

MegaBACE DNA Analysis System

Enabling technology for DNA sequencing and genotyping laboratories

product code

63-0034-24 MegaBACE 1000 Sequencing System

product code

63-0034-25 MegaBACE 1000 Genotyping System

product code

63-0034-30 MegaBACE 500 Sequencing System

product code

63-0034-31 MegaBACE 500 Genotyping System

MegaBACE™ DNA Analysis System is a fluorescence-based system utilizing capillary electrophoresis (Fig 1). The system performs gel matrix replacement, sample injection, DNA separation, detection, and data analysis for DNA sequencing, microsatellite genotyping, and single nucleotide polymorphism (SNP) analysis.

For throughput flexibility, MegaBACE 1000 can be configured to use 16, 32, 48, 64, 80, or 96 capillaries, MegaBACE 500 can be configured to use 16, 32, or 48 capillaries. If sample throughput requirements increase, MegaBACE 500 can be field-upgraded to 96 capillaries for significantly less than the cost of a new instrument. The total turnaround time per sequencing run is less than 2 h from injection to injection.

	MegaBACE 500 8-h day	MegaBACE 1000 8-h day	MegaBACE 1000 24-h day
Sequencing	250+ samples	500+ samples	1100+ samples
Microsatellites	5760 genotypes	11 520 genotypes	21 120 genotypes
SNPs	1728 genotypes	3456 genotypes	6336 genotypes

Table 1. Throughput flexibility of MegaBACE 500 using 48 capillaries and MegaBACE 1000 using 96 capillaries. For genotypes, assumes 20-fold pooling per well and six injections per run.

Components

- Capillary electrophoresis instrument
- Capillary arrays
- Separation matrix for DNA analysis
- Reagents for DNA sequencing and genotyping
- MegaBACE Instrument Control Manager and data analysis software
- Computer workstation and monitor for instrument operation and data analysis



Fig 1. MegaBACE DNA Analysis System.

Capillary arrays

The capillaries are supplied in pre-assembled arrays of 16 capillaries. Arrays can be added or removed to accommodate throughput. Capillary lifetime is influenced by the type of samples used. Cleaner sample preparation methods result in longer array lifetimes.

- 200 µm o.d.
- 75 µm i.d.
- 40 cm detection length

Separation matrix

MegaBACE Long Read Matrix with linear polyacrylamide (LPA) produces long read lengths with tremendous accuracy. LPA matrix enables average read lengths of ≥ 500 bases with PHRED accuracy scores of ≥ 20 , and with a slower, 3-h run time, sequences of up to 1000 bases is possible. In genotyping applications, MegaBACE Long Read Matrix is optimized for resolutions of: 0.3 bp using MegaBACE ET400-R Size Standards; 0.5 bp using MegaBACE ET550-R Size Standards; and 2 bp using MegaBACE ET900-R Size Standards.

The gel matrix is replaceable, and each pack includes 96 pre-dispensed tubes plus running buffer.

Reagents

The following reagents are available for use with MegaBACE DNA Sequencing Systems:

- TempliPhi™ DNA Amplification Kit
- DYEnamic™ ET Terminator Kits with Thermo Sequenase™ II
- DYEnamic ET Primers
- Thermo Sequenase DNA Polymerase

Three size standards labelled with patented energy transfer dye technology for precision sizing of amplified fragments are available for MegaBACE DNA Genotyping Systems:

- MegaBACE ET400-R for sizing 60–400 bases
- MegaBACE ET550-R for sizing 60–550 bases
- MegaBACE ET900-R for sizing 60–900 bases

For MegaBACE SNP validation applications, the following reagents are available:

- MegaBACE SNUpe™ Genotyping Kit
- MegaBACE SNUpe Multiple Injection Marker
- MegaBACE SNUpe Control Kit

MegaBACE Instrument Control Manager

MegaBACE Instrument Control Manager (ICM) features an intuitive, graphical user interface that allows easy, manual input of run conditions at the beginning of each run. The ICM also recognizes input from a controlling database, such as a database integral to a laboratory information management system (LIMS), allowing complete automation of system setup. The ICM automates workflow with these features:

- Interfaces with controlling databases for complete setup, including naming samples and entering run parameters
- Integrates with MegaBACE Sequence Analyzer to automatically base call and export to third-party formats after completion of run, decreasing hands-on time
- Shuts down the instrument after the last run of the day, allowing the last run to be unattended

- Reads bar codes on sample plates
- Offers autofocus capability

MegaBACE Sequence Analyzer

MegaBACE Sequence Analyzer features a graphical user interface that is divided into three tabbed windows. The electropherogram window can display from one to five traces at a time, making it easy to compare data from several different runs. Both raw and processed data from the same sample can be displayed simultaneously for comparison.

With Molecular Dynamics base caller, users can sequentially process a trace file as well as stack and align output from background-subtracted, spectrally-separated, normalized, and base-called data.

This powerful software enable efficient optimization of any desired sequencing application. With MegaBACE Sequence Analyzer, users can:

- Process large amounts of data without manual intervention
- Batch print chromatograms
- Output processed data to SCF, ABD, FASTA, and ESD (MegaBACE format)
- Install up to 10 different base callers for easy future upgrades
- Validate output from Cimarron Slim Phredify base callers with PHRED—the industry standard in base calling quality assessment
- Identify heterozygotes at a user-defined threshold

MegaBACE Genetic Profiler

MegaBACE Genetic Profiler is used for fragment sizing and allele calling. This software can also be used to analyze data from haploid, diploid, or polyploid genomes. Users can define multinucleotide repeats for analysis.

- Features a user-friendly interface for editing, importing, and entering marker data
- Is ideal for polyploid analysis with any number of allele calls per marker
- Includes a differential display module and peak list comparison tool
- Offers more analysis control and flexibility for handling various repeat patterns for microsatellite markers
- Saves time with flexible and intuitive report generator

MegaBACE SNP Profiler

MegaBACE SNP Profiler is used for detecting SNP assays in the multiple injection protocol followed by allele calling. The workflow used for analysis is as follows:

- Create a project—define SNP marker profiles

- Add runs to the project
- Perform genotyping—process traces, identify injections, call alleles, assign quality scores to genotypes
- Display project results—allele histogram, table, and traces
- Present summary reports

Computer requirements

Each MegaBACE DNA Analysis System includes a workstation. To add additional workstations, the following are minimal computer requirements:

Hardware: Pentium™ 4, 2.4 GHz

Operating system: Windows™ 2000 SP2

Installed RAM: 512 MB

Specifications

Sequencing read length

Average read length with dye primer chemistry is 500 bases at 98.5% accuracy with M13 standard template.

Genotyping sizing and resolution

MegaBACE Long Read Matrix (LPA) has 0.3 bp resolution up to 400 nucleotides, 0.5 bp resolution up to 550 nucleotides, and 2 bp resolution up to 900 nucleotides. LPA sizing precision is a standard deviation of < 0.005 nucleotides up to 600 nucleotides.

Sample requirement

Minimal sample loading volume of 5 µl. The samples are introduced to the system in a 96-well format. Samples can be reinjected up to 10 times.

Excitation

- 488 nm for sequencing
- 488 and 532 nm for microsatellite genotyping

Detection

- Four channels with two PMTs
- Confocal optics with multiple filter sets
- Digital resolution is 16 bits linear to ≤ 5%

Electrophoresis voltage: Up to 320 V/cm

Chamber operating temperature: 27 °C to 44 °C

Environmental requirements

Temperature: 20 °C to 25 °C

Humidity: ≤ 80% noncondensing

Weight and dimensions

MegaBACE instrument: 272 kg

Power supply fan module: 26 kg

MegaBACE instrument dimensions: 81.2 cm (height) x 103.3 cm (width) x 87.4 cm (depth)

Voltage and current

Location	Volts (AC)	Frequency	Amps
US/Canada	208 ± 10% or 220 ± 10%	60 Hz ±1%	15
Japan	200 ± 10%	50/60 Hz ±1%	16
Europe	230 ± 10%	50 Hz ±1%	14
U.K. and Australia	240 ± 6% or -10%	50 Hz±1%	13

Ordering information

MegaBACE 500 Sequencing System

Blue laser only	63-0034-29
Blue and green laser	63-0034-30

MegaBACE 500 Genotyping System

63-0034-31

MegaBACE 1000 Sequencing System

Blue laser only	63-0034-23
Blue and green laser	63-0034-24

MegaBACE 1000 Genotyping System

63-0034-25

MegaBACE 1000 Flexible Upgrade Kit

63-0055-70

To upgrade a MegaBACE 500 to a MegaBACE 1000

MegaBACE Flexible Genotyping Upgrade

63-0034-65
Upgrade kit to add genotyping application to a sequencing system

MegaBACE Flexible Sequencing Upgrade

63-0034-64
Upgrade kit to add sequencing application to a genotyping system

MegaBACE SNP Upgrade

63-0041-61

Upgrade kit to add SNP application to a sequencing system

MegaBACE SNP Upgrade

63-0041-63

Upgrade kit to add SNP application to a genotyping system

related products:

LPA Array (MBCAPL)

1 array = 16 capillaries 63-0008-84

4-Color MegaBACE Sequencing Standard Set

4 × 96 reactions US79678

MegaBACE Long Read Matrix

96 vials US79676

MegaBACE LPA Focusing Solution

100 tubes 25-0206-00

Ordering information (continued)

TempliPhi DNA Amplification Kit

100 reactions	25-6400-10
500 reactions	25-6400-50

DYEnamic ET Terminator Kit

10 000 reactions	US81095
500 reactions	US81090

PCR Pre-Sequencing Kit

12 500 templates	US81012
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MegaBACE ET400-R Size Standard

500 µl	25-0205-01
2.5 ml	25-0205-02

MegaBACE ET550-R Size Standard

500 µl	25-6550-01
2.5 ml	25-6550-02

MegaBACE ET900-R Size Standard

500 µl	25-6900-01
2.5 ml	25-6900-02

MegaBACE SNUpe Genotyping Kit

1000 reactions	25-6001-01
10 000 reactions	25-6001-02

MegaBACE SNUpe Multiple Injection Marker

1000 reactions	25-6001-03
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MegaBACE SNUpe Multiple Injection Marker

10 000 reactions	25-6001-04
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MegaBACE SNUpe Control Kit

4 x 10 reactions	25-6001-05
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The TempliPhi DNA Amplification Kit, TempliPhi 100 Amplification Kit, and TempliPhi 500 Amplification Kit and the use thereof for DNA synthesis is covered by US patent application number 09/920,571 and US patents 5,854,003, 5,198,543, 5,576,204 and 5,001,050 license exclusively to Amersham Biosciences Corp.

Energy transfer dyes and primers are covered by U.S. patent numbers 5,654,419; 5,688,648; 5,707,804; and foreign equivalents.

Thermo Sequenase is a heat-stable DNA polymerase producing even band intensities in DNA sequencing. This product and/or its methods of use is covered by U.S. patent numbers 4,962,020; 5,173,411; 5,409,811; 5,498,523; and foreign equivalents.

Thermo Sequenase Dye Terminator Sequencing Kits are covered by U.S. patent numbers 4,849,513; 5,015,733; 5,118,800; 5,118,802; 5,151,507; 5,171,534; 5,332,666; 5,242,796; 5,306,618; 5,366,860; 4,855,225; and corresponding foreign patents and patent applications.



Asia Pacific Tel: +852 2811 8693 Fax: +852 2811 5251 **Australasia** Tel: +61 2 9899 0999 Fax: +61 2 9899 7511 **Austria** Tel: 01 576 0616 22 Fax: 01 576 0616 27 **Belgium** Tel: 0800 73 888 Fax: 03 272 1637 **Canada** Tel: +1 800 463 5800 Fax: +1 800 567 1008 **Central, East, and Southeast Europe** Tel: +43 1 982 3826 Fax: +43 1 985 8327 **Denmark** Tel: 45 16 2400 Fax: 45 16 2424 **Finland & Baltics** Tel: +358 (0)9 512 39 40 Fax: +358 (0)9 512 17 10 **France** Tel: 01 69 35 67 00 Fax: 01 69 41 96 77 **Germany** Tel: 0761 4903 291 Fax: 0761 4903 405 **Italy** Tel: 02 27322 1 Fax: 02 27302 212 **Japan** Tel: +81 3 5331 9336 Fax: +81 3 5331 9370 **Latin America** Tel: +55 11 3933 7300 Fax: +55 11 3933 7315 **Middle East and Africa** Tel: +30 210 96 00 687 Fax: +30 210 96 00 693 **Netherlands** Tel: 0165 580 410 Fax: 0165 580 401 **Norway** Tel: 2318 5800 Fax: 2318 6800 **Portugal** Tel: 21 417 70 35 Fax: 21 417 31 84 **Russia & other C.I.S. & N.I.S.** Tel: +7095 232 0250, 956 1137 Fax: +7095 230 6377 **Southeast Asia** Tel: +60 3 8024 2080 Fax: +60 3 8024 2090 **Spain** Tel: 93 594 49 50 Fax: 93 594 49 55 **Sweden** Tel: 018 612 1900 Fax: 018 612 1910 **Switzerland** Tel: 01 802 81 50 Fax: 01 802 81 51 **UK** Tel: 0800 616928 Fax: 0800 616927 **USA** Tel: +1 800 526 3593 Fax: +1 877 295 8102

Amersham Biosciences UK Limited

Amersham Place, Little Chalfont, Buckinghamshire, England HP7 9NA

Amersham Biosciences AB

SE-751 84 Uppsala, Sweden

Amersham Biosciences Corp

800 Centennial Avenue, PO Box 1327, Piscataway, NJ 08855 USA

Amersham Biosciences GmbH

Munzinger Strasse 9, D-79111 Freiburg, Germany

Amersham Biosciences (SV) Corp

928 East Arques Avenue, Sunnyvale CA 94085 USA

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