Multi-capillary DNA Sequencer

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Overview: In the middle of the 1980's, an automated DNA sequencer for analyzing the genetic code of deoxyribonucleic acid, or DNA, was established, initiating rapid progress in DNA sequence studies. Currently, several DNA research projects are being conducted not only to obtain fundamental knowledge, but also to learn the sequence data of a variety of biological genomes for diagnostics, therapeutics of diseases, or the design of new drugs. In this field, a fully automated instrument with high throughput is strongly desired for obtaining large amounts of data in a short time. Hitachi has been researching and developing new technologies for DNA sequencing, such as the use of multi-capillary arrays instead of conventional slab gel for the separation medium. High throughput and full automation are achieved with these new detection and sampling technologies.

INTRODUCTION

GENETIC information is recorded in deoxyribonucleic acid (DNA) in all forms of life. Each cell in an individual biological body contains the same DNA molecules. The linear order of the four kinds of base residues in the DNA molecule — adenine (A), guanine (G), thymine (T) and cytosine (C) — represent the genetic code. DNA sequencers can be used to identify this linear order.

In the middle of 1980's, Applied Biosystems Inc. introduced an automated sequencer to analyze DNA sequences.[1)] Several other DNA sequencers from other manufacturers followed. Before the introduction of these sequencers, all steps for analyzing DNA sequences had to be executed manually, took over a week for a single run. The appearance of the DNA sequencer brought a major change in the field of genetic research and use of the instrument has spread rapidly. The system is not fully automated, however, it contributes not only to reduce run time but also to work as a trigger toward large scale DNA sequencing like the human genome project.

To expand the scale of research and enable the sequencer to be used in routine work, a "new-generation" DNA sequencer with new features and functions is desired. This new sequencer should have high throughput and full automation. Conventional automated DNA sequencers use slab gel as the separation medium, but slab gel has certain technical limitations that cannot be overcome. Hitachi is developing DNA sequencing technology to establish a new-generation of DNA sequencers and contribute to international DNA research efforts.

CONVENTIONAL "SLAB GEL" SYSTEM

Conventional DNA sequencers use a slab gel plate as a separation medium, as shown in Fig. 1. Samples are prepared following sample preparation protocol. In the first step, a mixture of DNA fragments with different fragment lengths in every single base is prepared. During the preparation procedure, four kinds of fluorescent dyes are attached to the DNA fragments. The wavelength of the fluorescent light of each dye corresponds to each of the four bases. The DNA fragments are then separated according to their size through migration from one side of the gel plate to the other by electrophoresis. DNA fragments can be moved by high voltage through a separation medium because the fragment molecules have electrical charges. The prepared sample is laid on top of the slab

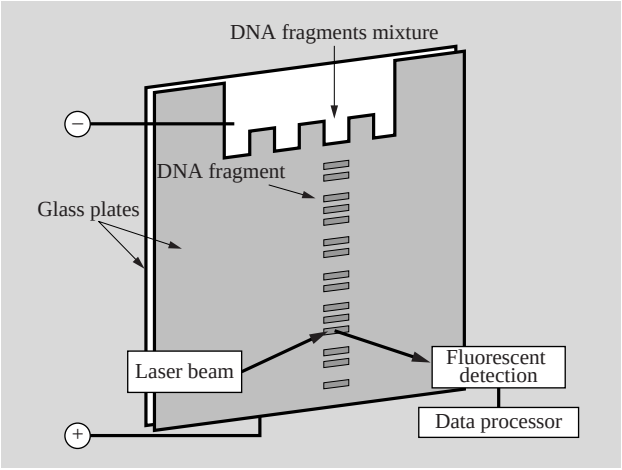

Fig. 1—"Slab gel" DNA Sequencer.
Conventional DNA sequencer uses slab gel. Separation gel is formed in the gap between two flat glass plates.

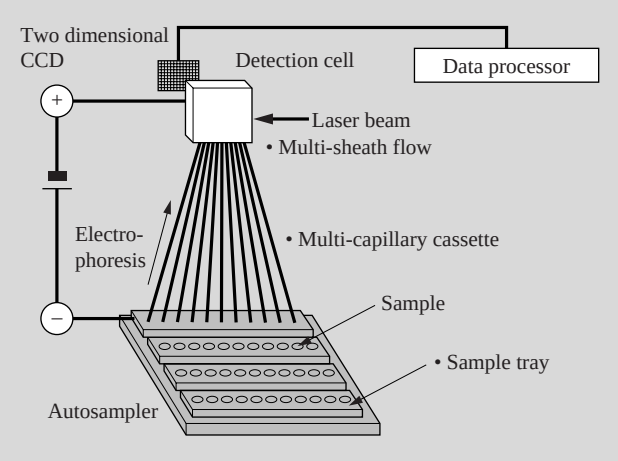

Fig. 2—Multi-capillary DNA Sequencer. Samples in sample tray are loaded into capillaries automatically. DNA fragments separated through the capillary by electrophoresis are detected by laser-induced fluorescent detection.

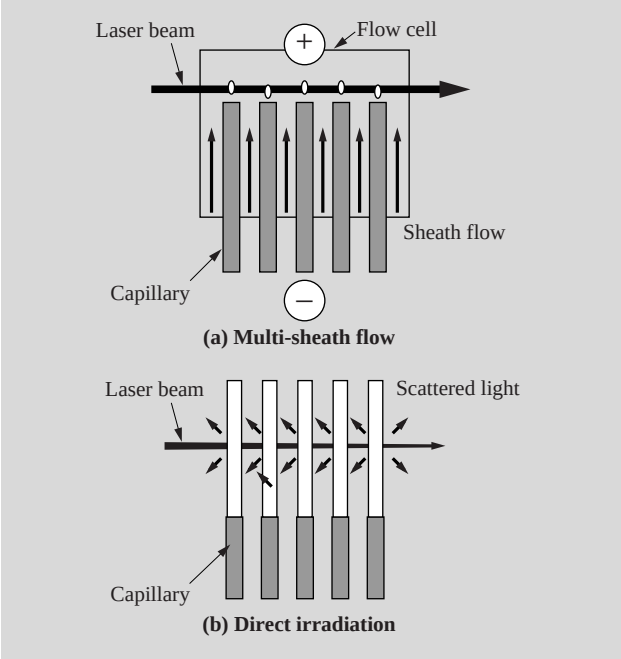

Fig. 3—Comparison of Laser Irradiation Methods. "Multi-sheath flow" provides simultaneous irradiation of samples from multiple capillaries. The same irradiation intensity for each capillary cannot be achieved by directly irradiating the array of capillaries.

gel and then a high voltage is applied to it. DNA fragments are driven from the negative side to the positive side as their charge becomes negative. Laser is irradiated on almost another end of gel plate. Fluorescent light is emitted when a fragment tagged with one of the fluorescent dyes passes through the laser beam. The type of base is identified by the fluorescent wavelength detected.

Slab gel is prepared between two flat glass plates placed as its gap is around 0.3 mm. This preparation process is time consuming, taking up to three hours or more from washing the glass plates until they are ready to use.

Sampling is also time consuming, and it requires a high level of skill to place a layer of a small aliquot part of a sample onto the surface of the fragile gel.

Then, gel preparation and sampling are the two critical paths to huge scale DNA sequencing.

MULTIPLE CAPILLARY SYSTEM

System Configuration

A multi-capillary system is shown in Fig. 2. Capillary gel is used as a separation medium instead of slab gel. The inner diameter of each capillary is as small as 0.1 mm or less. Separation gel is formed in the capillary inner cavity. A number of capillaries are bundled together and handled as a cassette. The prepared sample is placed on the sample tray of the autosample and loaded automatically from the sample tray by electrokinetic injection.

DNA fragments in the sample are separated by electrophoresis and arrive at the detecting flow cell. A laser irradiates light at the flow cell and the fluorescence emitted from sample fragments is detected. A newly developed "multi-sheath flow" technology,[2] explained below, is used in this detection process. The emitted light is focused on a two-dimensional charge-coupled device, or CCD, and the detected signal is processed using a data processor. The key technologies are described in the following sections.

Multi-capillary Cassette

Forty-eight capillaries are bundled together in a cassette, which can be easily set and exchanged. The gel is an easy-to-use ready-made gel that reduces operation time. For system evaluation, a cross-linked polyacrylamide gel was tested as the separation medium.

Sample Tray

An electrode has been fixed to the bottom of the sample tray so that it can be easily immersed in the tiny sample. At least 4 μ*l* of the sample can be applied. Multiple runs can be carried out without the presence of an operator as the autosampler contains up to five sample trays. The instrument can carry out a series of multiple runs automatically, which is a nice advantage, especially for overnight operation.

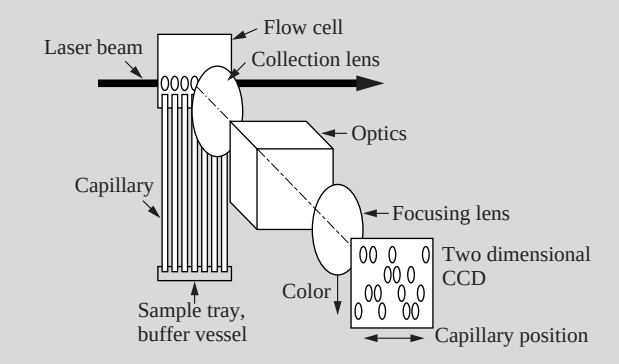

Fig. 4—Optics for Two-dimensional Detection.
Fluorescent emission from DNA fragments in the sheath flow cell is focused on a two-dimensional CCD. Four spots corresponding to four kinds of bases at each capillary position are detected.

Multi-sheath Flow

In general, simultaneous laser irradiation is effective for highly sensitive detection. In the newly developed "multi-sheath flow" technology,[2] shown in Fig. 3, the ends of the capillaries are located in the flow cell. Sheath flow is generated to carry the DNA fragments poured out from capillary according its continuous flow. The positive flow pressure of the sheath flow prevents DNA fragments from each capillary from mixing with each other. The laser irradiates the flow, not the capillary. The fluorescence is detected outside the capillaries, where the light scattering and reflection from the capillary walls are much less than that for direct on-capillary detection. The fluorescent emission from the DNA fragments from multiple capillaries can thus be detected with higher sensitivity.

Two-dimensional Detection Using CCD

The fluorescence signal obtained has two dimensions, one indicating the capillary position in the array, the other indicating the fluorescence wavelength. In combination with multi-sheath flow, this technology achieves simultaneous multi-capillary and multi-wavelength detection (see Fig. 4). As a total performance, high sensitivity detection has great merit to save reagents consumption.

System Advantages

The advantages of this system are summarized as follows.

(1) The ready-made gel capillary cassette reduces operation time by up to 3 hours.

(2) The sample tray, equipped with the electrode, provides automated sample loading. Multiple runs can be carried out without the presence of an operator.

(3) The combination of multi-sheath flow and two-dimensional detection provides highly sensitive detection for multiple capillaries.

CONCLUSIONS

In this report, the technologies developed for a multi-capillary DNA sequencer are described. These technologies provide high throughput for a fully automated DNA sequencer, and will contribute to large-scale DNA sequencing studies and fragment analyses, including genome research. In the near future, we believe the DNA sequencer will be routinely used in DNA analyses. For example, it may be used as a clinical routine analyzer, which would require total system integration, from sample preparation to reporting of analysis results. We will continue effort on DNA study to enhance toward future technology.

REFERENCES

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