MegaBACE

Instrument Operator's Guide
Version 2.4

um 375-685

Amersham Biosciences

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June 2002

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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.

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Preface

About this guide

The *MegaBACE Instrument Operator's Guide* describes how to use the MegaBACE™ DNA Analysis System to automatically set up plate definitions and perform runs.

- **Part one: Introduction and safety—**Provides introductory and safety information about the MegaBACE instrument.
- **Part two: Running the MegaBACE system—**Describes how to start the instrument and how to use the Instrument Control Manager software to run plates. Part two also describes how to store the capillaries when leaving the instrument idle or when shutting the instrument down completely.
- **Part three: Appendixes—**Provides a quick reference to the menu commands, windows, and protocols in the Instrument Control Manager software and procedures for sample preparation and spectral calibration runs for genotyping.

Related publications

In addition to the *MegaBACE Instrument Operator's Guide,* the following publications are available for the MegaBACE system:

- *MegaBACE Instrument Maintenance and Troubleshooting Guide* provides instructions on maintaining the instrument and guidelines on troubleshooting.
- *MegaBACE Instrument Administrator's Guide* provides information on how the instrument works and how to manually set up plate definitions, create plate setup and instrument parameter templates, and use the configuration files.
- *MegaBACE Planning Guide* provides instructions for setting up the installation site for the MegaBACE instrument. Reading the planning guide is a prerequisite for the installation of the MegaBACE system.
- MegaBACE analysis software user's guides provide instructions on how to use the software to analyze the data collected from the MegaBACE instrument.

- The MegaManual provides detailed troubleshooting guidelines for the sequencing application. The MegaManual is posted in the MegaBACE User Zone at the following Web site:

  www.amershambiosciences.com

  You need the serial number of your instrument to obtain a password to the User Zone.

In addition, electronic versions of most of the documents listed above are available on the software CD provided with your MegaBACE system.

Safety

Chapter 2 in this guide provides important safety information to be used in conjunction with your training. Read and understand it thoroughly before you begin operating the instrument.

Special safety text

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

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

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

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

Note: Identifies items of general interest.

Trained operator

Warning ⚠ **The operator of the MegaBACE instrument is assumed to be trained in the correct operation of the instrument and the safety issues. Throughout the MegaBACE instrument documentation, the word “you” refers to this trained operator.**

Assumptions

The software-related instructions in this user's guide assume you have basic computer skills. You should be familiar with the Microsoft™ Windows NT™ or Windows™ 2000 graphical user interface. If you do not have these skills, refer to the Windows NT or Windows 2000 documentation or refer to the Help for Windows NT or Windows 2000.

Safety standards

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

MegaBACE system site requirements

Electrical requirements

MegaBACE instrument

- Fuse rating: Total of 6 fuses—2A, 250V~ (quantity 2) and 5A, 250V~ (quantity 4)
- Fuse type: Type T (slow acting)
- Electrical rating: 200–240V~ 6A 50/60Hz

Power supply fan module

Electrical rating: 180–229V~ or 230–264V~ 10A 50/60Hz

Environmental conditions

- Ambient temperature range: 20–25 °C (68–77 °F)
- Humidity condition: ≤ 80% noncondensing
- Pollution degree: 2
- Installation category: II

Assistance

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 (figure 2-2). For contact by phone or fax, please use one of the numbers below.

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 3667 5700
Fax: +55 11 3667 87 99

Middle East and Africa
Tel: +30 (1) 96 00 687
Fax: +30 (1) 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: +7 (095) 232 0250, 956 1137
Fax: +7 (095) 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

Web site

http://www.amershambiosciences.com

Part one

Introduction and safety

Chapter 1 Introduction to the MegaBACE system

The MegaBACE DNA Analysis System is a high-throughput automated gene analysis system.

This chapter describes—

- System hardware components (section 1.1)
- System functions (section 1.2)
- Overview of instrument operation (section 1.3)
- Overview of the Instrument Control Manager software (section 1.4)
- Before you begin (section 1.5)

Table 1-1 lists the available models of the MegaBACE instrument. Throughout this guide, some section titles and some paragraphs use the model name to highlight model-specific topics.

Table 1-1. Available models of the MegaBACE instrument

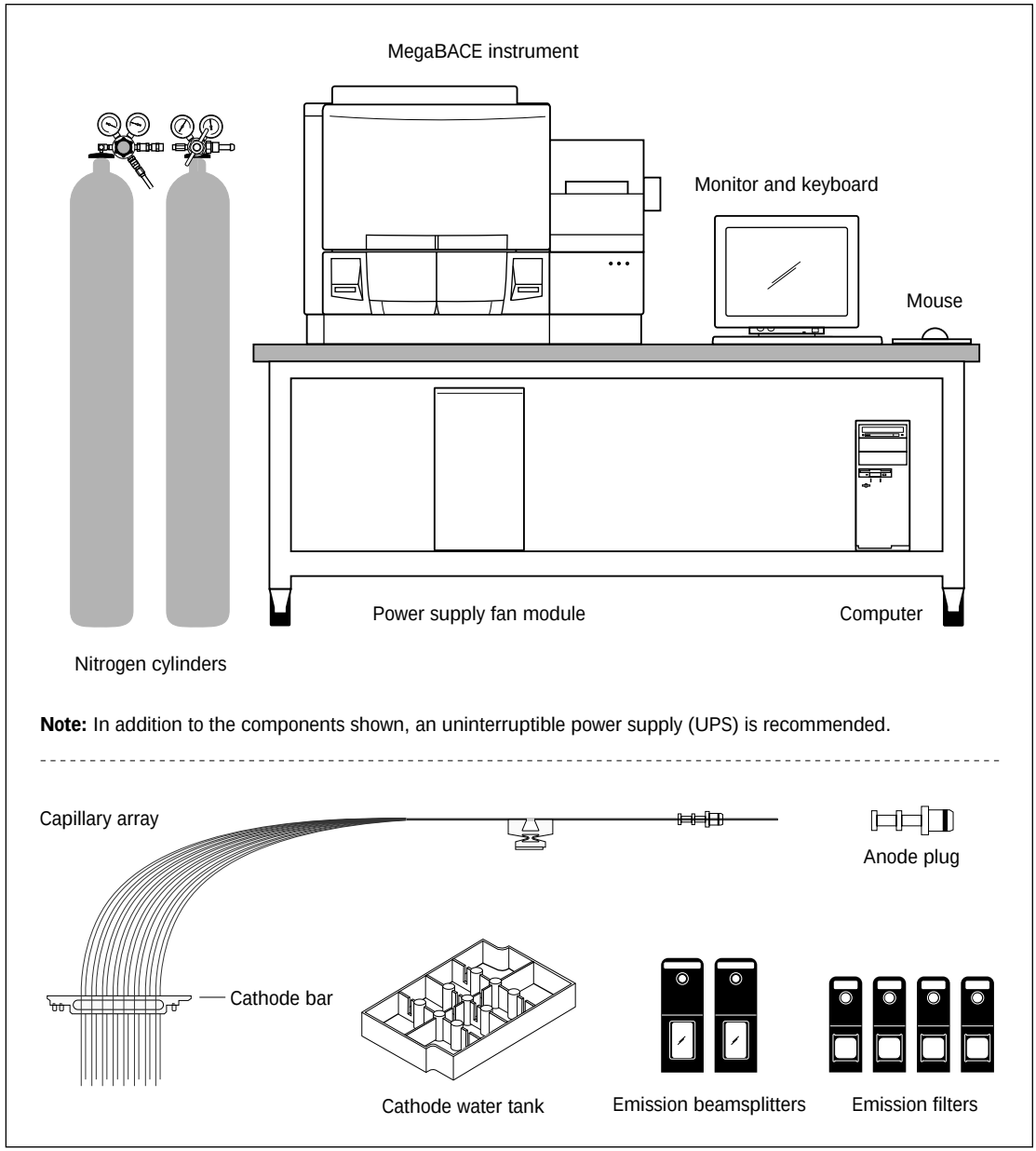
Model	Description
MegaBACE 500	A modified MegaBACE 1000 that accepts only one, two, or three 16-capillary arrays for a capacity of 16, 32, or 48 capillaries.
Flexible MegaBACE 1000	A modified MegaBACE 1000 that accepts up to six 16-capillary arrays for a capacity of 16, 32, 48, 64, 80, or 96 capillaries.
MegaBACE 1000	The standard model with 96 capillary capacity, grouped in six 16-capillary arrays.

1.1 System hardware components

The MegaBACE system consists of the following hardware components (figure 1-1):

- **MegaBACE instrument—**Electrophoresis components and temperature regulation system, lasers and light-collection system, and scanner electronics.
- **Power supply fan module—**Blue laser power source and fan for cooling the laser.
- **Computer—**Computer, monitor, keyboard, and mouse.
- **Other components included with the instrument—**Cathode water tank, anode plugs, emission beamsplitters and filters, and capillary arrays.
- **(MegaBACE 500 and flexible MegaBACE 1000 instruments only) Additional components—**MegaBACE 500 anode cover, and for both modes, array placeholder, which consists of an anode blocker, an anode sleeve, a window blank, and a cathode plunging tool (figure 1-2).
- **Accessory kit—**Hoses, cables, fittings, tools, documents, and software CD.
- **Optional items not included—**
  - Bar-code reader
  - Uninterruptible power supply (UPS) with battery storage (recommended)

The system uses a **nitrogen pressure source** (cylinder with regulators or multiunit manifold). The *MegaBACE Planning Guide* provides the specification for the nitrogen system. For more information on the nitrogen pressure source, see the *MegaBACE Instrument Maintenance and Troubleshooting Guide.*



Note: In addition to the components shown, an uninterruptible power supply (UPS) is recommended.

Figure 1-1. The MegaBACE system and components.

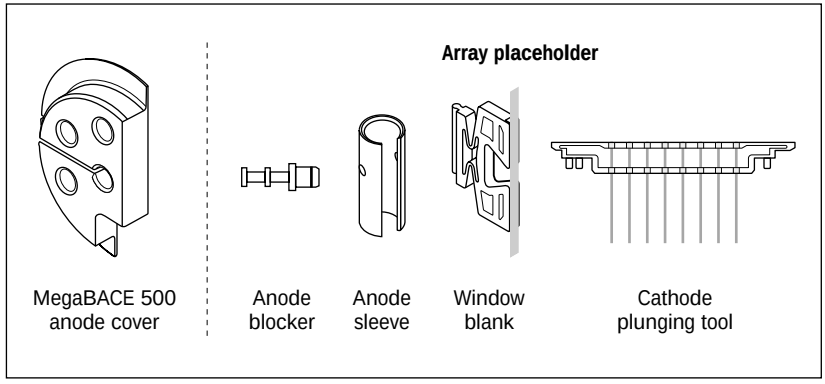


Figure 1-2. Additional components (MegaBACE 500 and flexible MegaBACE 1000 instruments only).

1.2 System functions

The MegaBACE instrument uses capillary array electrophoresis to perform fragment size separation on the DNA samples and to produce electropherograms. The MegaBACE instrument and computer work interactively to—

- Automatically fill the capillaries with matrix, remove spent matrix, and maintain the capillaries between runs and during inactive periods.
- Automatically inject and electrophorese DNA samples, continuously scan the capillaries with laser excitation of the fluorescently labeled samples, and collect and record electrical current data and fluorescence readings from each capillary.
- Display the capillary array four-color fluorescence and electrical current data in real time during the run.

In addition, the MegaBACE software allows you to—

- Analyze the run data and view the raw or spectrally separated and analyzed four-color electropherograms of the samples.
- Store the raw and analyzed data, export the data and results (for example, to other software for further analysis), and print the results.

For additional detailed information on the MegaBACE instrument, see the *MegaBACE Instrument Maintenance and Troubleshooting Guide* and the *MegaBACE Instrument Administrator's Guide.* For detailed information on the analysis software, see the appropriate MegaBACE analysis software user's guide.

1.3 Overview of instrument operation

The MegaBACE instrument is used to inject and scan a plate of samples. This process is called a run. During a run—

1. The MegaBACE instrument pressure fills the capillaries with sieving matrix.
2. The instrument applies a voltage pulse to electrokinetically inject a portion of the fluorescently labeled sample from each well in the plate simultaneously into the capillaries. The DNA fragments in the sample separate by size, with the shorter fragments moving faster through the matrix than the longer fragments.
3. The instrument uses laser light to scan the capillaries containing the fluorescently labeled samples. The laser light excites the fluorescent dyes in the samples (up to four colors per capillary), which in turn emit fluorescent light.
4. The instrument uses two beamsplitters to split the emitted fluorescent light, and then filters the light using four filters. Each filter permits only a specific range of light, corresponding to the emissions of one of the dyes, to pass through to a photomultiplier tube (PMT).
5. Two PMTs detect the filtered light and convert the light into an electrical current, which is digitized to produce an electropherogram for each capillary.

For details on how to perform a run, see chapter 4.

1.4 Overview of the Instrument Control Manager software

This section provides a brief overview of the Instrument Control Manager software, which automates the process of plate setup, data collection, and data analysis.

The Instrument Control Manager allows you to—

- Set up plate definitions and import plate setup parameters and sample names automatically.
- Collect data.
- (Sequencing only) Automatically call bases and export the data to other file formats after base calling.

You use the Plate Setup window (figure 1-3) to define a plate. You first type a plate ID or scan a bar code. Then the Instrument Control Manager automatically—

- Loads the parameters from a default parameter template.
- Imports an existing parameter file.

The Instrument Control Manager uses two different kinds of parameters—

- The plate setup parameters, which become a part of the plate definition when you enter a plate ID.
- The instrument parameters, which are used when you perform a run.

See chapter 4 for details about automatic plate setup and instructions on performing runs.

(Sequencing only) You can increase the sequencing throughput by performing automatic base calling and automatically exporting the data to ABD, FASTA, SCF, and ASCII file formats. This feature allows you to continuously collect data, and then analyze the data and create the various types of export files without manual intervention.

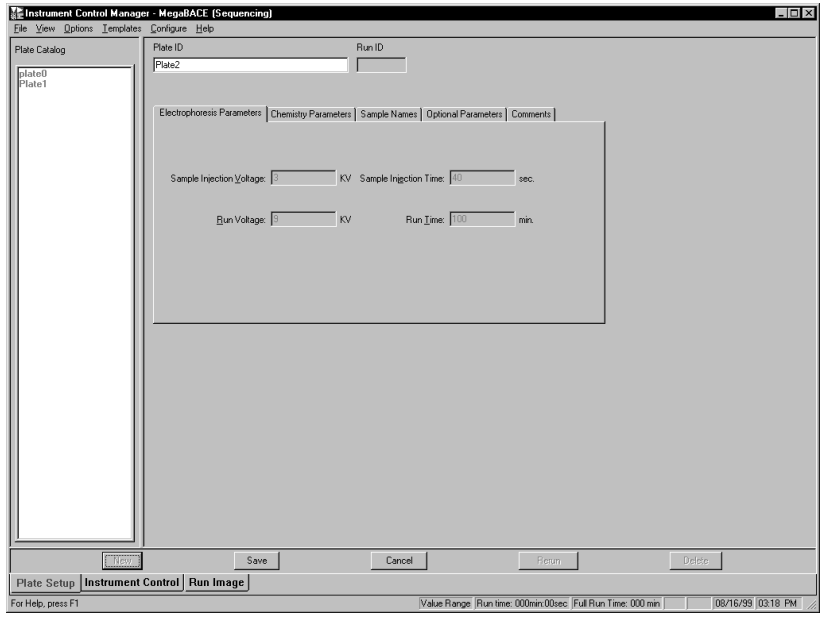


Figure 1-3. The Plate Setup window for sequencing.

1.5 Before you begin

Before using the MegaBACE system, become familiar with—

- Chapter 2: Safety precautions
- Chapter 3: Starting the MegaBACE system
- Chapter 4: Instructions on how to use the MegaBACE instrument and the Instrument Control Manager software to perform a run.

Chapter 2 Safety precautions

The MegaBACE instrument and its accessories have been designed for safe operation. It is imperative that you follow the precautions in this chapter. The topics are—

- General safety precautions (section 2.1)
- Locations of important labels (section 2.2)
- Cathode and anode compartments and instrument displays (section 2.3)
- Electrophoresis compartment (section 2.4)
- Filter compartment (section 2.5)
- Internal electronics (section 2.6)
- Chemicals (section 2.7)
- Nitrogen cylinders and pressure regulators (section 2.8)
- Lasers (section 2.9)
- PMTs (section 2.10)
- Power supply fan module, computer, and monitor (section 2.11)
- System electrical connections (section 2.12)
- Serial number labels (section 2.13)
- Service for the MegaBACE instrument (section 2.14)

2.1 General safety precautions

While using the MegaBACE instrument, you should follow the laboratory procedures appropriate for the experiments you are performing.

Warnings

The operator of the MegaBACE instrument is assumed to be trained in the correct operation of the instrument and the safety issues. Throughout the MegaBACE instrument documentation, the word "you" refers to this trained operator.

Using controls, making adjustments, or performing procedures other than those specified in this guide may result in hazardous exposure to laser light, high voltage, high pressure, or moving parts. Such exposure can cause severe or fatal injury.

Under normal operating conditions, you are protected from laser light, high voltage, high pressure, and moving parts. The cathode and anode drawers and the electrophoresis compartment lid are fitted with sensors and interlocks. The access lid of the filter compartment has a safety switch. Figure 2-1 shows the locations of the drawers and lids used during routine operation of the instrument.

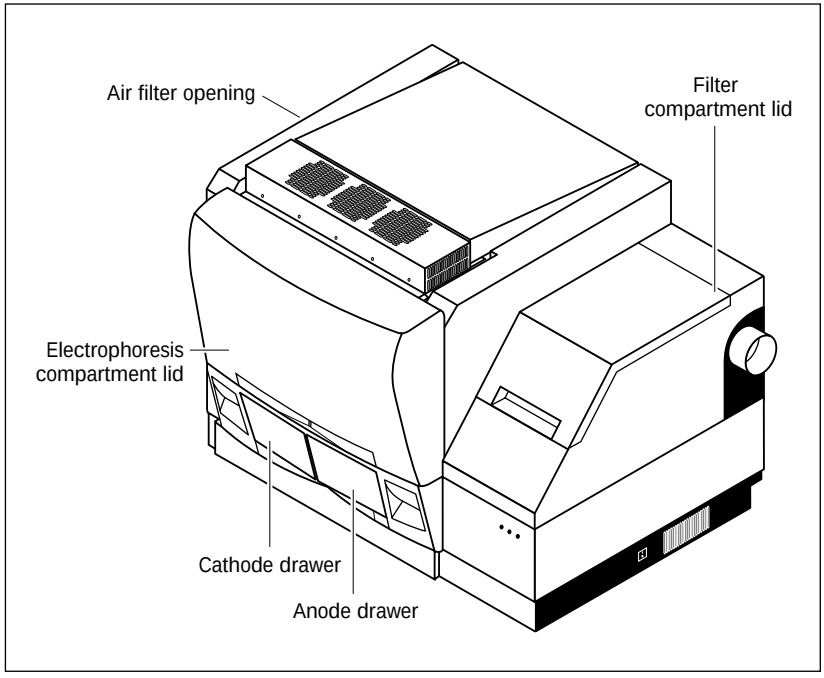


Figure 2-1. Locations of the drawers and lids used during routine operation of the MegaBACE system. The air filter opening is used infrequently.

Warnings

Do not defeat the sensors and interlocks or try to gain access to the interior of the instrument through any other opening. Do not remove panels for any reason. Exposure to laser light, high voltage, high pressure, or moving parts inside the instrument can cause severe or fatal injury.

To prevent hazardous exposure to laser light, check the cover panels all around the instrument regularly. If laser light is visible in the electrophoresis compartment, you should immediately turn off the instrument and call MegaBACE System Technical Support. See Assistance in the preface for contact information.

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.

2.2 Locations of important labels

The locations of important labels on the MegaBACE instrument are shown in figures 2-2 and 2-3. Figure 2-4 shows the location of the serial number certification label on the power supply fan module.

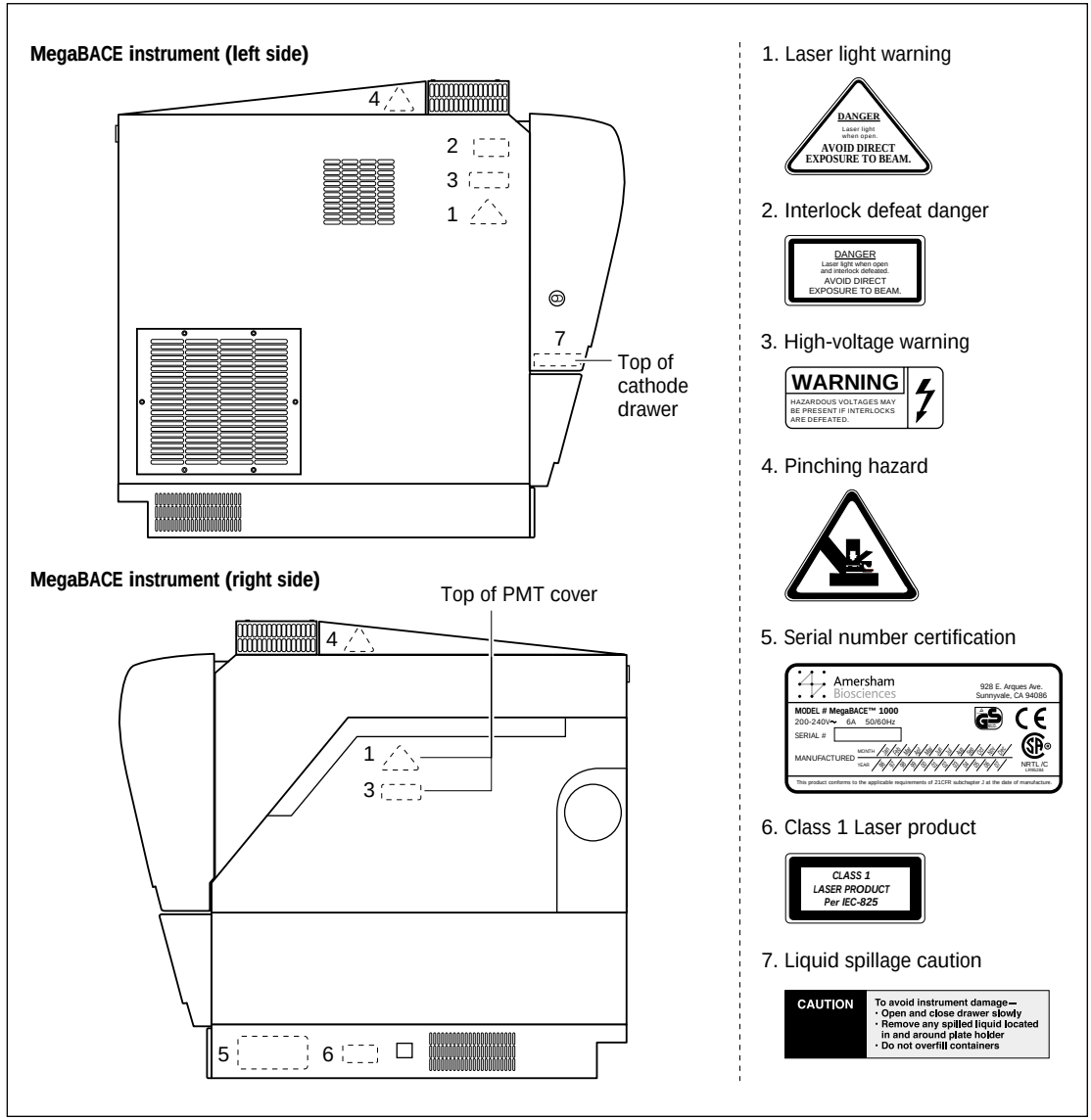
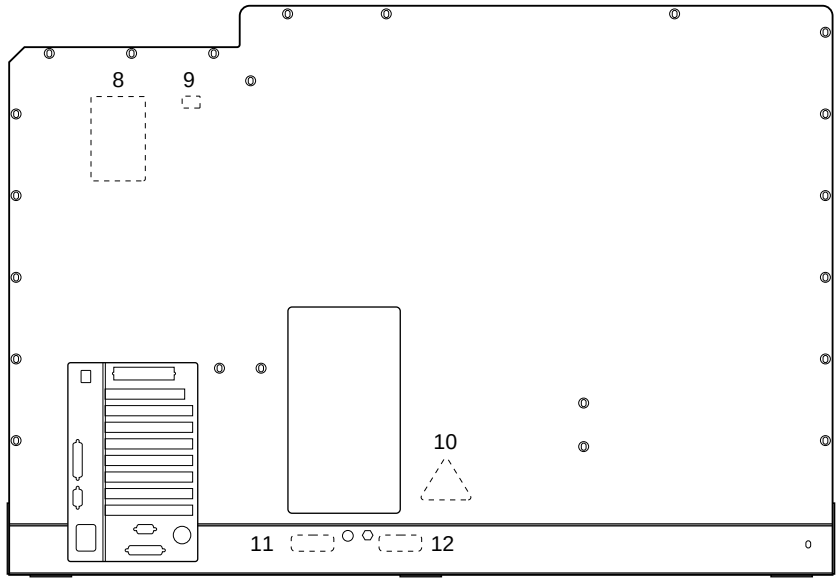


Figure 2-2. Locations of important labels on the MegaBACE instrument (side views).



MegaBACE instrument (back view)

8. License agreement

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AMERSHAM IS A LICENSED VENDOR FOR AUTHORIZED REAGENTS

9. Made in U.S.A.

10. Nitrogen pressure general hazard

11. Low-pressure nitrogen

100 PSI
(6.89 X 10^2 kPa)

12. High-pressure nitrogen

1000 PSI
(6.89 X 10^3 kPa)

Figure 2-3. Locations of important labels on the MegaBACE instrument (back view).

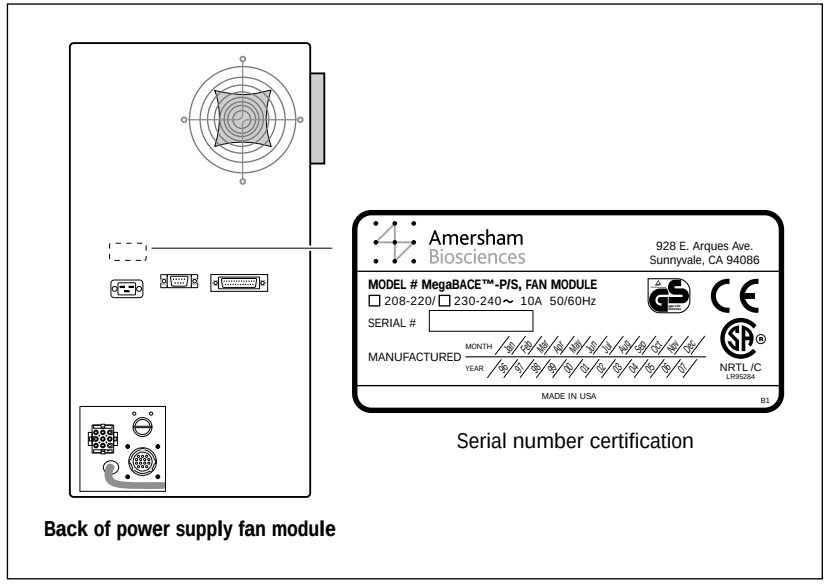


Figure 2-4. Location of the serial number certification label on the power supply fan module.

If a label becomes illegible for any reason, please contact MegaBACE System Technical Support for a free replacement label. While waiting for the replacement label, copy the label from the appropriate figure in this chapter and attach the copy to the instrument.

2.3 Cathode and anode compartments and instrument displays

When the workflow requires you to access the cathode or anode compartment, the system shuts off the high voltage and nitrogen pressure and lowers the cathode or anode stage before unlocking the corresponding drawer.

Caution **Do not overfill the water tank. Open and close the cathode drawer slowly. Remove any liquid that has been spilled in and around the plate holder. Failure to remove the spilled liquid can result in damage to the instrument.**

Figure 2-5 shows the liquid spillage caution label. Figure 2-2 shows the location of the label on top of the cathode slider inside the cathode drawer.

After you open the cathode or anode drawers, the displays on the front of the instrument instruct you to perform the next step.

No voltage, pressure, or laser light can be applied as long as either drawer remains open. When you close the cathode or anode drawer, the software assumes that you have performed the step shown on the instrument display. The drawer locks, and the system raises the stage. The system automatically moves to the next step.

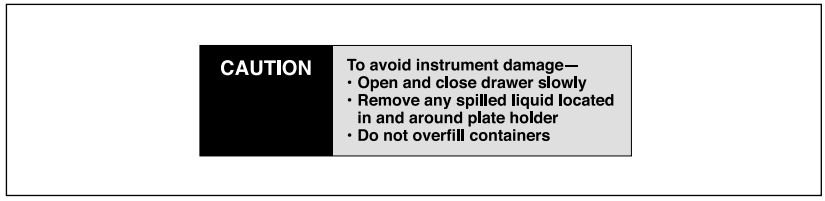


Figure 2-5. The liquid spillage caution label.

2.4 Electrophoresis compartment

You may occasionally need to open the electrophoresis compartment lid.

Warning ⚠

When the electrophoresis compartment lid is open, do not place your hands on or near the two support bars on each side of the lid. If the lid moves, your fingers can be pinched.

Achtung

WENN DER DECKEL DER ELEKTROPHORESE-KAMMER GEÖFFNET IST, FINGER NICHT AN ODER AUF DIE BEIDEN HALTESCHIENEN AUF JEDER SEITE DES DECKELS LEGEN. WENN DER DECKEL SICH BEWEGT, KÖNNEN FINGER EINGEKLEMMT WERDEN.

The label shown in figure 2-6 warns of this pinching hazard. Figure 2-2 shows the locations of two pinching hazard labels, one on each side of the top portion of the instrument.

Figure 2-6. The pinching hazard label.

Under normal operating conditions, you are protected from high voltage. Nevertheless, during the prerun and sample electrophoresis, voltages up to 20 kV are present in the electrophoresis compartment. The label in figure 2-7 warns of this danger and is located on the left side of the instrument on the side wall inside the electrophoresis compartment and on the photomultiplier tube (PMT) cover. Figure 2-2 shows the locations of the label.

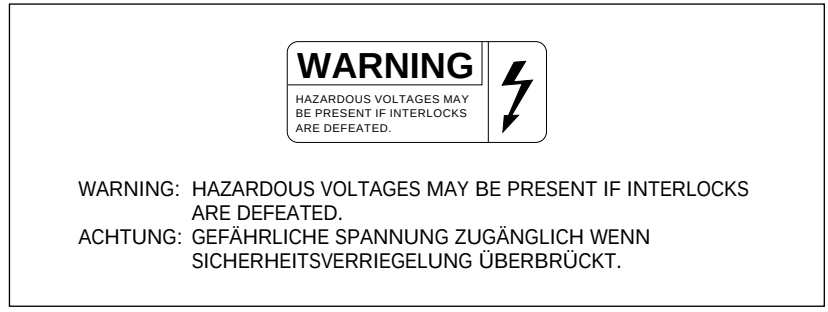


Figure 2-7. The high-voltage warning label.

Warnings

The instrument has sensors and interlocks that are designed to protect you from moving parts, high pressure, hazardous voltage, or laser light. Do not defeat the sensors or interlocks. Do not remove panels for any reason. Exposure to these hazards can cause severe or fatal injury.

Check the operation of the interlock on the electrophoresis compartment lid periodically to make sure the interlock is functioning properly.

When you replace capillary arrays, do not pull on the capillaries to release the cathode bar or the anode plug. The capillaries are fine glass tubes and can break, leaving sharp ends or fragments, which can damage the instrument or cause injury.

Cautions

Do not leave any objects inside the electrophoresis compartment or on the stages. Metal objects can cause arcing when high voltage is applied during electrophoresis, possibly damaging the instrument.

Always avoid touching the windows of the capillaries. Oils and salts from your skin could result in arcing between capillaries during high-voltage electrophoresis, which could damage the instrument.

Avoid spills in the chamber and below the cathode stage. Clean all spills immediately and call MegaBACE System Technical Support for information on how to clean any large internal spills below the anode and cathode stages. A spill in the high-voltage area can cause arcing and damage the instrument.

Caution **Opening the electrophoresis compartment lid during an electrophoresis run interrupts the data recording. Open the lid between runs only. If you need to open the lid during a run, stop the run before opening the lid to protect the data you have already collected.**

Note: The capillaries become warm during electrophoresis.

For your protection, sensors make sure that when the lid opens—

- If the electrophoresis voltage is on, the high-voltage power supply shuts off, and the voltage drains.
- If the laser shutter is open, the shutter closes and blocks the laser light from entering the compartment.
- If nitrogen pressure is present in the anode vessel, the pressure shuts off, and the pressure vents.

In addition, the temperature control for the electrophoresis compartment turns off. You cannot scan until you close the lid.

2.5 Filter compartment

To make sure data is recorded properly, you should check that the appropriate filters and beamsplitters are installed before starting an electrophoresis run. (For details on changing filters and beamsplitters, see the *MegaBACE Instrument Maintenance and Troubleshooting Guide*.)

Caution **Opening the filter compartment lid during an electrophoresis run interrupts data recording. Open the lid between runs only. If you need to open the lid during a run, stop the run before opening the lid to protect the data you have already collected.**

When you open the filter compartment lid, the system shuts off the voltage to the PMTs, which protects the PMTs and stops the data collection.

2.6 Internal electronics

Under normal operating conditions, you are protected from high voltage within the instrument electronics. Nevertheless, voltages up to 20 kV are present in the instrument during a scan. The label in figure 2-7 warns of this danger. Figure 2-2 shows the locations of the label on the left side of the instrument on the side wall inside the electrophoresis compartment and on the PMT cover.

Warning

The instrument has sensors and interlocks that are designed to protect you from moving parts, high pressure, hazardous voltage, or laser light. Exposure to such hazards can cause severe or fatal injury. Do not remove panels for any reason. Do not defeat the sensors or interlocks or try to gain access through any other opening.

Note: You can, however, remove the air filter panel on the left side of the instrument to clean the air filter (see the *MegaBACE Instrument Maintenance and Troubleshooting Guide*).

2.7 Chemicals

Warning

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.

2.8 Nitrogen cylinders and pressure regulators

The MegaBACE system requires the use of high-pressure nitrogen sources.

2.8.1 Handling high-pressure cylinders and tubing

Always use good laboratory procedures when handling a high-pressure cylinder and follow any instructions provided with the cylinder.

Warning

High-pressure connection. Do not disconnect tubing without bleeding the tubes. Disconnecting without bleeding can cause injury.

Achtung

HOCHDRUCKVERBINDUNG. SYSTEM ENTLÜFTEN BEVOR SCHLAUCHVERBINDUNG GELÖST WIRD. LÖSEN DER VERBINDUNG OHNE ENTLÜFTEN KANN ZU VERLETZUNGEN FÜHREN.

The label in figure 2-8 warns of this danger. Figure 2-3 shows the location of the label on the back of the instrument.

Figure 2-8. The nitrogen pressure general hazard label.

Warning

Make sure a standard cylinder bracket is bolted to a solid permanent structure in a manner that meets or exceeds all local seismic and safety code requirements.

2.8.2 Regulating the nitrogen pressure

The regulators on the external nitrogen cylinder(s) control the amount of nitrogen pressure applied within the instrument. The hose size, the hose characteristics, and the fittings inside the instrument are designed to withstand the working pressures.

Warnings

Do not attempt to adjust the regulators to pressure settings above those described in this guide. 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 6.89×10^3 kPa (1000 psi) of pressure. Never apply high pressure to the low-pressure line. This can damage the instrument or the low-pressure line and can cause injury.

Figure 2-9 shows the labels that are placed on the back of the instrument next to the high- and low-pressure nitrogen line connections. Figure 2-3 shows the locations of the labels.

Warning

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

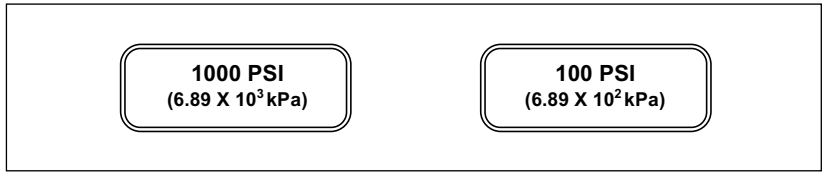


Figure 2-9. The high- and low-pressure nitrogen labels.

2.9 Lasers

Warning

Changing controls, making adjustments, or performing procedures other than those specified in the MegaBACE instrument documentation can result in hazardous laser light exposure.

2.9.1 Class 1 Laser Product label

The MegaBACE instrument satisfies the Class 1 requirements of IEC 825-1:1993 and EN 60825-1. Figure 2-10 shows the Class 1 Laser Product label. Figure 2-2 shows the location of the label on the lower-right side of the instrument.

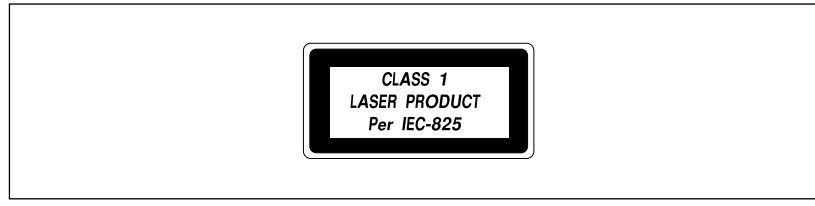


Figure 2-10. The Class 1 Laser Product label.

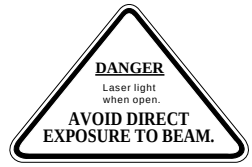
2.9.2 Laser light warning label

The instrument does not allow operator exposure to laser light. Nevertheless, the instrument contains a blue argon-ion laser with power up to 25 mW at 488 nm with a 0.95-mrad divergence. The instrument can also contain a green solid-state laser with power up to 50 mW at 532 nm with 1.2-mrad divergence.

Warning

Do not remove any of the inner covers of the MegaBACE instrument. The laser power specified in the paragraph above could be accessible if you remove the inner covers.

The label in figure 2-11 warns of laser light danger. Figure 2-2 shows the locations of the label on the PMT cover and in the electrophoresis compartment of the instrument.



DANGER: LASER LIGHT WHEN OPEN. AVOID DIRECT EXPOSURE TO BEAM.
GEFAHR: LASER! NACH DEM ÖFFNEN DIREKTEN KONTAKT ZUM LASERSTRAHL VERMEIDEN.

Figure 2-11. The laser light warning label.

2.9.3 Safety interlock danger label

The label in figure 2-12 warns of the laser danger from defeating the interlock on the electrophoresis compartment. The label is located on the left side of the instrument on the side wall inside the electrophoresis compartment. Figure 2-2 shows the location of the label.

Warning

Do not defeat the interlocks or try to gain access to the interior of the MegaBACE instrument through any other opening. Exposure to laser light can cause injury.

DANGER: LASER LIGHT WHEN OPEN AND INTERLOCK DEFEATED.
AVOID DIRECT EXPOSURE TO BEAM.
GEFAHR: LASERSTRAHLUNG, WENN ABDECKUNG GEÖFFNET UND SICHERHEITSVERRIEGELUNG ÜBERBRÜCKT.

Figure 2-12. The interlock defeat danger label.

2.9.4 Light leaks

If a panel becomes damaged and the MegaBACE instrument is no longer light-tight, do not continue to use the instrument.

Caution

Ambient light can damage electrical components in the MegaBACE instrument, such as the PMTs. Call MegaBACE System Technical Support immediately to arrange for repair. See Assistance in the preface for contact information.

2.10 PMTs

The PMTs are covered by a protective housing and are not accessible by the operator. During a prerun or electrophoresis run, the PMTs carry a high voltage, which can cause injury if you touch them.

Warning

Do not try to gain access to the PMTs or remove the protective panels for any reason. Exposure to high voltage from the PMTs can cause severe or fatal injury.

2.11 Power supply fan module, computer, and monitor

Voltages are exposed inside the power supply fan module, computer, and monitor. See the computer manufacturer documentation for the high-voltage hazard warning. Make sure you follow the instructions for the safe operation of the computer.

Warning

Do not open the power supply fan module or monitor. Open the computer only by following the computer manufacturer's instructions.

2.12 System electrical connections

The MegaBACE system includes four devices that require electrical power: the instrument, the power supply fan module, the computer, and the monitor. A total of four electrical power cords are supplied with each system, one for each of these main components. See your *MegaBACE Planning Guide* for detailed electrical requirements.

Important

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

Warning

Use only the power cords supplied. 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.

You should use an uninterruptible power supply (UPS) rated for at least 4 kVA to protect the instrument, the capillaries, and your data from damage or loss caused by unexpected power failures, surges, or AC line fluctuations. A UPS also acts as a power line regulator, line conditioner, and surge suppressor and works to protect against all power line problems.

Cautions

In the event of a power failure (chapter 6), a UPS might not contain enough stored power to finish the run and allow the capillaries to be flushed for storage. Contact MegaBACE System Technical Support for information about a qualified UPS. See Assistance in the preface for contact information.

Plug the computer and monitor into the UPS. Make sure the voltage selection switch on the back of the computer correctly matches the voltage at the outlet.

2.13 Serial number labels

2.13.1 Instrument serial number label

You can find the serial number and model number of your MegaBACE instrument on the serial number label (figure 2-13). The label is located on the lower right side of the MegaBACE instrument. Figure 2-2 shows the location of the label. You will need the serial number when contacting MegaBACE System Technical Support about your instrument.

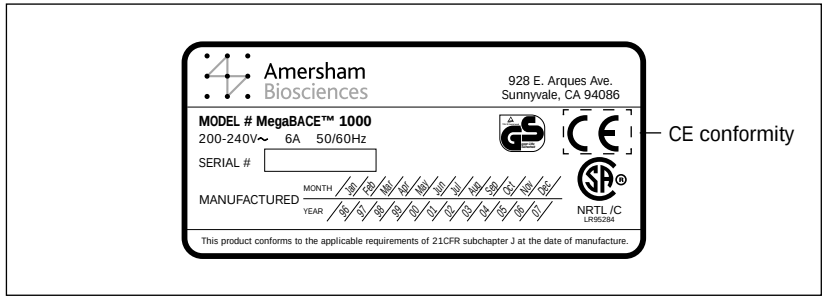


Figure 2-13. The MegaBACE instrument serial number certification label.

2.13.2 Power supply fan module serial number label

You can find the serial number and model number of the power supply fan module on the serial number certification label (figure 2-14). The label is located on the back of the power supply fan module. Figure 2-4 shows the location of the label. You will need the serial number when contacting MegaBACE System Technical Support about your instrument.

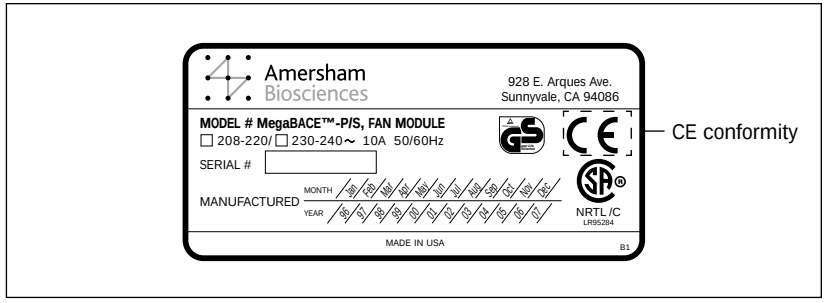


Figure 2-14. The power supply fan module serial number certification label.

2.14 Service for the MegaBACE instrument

To protect your warranty and for proper operation, the instrument should be serviced only by an authorized service representative. If the instrument is not working correctly, call MegaBACE System Technical Support. See Assistance in the preface for contact information.

When you call MegaBACE System Technical Support, be prepared to give the serial number of your instrument or power supply fan module. You can find the serial numbers on the serial number certification labels (figures 2-2, 2-4, 2-13, and 2-14).

Part two

Operating the MegaBACE system

Chapter 3 Starting the MegaBACE system

The MegaBACE system requires specific startup procedures to prepare the instrument for operation, warm up the laser(s) and electrophoresis compartment, and prepare the capillaries for matrix fill and sample injection.

Important

In general, you leave the instrument power on unless the instrument is being serviced or stored. The instrument can be left on and idle for 3 days (including 3-day weekends). For more than 3 days, you shut the instrument down and store the capillaries dry.

For detailed instructions on leaving the instrument idle overnight or for up to 3 days, see section 6.1. For detailed instructions on leaving the instrument shut down for more than 3 days, see section 6.2. For instructions on rehydrating the capillaries after they have been stored dry, see section 6.5.

This chapter describes the procedures you use to start the instrument. The topics in this chapter are—

- Preparing the instrument for operation (section 3.1)
- Starting the system (section 3.2)
- Starting the Host Scan Controller software (section 3.3)
- Starting the Instrument Control Manager software (section 3.4)
- Changing the application (section 3.5)
- Changing the storage location for the raw sample data files (section 3.6)
- Using the Automatic Base Calling feature (sequencing only) (section 3.7)

For a description of the various workflows, see chapter 4. For the shutdown procedures, see chapter 6.

3.1 Preparing the instrument for operation

Before you turn on the MegaBACE instrument, see chapter 2 for important safety information and check the following:

Nitrogen pressure system

- The nitrogen cylinder(s), regulators, and tubing are connected correctly and in good condition.
- The cylinder(s) contain sufficient pressure to complete one run (based on usage in your lab).
  - High pressure: 6.89×10^3 kPa (1 000 psi)
  - Low pressure: 6.89×10^2 kPa (100 psi)

Laser cooling and cables

- The laser cooling air hoses and the control cables are connected correctly and in good condition.
- Nothing is blocking free air access to the air vents on the sides and top of the MegaBACE instrument and on the back of the power supply fan module (figure 3-1). The exhaust on the side of the power supply fan module can be connected to an exhaust hose that is vented out of the room. If the exhaust fan is external to the power supply fan module, make sure the fan is on. You should plug the power supply fan module into an uninterruptible power supply (UPS).

Power connections

- The MegaBACE instrument, computer, and monitor are plugged in. You should plug these components, including the power supply fan module, into a UPS. Make sure that the UPS is plugged in and turned on (section 2.12).
- The power supply fan module is plugged in, and the key on the back of the unit is in the horizontal (on) position (figure 3-1).

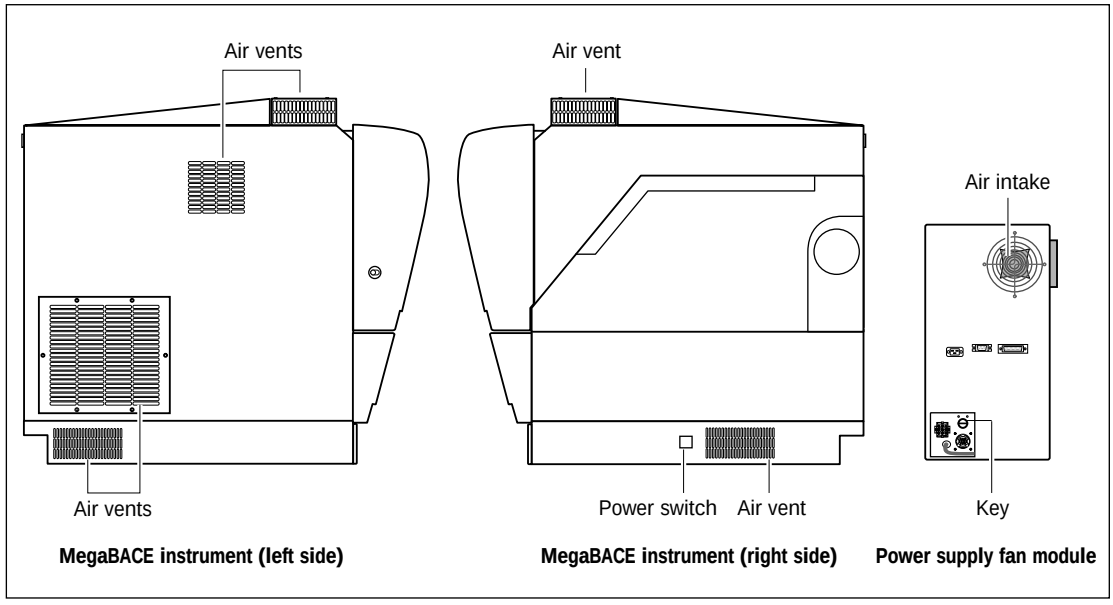


Figure 3-1. Make sure the airflow openings on the MegaBACE instrument and power supply fan module are free of obstructions.

3.2 Starting the system

Normally, you leave the instrument turned on, even if you are leaving it idle for a period of time (overnight or on the weekend). You should turn off the instrument before servicing or before storing for more than 3 days.

Caution **If you are turning off the instrument for a period of more than 3 days, be sure you store the capillaries dry. See chapter 6 for instructions on storing the capillaries dry, shutting down the system, and rehydrating the capillaries after they have been stored dry.**

To start the system for first-time operation, use the Prepare Capillaries protocol described in section 6.5 before running a plate of samples.

3.2.1 Turning on the nitrogen pressure system

If the nitrogen pressure system is not already on, turn on the valve at the top of the cylinder. Next, set—

- The high-pressure nitrogen regulator gauge to 6.89×10^3 kPa (1 000 psi)
- The low-pressure nitrogen regulator gauge to 6.89×10^2 kPa (100 psi)

3.2.2 Turning on the instrument and computer

Always turn on the instrument and computer in the following order:

1. Turn on the power switch on the right side of the instrument (figure 3-1). The instrument starts up and—
   - The power light on the front of the instrument turns on.
   - The electronics in the instrument begin to warm up.
   - The instrument beeps five times.
   - After the internal diagnostics are complete, a MegaBACE message appears in the displays on the front of the instrument.
2. After turning on the instrument, wait at least 45 seconds. Then turn on the computer and the monitor.

Note: After you turn on the computer, it checks for connected instruments that have been turned on. The computer then tracks these instruments and their SCSI (small computer system interface) locations as long as the computer remains on.

If you leave the computer on and turn on or turn off the instrument, the computer still recognizes and can communicate with the instrument. However, if you turn off the computer, you must repeat steps 1 and 2 above.

3.2.3 Warming up the system

After you turn on the instrument power switch, the internal electronics take approximately 5 minutes to warm up. The blue laser warms up in 5 minutes and remains in the idle mode until you perform a scan. If the instrument also contains a green laser, the green laser warms up in 10 minutes. The green laser does not have an idle mode. If the shutter fails, the software turns off the laser(s) and does not allow you to turn them on until the shutter is fixed.

The air in the electrophoresis compartment reaches the temperature set in the Instrument Control window in a few minutes. The components in the compartment stabilize at the set temperature in approximately 3 hours, depending on the temperature change involved.

Table 3-1 provides examples of approximate warmup times from room temperature or for changes in setpoint.

Table 3-1. Examples of warmup times from room temperature or for changes in setpoint. **Note:** The instrument always shows temperatures in °C.

Temperature change	Wait time
<5 °C (<9 °F)	1 minute
5–10 °C (9–18 °F)	10 minutes

Caution **Opening the electrophoresis compartment lid causes the temperature in the compartment to drop. You must allow time for the electrophoresis compartment to rewarm to the temperature you set for the run. Insufficient temperature can cause unreliable data collection results.**

Table 3-2 provides examples of the warmup times you should allow before you perform a plate run. If you perform a plate run before the compartment has stabilized to the set run temperature, the quality of the data collected will be unpredictable.

Table 3-2. Examples of warmup times for the electrophoresis compartment for a set temperature of 44 °C (111.2 °F)

Time open	Warmup time
1 minute	1 minute
1–15 minutes	10 minutes
> 15 minutes	30 minutes

3.3 Starting the Host Scan Controller software

You use the Host Scan Controller software to start communication between the MegaBACE instrument and the Instrument Control Manager software. After you start the Instrument Control Manager, you can minimize the Host Scan Controller and use the Command Log tab on the Instrument Control window to monitor the running of the system (section 5.1).

To start the Host Scan Controller software—

1. Double-click the MegaBACE folder on the Windows NT or Windows 2000 desktop (figure 3-2) to open the folder and display the icons for the MegaBACE software. (Alternatively, you can start the Host Scan Controller software using the Start menu.)

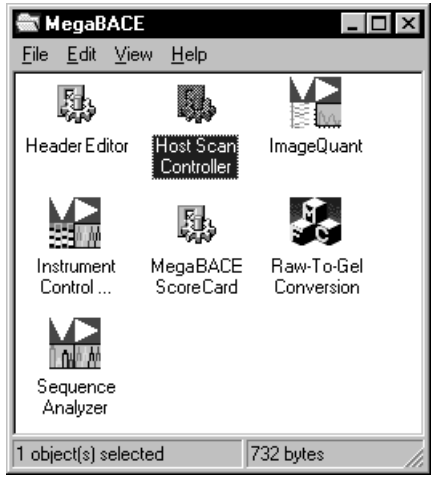


Figure 3-2. The MegaBACE folder. Note that the icon for the applicable analysis software appears in this folder (Sequence Analyzer or Genetic Profiler).

2. Double-click the **Host Scan Controller** icon. The Host Scan Controller window appears.
3. (Optional) You can minimize the Host Scan Controller and monitor the run using the Command Log tab on the Instrument Control window.

Note: After starting, the Host Scan Controller downloads the firmware, and displays the instrument model number (table 1-1).

Important **Wait about 10 seconds for the Host Scan Controller to complete initialization before starting the Instrument Control Manager.**

3.4 Starting the Instrument Control Manager software

Important **The Host Scan Controller must be running to start the Instrument Control Manager (section 3.3).**

The Instrument Control Manager software provides the various protocols that step you through using the instrument.

To start the Instrument Control Manager, open the MegaBACE folder and double-click the **Instrument Control** icon. The Instrument Control Manager opens and displays the Plate Setup window (figure 3-3).

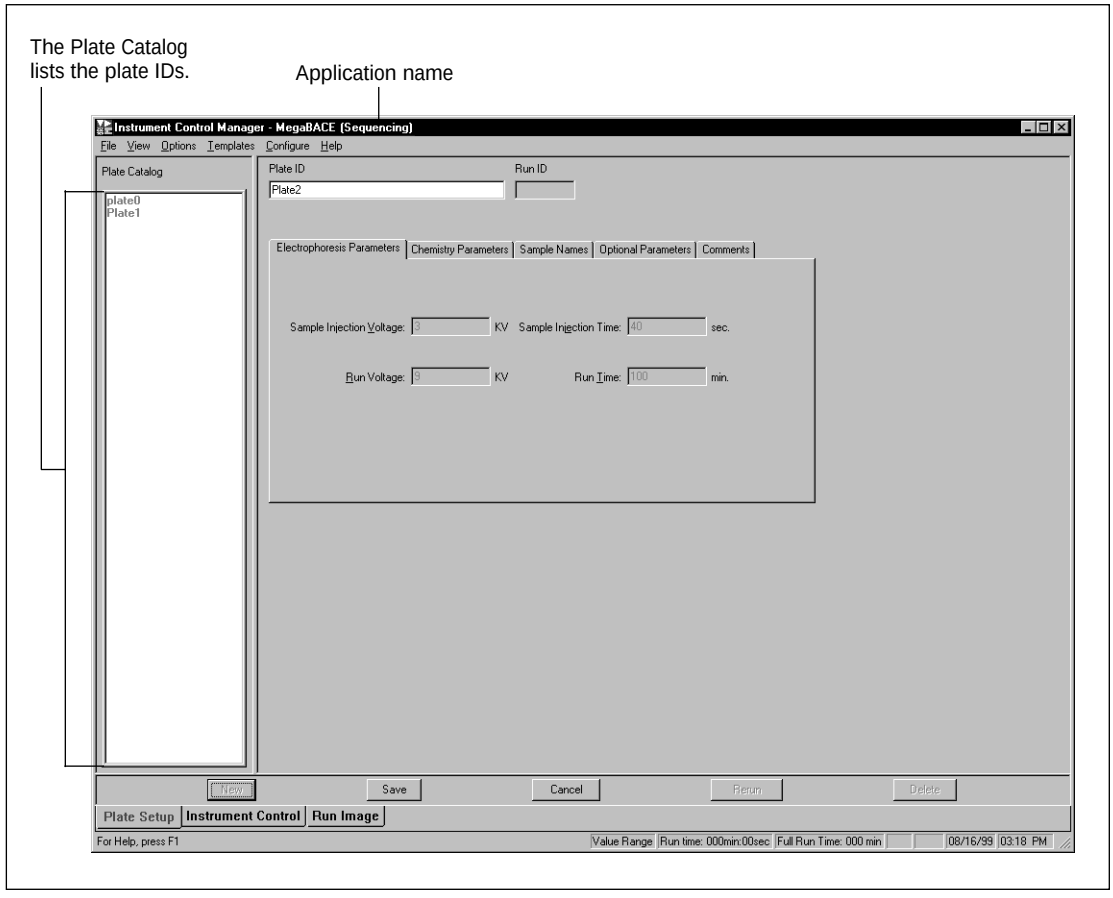


Figure 3-3. The Instrument Control Manager software displaying the Plate Setup window and the Plate Catalog. Note that sequencing is selected as the application and displayed in the title bar.

To use the protocols for running the instrument, click the **Instrument Control** tab to display the window. The Instrument Control window appears with the list of protocols (figure 3-4).

For details on performing runs, see chapter 4.

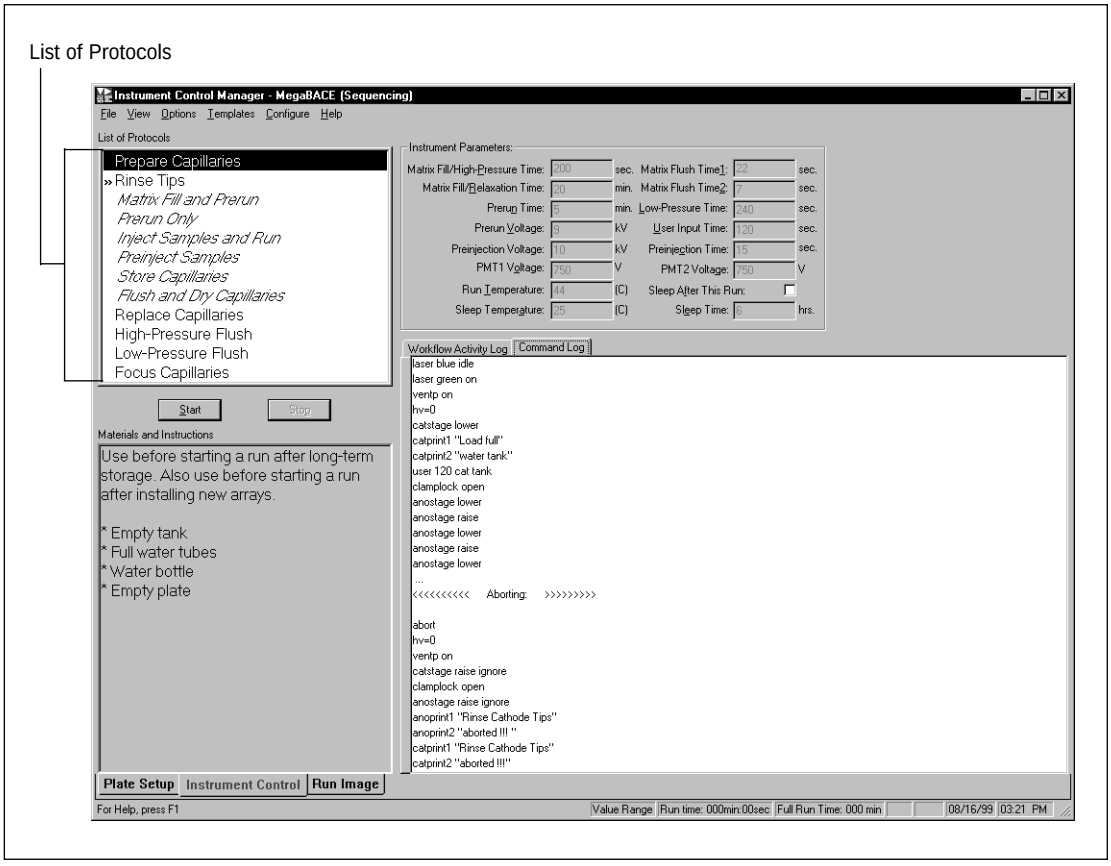


Figure 3-4. The Instrument Control window for sequencing.

3.5 Changing the application

The Instrument Control Manager allows you to use multiple applications. Currently, sequencing and genotyping are the two applications that are available.

Caution **Check the filter compartment to make sure the correct filters are installed for the application you are selecting and the dye set in your plate. See the instrument maintenance and troubleshooting guide and the administrator's guide for instructions.**

To change to a different application—

1. From the Configure menu, point to **Applications** and then choose **the name of the application** you want to use (sequencing or genotyping). A check mark appears in front of the selected application (figure 3-5).

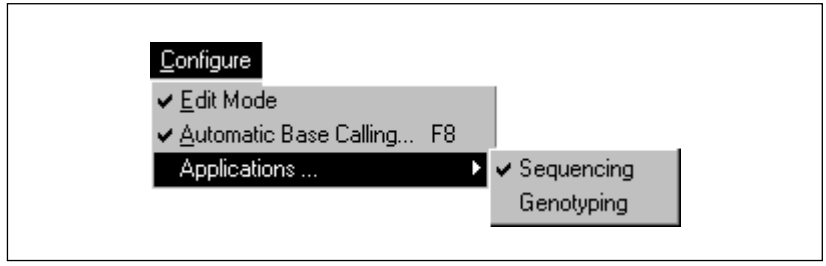


Figure 3-5. The Configure menu with the sequencing application selected.

2. In the Instrument Control Manager window, click the **Plate Setup** tab, and then click **New** to set up a new plate.
3. From the Templates menu, point to **Plate Setup Templates** and then choose **Select Template.** The Select Template window appears.
4. In the Select Templates window, choose the appropriate plate setup template (.tpl), and then click **Open.** The Plate Setup window displays the parameters for the selected template.

   (For genotyping, the default is **StdGenotyping.tpl.**)
5. Click the **Instrument Control** tab.
6. From the Templates menu, point to **Instrument Templates** and choose **Select Template.** The Select Template window appears.
7. In the Select Template window, choose the **.icp file** that you want to use.
   - For sequencing, the default is **Normal.icp.**
   - For genotyping, the default is **Genotyping.icp.**
8. Click **Open.** The Instrument Control window displays the parameters for the selected template.

The name of the selected application changes in the title bar of each of the Instrument Control Manager windows (figure 3-6). The Instrument Control Manager displays only the plate IDs and plate setups for the selected application in the Plate Setup window.

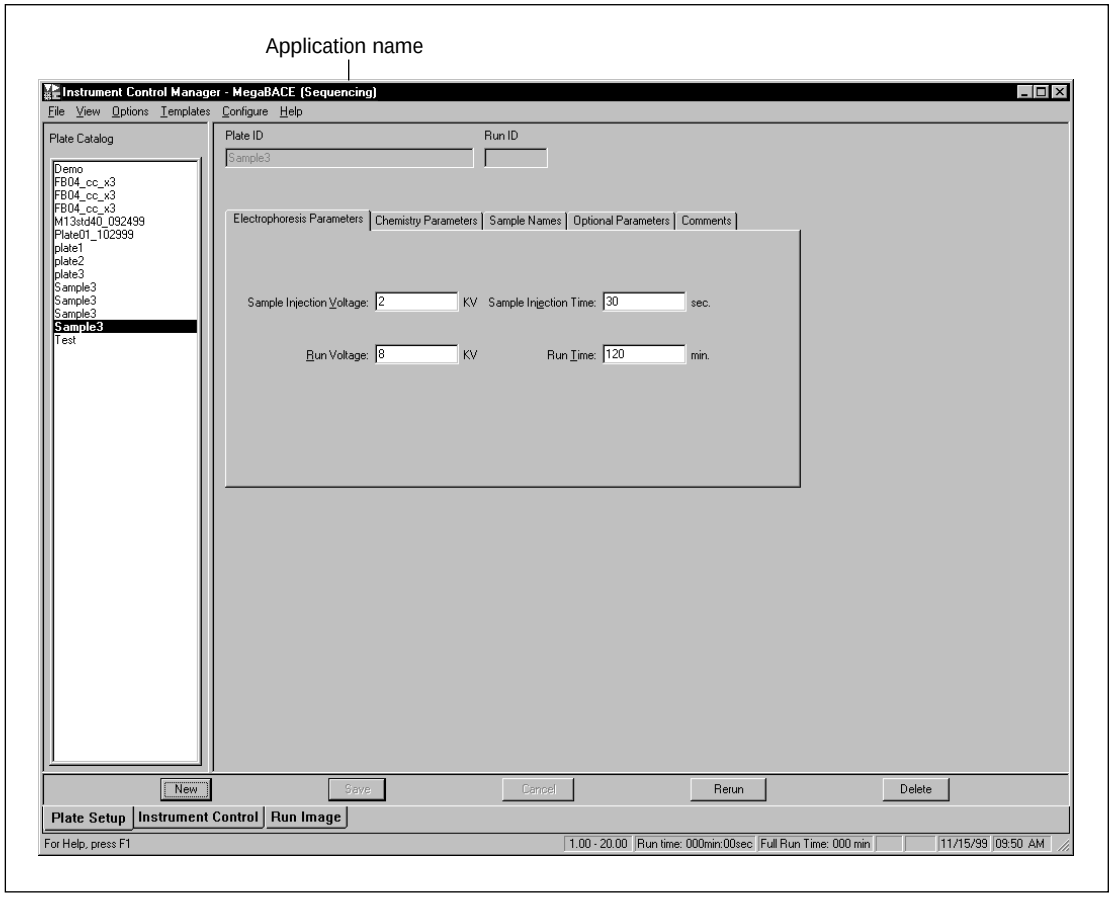


Figure 3-6. The Plate Setup window. The name of the selected application appears in the title bar of each of the Instrument Control Manager windows.

3.6 Changing the storage location for the raw sample data files

After you run a plate, the Instrument Control Manager stores the raw sample data files (.rsd) in a raw run folder in the default location or the folder you specified. Unless you specify a different location, the software stores the run data in the …\MegaBACE\Data folder.

Important **The Instrument Control Manager stores the raw run folders from any MegaBACE application (sequencing or genotyping) in a single Data folder.**

Important **Changing the storage location affects only future files that are created on the data collection workstation.**

Before running a plate, you can specify a different storage location for the raw sample data files (.rsd) on the instrument control workstation. For instance, you can store the files on another hard drive on the instrument control workstation if the computer is running out of space on the C drive.

Important **You cannot use File Storage to select a storage location on a remote workstation.**

To change the file storage location—

1. Choose **File Storage** from the Options menu. The File Storage window appears (figure 3-7).

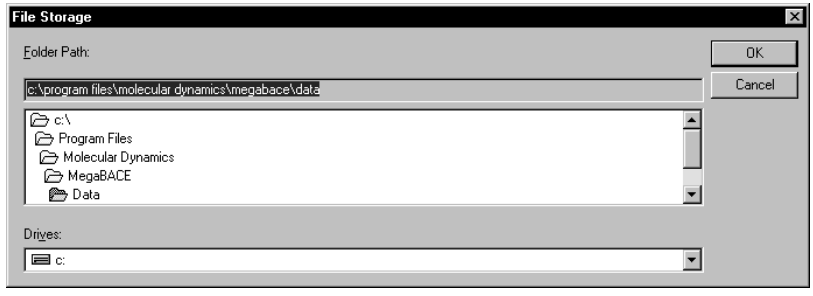


Figure 3-7. The File Storage window.

2. Select the hard drive and folder you want to use and click **OK.** The window closes. After you run the samples, the software creates a folder for the raw sample data with a name based on the plate ID and run ID (for example, Plate ID_Run ID) and stores it in the folder you selected.

3.7 Using the Automatic Base Calling feature (sequencing only)

You can use the Automatic Base Calling feature only if your administrator has enabled this feature.

If you want the software to automatically call bases and export the data, you use the Automatic Base Calling feature. The Automatic Base Calling feature allows you to select the base caller to use when calling bases and select the file formats you want to use when exporting the base-called data.

3.7.1 Selecting the base caller (sequencing only)

To select the base caller to use for automatic base calling—

1. Choose **Automatic Base Calling** from the Configure menu. The Automatic Base Calling window appears (figure 3-8).

Figure 3-8. The Automatic Base Calling window.

2. Click the **Automatic Base Calling** check box to select automatic base calling.
3. Choose a **base caller** from the Default Base Caller list.
4. Click the **check box(es)** for the file format(s) to use when exporting the data.
5. Click **OK.** The window closes.

3.7.2 Exporting the base-called data files (sequencing only)

The Instrument Control Manager can create a subfolder for each export file format that you use after automatic base calling or can export to the same folder. Your administrator turns on this feature. The Instrument Control Manager names each subfolder with the name of the file format (Abd, Fasta, Scf, or Text).

You can export both the raw and analyzed data to ASCII (text) files, and these files are stored in a subfolder within the plate folder in either the raw or analyzed run folder. All the other file formats are used only for exporting base-called data and are stored in subfolders within the analyzed run folder in the …\MegaBACE\AnalyzedData folder (default) or the location you specify (figure 3-9).

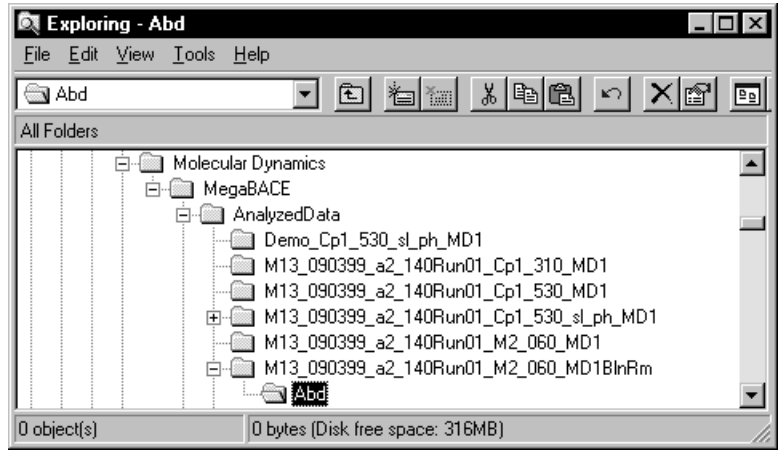


Figure 3-9. A subfolder (highlighted) for an exported file format.

Chapter 4 Performing runs

This chapter describes how you prepare for a run and how you set up plate definitions and run plates. The topics are—

- Choosing a protocol workflow (section 4.1)
- Preparing for a run (section 4.2)
- Materials required before performing a run (section 4.3)
- About the list of protocols (section 4.4)
- Rinsing the capillary tips (section 4.5)
- Filling the capillaries with matrix and performing a prerun (section 4.6)
- Performing automatic plate setup before the Inject Samples and Run protocol (section 4.7)
- Materials required for a run (section 4.8)
- Checking the instrument control parameters (section 4.9)
- Starting the Inject Samples and Run protocol (section 4.10)
- How the raw data are stored (section 4.11)
- Suppressing raw data file creation for empty or bad capillaries (section 4.12)
- Preinjecting samples (optional) (section 4.13)
- Automatically storing the capillaries after a run (section 4.14)

4.1 Choosing a protocol workflow

Figure 4-1 shows the alternative protocol workflows for setting up plates and performing runs using the Instrument Control Manager. Workflow A requires entering a plate ID and importing plate setup information before starting the Inject Samples and Run protocol.

Workflow B allows you to start the Inject Samples and Run protocol and then enter a plate ID (bar code) for a plate that has not yet been defined. Entering the plate ID automatically imports the precreated plate setup information and enters the plate ID in the Plate Catalog.

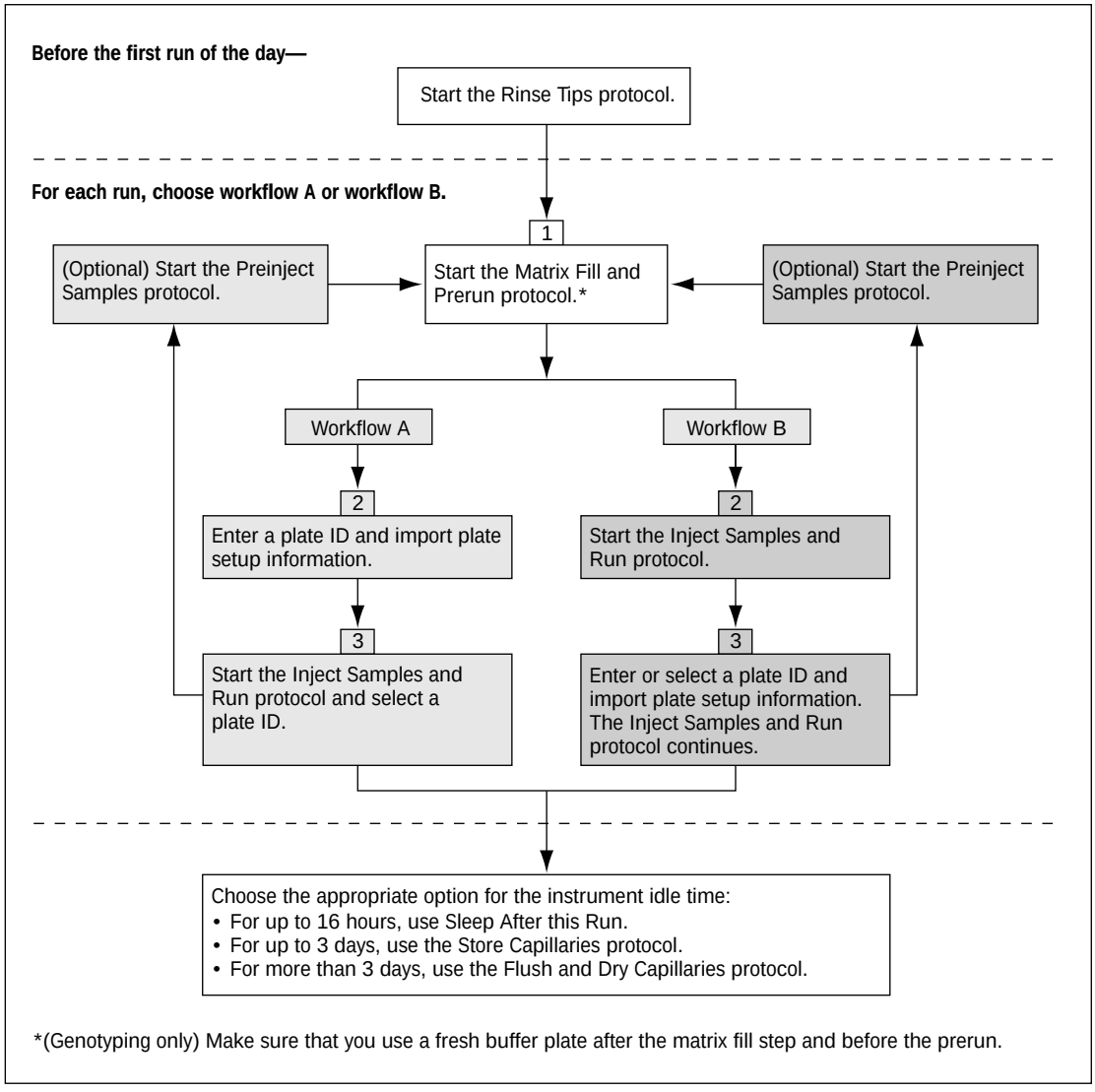


Figure 4-1. Alternative protocol workflows for performing runs using the Instrument Control Manager.

After the last run, you choose the appropriate option for the instrument idle time.

- If you are planning to leave the instrument idle overnight, the software allows you to set the Sleep After This Run parameter to a maximum of 16 hours, which is the maximum time for storing capillaries in matrix without evaporation.
- If you need to leave the instrument idle for longer than 16 hours, you should use the Store Capillaries protocol to store the capillaries in water for up to 3 days.
- For storage more than 3 days, you use the Flush and Dry Capillaries protocol to store the capillaries dry.

See chapter 6 for instructions for using these protocols.

Table 4-1 describes the main tasks necessary to prepare for and perform a run using Workflow A.

Table 4-1. Description of Workflow A

Task	Description	See section
1. Prepare the materials you need for the run.	For the Rinse Tips and Matrix Fill and Prerun protocols, you need a squirt bottle filled with fresh deionized filtered water, a clean water tank, tubes (one for each array installed) containing fresh deionized filtered water, a plate containing buffer, and tubes containing linear polyacrylamide (LPA) matrix.	4.3
2. Rinse the capillary tips.	Before the first run of the day, use the Rinse Tips protocol. Follow the instructions on the instrument displays to load a clean water tank and water tubes into the instrument.	4.5
3. For each run, fill the capillaries with matrix and perform a prerun.	Start the Matrix Fill and Prerun protocol and follow the instructions on the instrument displays to load the buffer plate and the matrix tubes into the instrument.	4.6

Table 4-1. Description of Workflow A (continued)

Task	Description	See section
4. Enter a plate ID or bar code in the Plate Setup window and import the plate setup information for each plate you are going to run.	Click **New**. Type a plate ID or scan the bar code for each plate you will run. The software automatically imports plate setup parameters from a plate setup data file (.psd), if it exists, or uses the default plate setup parameter template file.	4.7
5. Bring the plate containing the samples to the instrument.	Before starting the Inject Samples and Run protocol, have the plate containing the prepared samples and the plate containing buffer available at the instrument.	4.8
6. Inject the samples and perform a run.	Start the Inject Samples and Run protocol. Follow the instructions on the displays.	4.10
7. (Optional) Preinject the samples.	Start the Preinject Samples protocol to preinject samples from a new plate using the matrix remaining in the capillaries from the previous run. Go to step 3 for the next run.	4.13
8. After the last run of the day, store the capillaries up to 16 hours.	Click the Sleep After This Run check box in the Instrument Parameters area.	4.14
For up to 3 days, store the capillaries wet.	Use the Store Capillaries protocol. This protocol reduces the temperature in the electrophoresis compartment to 25 °C (77 °F) and turns off the lasers.	6.1
For storage of more than 3 days, store the capillaries dry.	Use the Flush and Dry Capillaries protocol if you are shutting down the instrument for more than 3 days.	6.2

Table 4-2 describes the main tasks to prepare for and perform a run using Workflow B. If you want to use the Select a Plate window, you must have a .psd file that defines the plate setup parameters.

Table 4-2. Description of Workflow B

Task	Description	See section
1. Prepare the materials you need for the run.	For the Rinse Tips and Matrix Fill and Prerun protocols, you need a squirt bottle filled with fresh deionized filtered water, a clean water tank, tubes (one for each array installed) containing fresh deionized filtered water, a plate containing buffer, and tubes containing LPA matrix.	4.3
2. Rinse the capillary tips.	Before the first run of the day, use the Rinse Tips protocol. Follow the instructions on the instrument displays to load a clean water tank and water tubes into the instrument.	4.5
3. For each run, fill the capillaries with matrix and perform a prerun.	Start the Matrix Fill and Prerun protocol and follow the instructions on the instrument displays to load the buffer plate and the matrix tubes into the instrument.	4.6
4. Bring the plate containing the samples to the instrument.	Before starting the Inject Samples and Run protocol, have the plate containing the prepared samples and the plate containing buffer available at the instrument.	4.8
5. Start the Inject Samples and Run protocol.	With the Inject Samples and Run protocol selected, click Start.	4.10
6. Enter a plate ID or bar code and import the plate setup information for the plate you are going to run.	In the Select a Plate window, type a plate ID or scan a bar code. The plate setup parameters are imported from the .psd file. Click OK. The Inject Samples and Run protocol continues. Follow the instructions on the displays.	4.10
7. (Optional) Preinject samples.	Start the Preinject Samples protocol to preinject samples from a new plate using the matrix remaining in the capillaries from the previous run. Go to step 3 for the next run.	4.13

Table 4-2. Description of Workflow B (continued)

Task	Description	See section
8. After the last run of the day, store the capillaries up to 16 hours.	Click the Sleep After This Run check box in the Instrument Parameters area.	4.14
For up to 3 days, store the capillaries wet.	Use the Store Capillaries protocol. This protocol reduces the temperature in the electrophoresis compartment to 25 °C (77 °F) and turns off the lasers.	6.1
For storage of more than 3 days, store the capillaries dry.	Use the Flush and Dry Capillaries protocol if you are shutting down the instrument for more than 3 days.	6.2

4.2 Preparing for a run

Preparing for a run involves gathering the materials you need for each instrument control protocol (section 4.3) and preparing the instrument and the capillaries for the run (section 4.5).

Note: The Instrument Control window displays the list of instrument control protocols (section 4.4).

Before the first run of the day

- Gather the materials you need to rinse the capillary tips and fill the capillaries with matrix (section 4.3).
- Rinse the capillary tips (section 4.5).

Before each plate run

- Fill the capillaries with matrix and perform a prerun (section 4.6).
- Set up a plate definition in the Plate Setup window (section 4.7).
- Bring a plate containing samples to the instrument. The sample plate should be prepared according to the appropriate reagent protocol and should be stored when not in use.

Note: You are not required to set up a plate definition in the software before you start a run (section 4.7).

4.3 Materials required before performing a run

You should have available at the instrument adequate supplies of tanks, water tubes, and matrix tubes for the run. The next sections describe the materials you should have available for the Rinse Tips and Matrix Fill and Prerun protocols.

4.3.1 Materials required for the Rinse Tips protocol

Important **Make sure you replace the used water tank with a clean water tank each time you use a protocol that requires water.**

- A squirt bottle filled with fresh deionized filtered water
- A clean tank filled with fresh deionized filtered water
- One 2-ml tube for each array installed in the instrument, each containing 1.8 ml fresh deionized filtered water

Note: You will use these materials also for the Inject Samples and Run protocol and other protocols that require water (figure 4-2).

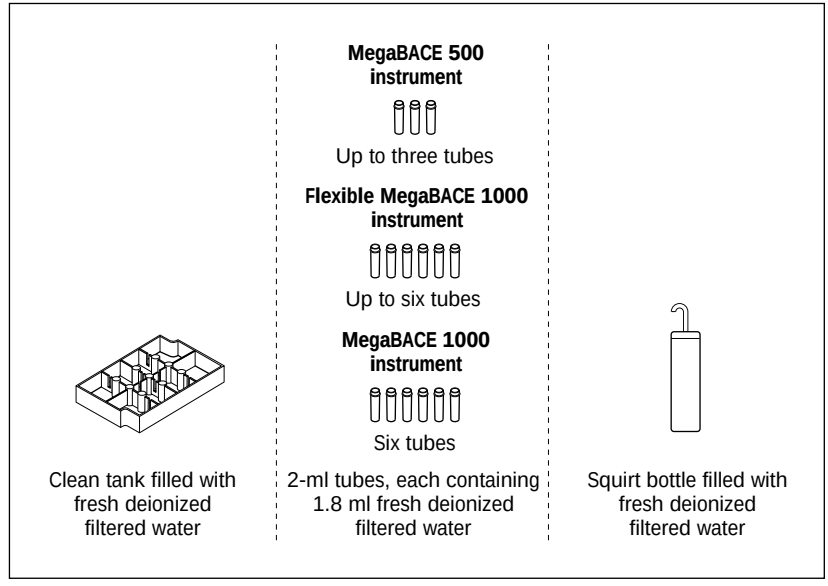


Figure 4-2. The materials for the Rinse Tips protocol. **Note:** You use one tube of water for each array installed in the instrument.

Caution **Do not fill the water tank too full. Open the cathode drawer slowly to prevent spilling the water on the cathode stage. Spilled water (or other material) can damage the electrodes on the cathode stage.**

4.3.2 Materials required for the Matrix Fill and Prerun protocol

Caution **Verify that you have the correct plate for the instrument's cathode assembly. See the *MegaBACE Planning Guide* for a list of qualified plates. Using the wrong plate can damage the instrument.**

You should have the following materials available in sufficient quantity to complete the number of runs you plan to perform (figure 4-3):

- A plate containing 200 µl LPA buffer per well (diluted to 1×). For genotyping, you need two buffer plates.
- One 2-ml tube for each array installed in the instrument, each containing 0.7 ml LPA matrix. Centrifuge the matrix at the rpm and time listed in the LPA package instructions.

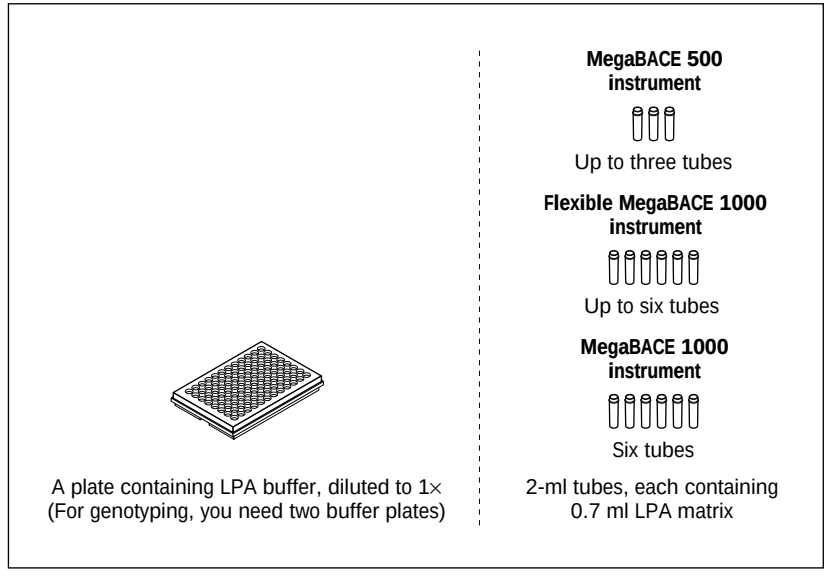


Figure 4-3. The materials for the Matrix Fill and Prerun protocol. **Note:** You use one tube of matrix for each capillary array installed in the instrument.

4.4 About the list of protocols

The Instrument Control window contains a list of the protocols you use to run the instrument. You use this window to step through the list of protocols (figure 4-4). Double arrows (>>) indicate the selected protocol. Protocols that are available for use any time are displayed in regular text. Protocols that are available only in a specified sequence are displayed in italics.

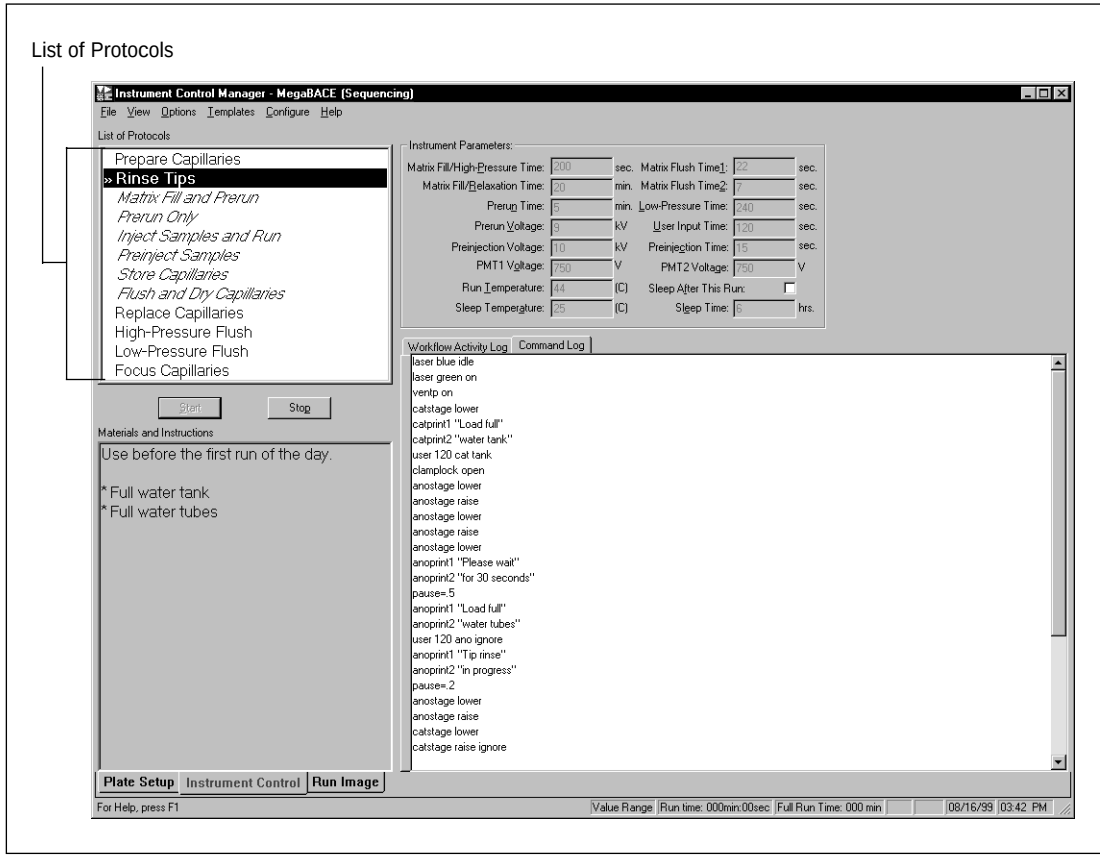


Figure 4-4. The List of Protocols in the Instrument Control window.

At the start of the day, the first protocol you use is the Rinse Tips protocol (figure 4-4), which rinses the tips of the capillaries. Use the Rinse Tips protocol at least once a day. You use the Matrix Fill and Prerun protocol for each plate you run.

4.5 Rinsing the capillary tips

Use the Rinse Tips protocol before starting the first run of the day to prevent clogging the holes in the circuit board and to minimize contamination of the upper portions of the electrodes. You can use the Rinse Tips protocol after any protocol in the List of Protocols.

Important **Make sure you have the materials listed in section 4.3 available before you start the Rinse Tips protocol. Use a clean tank filled with fresh deionized filtered water.**

After you start a protocol, observe the two displays on the front of the instrument for instructions (figure 4-5). The displays provide instructions for your interactions with the instrument while you are using the protocols.

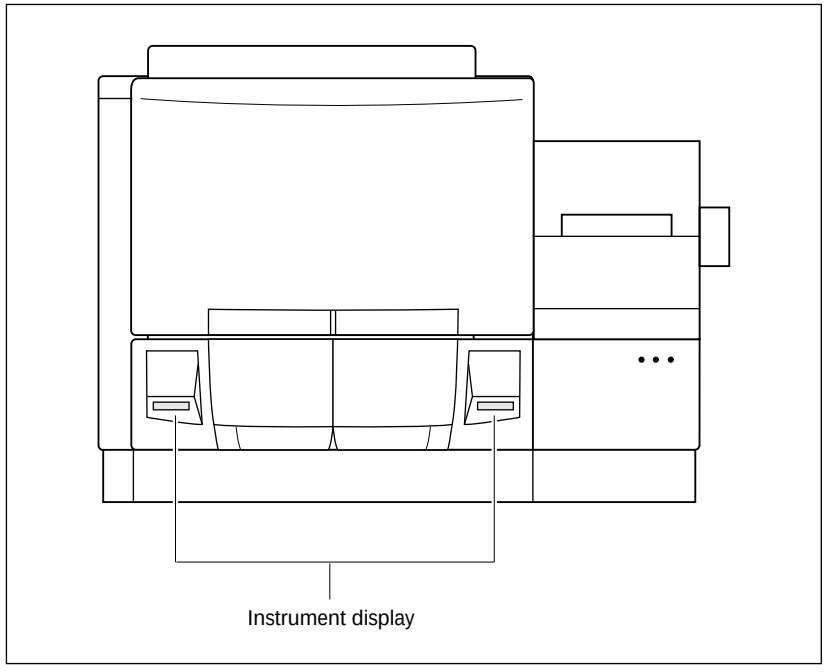


Figure 4-5. The displays on the front of the instrument.

To start the Rinse Tips protocol—

1. Click the **Instrument Control** tab to display the Instrument Control window. If the time you specified in the Store Capillaries protocol has elapsed, the Rinse Tips protocol is selected.

2. Click **Start** and follow the instructions on the instrument displays to load a **clean water tank** into the left (cathode) side of the instrument and **clean water tubes** into the right (anode) side of the instrument.

Caution **Do not overfill the water tank. Close the cathode drawer slowly.**

Note: For a complete list of messages and actions, see appendix D.

The displays tell you the tip rinsing is in progress. When the protocol is finished, the software selects the Matrix Fill and Prerun protocol as the next protocol to use.

4.6 Filling the capillaries with matrix and performing a prerun

Before you perform a run, you must fill the capillaries with matrix and perform an electrophoresis prerun. You use different procedures for the sequencing and genotyping applications:

- For sequencing, use the procedure in section 4.6.1.
- For genotyping, use the procedure in section 4.6.2.

Important **Make sure you have the materials listed in section 4.3 available before you start the Matrix Fill and Prerun protocol.**

4.6.1 Performing Matrix Fill and Prerun protocol (sequencing only)

1. In the Instrument Control window, with the Matrix Fill and Prerun protocol selected, click **Start.**
2. Follow the instructions on the instrument displays to load the **buffer plate** into the left side of the instrument and the **matrix tubes** into the right side of the instrument.

After the prerun begins, the displays tell you that equilibration is in progress. The prerun progresses according to the time you specified in the instrument parameters.

Note: For a complete list of messages and actions, see appendix D.

When the protocol is finished, the software selects the Inject Samples and Run protocol as the next protocol you should use.

Note: After the completion of the Matrix Fill and Prerun protocol, you must use the Inject Samples and Run protocol within 15 minutes. If more than 15 minutes elapses, the software selects the Prerun Only protocol as the next protocol to use, and you must perform another prerun before you inject the samples.

4.6.2 Performing Matrix Fill and Prerun protocol (genotyping only)

To use the Matrix Fill and Prerun protocol—

1. In the Instrument Control window, with the Matrix Fill and Prerun protocol selected, click **Start. Note:** If this is not the first run of the day, you may be able to use the buffer plate that is already in place in the instrument from the previous Inject Samples and Run protocol.
2. Follow the instructions on the instrument displays to load the **buffer plate** into the left side of the instrument and the **matrix tubes** into the right side of the instrument. The displays tell you that equilibration is in progress, and ask you to be ready to put in the new buffer plate.
3. Make sure you load a new buffer plate when the display prompts you to do so. After you load the new buffer plate, the prerun begins and progresses according to the time specified in the instrument parameters.

Important **You must use a plate of fresh buffer after the matrix fill step finishes and before the prerun begins. Make sure you centrifuge both buffer plates according to the LPA package instructions to prevent air bubbles from forming in the capillaries. During the protocol, make sure you follow the instructions on the instrument displays. Using a fresh buffer plate between the matrix fill and the prerun steps improves the quality of the genotyping data.**

Note: For a complete list of messages and actions, see appendix D.

When the protocol is finished, the software selects the Inject Samples and Run protocol as the next protocol you should use.

Note: After the completion of the Matrix Fill and Prerun protocol, you must use the Inject Samples and Run protocol within 15 minutes. If more than 15 minutes elapses, the software selects the Prerun Only protocol as the next protocol to use, and you must perform another prerun before you inject the samples.

4.7 Performing automatic plate setup before the Inject Samples and Run protocol

Before using the Inject Samples and Run protocol, you can use the Plate Setup window (figure 4-6) to create a plate definition. The plate definition includes the plate ID, electrophoresis parameters, chemistry parameters, sample names, optional parameters if any, and comments.

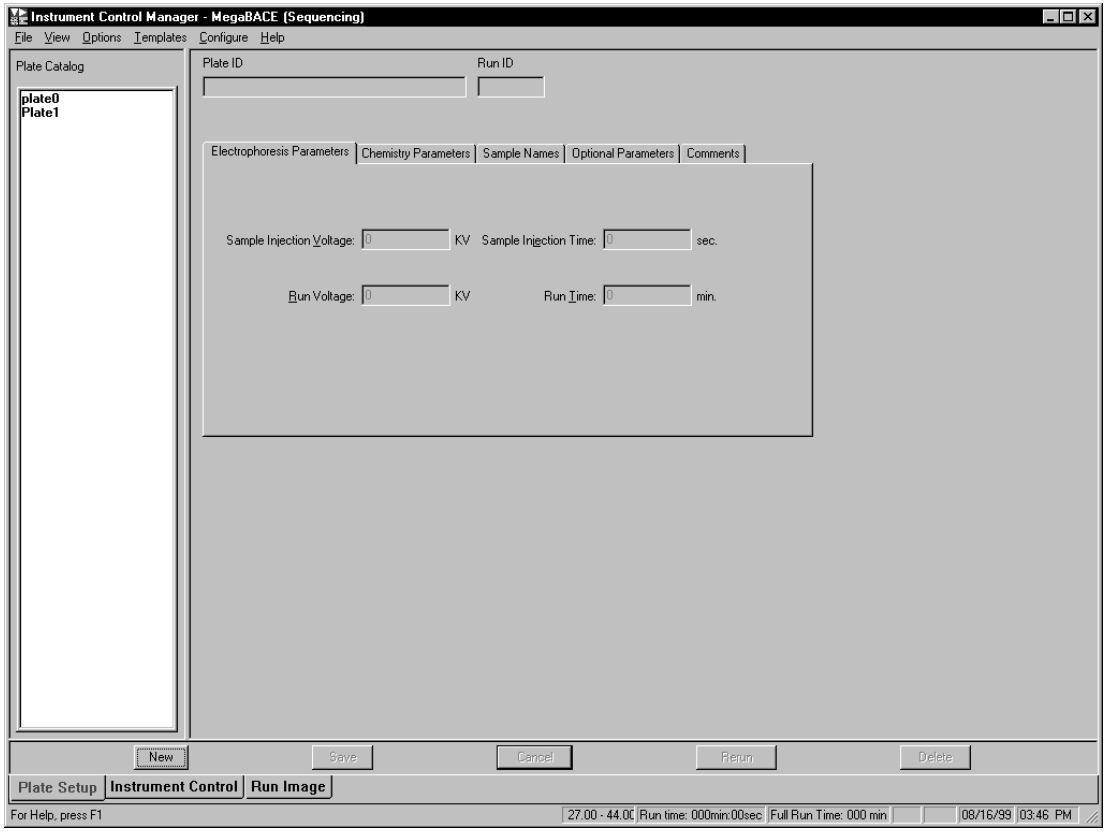


Figure 4-6. The Plate Setup window for sequencing.

4.7.1 About the plate ID

You provide a plate ID by typing the text into the Plate Setup window (figure 4-6) or scanning the bar code on the plate.

The plate ID (along with the run ID) is used to name the folder in which the Instrument Control Manager stores the raw sample data files for each run. So, a plate ID of a manageable size, such as 32 characters or fewer, is advisable.

4.7.2 Performing automatic plate setup

The Instrument Control Manager allows you to set up plates automatically. Your administrator can specify the plate setup parameters so that you can perform automatic plate setup.

You can set up plate definitions one at a time, as you run each plate, or you can set up multiple plate definitions and then run the plates later. Alternatively, you can set up plate definitions after you start the Inject Samples and Run protocol (section 4.10, step 2).

To perform automatic plate setup—

1. In the Plate Setup window (figure 4-7), click **New.**

   If a default plate setup template has been specified, the Instrument Control Manager loads the values of the template into the parameter boxes in the Plate Setup window. The Plate Setup window contains five tabs, which display the electrophoresis parameters, chemistry parameters, sample names, optional parameters, and comments.

   If no default plate setup template has been specified, the values in the parameter boxes are blank.

2. In the Plate ID box, enter a plate ID (type the text or scan a bar code). The Instrument Control Manager loads the values from a plate setup data file (.psd), if one exists.

   Note: The plate ID you type must match the file name of the .psd file. If you scan a bar code, the bar code must match the .psd file name. If you receive a message that indicates the Instrument Control Manager cannot find the .psd file, see your administrator. The default path to the .psd file is …\MegaBACE\Psd.

   The Instrument Control Manager automatically saves the new plate definition, and the plate ID appears in the Plate Catalog (figure 4-7).

3. (Optional) Repeat steps 1 and 2 for as many plate IDs as you want to create with imported plate setup parameters.

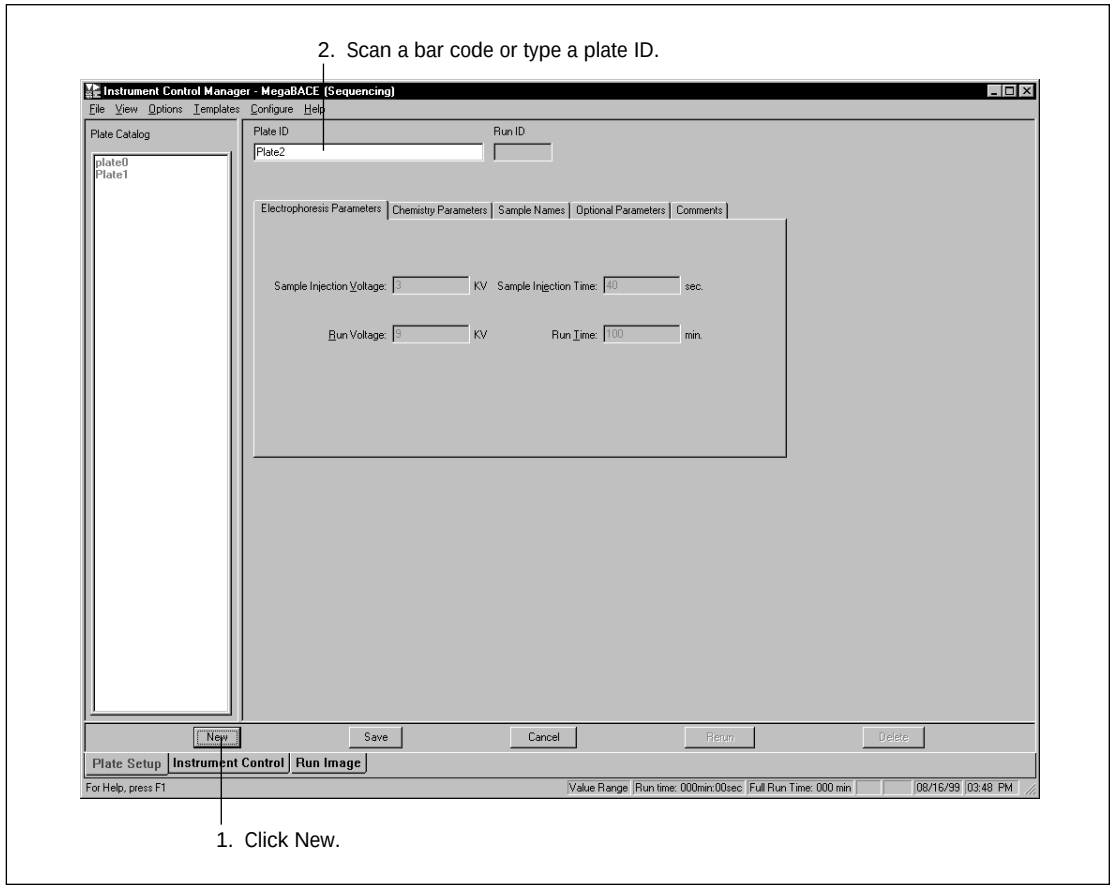


Figure 4-7. The Plate Setup window with the electrophoresis parameters for sequencing displayed.

4.7.3 Checking the plate setup parameters

Important

You can edit the instrument control parameters only if the edit mode is turned on and no protocol is running. If the edit mode is turned on, the Edit Mode command on the Configure menu has a check mark in front of it. If the edit mode is turned off, the command is gray and cannot be selected. See your administrator for information about the edit mode.

In the Plate Setup window, you can click the tabs to view the electrophoresis parameters, chemistry parameters, sample names, optional parameters, and comments that the Instrument Control Manager loaded when you set up the plate. Table 4-3 describes the contents of the tabs in the Plate Setup window.

Table 4-3. Contents of the tabs in the Plate Setup window

Plate Setup window tabs	Description
Electrophoresis Parameters	Contains the sample injection voltage and time and the run voltage and time.
Chemistry Parameters	Contains the chemistry name, the associated laser mode, the dyes, the channels, the filters, and the beamsplitters used for the run.
Sample Names	Contains the list of sample names you assigned to each sample in each well of the plate.
Optional Parameters	Contains the PMT1 and PMT2 voltages, the run temperature, and a base caller. Used only for nondefault plate definitions that are specific for the selected plate ID.
Comments	Contains a text box where you can provide information about the run.

4.8 Materials required for a run

Important **Make sure you have the plates containing the prepared samples available before you start the Inject Samples and Run protocol so that you can perform the steps in the protocol quickly. This preparation minimizes sample diffusion, which can decrease the image resolution of the data collection.**

Caution **Verify that you have the correct plate for the instrument's cathode assembly. See the *MegaBACE Planning Guide* for a list of qualified plates. Using the wrong plate can damage the instrument.**

For the Inject Samples and Run protocol, you should have the following materials available for each plate run (figure 4-8):

- A clean tank filled with deionized filtered water.
- A plate containing the samples prepared according to the applicable reagent protocol. At a minimum, you need 5 µl per well.
- A plate containing buffer (diluted 1×), 200 µl per well.
- The tubes of matrix already in place in the anode side of the instrument.

Caution **Do not fill the water tank too full. Open and close the cathode drawer slowly to prevent spilling the water on the cathode stage. Spilled water (or other material) can damage the electrodes in the cathode stage.**

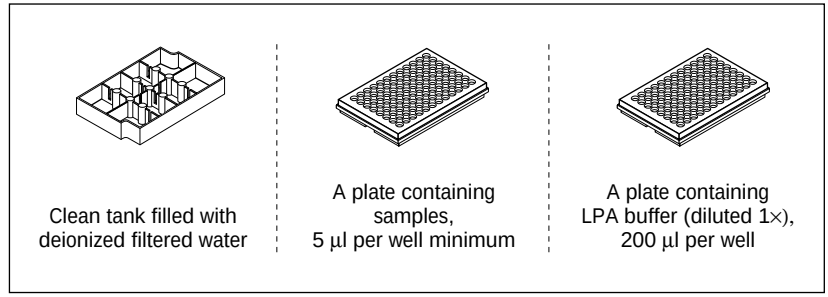


Figure 4-8. Materials for the Inject Samples and Run protocol.

4.9 Checking the instrument control parameters

Important

You can edit the instrument control parameters only if the edit mode is turned on and no protocol is running. If the edit mode is turned on, the Edit Mode command on the Configure menu has a check mark in front of it. If the edit mode is turned off, the command is gray and cannot be selected. See your administrator for information about the edit mode.

To view the instrument control parameters, click the **Instrument Control** tab. The Instrument Control window appears and displays the parameters in the Instrument Parameters area (figure 4-9).

If the edit mode is turned off (no check mark appears in front of the Edit Mode command), the only instrument control parameters you can change are **Sleep After This Run, Sleep Time,** and **Sleep Temperature.** You can select the Sleep After This Run check box so that the Instrument Control Manager automatically stores the capillaries in matrix and buffer after the current run has finished (section 4.14).

Note: During a run, you can select the Sleep After This Run check box at any time until the Inject Samples and Run protocol has finished. Make sure you set the Sleep Time correctly before selecting the Sleep After This Run check box. This will store the capillaries in matrix and buffer after the run is finished. If you select the Sleep After This Run check box after the Inject Samples and Run protocol has finished, the software stores the capillaries in matrix and buffer after the next run.

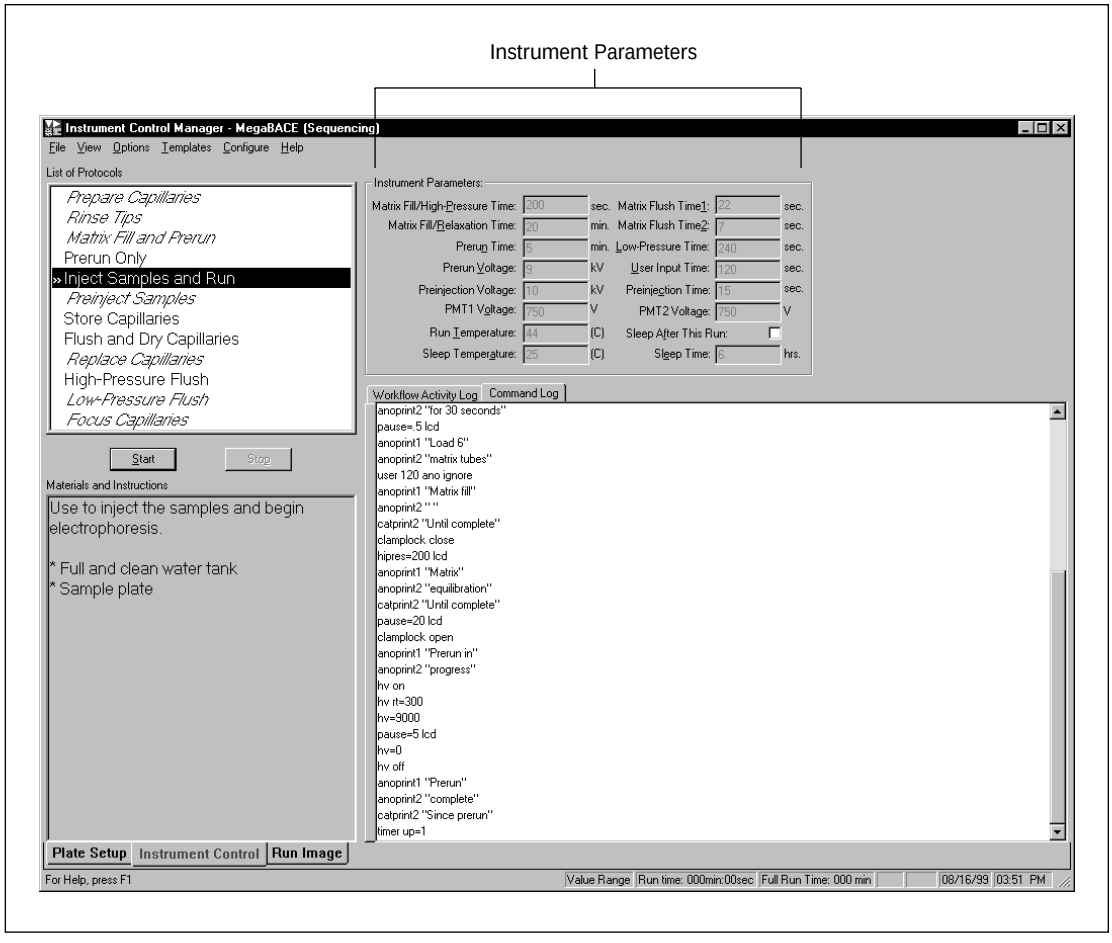


Figure 4-9. The Instrument Control window with the instrument parameters displayed for a sequencing run.

When you start the Inject Samples and Run protocol, the Instrument Control Manager uses the instrument parameters that are displayed. If no default template has been specified, the parameters are blank.

4.10 Starting the Inject Samples and Run protocol

Warning

Do not open the electrophoresis lid or the filter lid during a run. Opening these lids will cause a loss of data and may lead to injury.

Cautions

Before starting this protocol, make sure your sample plate is ready to run, as defined in the reagent protocol. At a minimum, you need 5 µl volume per well.

Before you begin a run, check the free disk space on the drive where your system is storing the raw sample data files (.rsd). For a 96-capillary run, you should have at least 150 MB of free disk space. If your system is set up for automatic base calling and export to other file formats, you should allow 50 MB of free disk space per hour of running plates.

To start a run—

1. In the Instrument Control window, with the Inject Samples and Run protocol selected, click **Start.** The Select a Plate window appears (figure 4-10).

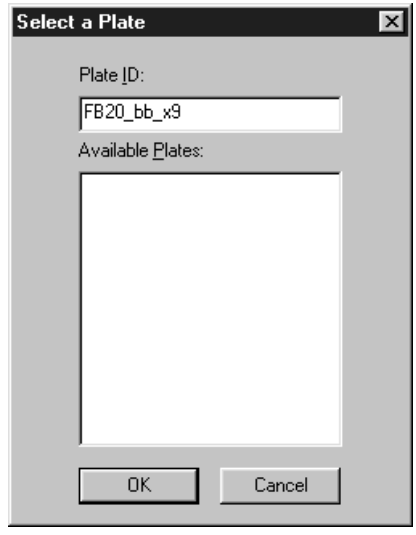


Figure 4-10. The Select a Plate window.

2. In the Select a Plate window, either—
   - Select the **plate** in the Available Plates list you want to run and click **OK** to confirm that this is the correct plate ID. The Inject Samples and Run protocol starts.

- In the Plate ID box, type the **plate ID** or scan a **bar code** for the plate you want to run and click **OK.** The Instrument Control Manager selects the plate definition if it is already in the list (otherwise, imports the plate setup information and creates the plate definition first), and starts the Inject Samples and Run protocol.

3. Follow the instructions on the instrument displays. After the first tip rinse is finished, the Confirm to continue window appears in the Instrument Control Manager (figure 4-11).

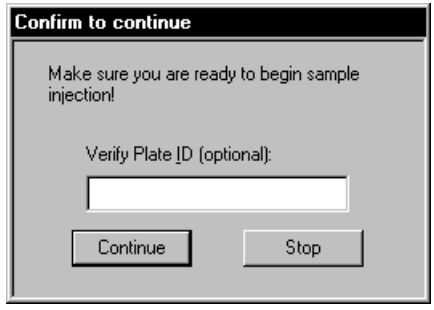


Figure 4-11. The Confirm to continue window with optional plate ID verification.

4. (Optional) You can confirm the ID of the plate you are running by scanning the bar code or typing the plate ID in the **Verify Plate ID (optional)** box.
5. Click **Continue.**
6. After the instrument injects the samples, load a buffer plate when the instrument displays prompt you to do so. A message appears and tells you the sample run is in progress.

For a complete list of messages and actions, see appendix D. If you want to monitor the run, see chapter 5 for instructions.

After the Inject Samples and Run protocol finishes, the software selects the Matrix Fill and Prerun protocol as the next protocol to use, unless you selected the Sleep After This Run check box in the Instrument Parameters area of the Instrument Control window (figure 4-12).

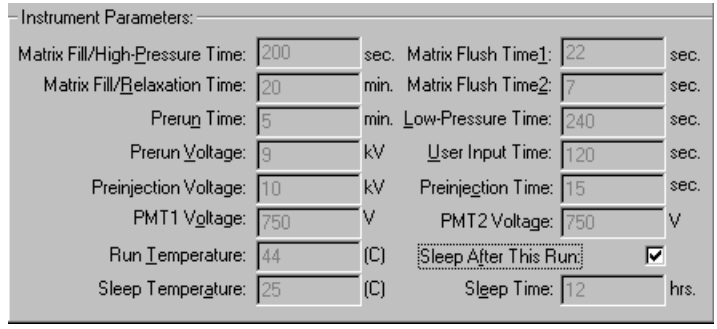


Figure 4-12. Instrument Parameters area with Sleep After This Run selected.

Important **(Sequencing only) If automatic base calling is selected, a window appears (figure 4-13) that shows the progress of the base calling. The Automated Base Calling command on the Configure menu has a check mark in front of it to show it is selected.**

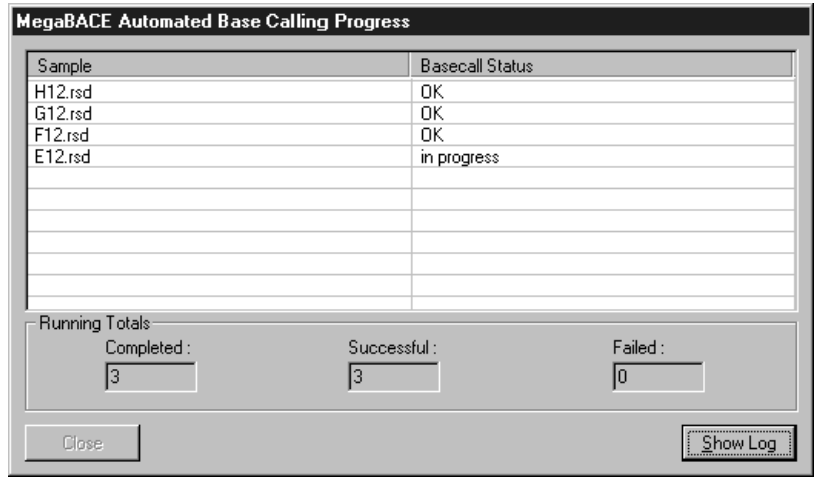


Figure 4-13. The Automated Base Calling Progress window (sequencing only).

4.11 How the raw data are stored

During data collection, the Instrument Control Manager creates raw sample data files (.rsd) and stores them in a raw run folder. The raw run folders are stored in the …\MegaBACE\Data folder (default) or the folder you specified (section 3.6).

The software uses the following file name conventions for the .rsd files:

- If no sample names are provided, the software uses the well IDs as the .rsd file names, for example, A01.rsd.
- If sample names are provided, the software uses the sample names as the .rsd file names (default). If duplicate sample names occur for a given plate, the software automatically appends the well IDs to the file names to generate unique file names. **Note:** You can use a plate setup data file (.psd) to specify file names that are different from the sample names.

4.12 Suppressing raw data file creation for empty or bad capillaries

The Instrument Control Manager does not create .rsd files for capillaries that are empty, broken, or clogged. Your administrator sets up this feature. If you know that a capillary is broken or clogged, notify your administrator. See section 5.5 for details on checking for empty or bad capillaries.

4.13 Preinjecting samples (optional)

If your application requires you to preinject samples, you can use the Preinject Samples protocol as many times as necessary for your application.

The Preinject Samples protocol is available only after you have used the Inject Samples and Run protocol. If you use the Preinject Samples protocol, your sample preinjection uses the matrix remaining in the capillaries after the completion of the Inject Samples and Run protocol.

If you decide you must use the Preinject Samples protocol after another protocol, you must use the Override command to start the protocol. See your administrator to use this command.

4.13.1 Materials required

Caution **Verify that you have the correct plate for the instrument's cathode assembly. See the *MegaBACE Planning Guide* for a list of qualified plates. Using the wrong plate can damage the instrument.**

For the Preinject Samples protocol, you need (figure 4-14)—

- A clean water tank
- A squirt bottle filled with deionized filtered water
- A plate containing the samples

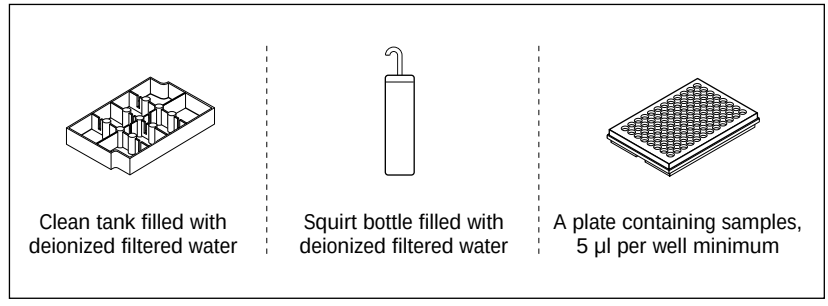


Figure 4-14. Materials for the Preinject Samples protocol.

4.13.2 The Preinject Samples protocol

To use the Preinject Samples protocol—

1. In the Instrument Control window, select the Preinject Samples protocol and click **Start.**
2. Follow the instructions on the left instrument display to complete the Preinject Samples protocol.

   Note: For a complete list of messages and actions, see appendix D.

The instrument injects the samples for the length of time and at the voltage set in the Instrument Parameters area of the Instrument Control window (figure 4-9).

Note: If you do not need to preinject the samples, you can start the Matrix Fill and Prerun protocol to prepare for running another plate (section 4.6). See figure 4-1 for a typical run workflow.

4.14 Automatically storing the capillaries after a run

The Instrument Control Manager allows you to store the capillaries after the run. To do this, you select the Sleep After This Run check box in the Instrument Parameters area of the Instrument Control window.

Important **Because this protocol allows storing the capillaries in matrix and buffer, you use this feature only for short-term storage of less than 16 hours. You cannot set the temperature higher than 25 °C (77 °F). Higher temperatures will cause evaporation and clog the capillaries.**

To store the capillaries after a run—

1. In the Instrument Parameters area of the Instrument Control window (figure 4-9), type a **temperature** in the Sleep Temperature box.
2. Type a **time** in the Sleep Time box.
3. Click the **Sleep After This Run** check box to select it. A check mark appears.

   Note: You can select the Sleep After This Run check box at any time during a run until the Inject Samples and Run protocol is finished. If you select the Sleep After This Run check box after the Inject Samples and Run protocol is finished, the software stores the capillaries in matrix and buffer after the next run.

   After the next run (or the current run), the Instrument Control Manager reduces the temperature in the electrophoresis chamber to 25 ºC (77 ºF) or the temperature you set and turns off the lasers.

   When the time you set has elapsed, the instrument starts warming up the electrophoresis compartment to the operating temperature. The display on the instrument counts up the time since the temperature has reached the proper level.

Chapter 5 Monitoring the run

This chapter describes how you use the Instrument Control window and Run Image window to monitor and assess the quality while you are performing a run.

The topics are—

- Viewing the status of the run (section 5.1)
- Setting the electropherogram attributes (section 5.2)
- Setting the run image attributes (section 5.3)
- Assessing the quality of the run (section 5.4)
- Checking for empty or bad capillaries (section 5.5)
- Checking the PMT voltage (section 5.6)
- Displaying an electropherogram (section 5.7)
- Modifying the Fluorescence Image Display area (section 5.8)
- Checking the capillary current (section 5.9)
- Changing the run time (optional) (section 5.10)

5.1 Viewing the status of the run

The Instrument Control window (figure 5-1) contains five areas that allow you to view the status of the run. The window also displays information about what you need to do to complete each protocol used in the run, including the materials that you will need to assemble.

- **List of Protocols area—**During the run, you can check the list to determine which protocol is currently being used by the instrument. The protocol being used is highlighted.
- **Materials and Instructions area—**You can check this area to make sure you have all the required materials assembled for the protocol.
- **Instrument Parameters area—**You can view the current instrument parameters in this area. If necessary, you can make adjustments to the parameters. For example, you could turn on the Sleep After This Run feature.

- **Workflow Activity Log tab—**The tab displays a record of the protocol, plate ID, run ID, status, and date and time for each protocol that you run. You can see such status information as whether a protocol was stopped, sent, expired, or completed. The Application column lists the application used for each protocol: genotyping or sequencing. If the Workflow Activity Log tab is not displayed, click the tab to change the view.
- **Command Log tab—**The tab displays the commands as they are sent from the Instrument Control Manager software to the Host Scan Controller software and then to the instrument. This allows you to monitor the real-time running of the system. If the Command Log tab is not displayed, click the tab to change the view.

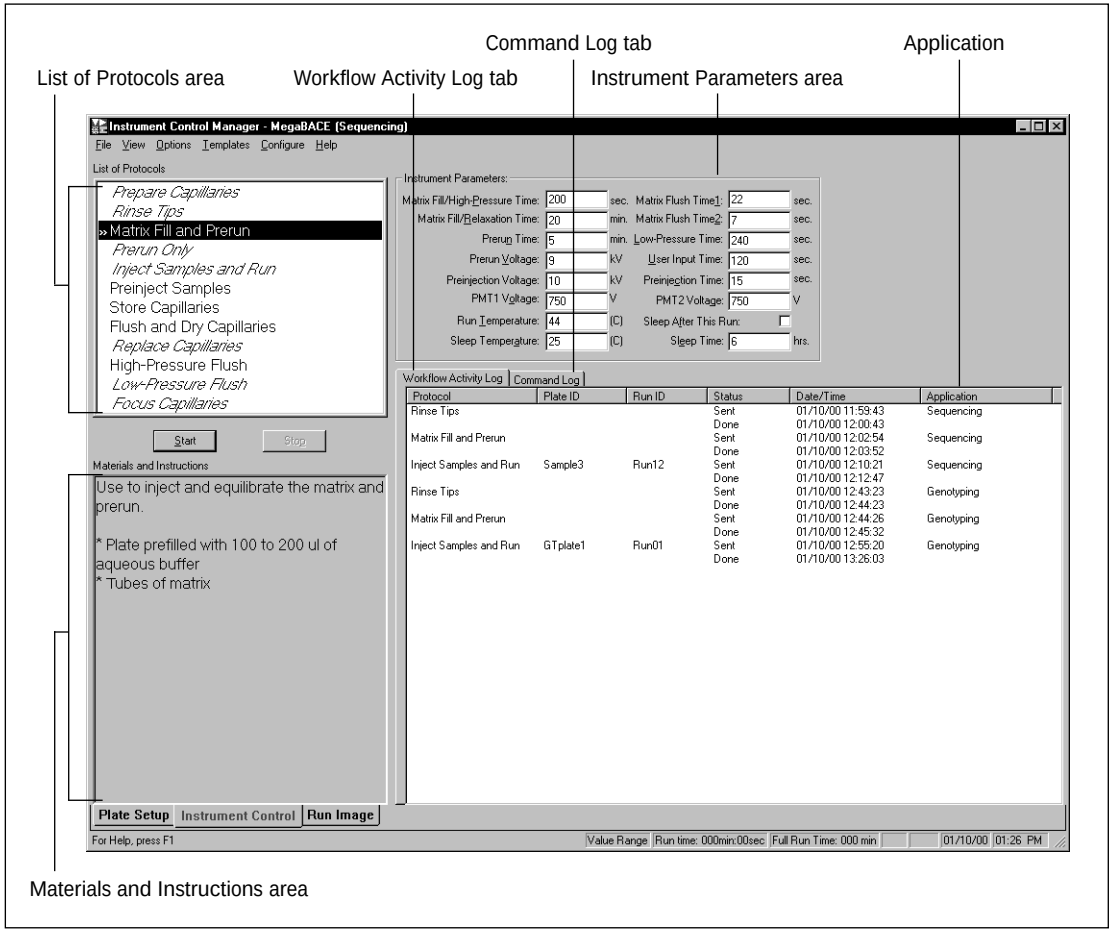


Figure 5-1. The Instrument Control window.

5.2 Setting the electropherogram attributes

The Electropherogram Display area displays a four-color electropherogram of the selected well. To select a well, click a well button in the Run Image window (figure 5-2).

Figure 5-2. The Run Image window displaying data for a sequencing run. For a selected well, the electropherogram area displays a different colored trace for each type of base (sequencing) or each dye label (genotyping).

You use the Electropherogram Attributes window (figure 5-3) to set an intensity range in relative fluorescence units (rfu) for the y-axis in the Electropherogram Display area. The upper limit is 65 535. Instead of setting the maximum and minimum values, you can choose to have the software determine the scale for you. To do this, click the **Autoscale** check box.

Important **The changes you make using the Electropherogram Attributes window are retained until you change them again.**

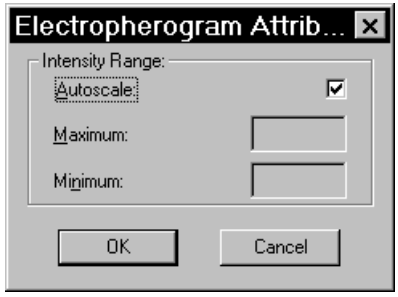


Figure 5-3. The Electropherogram Attributes window.

5.3 Setting the run image attributes

You use the Run Image Attributes window to choose how you want to view the real-time run data and to define which portion of the run you want to view in the Electropherogram Display area of the Run Image window (figure 5-2).

Important **The changes you make in the Run Image Attributes window affect the display only. They have no effect on the data.**

To set the run image attributes—

1. Choose **Run Image Attributes** from the Options menu. The Run Image Attributes window appears (figure 5-4).
2. (Optional) If you have previously saved settings, choose the file containing the settings from the Default Settings list and skip to step 11.
3. In the **Update Frequency** box, type a value in seconds.

   The update frequency determines how often the software updates the Electropherogram Display in the Run Image window (figure 5-2). To activate the update frequency in the Run Image window, click the **Update Frequency** button ( ) in the Capillary Information area above the electropherogram.
4. Type a value in the **For Color Scaling Take Last… Rows** box. The valid range is 10 to 500 rows.

   The default is the last 300 rows, which the software uses to calculate the minimum and maximum intensity values for every channel for the Fluorescence Image Display in the Run Image window (figure 5-2).

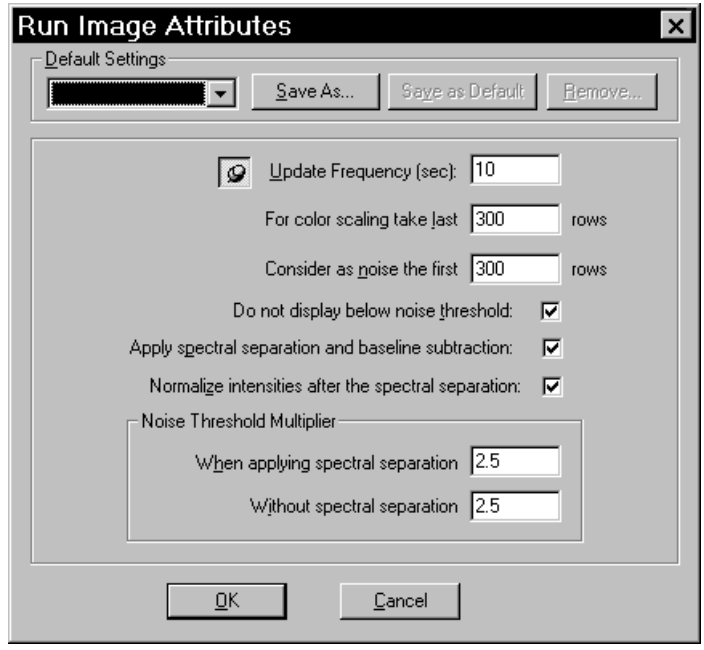


Figure 5-4. The Run Image Attributes window.

5. Type a value in the box for **Consider as Noise the First… Rows.**

   Note: During the first part of the run, the image is noise. The instrument does not read data for the first few seconds of the run because it takes time for the first samples to electrophorese to the window area of the capillaries.

6. If you do not want to display the noise below the threshold, click the check box for **Do Not Display Below Noise Threshold.**

7. If you want to **Apply Spectral Separation and Baseline Subtraction,** click the check box**.**

8. If you want to **Normalize Intensities After the Spectral Separation,** click the check box.

9. If you selected Apply Spectral Separation and Baseline Subtraction in step 7, in the Noise Threshold Multiplier area, type the multiplier you want to use in the **When Applying Spectral Separation** box.

   Note: The software calculates the maximum value of the noise within the first number of rows you selected in step 5 and multiplies the value by this multiplier. The default multiplier is 2.5.

10. If you deselected Apply Spectral Separation and Baseline Subtraction in step 7, type the multiplier you want to use in the **Without Spectral Separation** box. The default multiplier is 2.5.
11. Click **OK.** The electropherogram in the Run Image window (figure 5-5) displays the attributes you selected.
12. (Optional) If you want to save the settings, you can click **Save As** and provide a name. If you want to save the settings as the default settings, you can click **Save as Default.**

After you have checked or changed the display attributes, you monitor the run by checking the various areas on the Run Image window.

5.4 Assessing the quality of the run

You check the Fluorescence Image Display area in the Run Image window (figure 5-5) to assess the quality of the run. This pseudocolor display represents the capillaries from which the instrument is currently collecting data and indicates the dominant channel.

Note: The Run Image window is a split window that allows you to enlarge or reduce either the Fluorescence Image Display area or the Electropherogram Display area. To do this, place the pointer on the border between the two areas and drag the pointer up or down.

In the Fluorescence Image Display area, the new scans appear at the bottom of the view and move up the screen as new scans are added. When the display is full, the oldest scan lines disappear off the top of the Fluorescence Image Display.

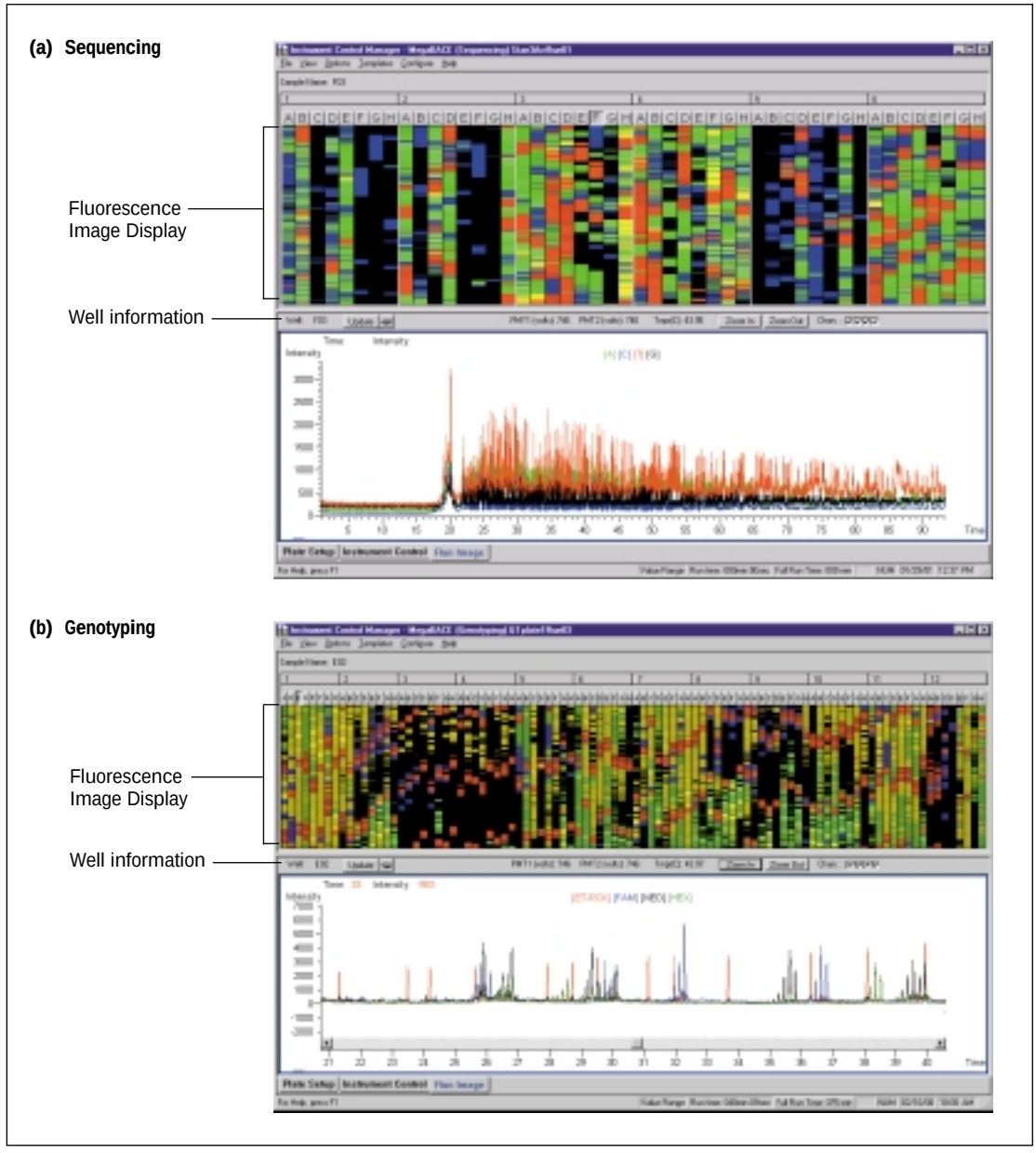


Figure 5-5. Assessing the quality of the run in the Run Image window. (a) A sequencing run in the 48-capillary configuration (MegaBACE 500). (b) A genotyping run in the 96-capillary configuration.

5.5 Checking for empty or bad capillaries

The Instrument Control Manager does not create .rsd files for capillaries that are empty, broken, or clogged. Your administrator sets up this feature. If you know that a capillary is broken or clogged, notify your administrator.

The software indicates an empty or bad capillary in the Run Image window of the Instrument Control Manager. Check the Run Image window to determine which capillaries are empty or bad. In the Fluorescence Image Display, a bad capillary appears black, and a red arrow appears above the well button (figure 5-6). An empty capillary also appears black, and a yellow arrow appears above the well button (figure 5-6).

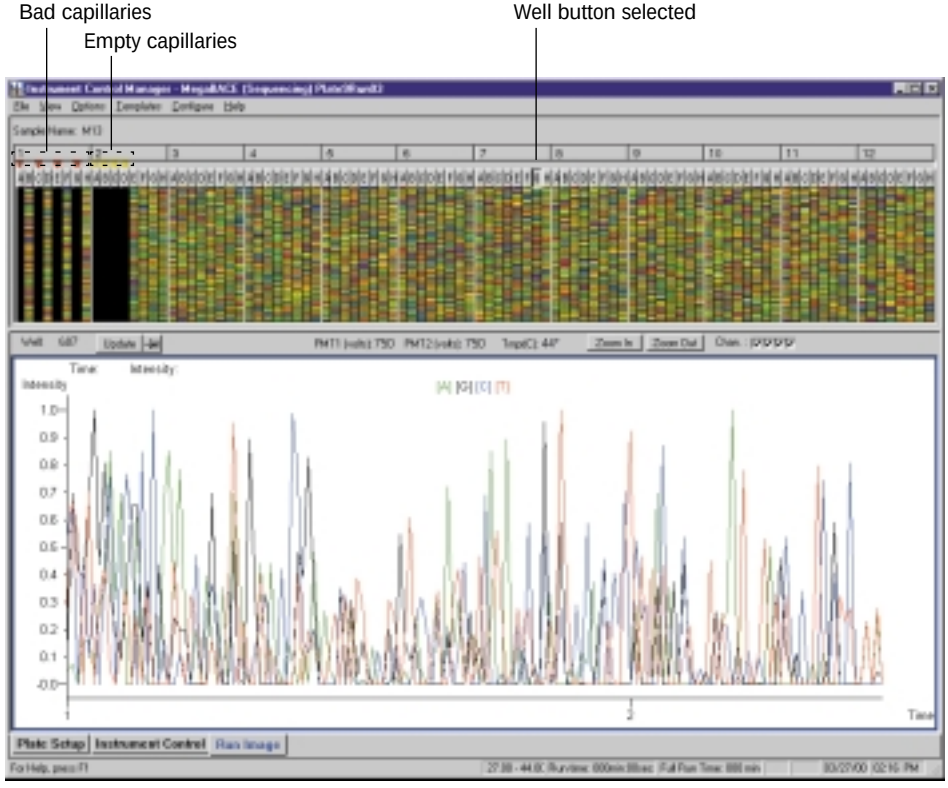


Figure 5-6. The Run Image window for a sequencing run. Empty capillaries are indicated by yellow arrows above the well buttons, and bad capillaries are indicated by red arrows above the well buttons.

5.6 Checking the PMT voltage

You can check the area labeled Well Information (figure 5-5) to see that the photomultiplier tube (PMT) voltage and temperature match the settings in the selected instrument parameters set.

5.7 Displaying an electropherogram

To display an electropherogram of the well data in real time, click a **well** button above the Fluorescence Image Display (figure 5-6). The Electropherogram Display updates at the intervals set in the Run Image Attributes window (section 5.3). You can scroll through the data using the scroll bar.

5.7.1 Navigating from well to well

To navigate—

- One well to the right: press the **right arrow key**
- One well to the left: press the **left arrow key**
- One row to the right: press **CTRL+right arrow key**
- One row to the left: press **CTRL+left arrow key**

5.7.2 Zooming in and out

You can use the **Zoom In** button to magnify the electropherogram so that you can better assess the quality of the data during the run. The **Zoom Out** button returns the display to the previous level of magnification.

5.8 Modifying the Fluorescence Image Display area

You can modify the appearance of the Fluorescence Image Display area in the Run Image window. The Fluorescence Image Display area represents the wells from which the instrument is currently collecting data. You can enlarge the width of the wells in a group by placing the pointer on the dividing line between two well groups (for example, between well group numbers 3 and 4) above the well buttons. The pointer changes to (↔). Drag the pointer to the **right** to enlarge the width of each well in the group to the **left** (for example, group number 3 as shown in figure 5-7).

If you want to enlarge all the wells in the display, click once on the well group number you just expanded (group number 3). All the wells in the display become wider. You use the scroll bar to display the fluorescence data in different wells.

You can reduce the width of the wells in a group by placing the pointer on the dividing line between two well group numbers (for example, between well group numbers 3 and 4). The pointer changes to (↔). Drag the pointer to the **left** to reduce the width of each well in the group to the **left** (for example, group number 3). The width of the wells is only reduced in group number 3.

If you want to return the wells in the display to the default sizing, click the well group number you reduced (number 3). The well widths are resized to the default sizing.

Figure 5-7. Modifying the Fluorescence Image Display area of the Run Image window.

5.9 Checking the capillary current

To check the electrical current in the capillaries, choose **Current Monitor** from the Options menu. The Current Monitor window appears (figure 5-8a and b) and displays the electrical current values for each capillary. For a typical 9–10 kV run, a good range for the current in each capillary is 4–7 µAmp**.**

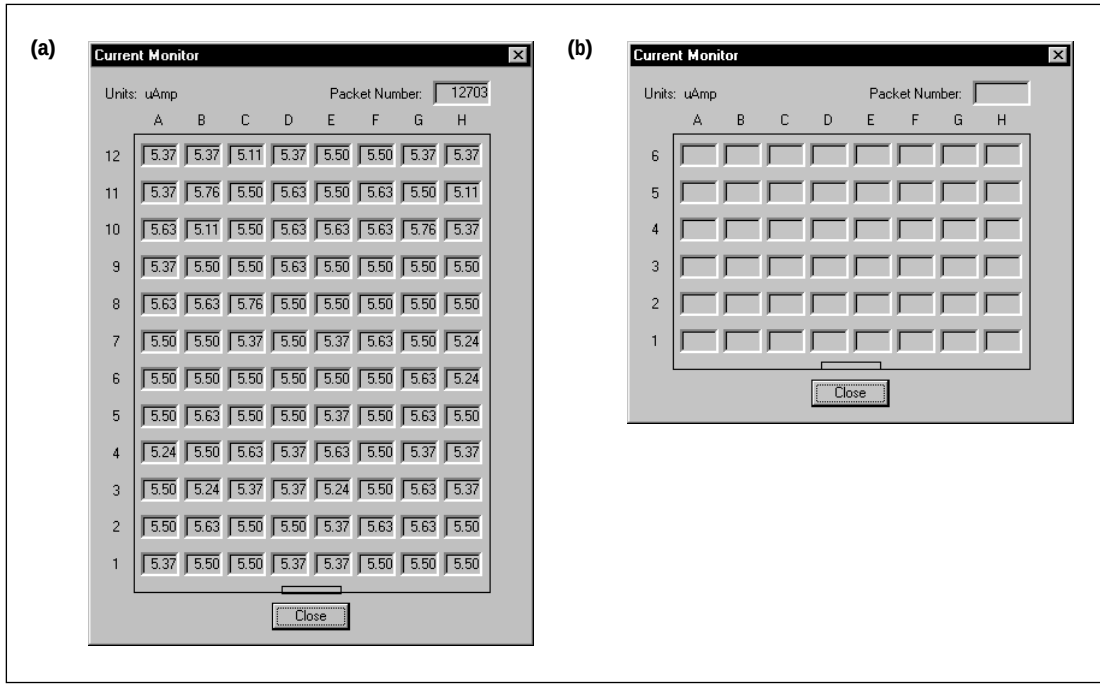


Figure 5-8. The Current Monitor window. (a) The window that appears for an instrument with 96 capillaries. (b) The window that appears for an instrument with 48 capillaries.

You can leave the Current Monitor window open during the run and move the window to the side of the display.

When you finish checking the capillary current, click **Close** to close the window.

5.10 Changing the run time (optional)

During a run, to increase or decrease the remaining time for the run—

1. Click the **Full Run Time** button in the status bar of the Instrument Control Manager window. The Run Length window appears (figure 5-9).

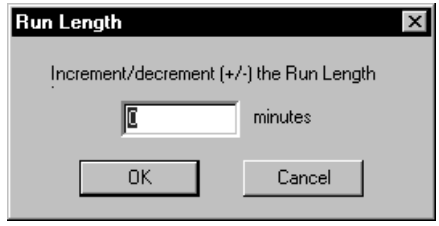


Figure 5-9. The Run Length window.

2. To increase the time, type a positive value in the **minutes** box. To decrease the time, type a negative number in the **minutes** box. Type a hyphen (-) before the number to indicate a negative value.
3. Click **OK.** The left instrument display changes to the new run time and continues to count up the elapsed time.

After the run is finished, you are ready to—

- Use the Matrix Fill and Prerun protocol before running another plate (section 4.10).
- Use the Store Capillaries protocol to store the capillaries in water overnight or on the weekend (section 6.1.3).
- Use the Flush and Dry Capillaries protocol to store the capillaries dry and shut down the instrument for more than 3 days (section 6.2.1).

Caution **If you are shutting down the instrument for more than 3 days, always flush the matrix from the capillaries at the end of the last run and store the capillaries dry. Leaving the matrix in the capillaries for prolonged periods can clog and ruin the capillaries. See section 6.2 for details on flushing and drying the capillaries for a complete shutdown.**

Chapter 6 Leaving the instrument idle or shutting down

This chapter contains instructions for leaving the instrument idle and for shutting down the system completely.

The topics are—

- Leaving the instrument idle overnight or over weekends (section 6.1)
- Shutting down the system for more than 3 days (section 6.2)
- Recovering from a power failure with a UPS (section 6.3)
- Recovering from a power failure without a UPS (section 6.4)
- Preparing the capillaries (section 6.5)

6.1 Leaving the instrument idle overnight or over weekends

Leaving the instrument idle means that the instrument power is on, but you are not using the instrument to run samples. If you are leaving the instrument idle overnight or over the weekend (including a 3-day weekend) and you are using linear polyacrylamide (LPA) matrix, you should store the capillaries wet. You do not need to flush the matrix from the capillaries.

You can store the capillaries two different ways when you are leaving the instrument idle for short periods of time—

- Use the Sleep After This Run check box in the Instrument Control window to store the capillaries in LPA matrix and buffer for up to 16 hours. See section 4.14 for instructions.
- Use the Store Capillaries protocol to store the capillaries in water for more than 16 hours, up to 3 days (section 6.1.1).

See section 6.2 for instructions on storing the capillaries for more than 3 days.

6.1.1 About the Store Capillaries protocol

The Store Capillaries protocol allows you to add water to the water tank on the cathode stage and place fresh water tubes in the reservoir on the anode stage to cover the tips of the capillaries. The protocol then turns off the lasers and reduces the temperature in the electrophoresis compartment to 25 °C (77 °F) or the temperature you set in the Sleep Temperature box in the Instrument Control window. The protocol stores the stages in the up position.

6.1.2 Materials required

For the Store Capillaries protocol, you need (figure 6-1)—

- A clean tank filled with deionized filtered water
- One 2-ml tube for each array installed in the instrument, each containing 1.8 ml deionized filtered water

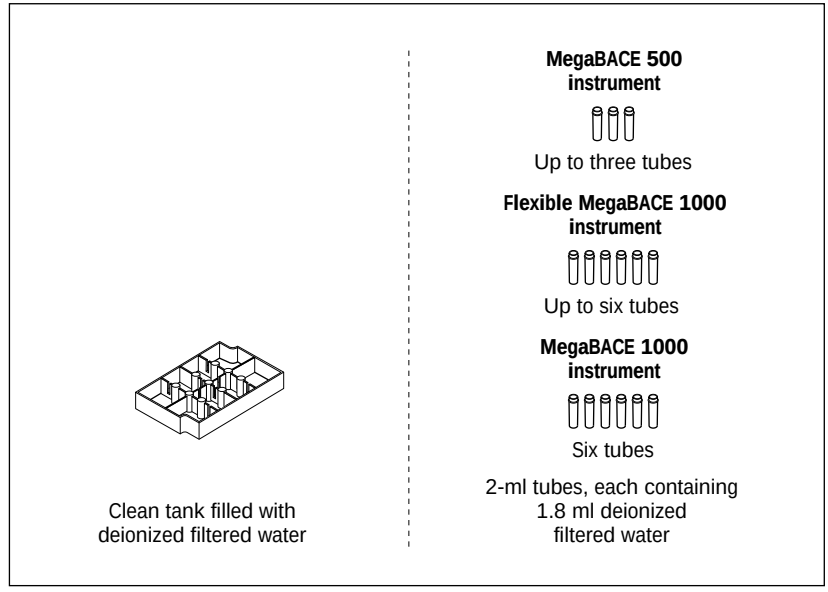


Figure 6-1. The materials for the Store Capillaries protocol. **Note:** You use one tube of water for each capillary array installed in the instrument.

Caution **Do not fill the water tank too full. Open the cathode drawer slowly to prevent spilling the water on the cathode stage. Spilled water (or other material) can damage the electrodes in the cathode stage.**

6.1.3 Using the Store Capillaries protocol

To use the Store Capillaries protocol—

1. Click the **Instrument Control** tab (figure 6-2) to display the Instrument Control window.
2. In the Instrument Parameters area, type a temperature in the **Sleep Temperature** box. The temperature range is 25–30 ºC (77–83 ºF), and the default temperature is 25 °C (77 ºF).

3. Type the length of time the instrument will be idle in the **Sleep Time** box. The time range is 1–72 hours, and the default time is 12 hours.

4. In the List of Protocols, click **Store Capillaries,** and then click **Start.**

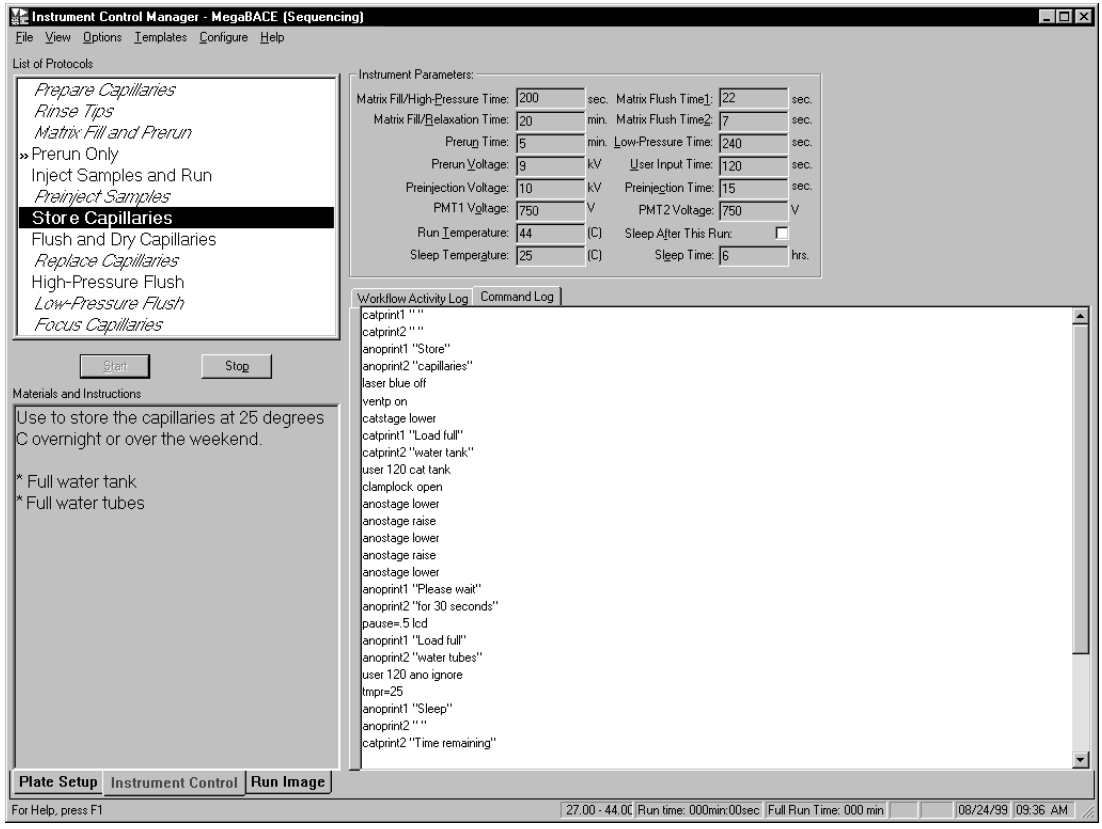


Figure 6-2. The Instrument Control window.

5. When instructed by the instrument displays, load a **full water tank** into the left side of the instrument and load the **water tubes** into the right side of the instrument.

After you close the drawers, the instrument raises the stages to cover the tips of the capillaries with water. The software turns off the laser and reduces the temperature of the electrophoresis compartment to 25 °C (77 ºF) or the temperature you set in the Sleep Temperature box in the Instrument Control window (figure 6-2).

Leave the nitrogen source, the instrument power, and the computer on. Leave the Instrument Control Manager and the Host Scan Controller software running.

Caution **The nitrogen pressure must remain on to keep the stages in the up position and keep the capillary tips covered with water, preventing the capillaries from clogging.**

When the sleep time elapses, the instrument starts warming up the electrophoresis compartment to the default temperature setting of 44 °C (111.2 °F). The left display counts up the time since the temperature was turned on.

Important **Before starting a run, you should allow approximately 3 hours to stabilize the components in the electrophoresis chamber at the set temperature.**

The software selects the Rinse Tips protocol (section 4.5) as the next protocol.

6.2 Shutting down the system for more than 3 days

In general, shut down the system completely only when you will be leaving the instrument continuously unattended for more than 3 days. Before shutting down the system, use the Instrument Control Manager to perform the Flush and Dry Capillaries protocol.

Although it is not required, after you use the Flush and Dry Capillaries protocol, you can log off or shut down the computer and turn off the nitrogen pressure (sections 6.2.2 and 6.2.4). Then you turn off the instrument.

Caution **If you are shutting down the instrument for more than 3 days and the instrument will be continuously unattended, always flush the matrix from the capillaries at the end of the last run and store the capillaries dry. Make sure you use the Flush and Dry Capillaries protocol to preserve the capillaries. If the capillaries are not properly flushed and stored dry, they will become clogged and will have to be replaced.**

6.2.1 Flushing and drying the capillaries

Before shutting down the system, you must flush the matrix from the capillaries with water and store the capillaries dry. This section contains detailed instructions on using the Flush and Dry Capillaries protocol.

Materials required

For the Flush and Dry Capillaries protocol, you need (figure 6-3)—

- An empty water tank
- One 2-ml tube for each array installed in the instrument, each containing 1.8 ml fresh deionized filtered water
- A squirt bottle filled with fresh deionized filtered water
- One empty 2-ml tube for each array installed in the instrument

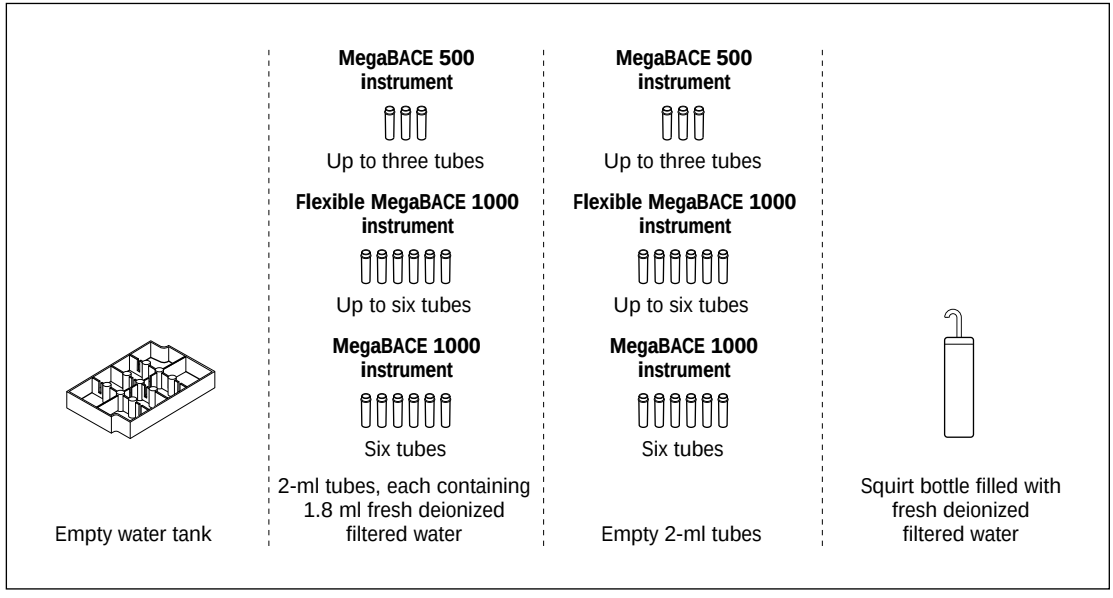


Figure 6-3. The materials for the Flush and Dry Capillaries protocol. **Note:** You use one tube of water for each capillary array installed in the instrument.

The Flush and Dry Capillaries protocol

To flush out the matrix and dry the capillaries—

1. Click the **Instrument Control** tab (figure 6-2) to display the Instrument Control window.
2. In the List of Protocols, click **Flush and Dry Capillaries,** and then click **Start.**
3. When instructed by the left instrument display, remove the **tank** from the left side of the instrument and replace it with an **empty water tank.**

Caution **When the instrument displays instruct you to load an empty water tank into the left side of the instrument, make sure the water tank is completely empty. Otherwise, the tank will overflow and spill inside the instrument.**

4. When instructed by the right instrument display, load **one water tube for each array installed in the instrument** into the right side of the instrument.

   The instrument rinses the capillary tips, and the instrument displays tell you the tip rinse is in progress. Then the instrument displays tell you that the first high-pressure flush is in progress.

Important **Always use fresh deionized filtered water to flush the capillaries. Stale water can cause damage to the capillaries.**

5. Follow the instructions on the instrument displays to complete the Flush and Dry Capillaries protocol.

When the protocol is finished, the instrument displays tell you that capillary drying is complete and display the time elapsed since completion. The Workflow Activity Log in the Instrument Control window lists the end time.

The software selects and places a double arrow in front of the Prepare Capillaries protocol in the List of Protocols (figure 6-2). See section 6.5 for instructions on how to prepare the capillaries.

Important **Remember to run the Prepare Capillaries protocol (section 6.5) when you start up normal operation after storing the capillaries dry. The Prepare Capillaries protocol hydrates the capillaries and prepares them for the matrix fill and sample injection protocols.**

6.2.2 Logging off or shutting down the computer

You can leave the computer running and log off so that another user can log on, or you can shut down the computer. If you decide to log off or shut down the computer, you should close the Instrument Control Manager (choose **Exit** from the File menu) and the Host Scan Controller (type **bye** in the command line). Next, shut down and then turn off the computer.

6.2.3 Turning off the instrument

Caution **Before leaving the instrument, make sure that the electrophoresis and filter compartments are closed. Closing the compartments protects the capillaries and filters and helps keep dust out of the system.**

To turn off the instrument, turn off the power switch on the right side of the instrument (figure 3-1).

6.2.4 Turning off the nitrogen pressure system

When storing the system for more than 3 days, you can leave the external nitrogen source on, or you can turn it off. To turn off the nitrogen pressure system, follow the procedure established in your laboratory.

6.3 Recovering from a power failure with a UPS

6.3.1 Brief power failure

If your instrument and computer are connected to an uninterruptible power supply (UPS), the battery power stored in the UPS should handle all brief power failures with a duration of less than 10 minutes without a problem. Because 90 percent of all power failures last less than 5 minutes, the UPS should allow the instrument to continue its activities without interruption.

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

6.3.2 Extended power failure

Caution **You should always stop the scan and shut off the power-consuming devices early enough to save enough battery power to store the capillaries.**

If the power does not return within several minutes, you should check the time left on the battery. The time remaining will help you decide whether you have time to finish the scan or if you should stop the scan immediately and use the Store Capillaries protocol (section 6.1.3) before the battery reserves are exhausted.

If the capillaries contain matrix and you experience a power failure that lasts more than 10 minutes (depending on the time on the battery backup), you should stop whatever activity the instrument is performing. Because the duration of a power failure is unpredictable, use the Store Capillaries protocol (section 6.1.3) to store the capillaries properly.

6.3.3 Storing the capillaries in the event of an extended power failure

To store the capillaries—

1. Click the **Instrument Control** tab to display the Instrument Control window.
   - If the **Flush and Dry Capillaries** protocol is running, allow the flushing and drying to continue until it is complete.
   - If another protocol is running, click **Stop** to end the activity and save whatever data the instrument has collected.
2. Use one of the two following protocols depending on how long you intend to leave the instrument idle:
   - If you plan to continue running the instrument after the power comes on, use the **Store Capillaries** protocol (section 6.1.3) to turn off the lasers and reduce the temperature of the electrophoresis chamber to 25 °C (77 ºF) or the temperature you specified in the Sleep Temperature box in the Instrument Control window. This protocol allows you to store the capillaries filled with matrix for short-term storage (overnight or over the weekend).
   - If you will be leaving the instrument for more than 3 days, use the **Flush and Dry Capillaries** protocol to clear the capillaries of the matrix (section 6.2.1). Then turn the instrument power switch to off. The switch is on the right side of the instrument (figure 3-1).

Important **Use the Prepare Capillaries protocol when you start up normal operation after flushing and storing the capillaries dry (section 6.5). The Prepare Capillaries protocol hydrates the capillaries and prepares them for matrix and sample injection.**

6.4 Recovering from a power failure without a UPS

If a power outage occurs during a run and you do not have your instrument and computer connected to a UPS, you will lose all the collected data.

Caution **To prevent damage to the instrument and computer, turn off the power switches immediately after losing the power.**

6.4.1 Brief power failure

If the power returns in less than 12 hours—

- Use the Rinse Tips protocol (section 4.5) when you start operation to rinse any excess matrix off the tips, and then use the Inject Matrix and Prerun protocol to fill the capillaries with new matrix. This should allow you to collect data using the Inject Samples and Run protocol.
- Use the Store Capillaries protocol (section 6.1.1) to store the capillaries wet and filled with matrix if you are leaving the instrument overnight or over the weekend. Then use the Rinse Tips protocol as you normally would at the start of operation.

6.4.2 Extended power failure

After a power failure of more than 12 hours—

- Use the Flush and Dry Capillaries protocol as soon as the power comes on and you are able to begin normal operation (section 6.2.1).
- Then use the Prepare Capillaries protocol. (See section 6.5 for instructions.) You can use extra high-pressure flushes to help clear any clogged capillaries.

6.5 Preparing the capillaries

The Prepare Capillaries protocol rinses the capillaries with water at high pressure to hydrate the capillaries and prepare them for matrix and sample injection. This protocol also allows you to inspect the plate to determine if any capillaries are clogged.

6.5.1 Materials required

For the Prepare Capillaries protocol, you need (figure 6-4)—

- An empty water tank
- One 2-ml tube for each array installed in the instrument, each containing 1.8 ml deionized filtered water
- A squirt bottle filled with deionized filtered water
- An empty plate

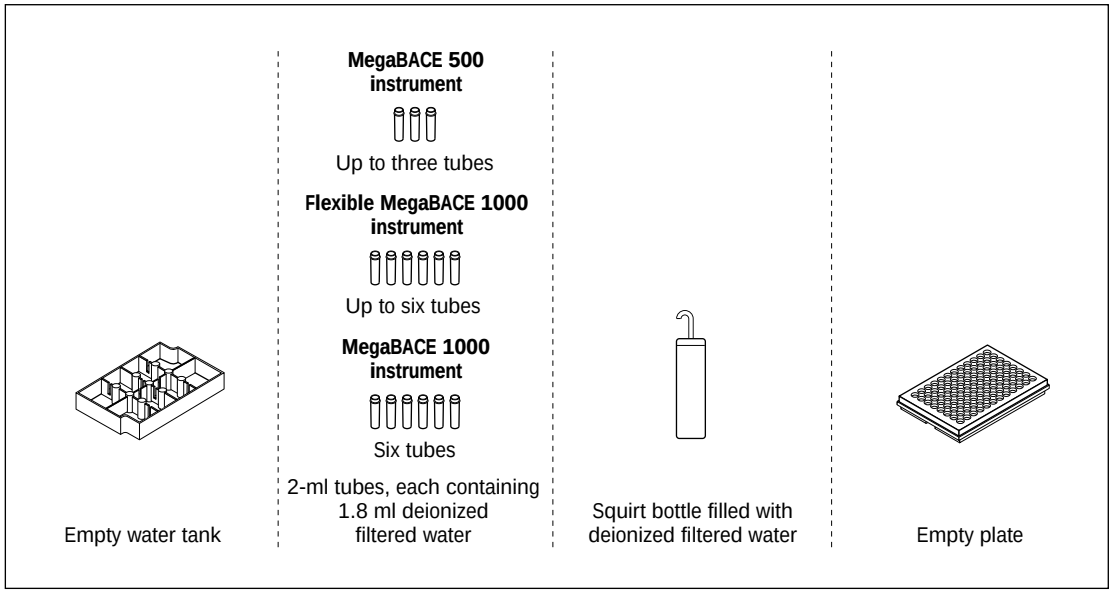


Figure 6-4. The materials for the Prepare Capillaries protocol. **Note:** You use one tube of water for each capillary array installed in the instrument.

6.5.2 The Prepare Capillaries protocol

To prepare the capillaries—

1. In the Instrument Control window, with the **Prepare Capillaries** protocol selected, click **Start.**

   The Prepare Capillaries protocol in the List of Protocols blinks to show that it is in progress, and the Workflow Activity Log lists the start time for the protocol.

2. Follow the instructions on the instrument displays to load the **empty water tank** into the left side of the instrument and the **water tubes** into the right side of the instrument.

   The instrument then applies high-pressure nitrogen to the right side to flush water from the water tubes through the capillaries. The instrument displays tell you that the high-pressure flush is in progress and displays the countdown of the time until the flush is complete.

3. Follow the instructions on the instrument displays to complete the Prepare Capillaries protocol.

The Workflow Activity Log in the Instrument Control window lists the end time for the Prepare Capillaries protocol. The Instrument Control Manager selects and places a double arrow in front of Matrix Fill and Prerun to indicate that this is the protocol that you should use next.

Caution **You must use the Prepare Capillaries protocol to flush any matrix from the capillaries. If any capillaries are clogged, use the High-Pressure Flush protocol and inspect the empty plate each time until you can determine that water can be flushed through all the capillaries.**

If you are unable to unclog some capillaries, you should replace the array that contains the clogged capillaries. See chapter 4 in the *MegaBACE Instrument Maintenance and Troubleshooting Guide* for instructions on replacing and focusing the capillaries.

Part three

Appendixes

Appendix A Quick reference to commands, windows, and buttons

This appendix contains a quick reference of—

- Menu commands and shortcut keys (section A.1)
- Parameters and functions for the Instrument Control Manager windows (section A.2)
- Functions of the buttons for the Instrument Control Manager (section A.3)

A.1 Menu commands and shortcut keys

A.1.1 File menu

Command	Shortcut keys	Function
Exit	CTRL+X	Closes the Instrument Control Manager software.

A.1.2 View menu

Command	Shortcut keys	Function
Tooltips		Displays or hides the Tooltips.
Refresh Screen	F5	Updates the screen.
Display Next Well	Right Arrow	Allows you to move to the next well to the right and display its electropherogram.
Display Previous Well	Left Arrow	Allows you to move to the previous well to the left and display its electropherogram.
Step One Group to the Right	CTRL+Right Arrow	Allows you to move to the next group of wells to the right and display an electropherogram.

Command	Shortcut keys	Function
Step One Group to the Left	CTRL+Left Arrow	Allows you to move to the previous group of wells to the left and display an electropherogram.
Sort Plates		Sorts the plate IDs in the Plate Catalog by plate ID or by plate creation time.

A.1.3 Options menu

Command	Shortcut keys	Function
Current Monitor		Allows you to view the values for the electrical current in each capillary.
Run Image Attributes	F2	Allows you to change the settings for the Run Image window.
Electropherogram Attributes	F4	Allows you to set the scaling for the intensity range of the electropherogram.
File Storage		Allows you to set the storage location for your raw sample data files.

A.1.4 Templates menu

Command	Shortcut keys	Function
Plate Setup Templates		Available only in the Plate Setup window.
Select Template	CTRL+T	Allows you to select a new template.
Set Default		Allows you to set a default template.
Clear Default		Allows you to clear the default template.
Save Template		Allows you to save a template.

Command	Shortcut keys	Function
Instrument Templates		Available only in the Instrument Control window.
Select Template	CTRL+I	Allows you to select a new template.
Save Template		Allows you to save a template.

A.1.5 Configure menu

Command	Shortcut keys	Function
Edit Mode		If enabled by the administrator, allows you to turn on or off the edit mode.
Automatic Base Calling	F8	If enabled by the administrator, allows you to use the Automatic Base Calling window to— • Turn on or off automatic base calling • Select a default base caller • Turn on or off automatic export to ABD, FASTA, SCF, or ASCII text file formats after automatic base calling
Applications		Allows you to select either the sequencing or the genotyping application.

A.1.6 Help menu

Command	Function
Help Topics	Displays the Help contents and index.
About MegaBACE Instrument Control Manager	Provides the software version number and copyright dates.

A.2 Parameters and functions for the Instrument Control Manager windows

This section lists the parameters and functions for each of the three windows in the Instrument Control Manager.

A.2.1 Plate Setup window

The Plate Setup window contains five tabs—

- Electrophoresis Parameters
- Chemistry Parameters
- Sample Names
- Optional Parameters
- Comments

Three of these tabs allow you to view or set parameters for the plate. The following tables list and define the parameters in the three parameters tabs.

Electrophoresis Parameters tab

Parameter	Function
Sample Injection Voltage	Allows you to set the voltage for the sample injection.
Run Voltage	Allows you to set the voltage for the run.
Sample Injection Time	Allows you to set the length of time for the sample injection.
Run Time	Allows you to set the length of time for the run.

Chemistry Parameters tab

Parameter	Function
Chemistry Name	Allows you to select the name for the chemistry parameter set.
Base (A, C, G, T)	(Sequencing only) Required to specify the bases-to-channel mapping, indicating which base is detected in each spectral channel.
Dye (1, 2, 3, and 4)	(Genotyping only) Required to specify the dye-to-channel mapping, indicating which dye is detected in each spectral channel.
Laser Mode	The laser mode specified for the run: Blue, Green and Blue, or Green.

Parameter	Function
Filter (1, 2, 3, and 4)	The names specified for the filters. Note that the filter number indicates the spectral channel. For example, filter 1 is used to detect the dye emission in spectral channel 1.
Beamsplitter (A and B)	The names specified for the beamsplitters. Beamsplitter A is used for spectral channels 1 and 2. Beamsplitter B is used for spectral channels 3 and 4.

Optional Parameters tab

Parameter	Function
PMT1 Voltage	Allows you to enter a voltage for PMT1 as a part of the plate definition. This value overrides the value entered in the Instrument Control window.
PMT2 Voltage	Allows you to enter a voltage for PMT2 as a part of the plate definition. This value overrides the value entered in the Instrument Control window.
Run Temperature	Allows you to enter the run temperature as a part of the plate definition. This value overrides the value entered in the Instrument Control window.
Base Caller	Allows you to specify a base caller that is different from the default base caller.

A.2.2 Instrument Control window

The Instrument Control window is divided into the following areas:

Area	Function
List of Protocols	Displays the list of protocols for running the instrument and maintaining the capillaries.
Instrument Parameters	Contains the fields for entering the instrument control parameters.
Materials and Instructions	Provides instructions for using the protocols and a list of the materials needed.
Command Log	Displays in real time the commands from the Host Scan Controller.

Area	Function
Workflow Activity Log	Displays a log of the protocol, plate ID, run ID, status, and date and time the protocol was used.
Status Bar	Displays the time elapsed and displays the full run time, which you can change during the run.

The Instrument Parameters area contains the following parameters:

Parameter	Function
Matrix Fill/High-Pressure Time	Allows you to set the length of time for the high-pressure matrix injection (range: 1–600 s).
Matrix Fill/Relaxation Time	Allows you to set the length of time to allow the matrix to equilibrate (range: 0–120 min).
Prerun Time	Allows you to set the length of time for the prerun (range: 0–120 min).
Prerun Voltage	Allows you to set the voltage for the prerun (range: 1–20 kV).
Preinjection Voltage	Allows you to set the voltage for the preinjection (range: 1–20 kV).
PMT1 Voltage	Allows you to set the voltage for PMT1 (range: 450–950 V).
Run Temperature	Allows you to set the operating temperature for the electrophoresis compartment (range: 27–44 °C).
Sleep Temperature	Allows you to set the temperature that you want to maintain in the electrophoresis compartment while the instrument is idle (range: 25–30 °C).
Matrix Flush Time1	Allows you to set the length of time for the first matrix flush (range: 0–60 s).
Matrix Flush Time2	Allows you to set the length of time for the second matrix flush (range: 0–60 s).
Low-Pressure Time	Allows you to set the length of time for the low-pressure flush (range: 1–300 s).
User Input Time	Allows you to set the amount of time you need to open and close the anode or cathode drawer after the instrument displays provide the instructions to load the plate, tank, or tubes in either the anode (right) or cathode (left) side of the instrument (range: 10–240 s).

Parameter	Function
Preinjection Time	Allows you to set the time for the preinjection (range: 1–600 s).
PMT2 Voltage	Allows you to set the voltage for PMT2 (range: 450–950 V).
Sleep After This Run	Allows you to select a check box to set the instrument in sleep mode after the current or next run.
Sleep Time	Allows you to set the time for the temperature to be reduced and the lasers to be turned off for storing the capillaries while the instrument is idle (range: 1–72 hr or 1–16 hr if used with Sleep After This Run).

A.2.3 Run Image window

The Run Image window is divided into the following areas:

Area	Function
Well Group Number	Displays the number for a group of eight wells.
Well Buttons	Displays the buttons for each of the wells.
Fluorescence Image Display	Displays the pseudo image of the fluorescence as it is scanned in the capillaries.
Well Information	Displays information about the selected well and the instrument, such as well ID, PMT voltage, temperature, and channel.
Electropherogram	Displays electropherogram data for the capillary you selected in the Well Buttons area.
Status Bar	Displays the Run Time count up and displays the Full Run Time, which you can change during the run.

The Well Information area of the Run Image window displays the following instrument parameters:

Parameter	Function
PMT1 and PMT2 (volts):	Displays the PMT voltage set in the Optional Parameters tab or the Instrument Control Manager window.
Temp(C):	Displays the temperature you set in the Instrument Parameters area of the Instrument Control window.
Chan:	Allows you to check a box for the channel(s) you want to display in the Electropherogram Display area.

A.2.4 Select a Plate window

The Select a Plate window includes the—

- **Plate ID box—**Allows you to enter the plate ID of a plate setup that has been defined in a plate setup data file (.psd).
- **Available plates list—**Contains the plate IDs that have already been entered in the Instrument Control Manager.

A.3 Functions of the buttons for the Instrument Control Manager

This section lists the buttons and their functions for each of the three windows in the Instrument Control Manager.

A.3.1 Plate Setup window

The Plate Setup window contains the following buttons:

Button	Function
New	Creates a new plate.
Save	Saves the current plate definition.
Cancel	Cancels all modifications to the current or new plate definition.
Rerun	Allows the selected plate to be rerun.
Delete	Removes the selected plate/run from the Plate Catalog List and allows deleting of the .rsd files (if any).

A.3.2 Instrument Control window

Button	Function
Start	Starts the series of commands in the selected protocol.
Stop	Stops the commands in the selected protocol.

A.3.3 Run Image window

Button	Function
A B C D E F G H Cathode assembly configuration	Each button allows you to display an electropherogram for the corresponding well.
Update	Updates the electropherogram display each time you click the button.
	Automatically updates the electropherogram display at the frequency you set in the Run Image Attributes window.
Zoom In	Magnifies the electropherogram.
Zoom Out	Returns the electropherogram to the previous magnification level.

Appendix B Sample preparation (genotyping only)

This section describes the sample preparation for genotyping runs on the MegaBACE instrument. The topics are—

- About the MegaBACE ET size standards (section B.1)
- PCR guidelines (section B.2)
- Why you need to desalt the DNA samples (section B.3)
- Using dialysis to desalt the DNA samples (section B.4)
- Preparing the samples for loading (section B.5)

B.1 About the MegaBACE ET size standards

For genotyping, you can use a MegaBACE energy transfer (ET) size standard as an internal DNA standard of known fragment sizes. The ET dye label on the MegaBACE size standard uses FAM as a donor dye and ROX as an acceptor dye. The blue laser emits light at 488 nm, which is near the absorption maximum of FAM. FAM captures the blue laser energy and then transfers the energy to excite ROX. By using the energy transferred from FAM, you can excite ROX efficiently with the blue laser and use a smaller amount of labeled size standard. See the *MegaBACE Instrument Administrator's Guide* for details on the recommended dye sets for genotyping.

B.2 PCR guidelines

Perform the PCR* process and pooling of PCR products under the conditions you normally use. Because electrokinetic injection uses very small amounts of DNA, you may be able to reduce the pooled reaction volume to as low as 5 µl and still achieve good injection. Pool your PCR samples to achieve the desired level of marker multiplexing.

Caution **If you use oil to overlay the PCR products, be sure to follow the dialysis step specific for oil. Using oil in the PCR may complicate the dialysis procedure.**

* See the reverse side of the title page for patent information.

B.3 Why you need to desalt the DNA samples

Cautions

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 genotyping, you must desalt your samples.

Failure to desalt the samples may result in data with low signal intensity.

You desalt the PCR pools to reduce the ratio of anions in the buffer to DNA in the final sample that is loaded into the MegaBACE instrument. Fluorescent detection of DNA requires only very small amounts of DNA sample, generally 1–20 nM. Capillary array electrophoresis uses electrokinetic injection to inject the DNA samples into the capillaries. During electrokinetic injection, anions in the loading buffer compete with the negatively charged DNA in the samples. To inject the DNA samples into the capillaries successfully, the ratio of the anions in the buffer relative to the DNA must be 10 000:1 or less.

However, in the typical PCR samples for genotyping, the ratio of anions to the DNA is 100 000:1. Therefore, you must desalt any genotyping samples before you run them on the MegaBACE instrument to achieve sufficient signal intensity from the samples. Desalting the samples in an EDTA-containing buffer, such as TE, reduces the anions in the buffer while maintaining the pH.

Various methods exist for desalting the samples, including dialysis, ethanol precipitation, and the use of Sephadex™ separation resins or magnetic beads. The dialysis procedure (section B.4) is recommended, but alternative methods can also produce good results.

B.4 Using dialysis to desalt the DNA samples

This section describes how to desalt the samples using dialysis.

B.4.1 Materials required for dialysis

For the desalting protocol, you need—

- Tabletop centrifuge
- 96-well filtration plate for desalting (Millipore™ MAVM N05 10 or MAVM N05 50)
- 20–200 µl pipettor
- TE buffer (50x), ultrapure (Amersham Biosciences US75834-100 ml)
- Deionized filtered water
- 1-liter graduated cylinder

- A glass dish (such as Pyrex™) measuring 20.32 cm by 20.32 cm by 5.08 cm (8 inch by 8 inch by 2 inch)
- Magnetic stirring unit and stirring magnet
- Paper towels
- Appropriate storage container for the desalted samples (sample plate)

B.4.2 Dialysis procedure

Cautions

Acceptable sample volume is 15–50 µl per well to obtain a uniform salt concentration in each well.

Keep pointed objects away from the well membranes of the filtration plate. If a well membrane is pierced, you can lose part of the well contents.

(Oil users only) Prewet the filtration plate with TE buffer before adding your samples to avoid clogging the pores of the membranes with the oil.

1. Prepare the sample filtration plate as follows (figure B-1):
   - Spin down the PCR sample plate or sample tubes using a tabletop centrifuge.
   - Remove the plastic cover of the 96-well filtration plate and remove the filtration plate from the base. Place the plate on the base loosely. Using a 20–200 µl pipettor, transfer the DNA samples from the tubes into the membrane-lined wells of the filtration plate. For uniform coverage, place each sample at the center of the membrane.
   - Carefully cover the top of the filtration plate using sealing film.
2. To prepare the dish of desalting solution, measure 750 ml 0.1x TE buffer (1.5 ml 50x TE buffer and 748.5 ml deionized water). Pour the solution into a glass dish containing a stirring magnet.
3. Place the dish of desalting solution onto a magnetic stirring unit (figure B-1), and then set the speed to low for gentle stirring.

Caution

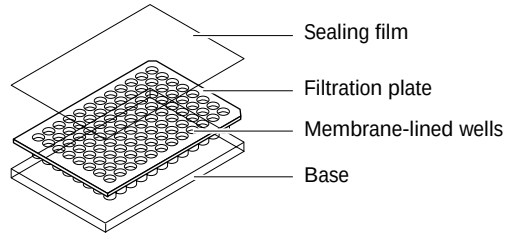
Make sure the desalting solution is gently stirring and not splashing on the sides of the dish.

4. Carefully remove the filtration plate from the base and place the plate on the liquid surface. Dialyze using gentle stirring for 15 minutes.
5. Turn off the stirring unit and carefully remove the filtration plate from the dish. Gently blot the bottom of the wells onto paper towels and return the plate to the base assembly.

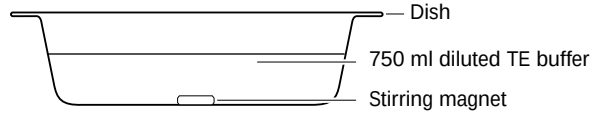
6. Transfer the desalted DNA samples into the plate for running samples in the instrument.

Caution **The dialysis process may change the reagent volumes.**

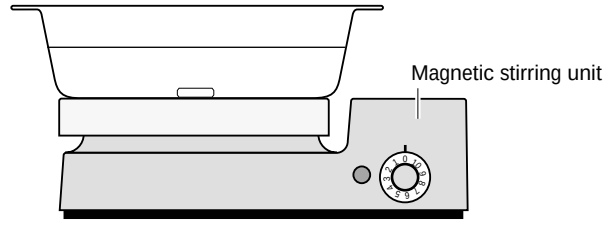
1. Put the DNA samples in the wells of the filtration plate, and then cover the plate with sealing film.



2. Prepare 750 ml diluted TE buffer in a dish. Place a stirring magnet in the dish.



3. Place the dish on a magnetic stirring unit, and set the speed to low for gentle mixing.



4. Remove the filtration plate from its base and carefully place the plate on the liquid surface in the dish. Gently mix for 15 minutes to dialyze the samples.

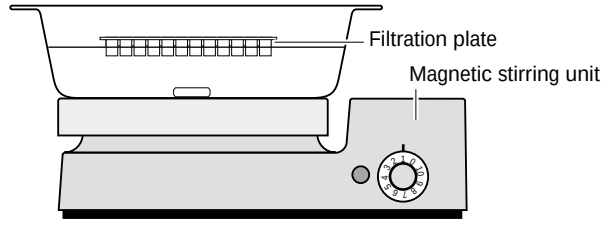


Figure B-1. The desalting protocol.

B.5 Preparing the samples for loading

This section describes how to prepare the spectral matrix standards and the desalted PCR pools for loading in the MegaBACE instrument.

B.5.1 Materials required for sample loading

- MegaBACE ET size standard from Amersham Biosciences. Use one of the following:
  - ET400-R, product code 250205-01 (500 µl) or 250205-02 (2.5 ml)
  - ET550-R, product code 256550-01 (500 µl) or 256550-02 (2.5 ml)
  - ET900-R, product code 256900-01 (500 µl) or 256900-02 (2.5 ml)
- Loading solution (Amersham Biosciences US79916, 40 ml)
- PCR pools, desalted
- Spectral matrix standards. Required only for a spectral calibration run (appendix C). You can order the standards from the supplier of your dye-labeled primers. Alternatively, you can use single-dye PCR reactions or dye-labeled size ladders in the relevant dyes. The Genetic Profiler software requires approximately five peaks in each color to create a spectral overlap matrix.

B.5.2 Procedure for preparing the samples for loading

Warning

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.

Caution

The minimum sample volume you can load successfully in the MegaBACE instrument is 5 µl.

For each sample—

1. Combine the loading solution with the other reagents using the following proportions:
   - PCR pools

     2.75 µl loading solution
     0.25 µl ET size standard
     2.00 µl PCR pools, desalted

     5.00 µl Total

- Spectral matrix standards (for a spectral calibration run only)

  2.5 µl loading solution
  2.5 µl spectral matrix standard (for ET size standard, use 2.5 µl diluted with deionized water 1:20)

  5.00 µl Total

Important **If you use a size standard other than one of the MegaBACE ET size standards, you may need to adjust the volume of the size standard.**

Note: You can adjust the proportions of the PCR pools and loading solution as necessary. Add less of the PCR pools if your signals are too high or more of the PCR pools if your signals are too weak.

2. Mix the prepared samples thoroughly and then spin down the samples in a tabletop centrifuge.
3. Denature the samples at 95 °C (203 °F) for 1 minute. Immediately, cool the samples on ice for several minutes. The samples should be kept on ice until they are loaded in the instrument.

Appendix C Performing a spectral calibration run (genotyping only)

You perform a spectral calibration run to collect the signal data needed to create a spectral overlap matrix. For the calibration run, you use the spectral matrix standards for your dye set, which may be run on the same plate as your PCR samples. This appendix describes—

- Why you need to perform spectral calibration runs (section C.1)
- Preparing a spectral calibration plate (section C.2)
- Performing the spectral calibration run (section C.3)

C.1 Why you need to perform spectral calibration runs

C.1.1 About spectral overlap

The MegaBACE instrument has four spectral channels that allow you to detect the emissions of up to four dyes per capillary. Each dye in a multicolor experiment emits fluorescent light of a characteristic wavelength that is filtered and detected through a specific spectral channel. However, portions of the fluorescent emission from each dye usually occur within the emission range of the other dyes in the dye set and can be present in other spectral channels. The overlap in the fluorescent emissions across the spectral channels is called spectral overlap. To perform reliable genotyping on the raw MegaBACE data, you must first remove the spectral overlap.

C.1.2 About spectral calibration runs

A spectral calibration run measures the signal present from each dye in all four spectral channels of the instrument. For the spectral calibration run, the plate should include spectral matrix standards for each dye in your dye set.

After the calibration run, you use the Genetic Profiler software to create a spectral overlap matrix from the run data, which measures the amount of unwanted signal detected in each spectral channel.

You should perform a spectral calibration run and recalculate the spectral overlap matrix approximately every 2–4 weeks. In addition, you should perform a spectral calibration run if you—

- Change the run conditions, such as the dye set or filter set, or the PMT, laser, or temperature settings.
- Replace the capillary arrays.
- Move the instrument.

C.1.3 About the spectral overlap matrix

The spectral overlap matrix is a mathematical correction matrix that you create using Genetic Profiler. The matrix measures the spectral overlap present in each spectral channel. During analysis, Genetic Profiler uses the matrix to remove the spectral overlap.

After a spectral overlap matrix is created, it can be used again for subsequent runs performed on the same instrument. The runs must share the same run conditions, such as the dye set or filter set, or the PMT, laser, and temperature settings. When any of these variables changes, you must perform a spectral calibration run and recalculate the spectral overlap matrix. You should periodically recalculate the spectral overlap of the system, approximately every 2–4 weeks.

C.2 Preparing a spectral calibration plate

You prepare a spectral calibration plate that contains spectral matrix standards for the dye set that you plan to use. You can use—

- One well on the plate for each dye in your dye set (minimum requirement). **Note:** You may want to use two wells for each dye, and then use the resulting sample file with the best signal from each pair.
- Two to five wells on the plate for each dye. Genetic Profiler allows you to average the results of up to five wells for each dye, which may provide a more accurate matrix.

Warning ⚠

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.

Caution

The minimum sample volume you can successfully load in the MegaBACE instrument is 5 µl per well.

To prepare the spectral calibration plate, combine 50% loading solution with 50% standard for each well as table C-1 shows. Make sure you mix and denature the samples.

Table C-1. Example of a spectral calibration plate using dye set 2

Wells*	Contents
A01, B01	2.5-µl FAM spectral matrix standard, 2.5-µl loading solution
C01, D01	2.5-µl HEX or JOE spectral matrix standard, 2.5-µl loading solution
E01, F01	2.5-µl NED or TAMRA spectral matrix standard, 2.5-µl loading solution
G01, H01	2.5-µl diluted MegaBACE ET size standard (diluted with deionized water 1:20), 2.5-µl loading solution

* You can use up to five wells for each dye if you want Genetic Profiler to average the results for each dye. Averaging the results may provide a more accurate spectral overlap matrix.

C.3 Performing the spectral calibration run

See chapter 4 for instructions on how to perform a run. During the run, you can use the Run Image window to monitor the signal in the wells that contain the spectral matrix standards. (See chapter 5 for details.)

After the run is complete, open the Genetic Profiler software and create a spectral overlap matrix from the collected data. (See the *MegaBACE Genetic Profiler User's Guide* or the Help available within Genetic Profiler for details.)

Appendix D Quick reference to the protocols

This appendix contains tables of the messages provided by the instrument or computer and the actions you perform while running the protocols. Some of the key instrument actions are also included.

The tables are—

- Messages and actions for the Prepare Capillaries protocol (table D-1)
- Messages and actions for the Rinse Tips protocol (table D-2)
- Messages and actions for the Matrix Fill and Prerun protocol (sequencing only) (table D-3)
- Messages and actions for the Matrix Fill and Prerun protocol (genotyping only) (table D-4)
- Messages and actions for the Inject Samples and Run protocol (table D-5)
- Messages and actions for the Store Capillaries protocol (table D-6)
- Messages and actions for the Flush and Dry Capillaries protocol (table D-7)

Table D-1 describes the step-by-step tasks you perform when you use the Prepare Capillaries protocol. The table also describes some of the key instrument actions. After you click **Start,** the displays on the front of the instrument (left and right sides) or computer messages prompt you to perform each task.

Table D-1. Messages and actions for the Prepare Capillaries protocol

Message	Your actions/instrument actions
Load empty tank.	Open the cathode drawer, remove the empty water tank, and then insert a clean empty water tank. Close the drawer. **Caution: Make sure the tank is completely empty.**
Load full water tubes.	Open the anode drawer, remove the empty tubes, and place full water tubes in the anode holder. Close the drawer. The instrument rinses the capillary tips on the cathode side. **Note:** Use one tube for each capillary array installed in the instrument.
High-pres flush 1 in progress.	The instrument applies high pressure to hydrate the capillaries.
Refill tubes with water.	Open the anode drawer and, using the squirt bottle, refill the tubes with water.
High-pres flush 2 in progress.	The instrument performs another high-pressure flush to thoroughly hydrate the capillaries.
Load empty plate.	Open the cathode drawer, remove the water tank, and place a clean empty plate in the cathode stand.
Refill tubes with water.	Open the anode drawer and, using the squirt bottle, refill the tubes with water.
High-pres flush 3 in progress.	The instrument uses high pressure to flush water through the capillaries into the empty plate.
Inspect plate. Load full tank.	Open the cathode drawer, remove the plate, and inspect it to see that each well has water. An empty well indicates a clogged capillary. Place a clean full water tank in the cathode stand. **Caution: Do not fill the water tank too full. Close the cathode drawer slowly.**
Refill tubes with water.	Open the anode drawer and, using the squirt bottle, refill the tubes with water.
Capillaries ready for matrix fill.	The instrument has finished the Prepare Capillaries protocol and is ready to fill the capillaries with matrix. The time counts up since the Prepare Capillaries protocol finished.

Table D-2 describes the step-by-step tasks you perform when you use the Rinse Tips protocol. The table also describes some of the key instrument actions. After you click **Start,** the displays on the front of the instrument (left and right sides) or computer messages prompt you to perform each task.

Table D-2. Messages and actions for the Rinse Tips protocol

Message	Your actions/instrument actions
Load full water tank.	Open the cathode drawer, remove the buffer plate or water tank, and then insert a clean full water tank. Close the drawer. **Caution: Do not fill the water tank too full. Close the drawer slowly.**
Load full water tubes.	Open the anode drawer, remove the tubes, and place full water tubes in the anode holder. Close the drawer. **Note:** Use one tube for each capillary array installed in the instrument.
Tip Rinse in progress.	The instrument rinses the capillary tips on both the cathode side and the anode side.
Rinse tips complete.	The instrument has finished rinsing the capillary tips and selects the Matrix Fill and Prerun protocol as the next protocol to run.

Table D-3 describes the step-by-step tasks you perform when you use the Matrix Fill and Prerun protocol for sequencing. The table also describes some of the key instrument actions. After you click **Start,** the displays on the front of the instrument (left and right sides) or computer messages prompt you to perform each task.

Table D-3. Messages and actions for the Matrix Fill and Prerun protocol (sequencing only)

Message	Your actions/instrument actions
Load prefilled buffer plate.	When a message appears in the Instrument Control Manager, **Load prefilled buffer plate,** open the cathode drawer and remove the old buffer plate or water tank. Then load a buffer plate. **Caution: Open the cathode drawer slowly to prevent spilling liquid on the cathode stage.**
Load matrix tubes.	Open the anode drawer, place tubes full of matrix in the anode holder, and then close the anode drawer. A message appears on the instrument display, **Matrix fill.** Then another message, **Matrix equilibration,** appears, and the time until completion counts down. **Note:** Use one tube of matrix for each capillary array installed in your instrument.
Prerun in progress.	The instrument performs a prerun for the length of time entered into the instrument parameters.
Prerun complete.	The Instrument Control Manager selects Inject Samples and Run as the next protocol to run.

Note: If more than 15 minutes elapses after the Matrix Fill and Prerun protocol has finished, the software selects the Prerun Only protocol as the next protocol to use, and you must perform another prerun before you inject the samples.

Table D-4 describes the step-by-step tasks you perform when you use the Matrix Fill and Prerun protocol for genotyping. The table also describes some of the key instrument actions. After you click **Start,** the displays on the front of the instrument (left and right sides) or computer messages prompt you to perform each task.

Table D-4. Messages and actions for the Matrix Fill and Prerun protocol (genotyping only)

Message	Your action/instrument action
Please load buffer plate.	Open the cathode drawer, remove the old buffer plate or water tank, and insert a fresh buffer plate. Alternatively, you can leave the buffer plate in place from a previous run. **Caution: Open the cathode drawer slowly to prevent spilling liquid on the cathode stage.**
Load matrix tubes.	Open the anode drawer, place tubes full of matrix in the anode holder, and then close the anode drawer. A message appears on the instrument display, **Matrix fill.** Then another message, **Matrix equilibration,** appears, and the time until completion counts down. **Note:** Use one tube of matrix for each capillary array installed in your instrument.
Click continue to change plate.	When a message appears in the Instrument Control Manager, **Please be ready to put in the NEW buffer plate,** click **Continue.**
Load NEW buffer plate.	Open the cathode drawer, remove the used buffer plate, and insert a *fresh* buffer plate. Close the drawer. The instrument starts the prerun, and a message appears on the instrument display, **Prerun in progress.**
Prerun complete.	The Instrument Control Manager selects Inject Samples and Run as the next protocol to run.

Note: If more than 15 minutes elapses after the Matrix Fill and Prerun protocol has finished, the software selects the Prerun Only protocol as the next protocol to use, and you must perform another prerun before you inject the samples.

Table D-5 describes the step-by-step tasks you perform when you use the Inject Samples and Run protocol. The table also describes some of the key instrument actions. After you click **Start,** the displays on the front of the instrument (left and right sides) or computer messages prompt you to perform each task.

Table D-5. Messages and actions for the Inject Samples and Run protocol

Message	Your actions/instrument actions
Load clean water tank.	Open the cathode drawer and remove the buffer plate and set aside for future use. Insert a clean full water tank. **Caution: Do not fill the water tank too full.** Slowly close the drawer. The instrument rinses the capillary tips on the cathode side.
Click continue to add samples.	A message appears in the Instrument Control Manager software, **Make sure you are ready to begin sample injection!** Click **Continue** in the message box.
Quickly load sample plate.	Carefully open the cathode drawer, remove the water tank, and insert the sample plate. The instrument starts sample injection. A message appears on the instrument display, **Sample injection in progress,** along with the time remaining to complete the injection.
Load buffer plate.	Slowly open the cathode drawer, remove the sample plate and load the buffer plate. A message appears on the instrument displays, **Sample run in progress.** The instrument starts to scan the samples.
Sample run complete.	The Instrument Control Manager selects Matrix Fill and Prerun as the next protocol to run.

Table D-6 describes the step-by-step tasks you perform when you use the Store Capillaries protocol. The table also describes some of the key instrument actions. This protocol allows you to store the capillaries in water for more than 16 hours, up to 3 days. After you click **Start,** the displays on the front of the instrument (left and right sides) or computer messages prompt you to perform each task.

Table D-6. Messages and actions for the Store Capillaries protocol

Message	Your actions/instrument actions
Load full water tank.	Open the cathode drawer, remove the buffer plate, place the full water tank in the cathode holder, and then slowly close the cathode drawer. **Caution: Do not fill the water tank too full.**
Load full water tubes.	Open the anode drawer, place tubes full of water in the anode holder, and then close the anode drawer. A message appears on the instrument display, **Sleep. Time remaining.** **Note:** Use one tube of water for each capillary array installed in your instrument. The blue laser is left in idle mode, and the temperature in the electrophoresis chamber is reduced to 25 °C (77 °F) or the temperature you set in the instrument parameters. Both the cathode and the anode stages rise to store the capillaries in water.
Store capillaries complete.	The temperature starts to increase to 44 °C (111.2 °F). The instrument display counts the time since the temperature has been at 44 °C (111.2 °F) or the temperature you set in the instrument parameters.

Table D-7 describes the step-by-step tasks you perform when you use the Flush and Dry Capillaries protocol. The table also describes some of the key instrument actions. This protocol allows you to store the capillaries dry for more than 3 days. After you click **Start,** the displays on the front of the instrument (left and right sides) or computer messages prompt you to perform each task.

Table D-7. Messages and actions for the Flush and Dry Capillaries protocol

Message	Your actions/instrument actions
Load empty tank.	Open the cathode drawer, remove the buffer plate, and then insert a clean empty water tank. Close the drawer. **Caution: Make sure the tank is completely empty.**
Load full water tubes.	Open the anode drawer, remove the matrix tubes, and place full water tubes in the anode holder. Close the drawer. **Note:** Use one tube of water for each capillary array installed in your instrument.
Tip rinse in progress.	The instrument rinses the capillary tips.
High-pres flush 1 in progress.	The instrument applies high pressure to flush the capillaries.
Refill tubes with water.	Open the anode drawer and, using the squirt bottle, refill the tubes with water. **Note:** Refill the tubes six times for six more high-pressure flushes.
High-pres flush [n] in progress.	The instrument performs high-pressure flushes to thoroughly cleanse the capillaries. Before each flush, the instrument displays tell you to refill the tubes with water.
High-pres flush 7 in progress.	A message appears in the Instrument Control Manager. **Do you want to dry the capillaries?** Click **Continue.**
Load clean emptied tank.	Slowly open the cathode drawer, remove the water tank, and place a clean, empty water tank on the cathode stand.
Load new empty tubes.	Open the anode drawer, remove the water tubes, and place clean empty tubes in the anode holder.
Capillary drying in progress.	The instrument applies high pressure to dry the capillaries.
Capillary flush complete.	The instrument has finished the flush and dry process and is ready for you to turn it off to shut down completely for more than 3 days.

Glossary

.abd files—(sequencing only) the base-called data files that can be viewed using ABD software and used by the Phred application. Each .abd file contains the data for a given well on a plate (for example, A01), including the raw and analyzed electropherogram data.

.esd files—(sequencing only) the base-called data files. Each .esd file contains the called sequence, read length, sequencing starting and ending points, quality values, current profile, and the analyzed electropherogram for a given ell on a plate (for example, A01). The sequenced data can be generated automatically by the Instrument Control Manager after a sequencing run or by the Sequence Analyzer software. Note that the Instrument Control Manager stores the .esd files for each run in an analyzed run folder.

.psd files—the plate setup data files that you can use to automatically import attributes for a plate and for individual wells on the plate, such as sample names. The Instrument Control Manager includes the information from the .psd file in the header of each raw sample data file (.rsd).

.rsd files—the raw sample data files, each of which contains the raw data for a given well on a plate (for example, A01). The storage location is in a corresponding raw run folder (plate ID_run ID) in the …\MegaBACE\Data folder (default) or the location you specify. Each .rsd file contains the plate ID, run ID, well ID, plate setup parameters, instrument parameters, raw electropherogram data, current intensities, and scan rate.

.scf files—(sequencing only) the base-called data files in SCF format. Each .scf file contains the raw data and electropherogram. If analyzed, each .scf file includes the raw and analyzed data and the called sequence.

.seq files—(sequencing only) the base-called data files in FASTA format. Each .seq file contains the .esd file name, .esd file location, and the called sequence for a given well on a plate. You can view these files in another application, such as Notepad.

analyzed run folder—(sequencing only) the folder that contains the base-called sample files for a given run. The Instrument Control Manager uses the plate ID and base caller ID to name the analyzed run folder. The Instrument Control Manager creates an analyzed run folder for the data from each plate for which the Instrument Control Manager performs automatic base calling. The software stores the resulting analyzed run folder in the …\MegaBACE\AnalyzedData folder (default) or the location you specify.

anode—the positive (+) end of the capillaries, where each reservoir tube contains an array of 16 capillaries and an electrode. The anode reservoir is located on the right side of the instrument. The negatively charged DNA ions migrate toward the anode. (MegaBACE 500 and flexible MegaBACE 1000 instruments only) each empty anode reservoir contains an anode sleeve to prevent you from loading a tube for this array position.

array placeholders—(MegaBACE 500 and flexible MegaBACE 1000 instruments only) the components that are installed in the empty array positions. Each array placeholder consists of a cathode plunging tool that is used to fill the empty wells of the sample plate, an anode blocker that is installed in place of an anode plug, an anode sleeve that is installed in the empty anode reservoir, and a window blank that is installed in place of the capillary window.

band-pass filters—optical filters that allow a band of selected wavelengths to pass through while rejecting both shorter and longer wavelengths.

base callers—(sequencing only) the software that identifies the candidate peaks and calls the bases in a sequence.

base colors—the display color of the base letter and trace associated with a given base: A = green, C = blue, T = red, G = black. Although the conventional color representation for G is yellow, the trace and letter are displayed in black for ease of viewing.

calibration run—see spectral calibration run.

cathode—the negative (–) end of the capillaries, where each capillary tip is inserted into one of the wells on the microplate along with an electrode. The cathode stage is located on the left side of the instrument.

cathode plunging tools—(MegaBACE 500 and flexible MegaBACE 1000 instruments only) the component used to fill the wells for the empty capillary array positions.

channels—see spectral channel.

chemistry parameters—the names of the dyes, the base order or dye-to-channel mapping, the names of the filters and beamsplitters, and the laser mode used for a given application (sequencing or genotyping).

dye set—the dyes used to label your experiment. The MegaBACE system is capable of detecting and separating the emissions of four dyes of different colors in a single capillary.

electropherograms—the digitized graphs the system produces from the fluorescent intensity detected from the capillaries during the run. The system produces an electropherogram for each capillary. Each electropherogram consists of four colored traces that represent the signals detected through the four spectral channels.

electrophoresis parameters—the settings for the sample injection voltage and time and the run voltage and time. The electrophoresis parameters are displayed in the Electrophoresis tab in the Plate Setup window of the Instrument Control Manager software.

empty array positions—(MegaBACE 500 and flexible MegaBACE 1000 instruments only) the instrument array positions that contain array placeholders instead of capillaries.

instrument parameters—a combination of settings defining the instrument run conditions and matrix fill and flush cycles. The instrument parameters are displayed in the Instrument Control window of the Instrument Control Manager software.

long-pass filters—optical filters that allow light of wavelengths longer than a specified cutoff to pass through to the PMT, while rejecting light of wavelengths shorter than the specified cutoff.

LPA (linear polyacrylamide)—see sieving matrix.

matrix—see sieving matrix or spectral overlap matrix.

Phred—sequence read editor program used to verify the accuracy of sequenced DNA. The program was developed by Phil Green at the University of Washington.

plate definition—includes the plate ID and plate setup parameters.

plate ID—the name you give to the plate when you create a plate definition in the Instrument Control Manager.

plate setup parameters—a combination of electrophoresis parameters, chemistry parameters, sample names, and optional parameters that define a plate.

raw data—the data collected by the instrument that have not been sequenced or genotyped. The Instrument Control Manager software creates a raw run folder for the raw sample data files (.rsd) for each plate you run.

raw run folder—the folder that contains the raw sample data files (.rsd) for a given run. The Instrument Control Manager software uses the plate ID and run ID to name the folder. The Instrument Control Manager stores the raw run folder containing the associated .rsd files in the …\MegaBACE\Data folder (default) or the location you specify.

run—the process of injecting and scanning a plate of samples on the MegaBACE instrument and detecting the resulting signal from each capillary. Each run has a unique date and user ID.

run folder—see analyzed run folder or raw run folder.

run ID—a unique designation the Instrument Control Manager software assigns to each run of a sample plate on the instrument.

s**ample files—**see .rsd files. For sequencing, see also .abd files, .esd files, .seq files, and .scf files.

sample names—the designation you provide for a given well in the sample names tab or in a plate setup template .psd file.

scan number—a number representing a sampling of the data during the run and describing the location of a data point. Instrument Control Manager samples the data continuously during a run at a rate of 1.75 Hz (105 times per minute).

sieving matrix—the sieving substance used to separate the DNA fragments in the sample by size, for example linear polyacrylamide (LPA).

spectral calibration run—(genotyping only) a genotyping run of a sample plate that contains the spectral matrix standards for the selected dye set. You use the data collected from the calibration run to create a spectral overlap matrix in Genetic Profiler.

spectral channel—the combination of laser, beamsplitter, optical filter, and PMT the system uses to detect the emission signals of a given dye. The MegaBACE instrument has a total of four spectral channels. The output of each spectral channel is represented by a different colored trace in the electropherogram.

spectral overlap matrix—the dye-to-channel mathematical matrix that defines which dye is detected through each channel and measures the amount of unwanted signal (spectral overlap) present in each spectral channel from the other dyes in the dye set. The Instrument Control Manager creates a real-time view of the spectrally separated data for display only.

- For sequencing, the base callers automatically perform spectral separation during data analysis.
- For genotyping, the operator uses the Genetic Profiler software to create a matrix that the software can use to perform spectral separation.

spectral separation—the software process that removes the unwanted signals present in each spectral channel. Each dye is detected through a specific spectral channel, but unwanted signal is always present in every channel from the emission of the other dyes in the dye set.

Stokes shift—the difference in wavelength between the apex of the excitation spectrum (shorter wavelength, higher energy) and the apex of the emission spectrum (longer wavelength, lower energy).

traces—the four curves in the electropherogram representing the signals detected through the four spectral channels of the instrument. Each trace is displayed in a specific color to represent a corresponding base or dye.

window blanks—(MegaBACE 500 and flexible MegaBACE 1000 instruments only) the replacement capillary windows installed in place of the missing capillaries for the empty array positions. The instrument optical system requires a capillary window or a window blank at each of the six array positions.

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