY]

A rapid assay for restricting endonuclease was developed using electrophoresis of DNA fragments in agarose gels and detection of DNA bands by staining with the fluorescence dye ethidium bromide. Using this assay, two different restriction endonucleases were purified from extracts of *Hemophilus parainfluenzae*. [The *SCI*® indicates that this paper has been cited over 1,040 times since 1973.]

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October 6, 1981

"Advances in molecular biology are frequently the product of the development of new methodology. Perhaps the most striking recent example of this is the impact that DNA recombinant technology has had. Publications that make novel contributions to the development of new methodology are widely read and frequently referenced. Bill Sugden, Joe Sambrook, and I described this type of methodology at a time when the molecular biological community was discovering the multiple uses of restriction endonucleases.

"In 1971, I arrived at Cold Spring Harbor in New York to begin studying the molecular biology of DNA tumor viruses. James D. Watson had just become director of the laboratory and Sambrook was initiating a research effort to study the molecular biology of SV40. The main goal was to define the genetic structure of DNA tumor viruses in general. Fortunately for the whole field, Ham Smith at Johns Hopkins[1] had just discovered type II restriction endonucleases that cleaved DNA at defined nucleotide sequences. Dan Nathans, chairman of Smith's department, returned from a sabbatical with Ernest Winocour at the Weizmann Institute in Israel and used Smith's purified restriction endonuclease from *H. influenzae* to break down SV40 DNA into 11 pure fragments.[2] The ability to dissect viral genomes with restriction endonucleases obviously offered the possibility of defining the genetic structure of SV40 DNA. Not to be left in the dust at Cold Spring Harbor, we began an intense program to isolate and use restriction endonucleases.

"Smith and colleagues had used a decrease in viscosity of a DNA solution as an assay for endonuclease activity. Nathans introduced gel electrophoresis as a means of fractionating endonuclease cleaved ^{32}P-labeled fragments; however, the distribution of fragments in the gel required radioautography, a time-consuming process. After having spent a postdoctoral fellowship at the California Institute of Technology and observing the enhanced fluorescence of the dye ethidium bromide upon binding to DNA, I decided to try to stain gels with ethidium bromide as a means of detecting the bands formed by different length fragments. The minimum concentration of ethidium bromide necessary for saturation of DNA was calculated and within three hours I had successfully detected DNA bands in gels with ethidium bromide.

"Staining of gels with ethidium bromide provided a rapid assay for detecting fragments produced by restriction endonucleases. Sugden was induced to help us purify the restriction endonucleases from *H. parainfluenzae* as this strain was thought to contain activities with different specificities than *H. influenzae*. We quickly discovered using the gel electrophoresis and ethidium bromide staining assay that there were two different endonucleases with different sequence specificities in these bacteria, Hpa I and Hpa II. Many other investigators have used these endonucleases or these methods in their experiments. In addition, a number of small companies have been established to supply such endonucleases to the molecular biological community."

1. **Kelly T J, Jr. & Smith H O.** A restriction enzyme from *Hemophilus influenzae*. II. Base sequence of the recognition site. *J. Mol. Biol.* **51**:393-409, 1970.
2. **Danna K & Nathans D.** Specific cleavage of simian virus 40 DNA by restriction endonuclease of Hemophilus influenzae. *Proc. Nat. Acad. Sci. US* **68**:2913-17, 1971.