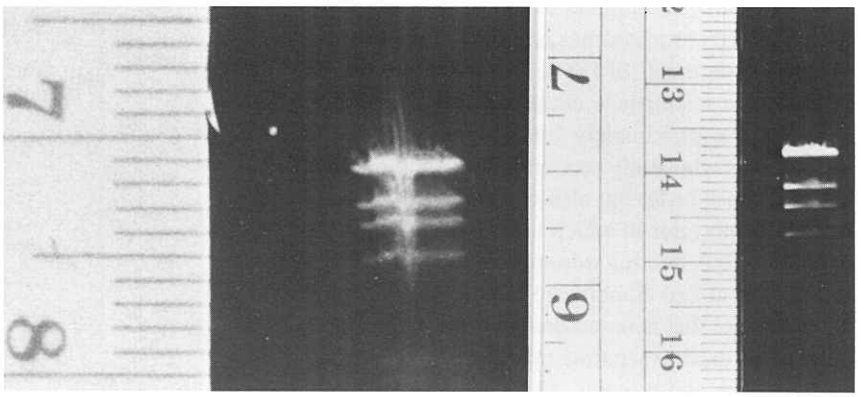
Fast Agarose Gel Electrophoresis (FAGE)

Inventor: Frederic R. Fairfield,* Theoretical Division



(a) *(b)*

(a) The electrophoresis of Lambda DNA fragments with the FAGE System lasted for four minutes. (b) The electrophoresis of Lambda DNA fragments with conventional techniques lasted for ninety minutes. The rulers in the two pictures show that, during electrophoresis, the DNA fragments have moved approximately the same distances.

When DNA or deoxyribonucleic acid—originally called "nuclein"—was discovered in 1869, scientists were unaware of its essential role in genetic inheritance. Until the early 1950s, biologists generally maintained that proteins were the chief carriers of heredity because proteins appeared to have a greater diversity of structure than nucleic acids did, a feature that made them more likely to be responsible for the diversity of the genes. But then, in the early 1950s, firm evidence was obtained that DNA, rather than proteins, serves as the physical basis of heredity.

Now scientists and lay people alike know that the basic material constituting the gene is made up of chainlike molecules of nucleic acids—DNA in most organisms and RNA (ribonucleic acid) in some viruses. Each human being carries an amazing amount of DNA that is coiled and folded inside cells; it has been estimated that if all the DNA in a human were stretched out, it would extend from the Earth to the Sun and back again. And it is in the molecules of DNA that the human genome resides and encodes chemical instructions for the production of thousands of proteins, which are largely responsible for the body's structure, functions, and development as well as for maintaining its biological systems.

Molecular biologists study DNA samples for such diverse purposes as genetic screening, forensic medicine, research in genetic diseases ranging from cancer to manic depression, and mapping of the entire human genome system. One analytical technique—gel electrophoresis—is dominant in all these studies; it allows scientists to separate different-size fragments of DNA by their movement through a gel under the influence of an electric field. However, this operation can take a long time, sometimes too long for the purposes of the specific run (in some cases it takes as long as 36 hours). The Fast Agarose Gel Electrophoresis (FAGE) System, developed in part at Los Alamos, dramatically shortens the time needed for the run (it can be ten to one hundred times faster than runs performed with other, similar techniques) and will therefore most likely become an indispensable tool of research worldwide. For developing this technology, the inventor won a 1990 R&D 100 Award; the awards are given each year by *Research and Development Magazine* for the one hundred most significant technical innovations of the year.

The FAGE System

The FAGE System includes the apparatus in which the electrophoresis is performed, the method for preparing the DNA and the gel, and the method for running the electrophoresis. The three components of the system are interconnected; a change in the apparatus implies a change in the gel that is produced in the apparatus as well as a change in the method for running the electrophoresis. The system separates DNA fragments by size when the fragments are placed in and then driven through a gel by an electric field that measures 20 to 40 volts per centimeter across the gel. During the experiment, the gel is thermostated to 4 degrees Celsius by a copper cooling plate and an insulating layer of polyethylene to prevent it from getting burned. After 4 to 60 minutes, depending on the experiment, the voltage is turned off and the gel is removed from the apparatus. The gel contains the DNA fragments, which are not visible; a dye is then used to stain the fragments and thus make them visible, and the result is photographed. This separation of the fragments provides a means for distinguishing among various types of DNA.

In aqueous solutions, the movement of the DNA is independent of the size of the fragments because

* *Frederic Fairfield started developing this technology while working at the University of Tennessee in Knoxville.*

For more information about the FAGE System, please contact Dr. Ronald E. Barks, Industrial Applications Office, Los Alamos National Laboratory, P.O. Box 1663, Mail Stop M899, Los Alamos, NM 87545, Telephone (505) 667-3839.

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April 1991

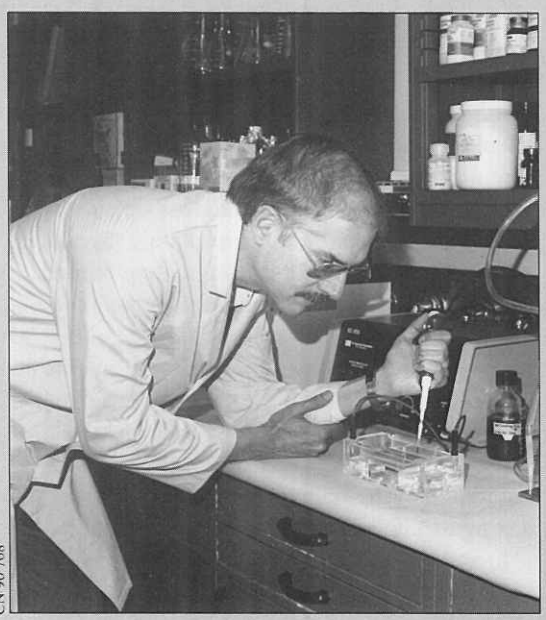
the ratio of the electrostatic and frictional forces on the DNA is constant. In contrast, when agarose gel is used in the solution, the movement of the DNA is dependent upon the size of the fragments; in this case, the DNA fragments have to move around the filaments of solidified agarose. Clearly, larger fragments will experience increased frictional forces that will slow down their motion. In conventional gel electrophoresis systems—at high voltages—the mobility of the DNA is controlled by the applied voltage, not by the size of the DNA fragments, although the agarose filaments are still present. FAGE contradicts established theory because, in this new technology, the mobility of the DNA in an agarose gel solution is determined by molecular weight (size of fragments) even at high voltages. Such an intriguing result calls for a new theory, and Los Alamos scientists are working to develop it.

Advantages and Applications

The FAGE System is inexpensive and highly reliable, but its main advantage is that it is fast: it can separate DNA fragments ranging in length from 200 base pairs (the chemical building blocks of the molecules) to 400,000 base pairs in 4 to 60 minutes. This remarkable speed makes our new product most attractive for biological and medical research, in which speed of separation or the stability of the particles is an important consideration.

Molecular biologists who clone DNA typically work with small fragments. By using FAGE, they will obtain results that are ten times faster than those obtained with conventional technologies. Biologists who research chromosomes and the human genome use larger fragments of DNA. Our technology will enable these scientists to cut processing time from 38 hours to approximately 2 hours. By using FAGE, biochemists who study protein-DNA complexes will not only work faster but also characterize, for the first time, mixtures in which individual complexes exist for a few, fleeting minutes. And finally, researchers at Los Alamos are interested in developing a product that is based on the same process and that can be used to separate other large molecules, such as RNA and proteins. The FAGE System clearly holds the promise of becoming a most influential analytical method in a variety of areas, from the mapping of the human genome to medical research.

A patent for the technology is pending. The invention is available for technology transfer.



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Frederic R. Fairfield

INVENTOR Frederic Fairfield's research in the field of gel electrophoresis led to the discovery of the Fast Agarose Gel Electrophoresis (FAGE) System that earned him a 1990 R&D 100 Award, one of seven such awards garnered by Los Alamos scientists in that year.

Fairfield obtained B.A. degrees in chemistry and mathematics in 1969 from Lehigh University in Bethlehem, Pennsylvania. After earning his Ph.D. in biochemistry in 1980 from the State University of New York at Stony Brook, Fairfield spent one year as a postdoctoral fellow at the same university. Between 1981 and 1985, he was a postdoctoral fellow at the University of Oregon. In November 1985, Fairfield became assistant professor in biochemistry at the University of Tennessee. In 1986, he started a two-year joint appointment with the University of Tennessee and the University of Oregon. He then continued at the University of Tennessee until July 1989, at which time he accepted a staff member position with the Theoretical Division at Los Alamos. In the past five years, Fairfield authored and co-authored numerous publications and gave thirty-seven invited talks on gels used in electrophoresis.

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