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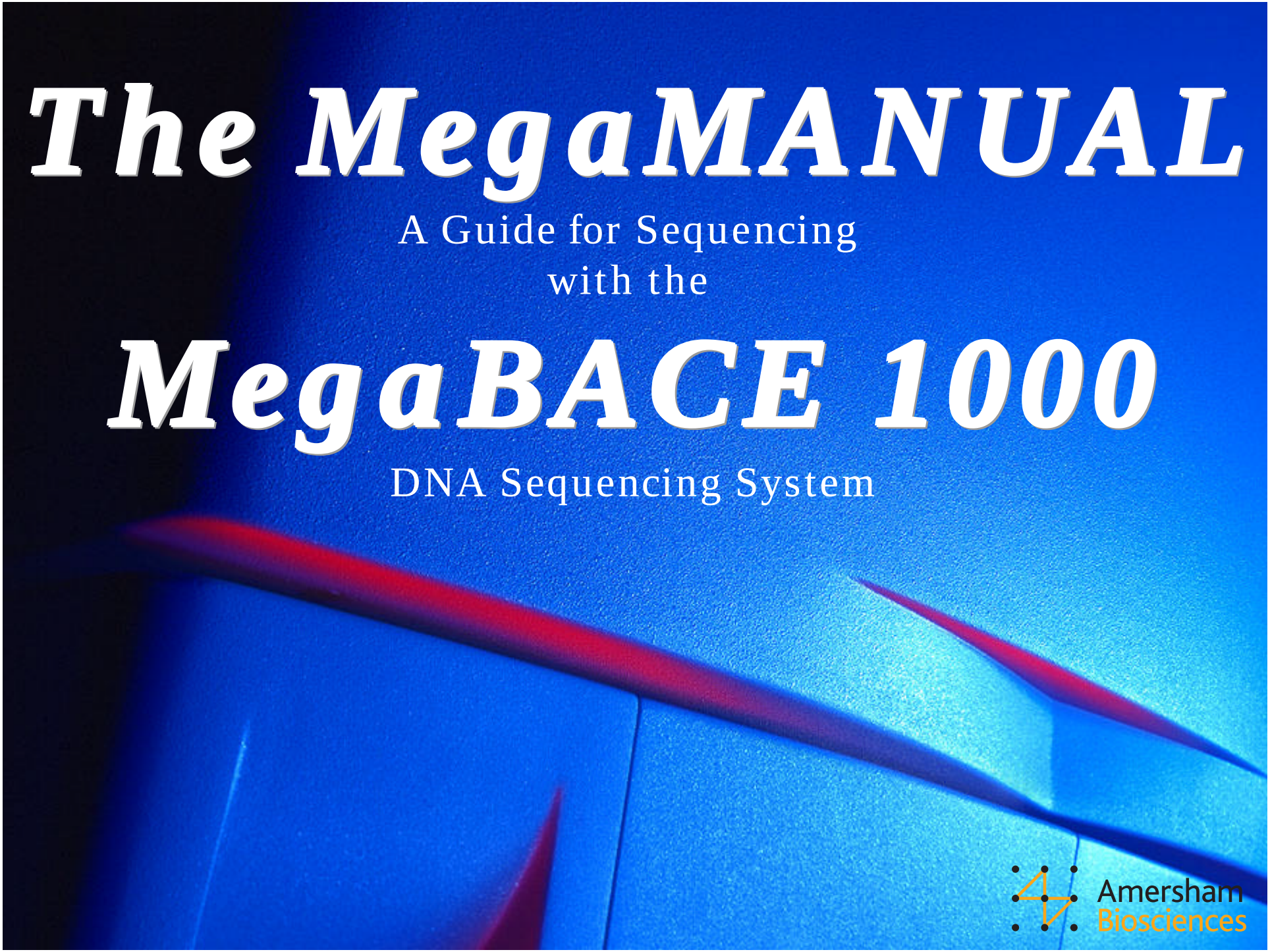
Find the *GE Healthcare / Amersham Biosciences MegaBACE 1000* at our website: ***Click HERE***

The MegaMANUAL

A Guide for Sequencing
with the

MegaBACE 1000

DNA Sequencing System



Amersham Biosciences

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Preface

About This Guide

TheMegaMANUAL is divided into two parts. Chapter 1 is an introduction to the MegaBACE™ 1000 DNA Sequencing System and includes a general overview of the integrated system components, which are the instrument, the reagents, and the software. This chapter provides general system background for people who are unfamiliar with the MegaBACE system.

Chapters 2 through 6 are technical and are designed to be used by people implementing the MegaBACE 1000 system for DNA sequencing. Most of the information in Chapters 2, 3, and 4 was used during system development to determine the parameters for the finished protocols. Chapter 5 provides extensive troubleshooting material. Chapter 6 provides a description of the optical system in the MegaBACE 1000. Throughout the MegaMANUAL, example chromatographs have been included as a visual reference against which to compare actual results.

The MegaMANUAL is a living, working document. Undoubedly not all circumstances encountered in production sequencing will be described. The APB sequencing team would like to receive your comments on this manual so that the manual can continue to evolve as a valuable tool for MegaBACE implementation and use.

How to Use the Guide

The MegaMANUAL should be used as a reference and a troubleshooting guide. If you have a specific technical question or problem that is not addressed in the MegaMANUAL, contact MegaBACE Technical Support.

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Chapter 1

The MegaBACE 1000 DNA Sequencing System

The MegaBACE™ 1000 DNA Sequencing System represents a milestone in automation. Designed for genetic analyses in production environments (**Figure 1.1**), the MegaBACE 1000 DNA Sequencing System is a high-throughput, fluorescence-based DNA sequencer that uses capillary electrophoresis. With the ability to sequence 96 samples in just 2 hours, the MegaBACE 1000 is the ideal choice for production-scale sequencing projects.

The MegaBACE 1000 DNA Sequencing System features:

- Capillary electrophoresis with automated gel matrix replacement, sample injection, DNA separation, and base calling. Unlike slab-gel systems, the automation of gel replacement eliminates the need to pour gels, wash glass plates, and re-track samples after electrophoresis.
- Electrokinetic sample injection automatically loads samples from a 96-well plate into the capillaries. Automated sample injection eliminates manual sample loading and sample mix up.
- Energy transfer (ET) dye chemistry kits are the most robust and sensitive kits available. DYEnamic™ ET primers and DYEnamic ET terminator kits are formulated with Thermo Sequenase™ DNA polymerase and Thermo Sequenase II DNA polymerase, respectively, and are optimized for use with the MegaBACE.
- The MegaBACE 1000 uses linear polyacrylamide (LPA) separation matrix. LPA is an innovation in capillary electrophoresis sieving matrices that enables read lengths in excess of 800 bases. With long read lengths; MegaBACE 1000 is ideal for finishing.
- Flexible, user-friendly analysis software produces accurate base-calling data in a PHRED-compatible file format.

With the capacity for long read lengths, as well as short run times, the MegaBACE 1000 DNA Sequencing System can be used for both production and finishing making it the most cost-effective, automated, yet versatile system available.

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Figure 1.1. The MegaBACE 1000 DNA Sequencing System automates gel matrix replacement, sample injection, electrophoresis, and data analysis. Sequences of over 800 bases can be obtained in less than 3 hours.

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Accelerated Genomic Research

The MegaBACE 1000 DNA Sequencing System delivers high-throughput sequencing. With a total turnaround time of less than 2 hours per run, it is possible to sequence more than 500 samples per 12-hour workday. During a 2-hour run, read lengths routinely exceed 600 base pairs per capillary, enabling one instrument to produce 300 kilobase pairs of unedited sequence per day. Superior read lengths and high productivity will accelerate your genomic research by reducing the number of subclones and sequencing passes required to complete your project.

Total System Automation

By automating capillary electrophoresis, including sample loading and gel matrix replenishment, the MegaBACE 1000 offers convenience and gains in productivity that cannot be matched by slab-gel-based sequencing systems. By eliminating the manual steps associated with slab gels, the MegaBACE 1000 ultimately improves the consistency and reliability of the results.

The high degree of automation and minimal hands-on time (less than 15 minutes per run) enables a single operator to manage an entire suite of MegaBACE systems (**Figure 1.2**).

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Figure 1.2. The high degree of system automation incorporated into the MegaBACE 1000 minimizes the hands-on time required to load the buffer and sample cassettes to less than 15 minutes per run.

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Innovative Instrumentation

The MegaBACE 1000 DNA Sequencing System combines innovation with convenience and reliability. This system features durable, capillary arrays, a highly sensitive detection method, and a user-friendly platform designed to fit into every type of high-throughput DNA sequencing facility.

Capillary Array Electrophoresis

Capillary array electrophoresis uses narrow-bore capillaries filled with a separation matrix to resolve DNA sequencing fragments (**Figure 1.3 and Figure 1.4**). An electric field is used to cause the DNA fragments to migrate into (electrokinetic injection) and through (electrophoresis) the capillaries. As in slab-gel electrophoresis, the DNA fragments are separated by size, with the shorter fragments moving faster (peaks earlier in the electropherogram) than the longer fragments (peaks later in the electropherogram). The large surface-to-volume ratio of the capillary efficiently removes the heat generated during electrophoresis. With less heat, electrophoresis can be performed at a higher voltage, which produces more rapid fragment migration and shorter separation times.

The MegaBACE system uses 6 arrays of 16 capillaries that provide rapid parallel separation of dye-labelled DNA fragments on a total of 96 samples. The capillaries are filled with a replaceable, non-cross-linked linear polyacrylamide (LPA) matrix. The instrument applies a variable time/variable voltage pulse to electrokinetically inject all 96 samples from a microtitre plate simultaneously. Each capillary has a clear detection window located at a fixed distance from the sample loading point through which the samples are scanned (**Figure 1.5**).

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Pre-assembled capillary array

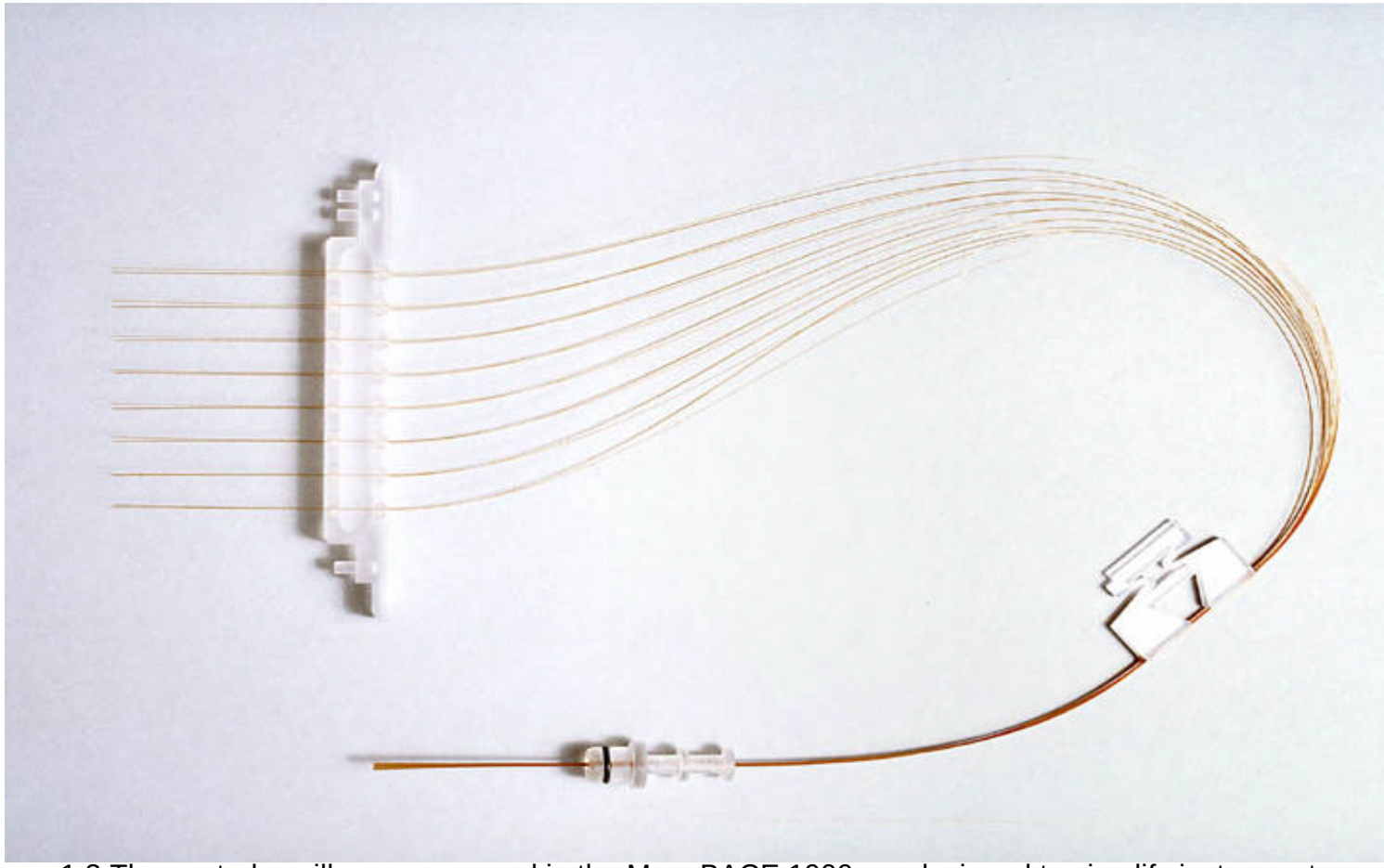


Figure 1.3 The coated capillary arrays used in the MegaBACE 1000 are designed to simplify instrument operation by providing long life times and easy replacement. When used in conjunction with the linear polyacrylamide long-read gel matrix, these capillaries deliver long read lengths and short run times. DNA sequence readings of 550 bases or more can be generated in just over 2 hours.

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Pre-assembled capillary array

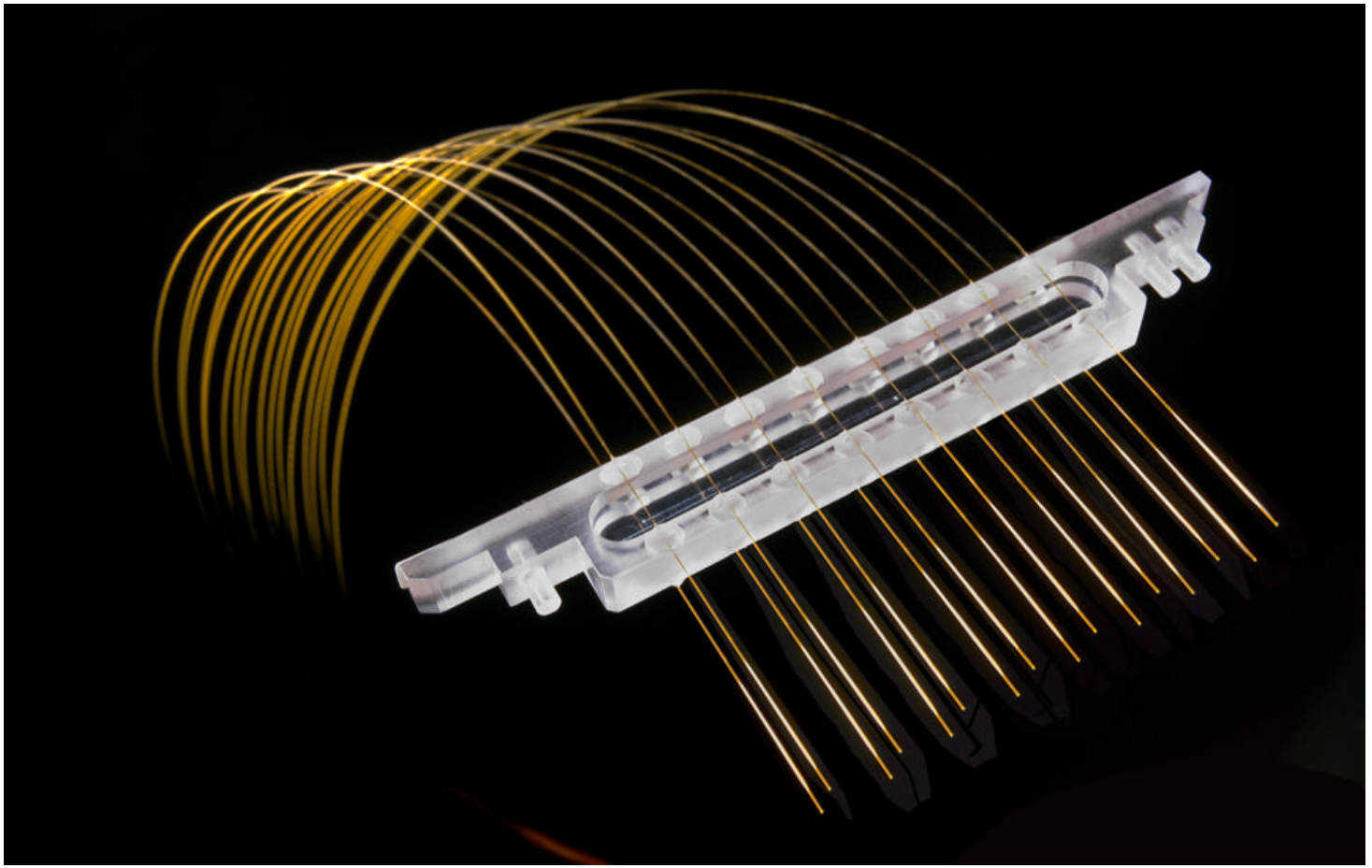


Figure 1.4 The coated capillary arrays used in the MegaBACE 1000 are designed to simplify instrument operation by providing long life times and easy replacement. When used in conjunction with the linear polyacrylamide long-read gel matrix, these capillaries deliver long read lengths and short run times. DNA sequence readings of 550 bases or more can be generated in just over 2 hours.

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Inside the MegaBACE 1000

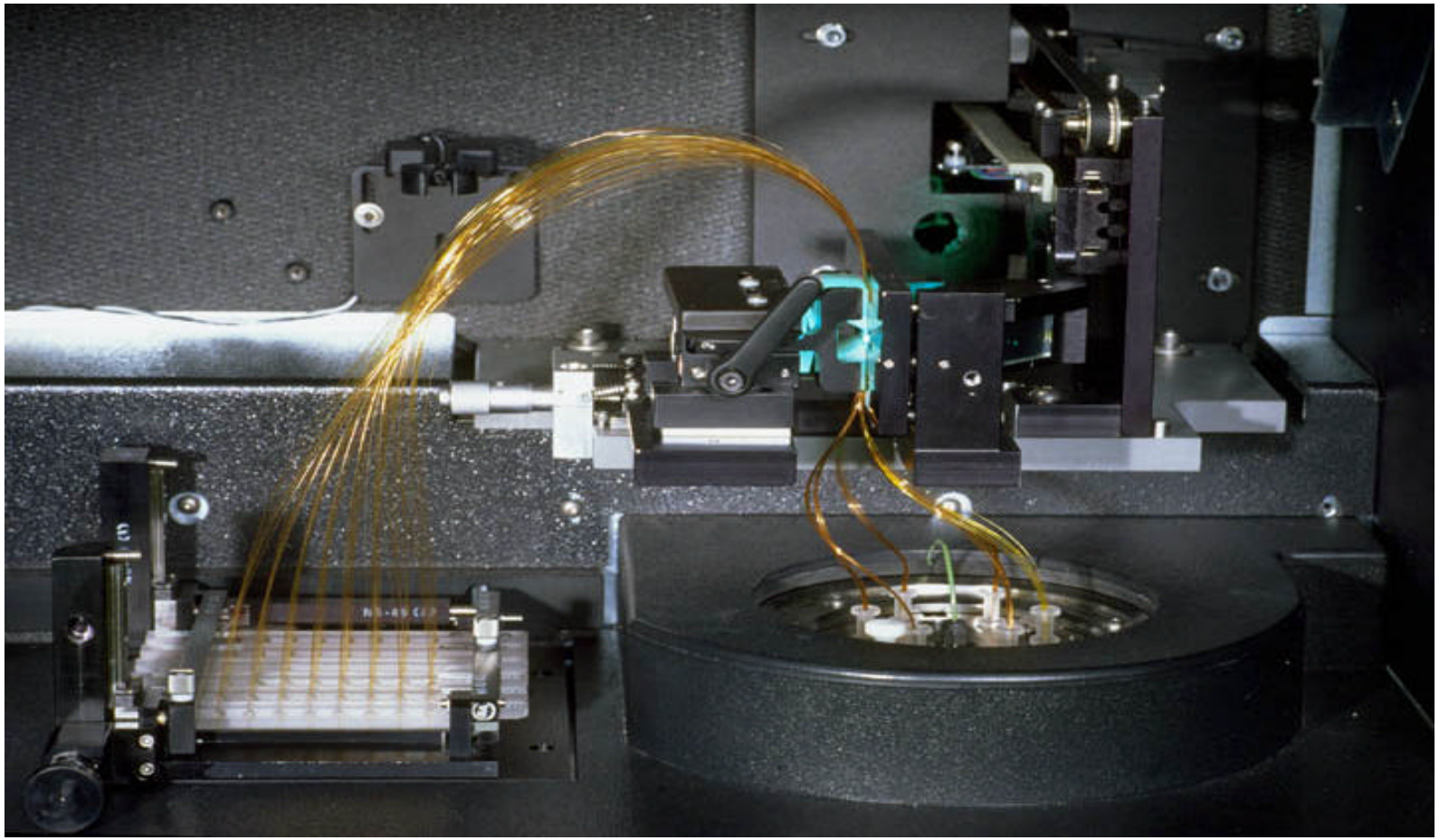


Figure 1.5. View of the MegaBACE 1000 system electrophoresis chamber.

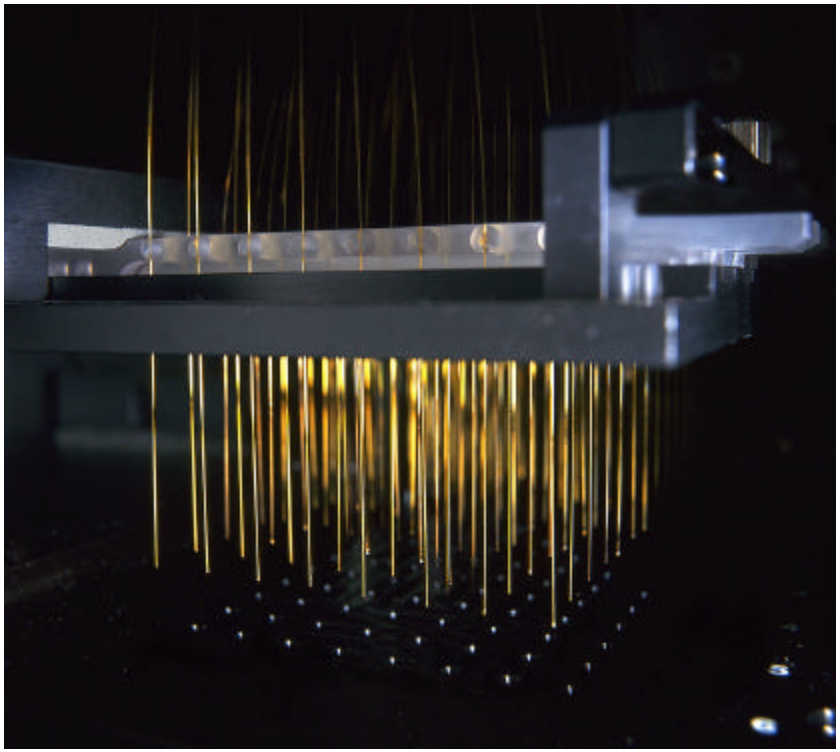
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Confocal Scanning for Sensitive, Accurate Detection

During electrophoresis the instrument uses laser excitation and a patented confocal optical detection system to both excite and detect the dye-labelled DNA fragments as they migrate past the detection window (**Figure 1.6**). The key to highly accurate and sensitive detection of the DNA sequencing products is the MegaBACE 1000's use of confocal laser scanning to focus on each sequencing product within the capillary. This technology optimizes dye excitation and fluorescence detection and eliminates light diffraction and scattering. Confocal scanning enables the production of data beyond 800 bases (in a 3-hour run) with very sensitive detection capabilities (1-8).

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Inside the MegaBACE 1000



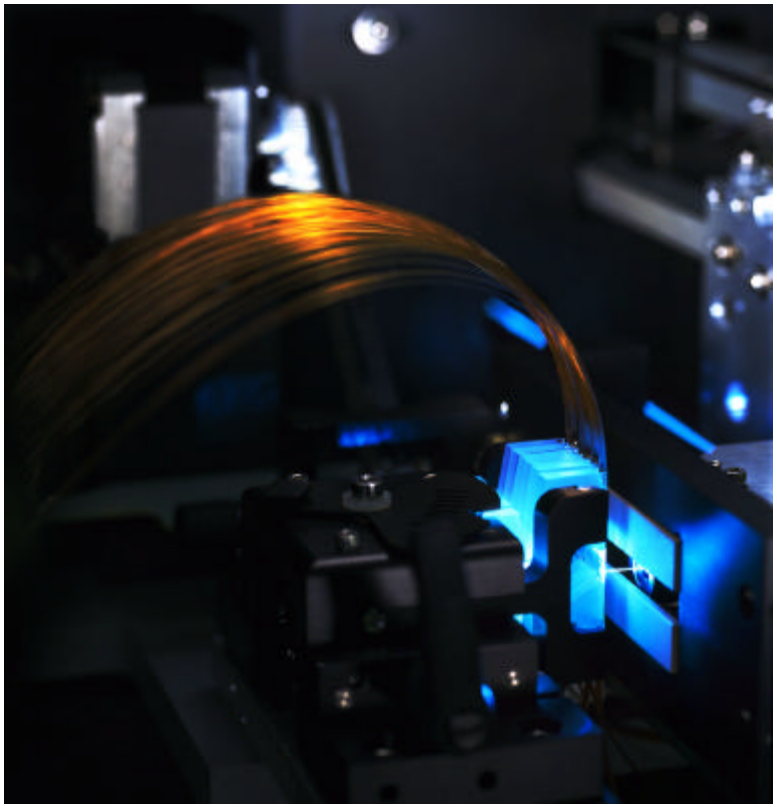


Figure 1.6 The MegaBACE 1000 uses confocal optics to scan back and forth across the capillary array read windows. During scanning, excitation and emission light is focused into the gel plane, enabling data collection with high sensitivity.

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Sequencing Reagents

With advancements such as Thermo Sequenase II DNA Polymerase and DYEnamic ET terminators, Amersham Biosciences continues to be an innovator in DNA sequencing enzymology and fluorescent dye chemistry. These innovations are brought together in DYEnamic ET terminator and dye primer kits for the MegaBACE 1000 Sequencing System. All MegaBACE DNA sequencing reagent kits offer unparalleled performance on a broad range of templates.

DYEnamic ET Terminator Kit for the MegaBACE

The DYEnamic ET terminator kit is an integral part of the MegaBACE 1000 DNA Sequencing System. This product exploits the benefits of the instrument, the superior properties of the LPA long-read matrix, the sensitivity of the DYEnamic ET terminators, and the robust performance of the Thermo Sequenase II DNA polymerase. The kit is designed to provide the high-throughput DNA sequencing laboratory with industry-leading data quality in a robust and flexible dye-terminator format.

To run a sequence with this kit, a reaction premix is combined with a laboratory's template DNA and primer. The single reaction mixture is thermally cycled. After cycling, the reaction products are precipitated with salt and ethanol, or they are passed through a gel-filtration resin and concentrated to remove the unincorporated dye-labelled terminators. The reaction products are resuspended in a formamide loading buffer, and then separated and detected on the MegaBACE 1000.

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DYEnamic ET Terminators

The dye terminators feature novel dye-labelled dideoxynucleotides and a new DNA polymerase. Each dideoxy terminator is labelled with two dyes. One of these dyes, fluorescein, has a large extinction coefficient at the wavelength (488 nm) of the argon-ion laser in the instrument. The fluorescein donor dye absorbs light energy from incident laser light and transfers the collected energy using radiationless energy transfer to an “acceptor” dye. Each of the four chain terminators, ddG, ddA, ddT, and ddC, has a different acceptor dye coupled with the fluorescein donor. The acceptor dyes then emit light at their characteristic wavelengths. The fluorescence is detected by the instrument, which allows the nucleotide that caused the termination event to be identified. Using energy transfer provides a more efficient excitation of the acceptor dyes than does using direct excitation by the laser, and results in a sequencing method that is very sensitive and robust.

The acceptor dyes are the same standard rhodamine dyes used in DYEnamic ET primers: rhodamine 110, rhodamine-6-G, tetramethyl rhodamine, and rhodamine X. By using the standard rhodamine dyes as acceptors, the reaction products can be detected using the same filter set as the DYEnamic ET primers.

Thermo Sequenase II DNA Polymerase

A major innovation of the DYEnamic ET terminator kit for the MegaBACE system is the introduction of a new enzyme specifically engineered for DNA sequencing. Thermo Sequenase II DNA polymerase is the latest enzyme from the Amersham Biosciences family of DNA sequencing enzymes. This enzyme has biochemical properties that make it a more efficient and robust sequencing enzyme. These properties include:

- tolerance to high salt conditions
- efficient utilization of dITP
- high processivity
- excellent performance on GC-rich templates

Amersham has used a recent discovery by Tabor and Richardson (10) to construct this exonuclease-free thermostable DNA polymerase. Like T7 Sequenase™ DNA polymerase (11, 12), Thermo Sequenase generates bands of uniform intensity that improve the data interpretation ability of the automated sequencing software. Thermo Sequenase II combines the thermal stability needed for cycle sequencing with accuracy comparable to that of T7 Sequenase.

The Thermo Sequenase II DNA polymerase formulation contains thermostable inorganic pyrophosphatase (TAP) cloned from the thermophile *Thermoplasma acidophilum*. TAP hydrolyzes the inorganic pyrophosphate product of nucleotide polymerization, which prevents pyrophosphorolysis, the reversal of polymerization, from occurring. Pyrophosphorolysis can result in sequence data with missing peaks.

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DYEnamic ET Primer Reagents for the MegaBACE System

The DYEnamic ET primer reagents are an integral part of the MegaBACE 1000 Sequencing System. These reagents exploit the benefits of the instrument, the superior properties of the LPA long-read matrix, the sensitivity of DYEnamic ET primers, and the robust performance of the Thermo Sequenase DNA polymerase. The reagents are designed to provide the high-throughput DNA sequencing laboratory with long read lengths, high pass rates, and industry-leading data quality.

These reagents are combined with the template DNA and the dye-labelled primers. The four termination reaction mixtures are thermally cycled, and the reaction products are precipitated with salt and ethanol. The reaction products are resuspended in a formamide loading solution, and then separated and detected on a MegaBACE 1000 sequencing instrument.

Energy Transfer Dye Primers

Energy transfer (ET) dye primers are oligonucleotide DNA sequencing primers that have two fluorescent dyes attached for improved detection properties (9). One dye, called the donor dye, was chosen because it efficiently absorbs light of the wavelength of the argon-ion laser. This dye transfers absorbed light energy to the second dye attached to the same primer molecule. This acceptor dye emits the absorbed energy as fluorescence at its normal emission wavelength for efficient detection by fluorescent sequencing instruments. Because the primers all absorb the laser light efficiently, the effective fluorescence intensities of the ET primers are 2 to 12 times greater than primers having single-dye labels. Depending on the application, the greater signal strength produces a savings in labor, time, or template.

The DYEnamic ET primers for the MegaBACE system are 5-carboxy-fluorescein (FAM) as the donor dye. The acceptor dyes are rhodamine 110 (R110) (C), 6-carboxyrhodamine (REG) (A), N,N,N’,N’,-tetramethyl-5-carboxyrhodamine (TAMRA) (G) and 5-carboxy-X-rhodamine (ROX) (T).

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Thermo Sequenase DNA Polymerase

Thermo Sequenase is a thermal stable DNA polymerase specifically engineered for DNA sequencing. Amersham Biosciences has used a recent discovery by Tabor and Richardson (10) to construct this exonuclease-free thermostable DNA polymerase. Like T7 Sequenase DNA polymerase (11, 12), Thermo Sequenase generates bands of uniform intensity that improve the data interpretation ability of the automated sequencing software. Thermo Sequenase combines the thermal stability needed for cycle sequencing with accuracy comparable to that of T7 Sequenase.

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LPA Long Read Matrix

MegaBACE 1000 long read matrix, with linear polyacrylamide (LPA), produces long read lengths with high accuracy. The LPA matrix produces average read lengths in excess of 500 base pairs with PHRED accuracy scores of greater than 20. In fact, when used in conjunction with a slower, 3-hour run time, read lengths of greater than 800 base pairs have been consistently achieved.

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The MegaBACE Software

The MegaBACE 1000 DNA Sequencing System features user-friendly software for instrument control and data analysis. The Instrument Control Manager software and the Sequence Analyzer software have been designed with the production DNA sequencing facility in mind.

The Instrument Control Manager Software

The Instrument Control Manager (ICM) software has an intuitive and flexible graphical user interface that provides total control of the MegaBACE 1000 DNA Sequencing System. The ICM software—

- Controls and optimizes all the run parameters, including sample injection, electrophoresis, instrument temperature, gel matrix replacement, and optics configuration
- Edits the run parameters and sample sheet information
- Saves multiple run conditions
- Accesses the saved runs easily using a pull-down menu
- Displays real-time run data for quick and easy instrument monitoring (**Figure 1.7**)

The Sequence Analyzer Software

The Sequence Analyzer software provides versatility and the power to handle genomic data. The software can be used to—

- Analyse data using features in the Sequence Analyzer software, or export the data to ABD file format for analysis by the PHRED software
- Retrieve data from as many as 20 runs and analyse up to 1 920 samples at a time
- Evaluate the quality of the base calling

Base calling is performed using the UTAH base-caller component from Cimarron Software Inc. After base calling, Sequence Analyzer calculates a quality index for the entire sequence that is displayed above each base as a curve within the electropherogram. The quality profile is a valuable diagnostic tool, eliminating the guesswork during editing.

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MegaBACE Run Image

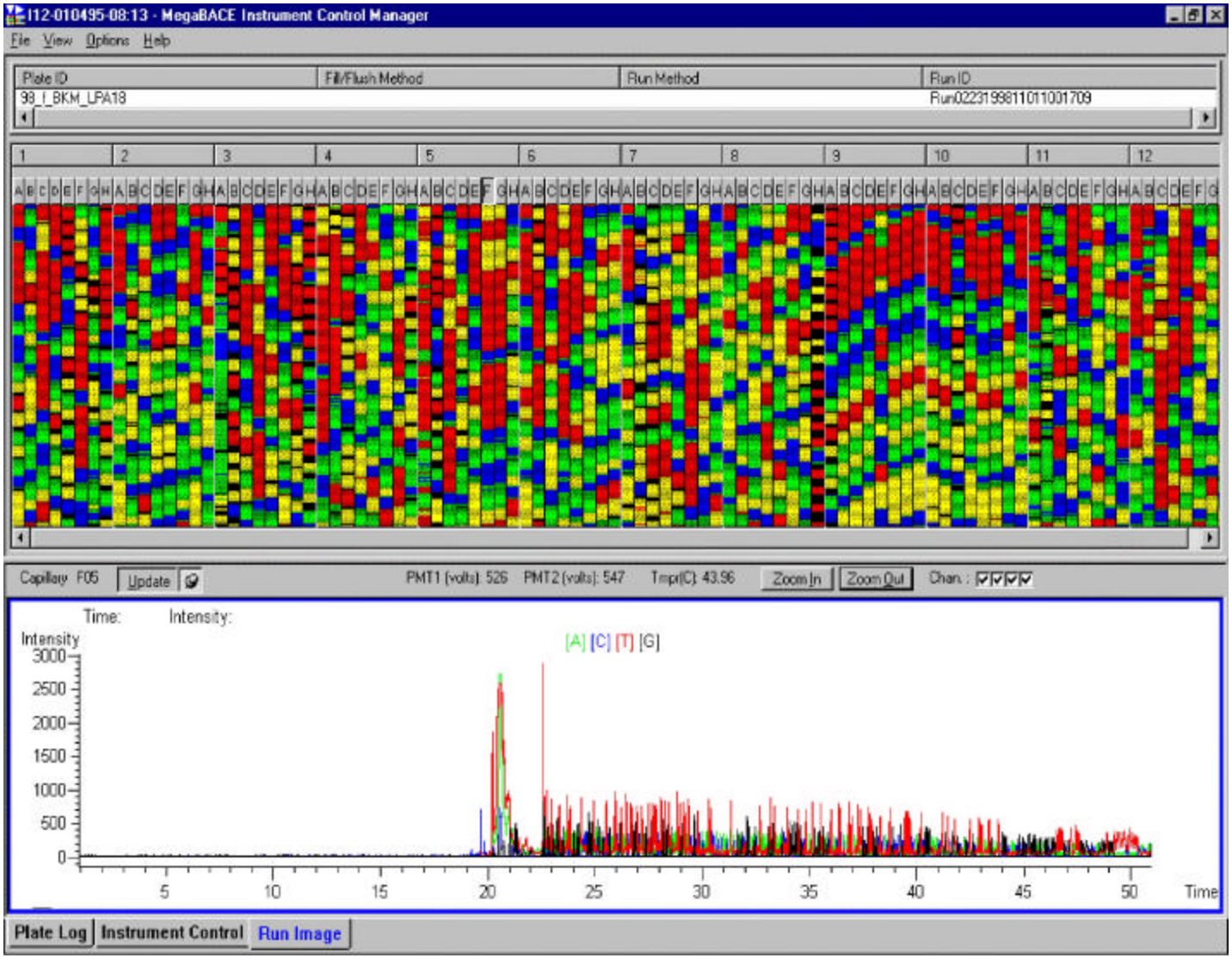


Figure 1.7. The MegaBACE Run Image as viewed during electrophoresis. All 96 capillaries can be viewed simultaneously in real time as a colour image, and the raw data can be viewed by selecting an individual capillary.

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