

Development of DS3000 Capillary Electrophoresis DNA Sequencer Designed for Compact Size

Capillary electrophoresis DNA sequencers are used to determine the sequence of bases in DNA, the blueprint of life, as well as the length of DNA fragments. These sequencers are in widespread use around the world in medicine, healthcare, and criminal investigation including DNA profiling. To supply the market for compact models that is expected to grow strongly in the future, Hitachi High-Tech Corporation has developed the DS3000 sequencer that is easy to use and suitable for the small-lot-size testing of diverse samples. The new sequencer has been launched on the Japanese and Asian markets. This article describes the DS3000 sequencer and its features, which include the adoption of a direct polymer injection mechanism and reagent cartridges for rapid analysis when used for small-lot-size testing of diverse samples, space-efficiency achieved through use of a panel computer and laser diode, and a remote monitoring system that significantly improves usability.

Motohiro Yamazaki, P.E.Jp.

Shohei Arai, Ph.D.

Isao Haraura

Hirokazu Kato

Noriyuki Sumida, Ph.D.

1. Introduction

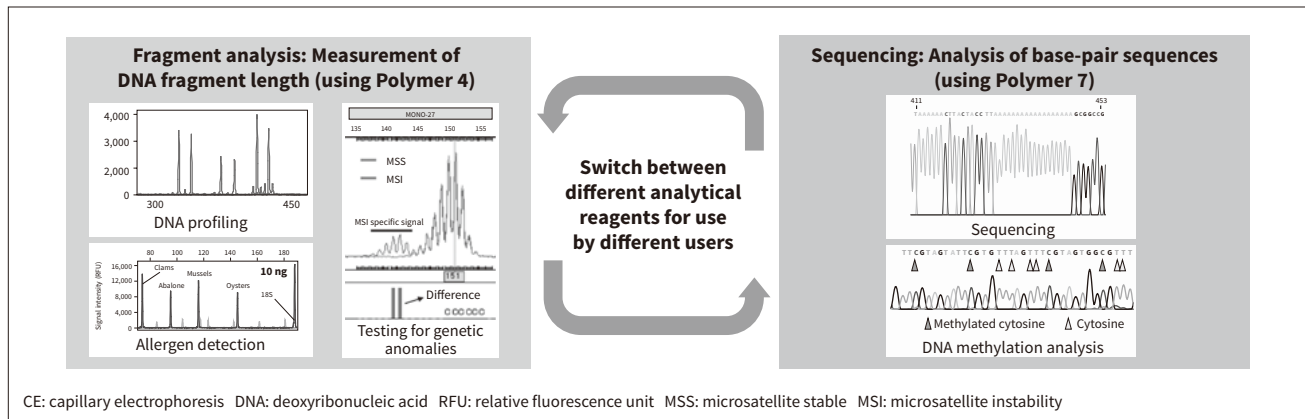
The foundations of today's genomic medicine were laid by the Human Genome Project of the early 2000s. The capillary electrophoresis (CE) deoxyribonucleic acid (DNA) sequencers (hereinafter "CE sequencer") developed by Hitachi made a significant contribution to this project by increasing the efficiency of the gold-standard Sanger sequencing method. The market for DNA sequencers grew considerably following the emergence from around 2005 of next-generation sequencers that worked by the massively parallel analysis of short DNA fragments, and a number of benchmark DNA databases (reference sequences) have since been established. Meanwhile, markets for specific applications in fields such as medicine and criminal investigation that involve the analysis of individual DNA differences have been expanding since about 2015.

CE sequencers are also used for forensic DNA profiling⁽¹⁾, where they take advantage of their ability to perform simple and low-cost analyses of particular sections of DNA. They also have an important role to play in healthcare such as in molecular diagnostics, where one application is the detection of microsatellite instabilities (MSIs)⁽²⁾, genetic abnormalities that can help identify which therapies to use on solid carcinomas such as colon cancer.

Strong growth is anticipated in the market for compact desktop sequencers with four or fewer capillaries. Working in collaboration with Promega Corporation, a company with expertise in the development and production of reagents, Hitachi High-Tech Corporation has developed a compact CE sequencer that is easy to use and suitable for small-lot-size testing of diverse samples. The companies have launched the new sequencer as the Hitachi-branded DS3000 in the Japanese and Asian markets, and under the Promega brand in the rest of the world. Promega operates a global sales and service network^{(3),(4)}.

Figure 1 — Use of Different Reagents (Polymers) for Different Types of Analysis

CE sequencers use different polymers to perform different types of analysis. A sequencer may have a number of users and have to alternate between performing fragment analysis (DNA profiling or testing for genetic anomalies) and DNA sequencing.



2. Market for Compact CE Sequencers

The demand for large high-throughput CE sequencers decreased significantly following completion of the Human Genome Project in 2003, with the emergence of next-generation sequencers also a factor. However, with CE sequencers finding widespread use in applications for medicine, healthcare, and criminal investigation such as DNA profiling at universities and other academic institutions or at government-run research institutes, there has been steady market demand for compact desktop sequencers intended for laboratory use in particular.

The compact sequencer market is characterized by the diversity of samples to be analyzed and the wide variety of applications, which include DNA sequencing and fragment analysis (measurement of the length or quantity of specific sections of DNA) (see **Figure 1**). This creates a need for instruments suitable for small-lot-size testing of diverse samples, with a user interface appropriate for non-experts and small enough to be installed in the confined space of a laboratory. Moreover, rather than contracting sequencing work out to a specialist provider or using a shared in-house laboratory, there is also demand from

customers who want to have their own machine in their own laboratory, perhaps because they require rapid results or have stringent requirements for results handling. While this led to the Applied Biosystems 310 Genetic Analyzer (a single-capillary sequencer) becoming widely used, the model was discontinued in 2011 leaving a need for an alternative compact sequencer.

To meet the needs of users in this market, Hitachi High-Tech launched a newly developed compact model with exceptional ease-of-use and the ability to switch between different reagents (polymers) quickly and easily for different analytical purposes.

3. New Sequencer and Technical Issues

For the DS3000, Hitachi High-Tech developed a direct polymer injection mechanism and new polymer cartridges to meet the needs of users wanting to conduct a variety of different measurements (see **Figure 2**). The new sequencer also featured an integrated panel computer for user operation and primary analysis, ensuring small size and keeping installation space requirements to a minimum. A remote monitoring function was also developed that works by connecting the sequencer to the user's network to enable

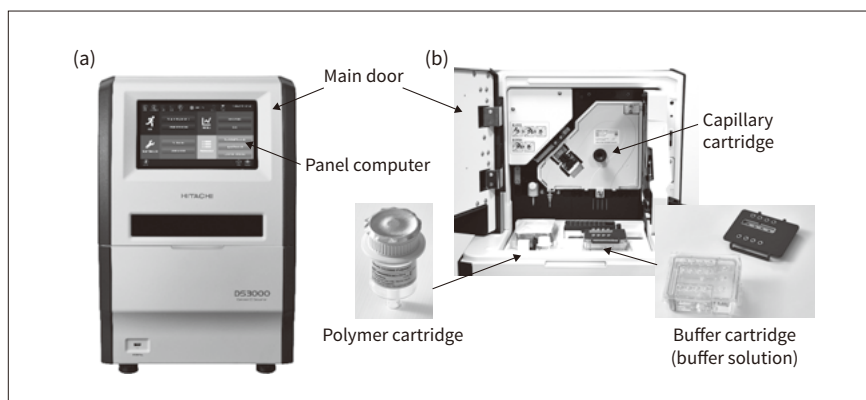


Figure 2 — DS3000 Sequencer and Consumables

The figure shows the sequencer unit (a) and an interior view with the main door open that also shows the consumables and where they are inserted (b). The panel computer that controls the sequencer is built into the front-side door. The consumable reagents are supplied in cartridges (disposable containers) and fitting the capillary array is also a simple task.

the collection of measurement data and the monitoring of instrument operation from a PC. This section describes these product features.

3.1

Suitability for Small-lot-size Testing of Diverse Samples

The main intended users of the sequencer are teams of researchers working in small laboratories engaged in things like clinical research or DNA profiling who share use of the instrument. What these users need, then, is an easy way to inject polymer into the capillaries as required for their particular purposes, whether it be DNA sequencing, fragment analysis, or something else.

Unfortunately, past models have required about 60 minutes to switch between polymers. The reason why this took so long was due to the need to replace the polymer in the flow channels and to bleed the pump block channels to eliminate bubbles.

To overcome this problem, Hitachi High-Tech developed a new polymer injection system that eliminates the pump block, instead using an actuator that connects the disposable polymer cartridge directly to the capillary (see **Figure 3**). The new system does not have to be bled to eliminate bubbles and makes it easy to switch to a different

polymer as needed by the application. Also, the syringe function that used to be part of the sequencer unit is now built into the polymer cartridge. As this was an area prone to being clogged by polymer crystallization, incorporating it into a consumable part eliminates the need for customer maintenance to clean out the polymer injection mechanism and associated parts.

3.2

Easier Operation and Remote Monitoring Function

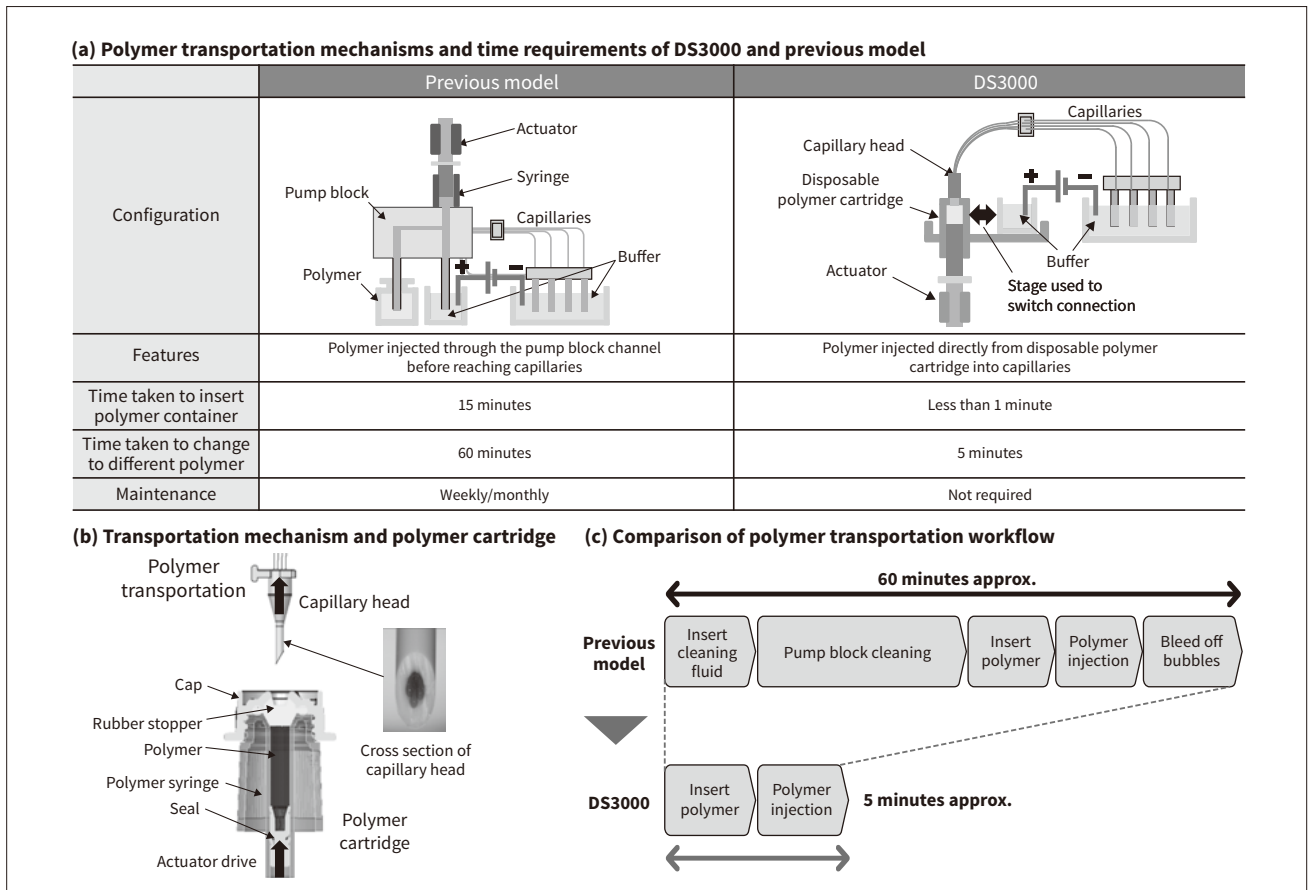
(1) Easier operation via user interface

The market for personal sequencers demands compact space-efficient design. Accordingly, the DS3000 has been equipped with a touch-screen panel computer that is built into the instrument. The concept was to provide an all-in-one design that combines all steps from electrophoresis to primary analysis in a single instrument.

Hitachi High-Tech also developed a wizard-based user interface to make the DS3000 easy to use for tasks such as DNA profiling by operators who are not experts in DNA sequencers. This enhances ease-of-use by having the system guide the user through the steps of an analysis, simplifying the processes from inserting the consumables and sample through to commencing measurement.

Figure 3 — Comparison of Polymer Transportation Mechanisms and Transportation Paths

The polymer transportation system for the DS3000 is made up of a polymer cartridge and an actuator. The polymer cartridge is a consumable item that also works as a syringe. The actuator pushes up on the bottom of the polymer cartridge to deliver polymer to all four capillaries at once. The new mechanism significantly reduces the time and effort required to change to a different polymer.



The sequencer can also be configured to meet the detailed requirements of expert users such as those at research institutions, including the ability to specify the electrophoresis, analysis, and other analysis conditions.

Assessing the quality of measurement data on the previous model required specialist expertise and procedures done using an external PC. On the new model, in contrast, it is a simple matter for even users who lack experience with DNA analysis to check the data quality as the calculations are performed automatically and the analysis results are output by software developed by Hitachi High-Tech for sequencing and for fragment analysis respectively.

(2) Enhanced convenience

In addition to standalone operation, the DS3000 can also connect to the user's local area network (LAN), with a Web server running on the panel computer providing browser access via the network. A remote user can use an external PC for preparatory tasks such as specifying analysis conditions or collating the sample list, and also to monitor sequencer operation. The only steps that have to be performed on the sequencer itself when initiating an analysis are insertion of the consumables and sample (see **Figure 4**).

3.3

Compact Design

The new sequencer takes up only about 60% as much space as the previous model. This was achieved by integrating the

panel computer used for instrument operation and sequencing of the measurement results and by miniaturizing the optics through the use of a laser diode light source (see **Figure 5**).

A new 505-nm semiconductor laser diode (LD) was adopted to reduce the size of the optics system. In contrast, the previous model used a diode-pumped solid-state (DPSS) light source. The DPSS was made up of the controller and a laser head unit to generate a continuous-wave from a laser crystal pumped by a laser diode as the excitation source. As a mechanical shutter was also used to block excitation light during charge-coupled device (CCD) data transmission, considerable space was required. On the new system, in contrast, the system size has been reduced by the laser beam pulsed from the LD, the single module combining laser head and controller, and the shutterless design.

4. Benefits

This section presents example uses of the sequencer to analyze a variety of different samples. As each of these analyses requires different polymers and sequencer settings, they provide a practical demonstration of the DS3000's features and its ability to handle different sample types (see **Table 1**). Use of the sequencer for DNA profiling⁽⁵⁾ and in medical research have also been reported elsewhere.

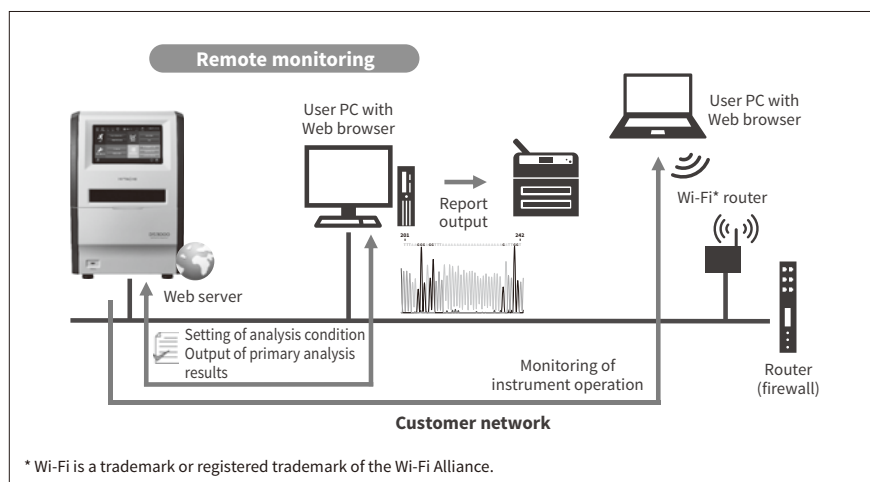


Figure 4 — Network Connection and Remote Monitoring

Accessibility is improved by functions for remote monitoring of the instrument during analyses, including real-time display of the electrophoretogram, and for downloading measurement results and reports from a browser.

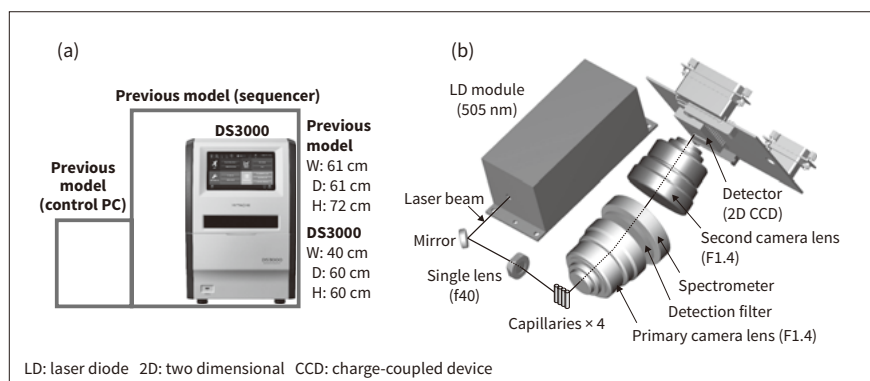


Figure 5 — Instrument Miniaturization

The image on the left (a) compares the size of the DS3000 to the previous model and that on the right (b) shows the miniaturized optics. The new optics and integration of a panel computer succeeded in reducing the size of the instrument and making more efficient use of space.

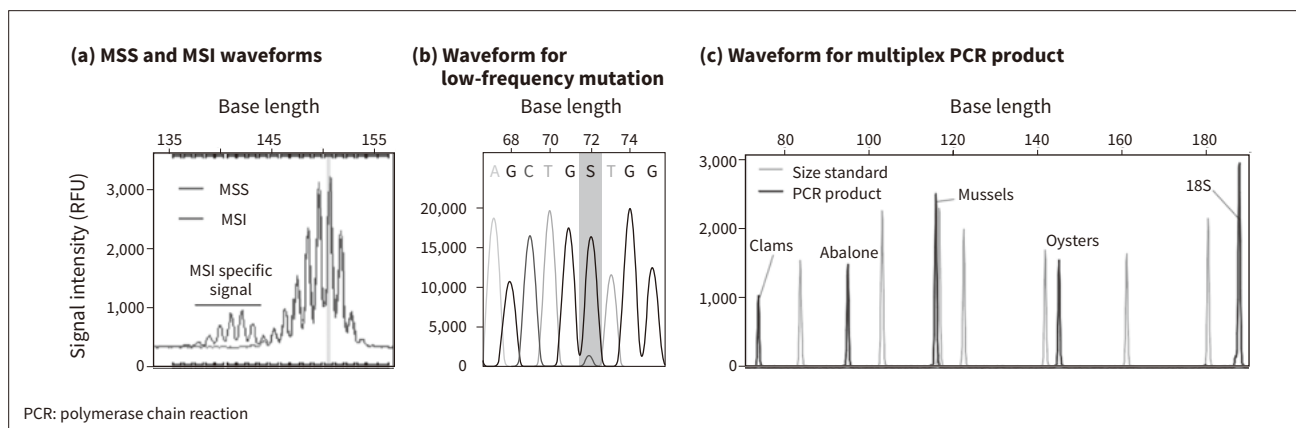
Table 1 — Instrument Settings for Different Types of Analysis

The DS3000 makes it easy to change the analytical reagents (polymers) and settings (assay and number of fluorescent dyes) shown in the table.

Type of Analysis	Polymer	Assay	Number of fluorescent dyes
MSI analysis	Polymer 7	Fragment analysis	4
Detection of low-frequency mutations	Polymer 7	Sequencing	4
Allergen detection	Polymer 4	Fragment analysis	5

Figure 6 — Analyses Conducted Using DS3000

The graph in (a) overlays the MSS and MSI waveforms. The peak waveform was plotted using GeneMarker v3.0.1 (from SoftGenetics, LLC). The graph in (b) shows the waveform for a KRAS mutation present at low frequency. Mixed base (S) indicates a guanine (G: peak in black) overlapping with cytosine (C: peak in grey) of which the frequency is 10%. The sensitivity of low-frequency mutation detection depends on the condition of the sample and the sequence contexts. The result presented here is an example obtained under restricted conditions. The graph in (c) shows the waveform for the multiplex PCR product obtained from 0.01 ng of shellfish genome DNA (using SoftGenetics GeneMarker HID v2.9.3).



4. 1

Medical Research Applications (1) Fragment Analysis

Genomes contain regions called microsatellites where a distinctive DNA sequence of between one to six base pairs is repeated several dozen times. Anomalies in these sections of DNA are called microsatellite instabilities (MSIs) and are known to be biomarkers predictive of defective mismatch repair proteins. As MSI analysis involves the detection and comparison of dispersed waveform peaks at the level of single base pairs, this is an application that demands a high level of sizing accuracy and replicability. This section describes an MSI analysis conducted on commercially available paraffin-embedded samples.

The samples in this case were a reference sample with a known MSI (MSI, HD830)¹ and a wild-type (non-mutated) sample [microsatellite stable (MSS) HD831]¹. The microsatellite region was amplified using Promega MSI Analysis System Ver. 1.2 and electrophoresis was performed using the resulting amplification product on Polymer 7.

Figure 6 (a) shows the results of analyzing the waveform data. This shows that the analysis has successfully detected all of the distinctive MSI peaks^{(6),(7)}.

4. 2

Medical Research Applications (2) Sequencing

Low-frequency mutations are mutations present in a sample derived from multiple cells that occur in only a subset of those cells. Such mutations are recognized for their utility in

cancer research as they can be a cause of the initiation and growth of tumors. As the signal from these low-frequency mutations is buried amid that of the wild-type DNA that makes up the bulk of the sample, their detection requires that sequencing have a high level of sensitivity and accuracy. This example describes the use of a DS3000 to detect low-frequency mutations in the KRAS proto-oncogene.

A genetic analysis reference sample was used for the demonstration¹. The sample contained DNA with a known number of mutations in specific sections, and these sections and frequencies were open to the public. The test sample containing low-frequency mutations was created by diluting this reference sample with wild-type DNA. Sequencing was then performed targeting the KRAS gene. The resulting waveform data was analyzed using Mutation Surveyor v5.1.2 (from SoftGenetics, LLC). Figure 6 (b) shows an example result in which a low-frequency mutation present in 10% of cells was successfully detected².

4. 3

Food Safety Application

Between 1 to 3% of adults and 4 to 6% of children suffer from food allergies, with severe reactions in certain cases that can be life-threatening. This makes allergen detection

¹ The reference and wild-type samples used in this analysis were both obtained from Horizon Discovery, Ltd.

² The sensitivity of detection for low-frequency mutations depends on factors such as sequence contexts and the condition of the sample. Results presented here are examples only and were obtained under restricted conditions.

and labeling an important food safety issue. This example describes a highly sensitive detection technique that uses a combination of multiplex polymerase chain reaction (PCR) and capillary electrophoresis to target the gene for tropomyosin, a known allergen widely present in shellfish⁽⁸⁾.

First, DNA was extracted from commercially purchased shellfish (clams, abalone, mussels, and oysters) and mixed in equal proportions. Next multiplex PCR was used to amplify the tropomyosin gene and 18S rDNA, the latter being used as a positive control. Electrophoresis was performed using Polymer 4 on the resulting product. The results are presented in **Figure 6** (c), showing a waveform peak that corresponds to a tiny 0.01 ng of shellfish DNA⁽⁹⁾.

5. Conclusions

This article has described Hitachi High-Tech's DS3000 compact CE sequencer, which is suitable for small-lot-size testing of diverse samples.

Along with seeking to identify customer issues and to foster and expand the market for CE sequencer applications that take advantage of advanced technological developments, Hitachi also intends to contribute to the creation of a safe and healthy society through the operation of its digital solutions business that integrates data information from such diverse sources as genomics, medicine, health, and the environment.

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Note: The DS3000 sequencer is marketed as a research device. If used for evidentiary purposes, the equipment operation and results interpretation must be done by a qualified expert with specialist knowledge.

Authors



Motohiro Yamazaki, P.E. Jp.

Bio Systems Design 1st Department, Life & Medical Systems Product Division, Hitachi High-Tech Corporation. *Current work and research:* Design and development of capillary DNA sequencer, and its production design. *Society memberships:* The Japan Society of Mechanical Engineers (JSME) and Hitachi Professional Engineers Association.



Shohei Arai, Ph.D.

Bio Systems Sales Department 2, Life & Medical Systems Business Division, Hitachi High-Tech Corporation. *Current work and research:* Domestic sales and marketing of biotechnology products.



Isao Haraura

Bio Systems Design 1st Department, Life & Medical Systems Product Division, Hitachi High-Tech Corporation. *Current work and research:* Development of capillary DNA sequencer and analysis software.



Hirokazu Kato

Bio Systems Design 1st Department, Life & Medical Systems Product Division, Hitachi High-Tech Corporation. *Current work and research:* Development of capillary DNA sequencer and its applications.



Noriyuki Sumida, Ph.D.

Bio Systems Design 1st Department, Life & Medical Systems Product Division, Hitachi High-Tech Corporation. *Current work and research:* Development of capillary DNA sequencer and its applications.