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## MegaBACE 500 and 1000

Planning Guide Revision 6 December 2002



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The PCR process for amplifying DNA is covered by US patent numbers 4,683,195 and 4,683,202 assigned to Hoffman-La Roche Inc and F Hoffmann-La Roche Ltd. Patents are pending or issued in other countries.

The MegaBACE DNA Analysis System is for research purposes only. It is not intended or approved for diagnosis of disease in humans or animals.

All goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Biosciences group that supplies them. A copy of these terms and conditions is available on request.

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December 2002

#### **Notice to Purchaser: Limited License**

The MegaBACE instrument is a confocal scanning system licensed under US Patent Numbers 5,091,652 and 5,274,240, and corresponding foreign patents and patent applications, including any continuations, continuations-in-part, and subdivisions and the like.

The instrument is also an Authorized DNA Sequencer. It is authorized under one or more US Patent Numbers 4,849,513; 5,171,534; 5,015,733; 5,118,800; 5,161,507; 5,118,802; 4,855,225; and 5,366,860, and corresponding foreign patents and patent applications. The purchase of this instrument includes limited, non-exclusive rights under the subject patents to use this instrument for sequencing and fragment length analysis when used with Authorized Reagents. The use of this instrument with Authorized Reagents provides a limited license to perform DNA sequencing and fragment length analysis in accordance with the label rights accompanying such reagents. Purchase of this instrument does not itself convey to the purchaser a complete license to perform DNA sequencing and fragment length analysis under the subject patents. Authorized reagents may be obtained from licensed vendors, or reagents may be authorized under separate license arrangements from PE Applied Biosystems. The above patent rights are granted solely for research and other uses that are not unlawful. No other licenses are granted expressly, impliedly, or by estoppel.

Further information on purchasing licenses to perform DNA sequencing and fragment length analysis may be obtained by contacting the Director of Licensing at PE Applied Biosystems, 850 Lincoln Center Drive, Foster City, California 94404.

PE Applied Biosystems does not guarantee the performance of this instrument.

Amersham Biosciences Limited is a licensed vendor for authorized reagents.

The MegaBACE SNuPe genotyping kit is supplied under license from Orchid BioSciences Inc and is covered by US patent numbers 5,888,819; 6,004,744, and foreign equivalents.

The method for serially injecting multiple plates during a single run on a capillary-array-electrophoresis instrument is covered by US patent number 6,156,178.

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#### **Preface**

#### **About this guide**

The MegaBACE 500 and 1000 Planning Guide describes how to plan and prepare your laboratory before you receive the MegaBACE<sup>TM</sup> DNA Analysis System. This guide describes the requirements for the MegaBACE 500, flexible MegaBACE 1000, and MegaBACE 1000 instruments.

- Chapters 1 through 5—Describe the system components, chemistry, and site requirements.
- Appendixes—Provide preinstallation forms, checklists, and a summary of the laboratory space requirements.

#### **Related publications**

In addition to the MegaBACE 500 and 1000 Planning Guide, the following publications are available for the MegaBACE system:

- MegaBACE instrument user's documentation provides safety information, operation instructions, and maintenance and troubleshooting guidelines for the instrument.
- MegaBACE instrument administrator's guide provides information on how to tailor the instrument control software for your laboratory and applications.
- MegaBACE analysis software user's guides provide instructions on how to use the analysis software to analyze the data collected from the MegaBACE instrument and guidelines for troubleshooting the software and application.

#### Special safety text

Make sure you follow the precautionary statements presented in this guide.

Indicates a possibility of severe or fatal injury to the user or other persons if the precautions or instructions are not observed.

Indicates that damage to the instrument, loss of data, or invalid data could occur if the user fails to comply with the advice given.

Highlights information that is critical for optimal performance of the system.

**Note:** Identifies items of general interest.

Warning <u>/</u>!\

Caution

Important

#### Safety standards

The MegaBACE instrument complies with CE and other applicable standards, such as UL and CSA. For the latest CE conformity information, contact MegaBACE Technical Support. See Assistance for contact infomation.

#### **Assistance**

For questions regarding the planning and preparation of your laboratory, contact MegaBACE System Technical Support.

When calling for assistance, be prepared to supply the serial number of your instrument. The serial number is located on the lower right side of the MegaBACE instrument. For contact by phone or fax, please use one of the numbers below.

Asia Pacific Latin America Tel: +852 2811 8693 Tel: +55 11 3933 7300 Fax: +852 2811 5251 Fax: +55 11 3933 7315 Australasia Middle East and Africa Tel: +61 2 9899 0999 Tel: +30 (1) 96 00 687 Fax: +61 2 9899 7511 Fax: +30 (1) 96 00 693 Netherlands Austria Tel: 01 576 0616 10 Tel: 0165 580 410 Fax: 01 576 0616 27 Fax: 0165 580 401 Belgium Norway Tel: 0800 73 888 Tel: 2318 5800 Fax: 03 272 1637 Fax: 2318 6800

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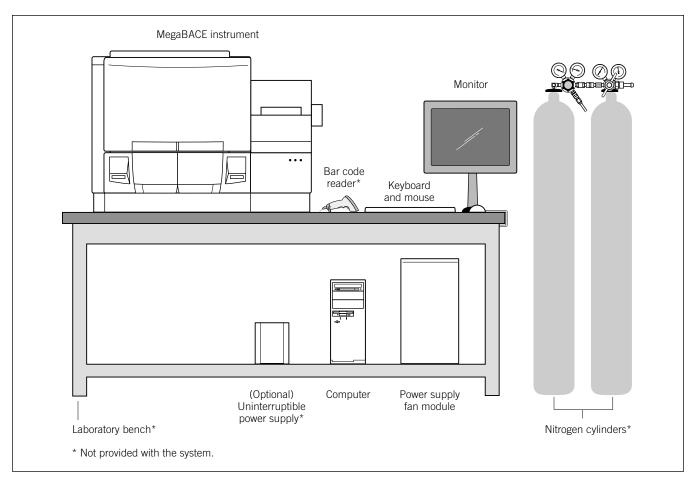
http://www.amershambiosciences.com

## **Chapter 1 Introduction**

The MegaBACE DNA Analysis System (figure 1-1) is a high-throughput automated gene analysis system. The system uses capillary array electrophoresis to perform fragment separation on DNA samples and then produces an electropherogram of the contents of each well in a microplate. The analysis software allows you to perform detailed analysis of the data.

This chapter provides a summary of—

- Preinstallation tasks (section 1.1)
- MegaBACE system components (section 1.2)
- Additional recommended components (section 1.3)
- Network (section 1.4)



**Figure 1-1.** The MegaBACE system.

#### 

#### 1.1 Preinstallation tasks

The MegaBACE system includes unpacking and installation by Amersham Biosciences. Before your MegaBACE system is installed, you should read this planning guide and prepare your laboratory space. If you have questions or concerns, contact MegaBACE System Technical Support. See Assistance in the preface for contact information.

Do not unpack or install the instrument and computer yourself. Only an Amersham Biosciences representative should unpack and install the MegaBACE instrument and computer. Improper unpacking and installation can cause personal injury and damage to the system components.

Before your MegaBACE system is scheduled for installation, make sure you complete the site information form and preinstallation checklist (appendix A). See appendix B for the training checklist.

#### 1.2 MegaBACE system components

The MegaBACE system consists of integrated hardware (figure 1-1), software, reagents (chapter 3), and capillary arrays. The basic MegaBACE system components are—

- MegaBACE instrument containing either one laser (488-nm argon-ion blue laser) or two lasers (488-nm argon-ion blue laser and 532-nm green solid-state laser)
- Pentium<sup>™</sup> workstation that uses Microsoft<sup>™</sup> Windows<sup>™</sup> and hosts the Instrument Control Manager and the analysis software.

**Note:** At a minimum, the MegaBACE software requires Windows 2000. Call MegaBACE System Technical Support for information on the exact configuration. See Assistance in the preface for contact information.

- Power supply fan module
- Capillary arrays
- Optical beamsplitter and filter set
- Accessory kit (cathode water tank, anode plugs, hoses, cables, fittings, and tools)
- Other components (user documents and software CD)
- Installation and training
- One-year warranty
- Liability statement for the MegaBACE system
- (Optional) Service contract

#### 1.3 Additional recommended components

The following components, which are not included as part of the basic MegaBACE system, are recommended:

- Analysis workstation—Allows you to dedicate the provided workstation for performing instrument runs. See chapter 4 for recommended configurations.
- Uninterruptible power supply (UPS)—Protects the instrument from power line fluctuations. See section 2.3.1 for details.

#### 1.4 Network

The MegaBACE instrument and the workstation are connected using a computer network—

- Instrument connection—PCI (peripheral component interconnect) SCSI (small computer system interface) connection from the MegaBACE instrument to the instrument control workstation
- Workstation network connection—10/100 Fast Ethernet<sup>TM</sup> adapter

See chapter 4 for recommended configurations. Contact MegaBACE System Technical Support for specifications. See Assistance in the preface for contact information.

## **Chapter 2 Site requirements**

This chapter describes the site requirements for the MegaBACE system. The topics include—

- Laboratory space requirements (section 2.1)
- Environmental requirements (section 2.2)
- System electrical requirements (section 2.3)
- Requirements for nitrogen cylinders and pressure regulators (section 2.4)

See appendix C for a summary of the laboratory space requirements.

#### 2.1 Laboratory space requirements

This section describes the laboratory space and laboratory bench requirements. The basic MegaBACE system includes one workstation for instrument control and analysis. If you want additional workstations, you need to plan the space accordingly. See chapter 4 for workstation configurations.

#### 2.1.1 Instrument space requirements

The dimensions of the MegaBACE instrument are—

```
Width 1.033 m (40.7 in)
Height 0.812 m (32 in)
Depth 0.874 m (34.4 in)
```

When planning laboratory space for your MegaBACE instrument, you should allow—

- 0.127 m (5 in) clearance for the laser exhaust port hose (figure 2-1)
- 0.127 m (5 in) clearance behind the instrument for service access
- 0.279 m (11 in) clearance above the instrument so that you can open the electrophoresis chamber door
- Room for the power supply fan module
- Laboratory bench space for the computer
- Wall space for the nitrogen cylinders
- Space for an uninterruptible power supply (UPS) if you plan to use one

See figure 2-1 for details on space requirements.

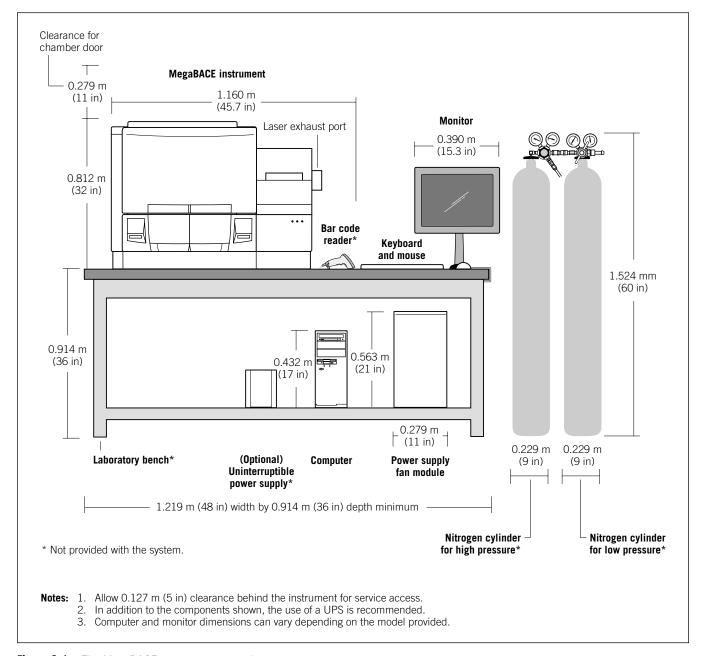


Figure 2-1. The MegaBACE system space requirements.

#### 2.1.2 Laboratory bench requirements

You must provide a level, stable laboratory bench or table space for the MegaBACE instrument and hardware components. The bench or table surface must be level within 0.635 cm over 0.914 m (0.25 in over 3 ft).

The laboratory bench or table you use must be rated to hold at least the combined weight of the components (shown in figure 2-1). The approximate weights of the components are—

MegaBACE instrument 272 kg (600 lb)

Computer monitor 5 kg (11.1 lb)

Computer tower 14 kg (30 lb)

Power supply fan module 26 kg (58 lb)

In addition, you should provide sufficient space to meet or exceed the requirements defined in figure 2-1.

(For Europe only) A customized table capable of holding the weight of the MegaBACE system is available for purchase. You can order the customized table (product number 94-9900-28) from the following site:

Amersham Biosciences Europe GmbH Munzinger Strasse 9, D-79111 Freiburg, Postfach 5480 D-790 21 Freiburg, Germany +49-761-4903-291

If the laboratory bench or table is not stationary, the legs should have a locking mechanism. Contact MegaBACE System Technical Support for a recommended supplier of benches that meet these requirements. See Assistance in the preface for contact information.

#### 2.2 Environmental requirements

This section describes the environmental requirements for the MegaBACE instrument.

#### 2.2.1 Temperature and humidity

For optimal performance of the MegaBACE instrument, the laboratory must meet the following requirements:

Ambient temperature: 20–25 °C (68–77 °F)

Humidity: ≤ 80% (noncondensing)

Pollution degree: 2

Installation category: II

Place the MegaBACE instrument away from heat and air conditioning ducts. Do not place the instrument in direct sunlight.

#### 2.2.2 Heat production

A standard power supply fan module is supplied with the instrument (figure 2-1). If you vent the laser exhaust into the room, the rate of heat energy production for each instrument is approximately 10 300 BTU. To reduce heat buildup in the instrument room to approximately 4 100 BTU per instrument, you can vent the exhaust into the ceiling space or outside the building if local safety and building codes permit.

If you vent the exhaust into the ceiling space, make sure that your building ventilation system can handle the approximately 6 200 BTU of heat energy that is vented. You should also take into account that the computer and monitor produce approximately 2 800 BTU of heat energy, depending on the model provided.

To reduce noise in the instrument room, you can use an external exhaust fan. Contact MegaBACE System Technical Support for more information. See Assistance in the preface for contact information.

You can choose to vent the hot air out the back of the power supply fan module into a duct. To do this requires a 0.102-m (4-in) flange. Contact MegaBACE System Technical Support for details.

The airflow requirement is  $1.557 \text{ m}^3/\text{min}$  ( $55 \text{ ft}^3/\text{min}$ ) minimum at 0.038 m (1.5 in) H<sub>2</sub>O static pressure at the laser exhaust port on the instrument (figure 2-1).

#### 2.3 System electrical requirements

The MegaBACE system includes four components that require electrical power: the instrument, the power supply fan module, the computer, and the monitor (sections 2.3.2 through 2.3.4). Four electrical power cords are included with each system, one for each of these main components. The use of a UPS is recommended to protect the instrument from power line problems (section 2.3.1).

Warning

Use only the power cords supplied with the MegaBACE system. Make sure the cords are in good condition and are not frayed. Use of incorrect power cords can cause damage to the instrument. Use of frayed or damaged power cords can cause injury.

Important

You must locate the right side of the MegaBACE instrument within 2.5 m (8 ft) of the electrical outlets.

#### Caution

#### 2.3.1 Uninterruptible power supply (UPS)

## Without the recommended UPS, the instrument is vulnerable to power line fluctuations.

In the event of a power failure, depending on the duration, the UPS might not contain enough stored power to finish a run that is in process and allow the capillaries to be stored properly.

Amersham Biosciences recommends the use of a UPS rated for at least 4 kVA. Using a UPS protects the instrument, the capillaries, and your data from damage or loss caused by unexpected power failures, surges, or AC line fluctuations. The UPS also acts as a power line regulator, line conditioner, and surge suppressor and works to protect against all power line problems.

If you are using a UPS, you must connect the instrument, power supply fan module, computer, and monitor to the UPS. If you have an external blower for laser cooling, you must also connect the blower to the UPS.

Contact MegaBACE System Technical Support for information about a qualified UPS. See Assistance in the preface for contact information.

**Note:** A UPS will most likely require electrical connections at the wall that differ from those of the MegaBACE instrument. Contact the UPS manufacturer or MegaBACE System Technical Support for details concerning site preparation when using a UPS. See Assistance in the preface for contact information.

## 2.3.2 Instrument and power supply fan module: North America and Japan

The MegaBACE instrument and power supply fan module are designed to run at 200–240  $V^{\sim}$  worldwide. In North America and Japan, the instrument and power supply fan module require a 200–220  $V^{\sim}$  circuit with a 20 A circuit breaker. See table 2-1 for detailed voltage requirements.

**Table 2-1.** Electrical requirements for the MegaBACE instrument and the power supply fan module

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
UK and Australia	240 +6% or -10%	50 Hz ±1%	13

Table 2-2 lists the plug and receptacle configurations that can be used for the MegaBACE instrument, power supply fan module, and computer. The MegaBACE system includes two power cords with standard NEMA 6-15P plugs for the instrument and power supply fan module and two 5-15P plugs for the computer and monitor.

You must provide a 20 A grounded circuit that terminates with two 6-15R or 6-20R receptacles. Termination can be at the wall, from a power strip, or at the output of a UPS. The 6-20R receptacle will accept either the 6-15P or 6-20P plug.

**Table 2-2.** Power plug and receptacle configurations

Location	Unit	Plug supplied	Receptacle required (customer supplied)
US/Canada and Japan	Instrument and power supply fan module	NEMA 6-15P (Quantity 2)	NEMA 6-15R or 6-20R (Quantity 2)
	Computer and monitor	NEMA 5-15P (Quantity 2)	NEMA 5-15R or 5-20R (Quantity 2)
Europe, UK, and Australia	Instrument, power supply fan module, computer, and monitor	Country-standard 230V (Quantity 4)	Country-standard 230V (Quantity 4)

**Note:** The receptacles are not provided. Contact MegaBACE System Technical Support for purchasing information. See Assistance in the preface for contact information.

#### 2.3.3 Instrument and power supply fan module: Europe

The MegaBACE instrument and power supply fan module are designed to run at 200–240 V<sup>~</sup> worldwide. (See tables 2-1 and 2-2 for detailed voltage and outlet requirements.) The MegaBACE system includes two power cords with European standard CEE 7/7 ("Schuko") plugs for the instrument and power supply fan module. This configuration allows the use of standard plugs and receptacles that are found in continental Europe.

Use of the recommended UPS (section 2.3.1) requires special high-current electrical hookups. Please refer to the manufacturer's guidelines and follow all applicable local electrical codes during the site preparation. Contact the UPS manufacturer or MegaBACE System Technical Support for more information. See Assistance in the preface for contact information.

## Without the recommended UPS, the instrument is vulnerable to power line fluctuations.

If you do not use a UPS, you can plug the MegaBACE instrument and the power supply fan module into separate grounded circuits capable of delivering at least 10 A each at 230  $V^{\sim}$ , using the standard country-accepted outlet. Alternatively, you can use a single grounded circuit capable of delivering at least 20 A at 230  $V^{\sim}$ .

#### 2.3.4 Computer and monitor

The computer and monitor can be connected to any appropriate AC outlet as long as you make sure the voltage selection switch on the back of the computer correctly matches the voltage at the outlet (table 2-3). See table 2-2 for plug and receptacle configurations.

Table 2-3. Voltage output by location

Location	Volts (AC)	
US/Canada	110–120	
Japan	100–120	
Europe	220–240	

#### 2.4 Requirements for nitrogen cylinders and pressure regulators

You can use a centralized nitrogen source or provide a separate nitrogen cylinder for each instrument. This section describes the requirements for a single locally installed nitrogen cylinder for both high- and low-pressure nitrogen, but the principles apply similarly to remote nitrogen sources.

Warning



When you install a nitrogen cylinder, make sure you bolt a standard cylinder bracket to a solid permanent structure in a manner that meets or exceeds all local seismic and safety code requirements. Failure to secure the nitrogen cylinder can cause injury to the operator.

#### 2.4.1 Regulator configuration

A single nitrogen source for both high- and low-pressure supplies—

- High pressure for injecting and removing the matrix.
- Low pressure for rinsing the capillaries and operating the cathode and anode stages and the service door.

Note: If you are using multiple instruments, contact MegaBACE System Technical Support for the recommended configuration. See Assistance in the preface for contact information.

Warnings



Do not attempt to adjust the regulators to pressure settings above those described in this guide and in the MegaBACE instrument user's documentation. If you are using separate cylinders for high and low pressure, make sure that the correct pressure is applied to each line.

The nitrogen pressure in the high-pressure line must not exceed  $6.89 \times 10^3$  kPa (1000 psi). Never apply high pressure to the low-pressure line. This can damage the instrument or the low-pressure line and can cause injury.

Use only hose types with ratings that exceed the required operating pressures. Do not use a frayed or damaged hose, which can rupture and can cause injury.

## 2.4.2 Ordering information for the high- and low-pressure nitrogen systems

You must provide the high- and low-pressure nitrogen systems that connect to the back of the MegaBACE instrument. You must also provide the high- and low-pressure cylinders, which you can obtain from your local supplier. Make sure you order 5.0 ultrahigh purity nitrogen.

The specifications for the nitrogen are—

- 99.999%
- $O_2 < 1$  ppm
- $H_2O < 3 \text{ ppm}$
- THC < 0.5 ppm (Ar and Ne Free Basis)

The following sections provide information for ordering the nitrogen systems.

#### Ordering nitrogen systems within the US

- Option 1: Ordering high- and low-pressure manifold assemblies—You can order a high- and a low-pressure nitrogen manifold assembly, each of which includes the high- and low-pressure regulators and tubing. Table 2-4 provides the ordering information for the manifold assemblies.
- Option 2: Ordering separate parts—If you are using multiple instruments, contact MegaBACE System Technical Support for assistance. See Assistance in the preface for contact information.

Table 2-4. High- and low-pressure nitrogen manifold assemblies

ltem	Description	Suggested supplier (company name, telephone number)	Part number
High-pressure manifold assembly	Includes 1/4-in O.D. flexible braided stainless steel tubing with male 1/4-in NPT at each end	Engineered Solutions (408) 617-2800 (or check with your local nitrogen supplier)	9G00294 (Rev. G)
Female adapter (for high-pressure manifold assembly)	1/4-in O.D. tube with 1/4-in NPT	Sunnyvale Valve and Fitting Company (408) 734-3145	Swagelok SS-4-TA-7-4
Low-pressure manifold assembly	Includes 1/8-in I.D. polyurethane tubing	Engineered Solutions (408) 617-2800 (or check with your local nitrogen supplier)	9G00293 (Rev. F)

Note: The female adapter comes in the instrument accessory kit.

#### Ordering nitrogen systems outside the US

Table 2-5 provides information for ordering nitrogen systems outside the US.

**Table 2-5.** Ordering information for nitrogen systems outside the US

Item	Description	Suggested supplier (company name, telephone number)	Product or Code number
Europe			
N <sub>2</sub> Panel for MegaBACE system	Customized nitrogen supply made for the MegaBACE, includes single-cylinder solution manifold.	Amersham Biosciences Amersham Labs, White Lion Road Amersham, Bucks HP7 9LL England +44-1494-545077	94-9900-29
N <sub>2</sub> Panel Connection Kit (Order the kit appropriate for the type of cylinder used.)	Gas cylinder connection kit to connect the nitrogen panel to the cylinders.	Amersham Biosciences Amersham Labs, White Lion Road Amersham, Bucks HP7 9LL England +44-1494-545077	
Kit DIN477 No. 10     (Scandinavia and Europe, except for France and the UK)			94-9900-30
• Kit BS341 No. 3 (UK)			94-9900-31
Kit DIN477 No. 1 (Air liquid type C)     (France)			94-9900-32
Outside of Europe			
Contact your local gas plumber for the appropriate nitrogen cylinder connections.			

#### 2.4.3 Requirements for the high-pressure nitrogen system

The following are the requirements for the high-pressure nitrogen system used with the MegaBACE instrument:

- Setpoint of high-pressure input— $6.89 \times 10^3$  kPa (1 000 psi)
- Maximum allowable pressure— $7.07 \times 10^3$  kPa (1 025 psi)
- Minimum allowable pressure— $6.72 \times 10^3$  kPa (975 psi)
- Flow rate—Depends on usage
- Pressure sensing—The instrument has a built-in pressure transducer that allows the system to monitor high pressure
  - **High setpoint**— $7.07 \times 10^3 \text{ kPa } (1025 \text{ psi})$
  - Low setpoint— $6.55 \times 10^3$  kPa (950 psi)
- Volume used (estimated)—
  - Single run— $0.0006 \text{ m}^3 (0.02 \text{ ft}^3)$
  - Five runs per day— $0.003 \text{ m}^3 (0.1 \text{ ft}^3)$

#### 2.4.4 Requirements for the low-pressure nitrogen system

The following are the requirements for the low-pressure nitrogen system:

- Standard low-pressure input— $6.89 \times 10^2$  kPa (100 psi)
- Pressure sensing—The instrument is equipped with a pressure switch to indicate low pressure. The pressure switch is set to trip at  $5.86 \times 10^2 \pm 20$  kPa (85  $\pm 3$  psi) as the pressure falls.

The pressure switch will trip in the range of  $5.65 \times 10^2$  kPa to  $6.07 \times 10^2$  kPa (82–88 psi). The pressure must be set above  $6.07 \times 10^2$  kPa (88 psi) to satisfy the pressure switch, or the system will identify a low-pressure condition.

- Volume used (estimated)—
  - **Single run**—0.013 m<sup>3</sup> (0.46 ft<sup>3</sup>)
  - Five runs per day—0.065 m<sup>3</sup> (2.3 ft<sup>3</sup>)

#### 2.4.5 Checking the available nitrogen pressure

To prevent pressure loss during a cycle, you should check the available nitrogen pressure on a regular basis to determine when to replace the cylinders.

#### 2.4.6 Installing a nitrogen cylinder

When you install a nitrogen cylinder, make sure you follow the local safety code requirements for the placement and mounting of the cylinder.

Follow the instructions provided with the cylinder for removal and installation. Always use good laboratory practices when handling a high-pressure cylinder.

Before you install the new cylinder, make sure the valve area and the passageway are free of dust or dirt. After you install the new cylinder, make sure you check that—

- The new connections have no leaks
- The high-pressure regulator gauge is set to the proper pressure:  $6.89 \times 10^3 \text{ kPa} (1\,000 \text{ psi})$
- The low-pressure regulator gauge is set to the proper pressure:  $6.89 \times 10^2 \text{ kPa} (100 \text{ psi})$

# Chapter 3 Emission filters, chemistry, and laboratory materials

The MegaBACE system can be used for various applications, including sequencing, single-nucleotide-polymorphism (SNP) genotyping, and short-tandem-repeat (STR) genotyping, also known as microsatellite genotyping. This chapter describes the emission beamsplitters and filters, chemistry, and laboratory equipment needed for the various applications. The topics are—

- Emission beamsplitters and filters (section 3.1)
- Chemistry (section 3.2)
- Laboratory equipment (section 3.3)
- Consumables (section 3.4)

#### 3.1 Emission beamsplitters and filters

#### 3.1.1 Filter set for sequencing or SNP genotyping

The MegaBACE sequencing system includes a set of beamsplitters and filters that are optimized for detecting the energy-transfer (ET) dye sets listed in tables 3-1 and 3-2. The same set of beamsplitters and filters can be used to detect the SNuPe<sup>TM</sup> dye set (table 3-3) for SNP genotyping.

**Table 3-1.** Beamsplitters and filters for the DYEnamic™ ET terminators

DYEnamic ET terminator dye	DYEnamic ET terminator base order	Spectral channel	Filter	Beamsplitter	
ET-R6G	Т	1	555DF20	A 540DDLD	
ET-R110	G	2	520DF20	A: 540DRLP	
ET-ROX	С	3	610LP	D COEDDID	
ET-TAMRA	А	4	585DF20	B: 595DRLP	

**Table 3-2.** Beamsplitters and filters for the DYEnamic ET primers

ET primer dye	ET primer base order	Spectral channel	Filter	Beamsplitter
ET-R6G	А	1	555DF20	A: 540DRLP
ET-R110	С	2	520DF20	A: 540DKLP
ET-ROX	T	3	610LP	B: 595DRLP
ET-TAMRA	G	4	585DF20	B: 5950KLP

**Table 3-3.** Beamsplitters and filters for the SNuPe dye set

SNuPe dye	SNuPe base order	Spectral channel	Filter	Beamsplitter
R6G	А	1	555DF20	A: 540DRLP
R110	G	2	520DF20	A: 540DRLP
ROX	С	3	610LP	B: 595DRLP
TAMRA	Т	4	585DF20	D: DSDUKLP

#### 3.1.2 Filter sets for STR genotyping

The MegaBACE STR genotyping system includes a set of emission beamsplitters and filters that are optimized for detecting a specific dye set. Table 3-4 lists the dyes detected by filter set 2. Table 3-5 shows an additional filter set that is required for detecting dye set 1.

**Table 3-4.** Genotyping filter set 2

Dye set 2*	Color	Spectral channel	Filter	Beamsplitter	
ET-ROX	Red	1	610DF20	A 540DDLD	
FAM	Blue	2	520DF20	A: 540DRLP	
NED	Yellow or black	3	580DF20	D EZODDID	
HEX or VIC	Green	4	555DF20	B: 570DRLP	

<sup>\*</sup> Alternatives for dye set 2 are ET-ROX, FAM, TAMRA, and HEX; or ET-ROX, FAM, TAMRA, and JOE.

**Table 3-5.** Genotyping filter set 1 (additional set required for dye set 1)

Dye set 1	Color	Spectral channel	Filter	Beamsplitter
ET-ROX	Red	1	610DF20	A 5400010
FAM	Blue	2	520DF20	A: 540DRLP
HEX	Yellow or black	3	565DF20	D EEEDDID
TET	Green	4	545DF20	B: 555DRLP

## Warning <a>\hat{\Lambda}</a>

#### 3.2 Chemistry

Use good laboratory procedures and follow the manufacturer's precautions when working with chemicals. Amersham Biosciences is not responsible or liable for any damages caused by or as a consequence of the use of hazardous chemicals.

Table 3-6 lists the chemistry requirements for sequencing. Table 3-7 lists the chemistry requirements for STR genotyping. Table 3-8 lists the chemistry requirements for SNP genotyping.

**Table 3-6.** Chemistry requirements for sequencing

Item	Storage conditions	Quantity	Part number*
MegaBACE DYEnamic ET Terminators			
DYEnamic ET Dye Terminator Set	−20 °C	500 reactions 10 000 reactions	US81090 US81095
MegaBACE DYEnamic ET Primers			
-40 M13 Forward ET Primer Set 5'-GTT TTC CCA GTC ACG ACG-3	−20 °C	2 000 reactions per pack	US79475
SP6 ET Primer Set 5'-ATT TAG GTG ACA CTA TAG-3	−20 °C	2 000 reactions per pack	US79850
T7 ET Primer Set 5'-TAA TAC GAC TCA CTA TAG GG-3	−20 °C	2 000 reactions per pack	US79855
-21 M13 Forward ET Primer Set 5'-TGT AAA ACG ACG GCC AGT-3'	−20 °C	2 000 reactions per pack	US79880
-28 M13 Reverse I ET Primer Set 5'-AGG AAA CAG CTA TGA CCA T-3	−20 °C	2 000 reactions per pack	US79970
-28 M13 Reverse II ET Primer Set 5'-AGG AAA CAG CTA TGA CAT G-3	−20 °C	2 000 reactions per pack	US79890
-29 M13 Reverse ET Primer Set 5'-TCA GGA AAC AGC TAT GAC C-3	−20 °C	2 000 reactions per pack	US79900
MegaBACE Thermo Sequenase™ reaction buffer	–20 °C	10.0 ml	US79607
MegaBACE Thermo Sequenase <sup>†</sup>	–20 °C	24 000 units	E79000M
MegaBACE dGTP sequencing blend <sup>†</sup>	–20 °C	2000 reactions per pack	US79655
MegaBACE 7-deaza dG sequencing blend <sup>†</sup>	−20 °C	2000 reactions per pack	US79657
Other Chemistry			
MegaBACE long-read matrix (LPA) and buffer	4 °C	96 tubes per pack	US79676
MegaBACE 4-color sequencing standard set	–20 °C	4 × 96 reactions per pack	US79678
M13 mp 18 (+) strand DNA	–20 °C	25 μg	27-1546-01
pUC18	–20 °C	25 μg	27-4949-01
pUC19	−20 °C	25 μg	27-4951-01

<sup>\*</sup> You can order the reagents from your local Amersham Biosciences sales office. For a complete listing, see the Web site: http://www.amershambiosciences.com.

<sup>†</sup> Choose the blend that is appropriate for your application and conditions.

**Table 3-7.** Chemistry requirements for STR genotyping

Item	Storage conditions	Quantity	Supplier	Part number*
MegaBACE long-read matrix (LPA) and buffer	4 °C	96 tubes per pack	Amersham Biosciences http://www.amershambiosciences.com	US79676
MegaBACE energy transfer (ET) size standard	4 °C (reduced exposure to light)		Amersham Biosciences http://www.amershambiosciences.com	
• ET400-R		500 μl 2.5 ml		25-0205-01 25-0205-02
• ET550-R		500 μl 2.5 ml		25-6550-01 25-6550-02
• ET900-R		500 μl 2.5 ml		25-6900-01 25-6900-02
MegaBACE loading solution (70% formamide, 1mM EDTA)	−20 °C	40 ml	Amersham Biosciences http://www.amershambiosciences.com	US79916
Spectral matrix standards; one for each dye in your dye set			Use the supplier who provides your primers.	
MegaBACE genotyping test plate kit	–20 °C	2 plates	Amersham Biosciences http://www.amershambiosciences.com	25-0207-01

<sup>\*</sup> You can order the reagents from your local Amersham Biosciences sales office. For a complete listing, see the Web site: http://www.amershambiosciences.com.

**Table 3-8.** Chemistry requirements for SNP genotyping

Item	Storage conditions	Quantity	Supplier	Part number*
MegaBACE long-read matrix (LPA) and buffer	4 °C	96 tubes per pack	Amersham Biosciences http://www.amershambiosciences.com	US79676
10× MegaBACE LPA buffer	4 °C	50 ml 1 liter	Amersham Biosciences http://www.amershambiosciences.com	US79677 US79686
MegaBACE loading solution (70% formamide, 1mM EDTA)	−20 °C	40 ml	Amersham Biosciences http://www.amershambiosciences.com	US79916
MegaBACE SNuPe genotyping kit (including premix, control primer, control template, and spectral matrix standards)	−20 °C	1 000-reaction pack 10 000-reaction pack	Amersham Biosciences http://www.amershambiosciences.com	25-6001-01 25-6001-02
MegaBACE SNuPe multiple-injection marker kit	−20 °C	1 000-reaction pack 10 000-reaction pack	Amersham Biosciences http://www.amershambiosciences.com	25-6001-03 25-6001-04
MegaBACE SNuPe control kit	−20 °C	4 × 10 reactions	Amersham Biosciences http://www.amershambiosciences.com	25-6001-05

<sup>\*</sup> You can order the reagents from your local Amersham Biosciences sales office. For a complete listing, see the Web site: http://www.amershambiosciences.com.

#### 3.3 Laboratory equipment

In addition to the chemistry, you need to supply the laboratory equipment listed below.

- Microcentrifuge for 1.5–2.0-ml microcentrifuge tubes (optionally refrigerated)
- Centrifuge rated to 3 100 g with microplate-format rotor (optionally refrigerated)
- Micropipettors with volume ranges of 1–10  $\mu$ l, 10–100  $\mu$ l, 20–200  $\mu$ l, and 100–1000  $\mu$ l and tips (optional 8-channel autopipettors in 10- $\mu$ l and 250- $\mu$ l volumes)
- Tube racks
- Pipettes with volumes of 5 ml, 10 ml, and 25 ml
- Heat block or 96-well thermal cycler able to maintain 95 °C
- Squirt water bottle
- -20 °C storage for the samples and reagents
- 4 °C storage for the linear polyachrylamide (LPA) matrix and buffer and the genotyping size standards
- Ice bucket
- Assorted beakers, flasks, and graduated cylinders

The following equipment is optional depending on your application protocol:

- Speed Vac<sup>TM</sup> concentrator with microplate-format buckets
- Balance, 1–500 ±0.1 g
- 1-liter graduated cylinder
- Pyrex<sup>TM</sup> or equivalent glass dish measuring 20.32 cm by 20.32 cm by 5.08 cm (8 in by 8 in by 2 in) (recommended for dialysis procedure)

#### 3.4 Consumables

#### 3.4.1 General consumables

You should have the consumables listed in table 3-9 available in your lab before instrument installation.

Caution

Make sure you use the specified microplates. Using an incorrect microplate can cause damage to the instrument cathode assembly.

 Table 3-9.
 Additional consumables required for the MegaBACE system

Item	Supplier	Product or part number
2-ml microcentrifuge tubes with screw caps	Sarstedt Inc http://www.sarstedt.com Within US: (1) (800) 257-5101 Outside US: (828) 465-4000 or refer to Web site	vial 72.609 cap 65.716.022
	VWR Scientific http://www.vwrsp.com Within US: (1) (800) 932-5000 Outside US: (908) 465-4045 or refer to Web site	20170-217
Capillary arrays (16-capillary arrays)	Amersham Biosciences (see the preface)	63-0008-84
Capillary window cleaners		
Liqui-Nox™	Alconox Inc http://www.alconox.com Within US: (1) (914) 948-4040 Outside US: See Web site	1232-1
Medium flexible head swabs, Texwipe™	VWR Scientific http://www.vwrsp.com Within US: (1) (800) 932-5000 Outside US: (908) 465-4045 or refer to Web site	TWTX709A
Nitrogen cylinders (5.0 ultrahigh purity)  99.999%  O <sub>2</sub> < 1 ppm  H <sub>2</sub> O < 3 ppm  THC < 0.5 ppm  (Ar and Ne Free Basis)	Local supplier	
Dow Corning™ high-vacuum silicone grease (lubricant for anode plug 0-ring)	Cole Parmer http://www.coleparmer.com Within US: (1) (800) 323-4340 Outside US: (847) 549-7600 or refer to Web site	E-79751-30

 Table 3-9.
 Additional consumables required for the MegaBACE system (continued)

Item	Supplier	Product or part number
Plates (96-well) for the skirted-plate cathode assemb	ly*	
<ul> <li>Microseal™ 96, skirted-plate, natural color or bar coded</li> </ul>	MJ Research, Inc. http://www.mjr.com Within US: (1) (888) 729-2165 Outside US: (775) 832-8000 or refer to Web site	MSP-9601 or MSP-9605
<ul> <li>Thermo-Fast™ 96, skirted thin-wall PCR plate, natural color</li> </ul>	ABgene http://www.abgene.com Within US: (1) (716) 241-2870 Outside US: (44) (0) (1372) 723456 or refer to Web site	AB-0800
<ul> <li>ABgene<sup>™</sup> Thermo-Fast 96, skirted thin-wall PCR plate, natural color</li> </ul>	Marsh Biomedical Products, Inc. http://www.biomar.com Within US: (1) (800) 445-2812 Outside US: (716) 654-4800 or refer to Web site	WAB-0800-N
Plates (96-well) for the Robbins-plate cathode assem	bly*	
<ul> <li>CyclePlate<sup>™</sup> -96ET thin-wall PCR plate (standard or sterile packaging)</li> </ul>	Robbins Scientific Corp http://www.robsci.com/contact.html Within US: (1) (800) 752-8585 Outside US: (408) 734-8500 or refer to Web site	1055-00-X (10 plates, standard) 1055-01-X (10 plates, sterile) 1055-90-X (50 plates) <b>Note:</b> X designates a number for plates of various colors.
BacePlate-96	Dot Scientific Inc http://www.dotscientific.com (810) 744-1478 or refer to Web site	96MGB-10 (10 plates) 96MGB-50 <sup>†</sup> (50 plates)
Plate sealers		
CycleSeal™ PCR plate sealer (for thermal cycling)	Robbins Scientific Corp http://www.robsci.com/contact.html Within US: (1) (800) 752-8585 Outside US: (408) 734-8500 or refer to Web site	1044-39-4
<ul> <li>CycleFoil™ plate sealer and roller (for post-reaction freezer storage)</li> </ul>	Robbins Scientific Corp http://www.robsci.com/contact.html Within US: (1) (800) 752-8585 Outside US: (408) 734-8500 or refer to Web site	1044-39-3
Tape pads     (for 4 °C storage and vortexing)	Qiagen http://www.qiagen.com or within US: (1) (800) 426-8157	19570

**Table 3-9.** Additional consumables required for the MegaBACE system (continued)

Item	Supplier	Product or part number
MegaBACE 1000 instrument: buffer tank (required for SNP genotyping)		
Tank for the Robbins-plate cathode assembly*	Amersham Biosciences (see the preface)	63-0035-88
Tank for the skirted-plate cathode assembly*	Amersham Biosciences (see the preface)	63-0035-89

<sup>\*</sup> Be sure to verify that you order the correct type of plate for your instrument's cathode assembly. The skirted-plate version of the cathode assembly is convertible to the Robbins plate design. If you want to use the Robbins CyclePlate only, you can have the cathode assembly converted. Contact MegaBACE System Technical Support for details. See Assistance in the preface for contact information.

#### 3.4.2 Additional consumables required for STR genotyping

#### Caution

Electrokinetic injection will not work properly if the ratio of the anion concentration to the DNA in the sample is greater than 10 000:1. To achieve this ratio for microsatellite genotyping, you should desalt your samples.

To achieve successful sample injection into the capillaries, you must desalt the genotyping samples. Failure to desalt the genotyping samples can result in data with low signal intensity. Various methods exist for desalting the samples, including dialysis, ethanol precipitation, and the use of Sephadex<sup>TM</sup> separation resins or magnetic beads. Amersham Biosciences recommends dialysis, but alternative methods can also produce good results. Table 3-10 lists the consumables required for dialysis.

Table 3-10. Additional consumables recommended for desalting the samples for STR genotyping

Item	Supplier	Product or part number
TE buffer concentrate (50x) (ultra pure)	Amersham Biosciences (see http://www.amershambiosciences.com for site listing)	US75834-100 ml
96-well filtration plate assembly	Millipore http://millipore.com Within US: (1) (800) 645-5476 Outside US: (781) 533-6000	MAVM NO5 50 (50 plates) MAVM NO5 10 (10 plates)
Sealing tape for the filtration plate	Dynex Technologies http://www.dynextechnologies.com Within US: (1) (800) 336-4543 Outside US: (703) 631-7800	3501

<sup>†</sup> To specify color, append the corresponding color code to the part number: N (natural), B (blue), G (green), R (red), or Y (yellow).

#### 3.4.3 Additional consumables required for SNP genotyping

Table 3-11 lists the additional consumables recommended for SNP genotyping.

 Table 3-11.
 Additional consumables for cleanup of the SNuPe reaction

Item	Quantity	Supplier	Product code
AutoSeq <sup>™</sup> 96 dye terminator cleanup kit	10 pack 100 pack	Amersham Biosciences (see http://www.amershambiosciences.com for site listing)	27-5340-10 27-5340-96
AutoSeq96 adapter plate (for use with non-skirted plates)	2	Amersham Biosciences (see http://www.amershambiosciences.com for site listing)	27-5341-01

## Chapter 4 Planning for computer network and data storage

This chapter describes the MegaBACE system computer, the potential configurations, and the data storage requirements. The topics are—

- Basic MegaBACE system configuration (section 4.1)
- Configuration for a high-throughput laboratory (section 4.2)
- Storage requirements (section 4.3)

Contact MegaBACE System Technical Support for the latest computer hardware and software specifications. See Assistance in the preface for contact information.

#### 4.1 Basic MegaBACE system configuration

The basic configuration for the MegaBACE system includes one MegaBACE instrument and one workstation (figure 4-1). A PCI SCSI connects the two components.

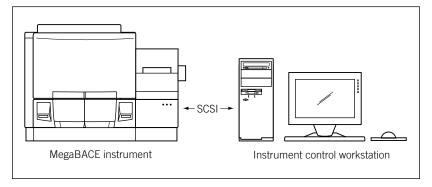


Figure 4-1. The basic MegaBACE system configuration.

The workstation uses the Windows operating system and hosts the instrument control software and the analysis software. The workstation contains a Fast Ethernet Adapter so that it can be connected to a supported network. Call MegaBACE System Technical Support for information on the exact computer configuration.

#### 4.2 Configuration for a high-throughput laboratory

If your laboratory requires high throughput, at least two workstations are recommended (figure 4-2):

- Instrument control workstation (provided)—Dedicated to controlling the
  operation of the instrument and collecting the data from the plates you run.
- Analysis workstation(s)—For performing analysis on collected data while scanning is in progress on another plate.

You can network multiple analysis workstations to the MegaBACE instrument. As figure 4-2 shows, the instrument control workstation is connected to the instrument and collects the data from a run. After data collection is complete, you transfer the collected data to an analysis workstation.

If you plan to have an additional analysis workstation that is not in the same room with the instrument control workstation, make sure you have the proper network drops for the computer connections.

For information about SCSI and network connections, contact MegaBACE System Technical Support. See Assistance in the preface for contact information.

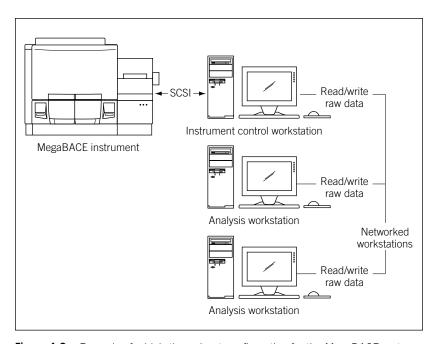


Figure 4-2. Example of a high-throughput configuration for the MegaBACE system.

#### 4.3 Storage requirements

The disk space required for each data collection run on the MegaBACE instrument depends on the application. A sequencing run of a 96-well plate requires about 150 MB of disk space. You should plan to archive or back up the large files to meet your requirements.

## **Chapter 5 Moving and reinstalling** the instrument

This chapter provides information about moving and reinstalling the MegaBACE instrument. Topics include—

- Moving the instrument (section 5.1)
- Required parts for reinstalling the instrument (section 5.2)
- Connecting a new computer (section 5.3)

An Amersham Biosciences representative installs your MegaBACE instrument after delivery. If you need to move the instrument, contact your Amersham Biosciences representative before you move it.

Warning



Do not connect or disconnect the power cords with the power on. Instead, turn off the instrument and computer by following the instructions in the MegaBACE instrument user's documentation. Connecting or disconnecting the power cords with the power on can damage the equipment and cause personal injury.

Important

To avoid loose or lost connections, make sure the screws holding the connectors in place are tight.

#### 5.1 Moving the instrument

Warning



The MegaBACE instrument weighs approximately 272 kg (600 lb). The instrument requires adequate physical support. Never attempt to lift the instrument without using proper equipment and trained personnel. Lifting the instrument without proper support can cause severe or fatal injury.

Caution

Do not attempt to move your MegaBACE instrument. Doing so will void your warranty. Instead, contact MegaBACE System Technical Support to set up an appointment. See Assistance in the preface for contact information.

If you need to move your MegaBACE instrument, review chapters 2 through 4 for information on selecting the new location, as well as the nitrogen pressure and electrical power requirements for your instrument. Make sure you have all the required parts for reinstallation (section 5.2).

#### 5.2 Required parts for reinstalling the instrument

Warning 

Do not install the instrument and computer yourself. Improper installation can cause damage to these components and personal injury.

Make sure you have the following components and parts for reinstalling the instrument.

#### **5.2.1 Major components and parts**

The MegaBACE system includes—

- MegaBACE instrument
- Power supply fan module
- Computer, mouse, monitor, and keyboard
- Accessory kit (includes hoses, cable clamps, cables, fittings, and documents as needed)

#### 5.2.2 Power cords

The MegaBACE system includes power cords for—

- MegaBACE instrument
- Power supply fan module
- Computer
- Monitor

Use only the power cords and cables supplied with the MegaBACE system. Make sure the power cords are in good condition and are not frayed. Use of incorrect power cords can cause damage to the instrument. Use of frayed or damaged power cords can cause injury.

Important

An uninterruptible power supply (UPS) is recommended to reduce the instrument's vulnerability to power line fluctuations.

#### 5.3 Connecting a new computer

To connect your MegaBACE instrument to a new computer, first turn off the MegaBACE instrument using the instructions in the MegaBACE instrument user's documentation.

Make sure that a PCI SCSI adapter card is installed in the computer. The MegaBACE software requires Windows 2000 at a minimum. See the instructions provided with the adapter card and in the Windows manuals. For information about SCSI and network connections, contact MegaBACE System Technical Support. See Assistance in the preface for contact information. See chapter 4 for networking configurations.

Install the MegaBACE Instrument Control Manager and analysis software using the instructions provided with the software.

# **Appendix A Preinstallation forms**

This chapter contains the—

- Site information form (section A.1)
- Preinstallation checklist (section A.2)

#### A.1 Site information form

Fill in the site and system information in the spaces provided below. You and the Amersham Biosciences MegaBACE representative will need this information to schedule your MegaBACE system installation.

Organization name:
User/manager name(s):
Telephone:
Email:
Address:
Additional storage location, if needed:
MegaBACE system serial numbers:
Instrument:
Power supply fan module:
Scheduled ship date:
Scheduled installation date:
Service engineer:
Technical support specialist:

#### A.2 Preinstallation checklist

The checklist below contains the preinstallation requirements. You must satisfy every item in the checklist that is applicable for your system before the system is installed. Please check all the applicable items and fax a copy of the checklist to MegaBACE System Technical Support. See Assistance in the preface for contact information. You will be contacted to verify that you have completed the checklist before system installation.

Check if completed	Task
	General
	Complete each item in this checklist and then schedule installation and training.
	<b>Electrical</b> (See section 2.3 for electrical specifications.)
	Provide an appropriate heating and cooling system to maintain a temperature of 20–25 °C (68–77 °F).
	Provide two dedicated, grounded circuits capable of delivering the appropriate amps and volts AC and terminated with receptacles appropriate for your country (sections 2.3.2 and 2.3.3).
	Provide a phone line in the lab (recommended for talking to MegaBACE Technical Support).
	Laboratory
	Provide a laboratory bench rated to hold the combined weight of the MegaBACE instrument and hardware components: about 331.1 kg (730 lb) (section 2.1). Dimensions: 2.146 m (84.5 in) length by 0.761 m (30 in) width, minimum.
	Provide the appropriate number of nitrogen cylinders for your laboratory configuration (section 2.4).
	Locate a vendor or contractor for the appropriate tubing and regulators for the nitrogen cylinders (section 2.4).
	Provide the appropriate ventilation for the power supply fan module (section 2.2).
	Provide 4 °C storage for the matrix and the LPA buffer.
	Provide –20 °C storage for the samples and loading solution.
	(Sequencing and SNP genotyping only) Provide $-20~^{\circ}\text{C}$ storage for the DNA sequencing reagents or the SNuPe reagents, multiple-injection marker, and spectral matrix standards.
	(STR genotyping only) Provide 4 °C storage for the size standard and spectral matrix standards.

#### Check if completed Task Laboratory equipment and consumables (See section 3.4 for ordering information.) ☐ Microcentrifuge for 1.5-ml or 2.0-ml microcentrifuge tubes (optionally refrigerated) ☐ Centrifuge with microplate-format rotor (optionally refrigerated) ☐ Micropipettors in various volume ranges ■ 8-channel autopipettors in 10-µl and 250-µl volumes ■ Pipettes in various sizes ☐ Tube racks ☐ 2-ml microcentrifuge tubes with screw caps ☐ Heat block or 96-well thermal cycler ☐ Microplates (96-well) □ Plate sealer □ Capillary arrays ■ Deionized filtered water ■ Squirt water bottle ☐ Ice bucket ■ Wet ice ■ Assorted beakers and flasks ☐ Uninterruptible power supply (optional, but recommended) ☐ High-vacuum silicone grease Sequencing only ☐ Speed Vac concentrator with microplate-format buckets ■ Balance ☐ STR genotyping only (recommended for dialysis) ☐ TE buffer concentrate (50×) ☐ 96-well filtration plate assembly ☐ Sealing tape for the filtration plate Pyrex or equivalent glass dish ☐ SNP genotyping only Consumables for PCR cleanup ☐ Consumables for cleanup of SNuPE reaction

#### Check if completed Task Sequencing chemistry (See section 3.2 for ordering information.) ☐ MegaBACE DYEnamic ET Dye Terminator Set ☐ MegaBACE DYEnamic ET primer chemistry (select all that apply) □ -40 M13 Fwd □ SP6 □ T7 ☐ -21 M13 Forward □ -28 M13 Reverse I ☐ -28 M13 Reverse II □ -29 M13 Reverse II ■ MegaBACE reaction buffer ■ MegaBACE Thermo Sequenase ☐ MegaBACE dGTP sequencing blend ☐ MegaBACE 7-deaza dG sequencing blend\* ☐ Other chemistry for sequencing (select all that apply) ☐ MegaBACE long-read matrix (LPA) ■ MegaBACE sequencing standards ☐ M13mp18 (+) strand DNA □ pUC18 □ pUC19 STR genotyping chemistry (See section 3.2 for ordering information.) ☐ MegaBACE long-read matrix (LPA) and buffer ■ MegaBACE loading solution ■ MegaBACE ET size standard ■ Spectral matrix standards ■ MegaBACE genotyping test plate kit SNP genotyping chemistry (See section 3.2 for ordering information.) ☐ MegaBACE long-read matrix (LPA) and buffer ■ Extra MegaBACE buffer ■ MegaBACE loading solution ■ SNuPe genotyping kit ☐ SNuPe multiple injection marker kit ■ SNuPe control kit

\* Choose the blend that is appropriate for your application and conditions.

# Appendix B Training checklist

The checklist below provides a list of items that should be covered when training the MegaBACE system users. You and the Amersham Biosciences MegaBACE representative will determine which tasks to assign to each MegaBACE system user.

Check if completed	Tasks and subtasks				
	Windows (workstation and server)				
	<ul><li>Logon and shutdown procedures</li><li>Features and multitasking</li><li>Windows workstation and server administration</li></ul>				
	Nitrogen cylinder				
	<ul><li>Valve operation</li><li>Tubing hookup</li><li>Cylinder changing</li></ul>				
	Protocols and procedures				
	nstrument hardware components				
	System startup and shutdown Workflow Capillary array installation and replacement Changing optical filters				
	System software				
	System startup  Setting the file storage locations Instrument Control Manager System configuration files Entering the plate ID and instrument parameters Performing a run Transferring plate run data files to an analysis wor Analysis software Retrieving data and performing analysis Evaluating the results of analysis	rkstation			
	System reagents and protocols				
	<ul> <li>Application instrument validation         (M13 standard or genotyping test plate)</li> <li>Sample preparation</li> <li>Injecting matrix</li> <li>Injecting samples</li> </ul>				

Check if completed	Tasks and subtasks		
	Maintenance and troubleshooting		
	☐ System specifications		
	☐ Cleaning the cathode and anode		
	☐ Cleaning the air filter		
	☐ Cleaning the water tanks and bottles		

# Appendix C Summary of laboratory space requirements

This appendix summarizes the space requirements for the MegaBACE system. The table below lists the space requirements for the various components of the system. Refer to the preceding chapters for specific details.

#### Warning



Do not attempt to lift the instrument. The MegaBACE instrument weighs approximately 272 kg (600 lb). Lifting the instrument can cause severe or fatal injury.

Hardware component	Width		Height		Depth	
MegaBACE instrument	1.033 m	(40.7 in)	0.812 m	(32 in)	0.874 m	(34.4 in)
	Allow an additional 0.127 m (5 in) on the side of the instrument for hose and behind the instrument for access.				strument for t	he exhaust
Power supply fan module <sup>*</sup>	0.279 m	(11 in)	0.563 m	(21 in)	0.393 m	(15.5 in)
Instrument control workstation <sup>†</sup>						
Computer tower*	0.178 m	(7 in)	0.432 m	(17 in)	0.425 m	(16.75 in)
Monitor	0.390 m	(15.3 in)	0.372 m	(14.6 in)	0.137 m	(5.4 in)
Keyboard	0.457 m	(18 in)	0.035 m	(1.375 in)	0.171 m	(6.75 in)
Mouse pad	0.203 m	(8 in)	0.006 m	(0.25 in)	0.178 m	(7 in)
Uninterruptible power supply (UPS) (recommended)*	See the manufacturer's specifications.					
Nitrogen cylinders	See the manufacturer's specifications.					
Analysis workstation (optional) $^\dagger$						
Computer tower	0.178 m	(7 in)	0.432 m	(17 in)	0.425 m	(16.75 in)
Monitor	0.390 m	(15.3 in)	0.372 m	(14.6 in)	0.137 m	(5.4 in)
Keyboard	0.457 m	(18 in)	0.035 m	(1.375 in)	0.171 m	(6.75 in)
Mouse pad	0.203 m	(8 in)	0.006 m	(0.25 in)	0.178 m	(7 in)

<sup>\*</sup> The power supply fan module and the computer tower can be located below the laboratory bench. The UPS, if used, can be located on the floor near the laboratory bench. Computer and monitor dimensions can vary depending on the model provided.

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