user manual

Now part of GE Healthcare

GE imagination at work

MegaBACE 4500

Instrument User's Guide Addendum
Version 4.0

um 0427-779

Amersham Biosciences

The polymerase chain reaction (PCR) is covered by patents owned by Roche Molecular Systems and F Hoffman-La Roche Ltd. A license to use the PCR process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers, such as Amersham Biosciences and affiliates, when used in conjunction with an authorized thermal cycler. The PCR process for amplifying DNA is covered by US patent numbers 4,683,195 and 4,683,202 assigned to Hoffman-La Roche Inc and F Hoffman-La Roche Ltd. Patents are pending or issued in other countries.

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

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

September 2004

Notice to purchaser: limited license

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

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

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

Applied Biosystems does not guarantee the performance of this instrument.

Amersham Biosciences is a licensed vendor for authorized reagents.

Amersham Biosciences UK Limited Amersham Place Little Chalfont Buckinghamshire HP7 9NA UK

Amersham Biosciences AB SE-751 84 Uppsala Sweden

Amersham Biosciences Corp 800 Centennial Avenue PO Box 1327 Piscataway NJ 08855 USA

Amersham Biosciences Europe GmbH Munzinger Strasse 9 D-79111 Freiburg Germany

Amersham Biosciences (SV) Corp 928 East Arques Avenue Sunnyvale CA 94085-4520 USA

Table of contents

Chapter 4 Performing sequencing runs

Chapter 5 Leaving the system idle or shutting down

Preface

About this addendum

The *MegaBACE 4500 Instrument User's Guide Addendum* and the *MegaBACE 4000 Instrument User's Guide v3.2* describe how to use and maintain the MegaBACE™ 4500 DNA Analysis System. This addendum describes how to use the MegaBACE 4500 system, describes the differences between the MegaBACE 4500 system and MegaBACE 4000 system, and specifies which topics in the *MegaBACE 4000 Instrument User's Guide v3.2* are relevant for the MegaBACE 4500 system.

Related publications

In addition to this addendum, the following publications are available for the MegaBACE 4500 system:

- *MegaBACE 4500 Site Preparation Guide* provides instructions for setting up the installation site for the MegaBACE 4500 instrument.
- The *MegaBACE 4000 Instrument User's Guide* describes how the instrument works and how to use and maintain the system.
- The *MegaBACE 4000 Instrument Administrator's Guide* describes how to configure the instrument control software for your laboratory workflow, and how to create plate definition files, plate setup templates, and instrument control parameter templates.
- The *MegaBACE 4500 64-Capillary Array v2 User's Guide* provides information on how to handle the capillary arrays and the criteria for returning damaged capillary arrays.
- The analysis software user's guides and/or the Help Topics within the software describe how to use the software to analyze the data collected from the MegaBACE instrument.

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 4500 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™ graphical user interface. If you do not have these skills, refer to the documentation or the Help for Windows.

Safety standards

The MegaBACE 4500 instrument complies with CE and other applicable standards, such as UL and CSA. For the latest conformity information, contact MegaBACE Technical Support. See the Assistance section for contact information.

MegaBACE system site requirements

Electrical requirements

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

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 4500 instrument. For contact by phone or fax, please use one of the numbers below. Alternatively, an updated list of contacts is available on the Web at www.amershambiosciences.com. Amersham Biosciences is now part of GE Healthcare. The GE Healthcare Web site is at www.gehealthcare.com.

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 10
Fax: 01 576 0616 27

Belgium
Tel: 0800 73 888
Fax: 03 272 1637

Canada
Tel: +1 800 463 5800
Fax: +1 800 567 1008

Central, East, and Southeast Europe
Tel: +43 1 982 3826
Fax: +43 1 985 8327

Denmark
Tel: 45 16 2400
Fax: 45 16 2424

Finland & Baltics
Tel: +358 (0)9 512 39 40
Fax: +358 (0)9 512 17 10

France
Tel: 01 69 35 67 00
Fax: 01 69 41 96 77

Germany
Tel: 0761 4903 291
Fax: 0761 4903 405

Italy
Tel: 02 27322 1
Fax: 02 27302 212

Japan
Tel: +81 3 5331 9336
Fax: +81 3 5331 9370

Latin America
Tel: +55 11 3933 7300
Fax: +55 11 3933 7315

Middle East and Africa
Tel: +30 (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

Chapter 1 Introduction to the MegaBACE 4500 system

The MegaBACE 4500 DNA Analysis System is a high-throughput automated sequencing system with a 384-capillary capacity. The MegaBACE 4500 system is based on the MegaBACE 4000 system. However, the MegaBACE 4500 system yields longer read lengths than the MegaBACE 4000 system for an equivalent run time. The MegaBACE 4500 system can also provide greater sensitivity than the MegaBACE 4000 system on samples that yield low signal.

This chapter describes—

- How to use this addendum (section 1.1)
- MegaBACE 4500 system enhancements (section 1.2)
- Sequencing workflow overview (section 1.3)
- Sequencing task overview (section 1.4)
- Overview of the Instrument Control Manager software (section 1.5)
- Overview of instrument operation (section 1.6)
- Before you begin (section 1.7)

1.1 How to use this addendum

You should use this addendum as your primary reference for information on the MegaBACE 4500 system. For the topics that are not covered by this addendum, see table 1-1 for a list of the applicable sections in the *MegaBACE 4000 Instrument User's Guide v3.2*.

Section 1.2 provides an overview of the primary differences between the MegaBACE 4500 system and the MegaBACE 4000 system.

Table 1-1. References for topics that are not covered by this addendum

Topic	*MegaBACE 4000 Instrument User's Guide v3.2* reference
How the MegaBACE instrument works	Chapter 3
Using the Automatic Base Calling feature	Chapter 5
Creating a sequencing plate definition	Chapter 6
Monitoring a run	Chapter 16
Quick reference to the Instrument Control Manager commands, windows, and buttons	Appendix A
Connecting a new computer to the instrument	Appendix C
Fluorescence imaging	Appendix D

1.2 MegaBACE 4500 system enhancements

The MegaBACE 4500 system has the following enhancements:

- **Solid-state blue laser and improved optical system—**The 100 mW solid-state laser provides pure 488-nm excitation light. Together, the laser and the improved optical system excite a wider area of the sample at greater intensity than the MegaBACE 4000 system. As a result, the MegaBACE 4500 system provides greater sensitivity for low-intensity signal detection.

  The solid-state laser has a longer lifetime, uses less power, and produces less heat than the argon-ion laser in the MegaBACE 4000 instrument. Because of the significantly lower heat production, the MegaBACE 4500 system does not include an external power supply fan module and is a quieter system. The low heat production also eliminates the need for the external ducts and hoses that are part of the MegaBACE 4000 instrument cooling system.

- **MegaBACE 4500 capillary arrays v2 and long-read matrix v2—**The 70-cm long capillaries and the new formulation of linear polyacrylamide (LPA) matrix are optimized for longer fragment separation. The v2 arrays and v2 LPA matrix are required for the MegaBACE 4500 system.

- **Enhanced instrument control software—**The Instrument Control Manager software v4.0 allows you to set the run temperature at 55 °C (131 °F). The elevated temperature increases the speed of the fragment separation, which allows you to achieve longer read lengths in the same run time as the MegaBACE 4000 system or the same read lengths in a shorter run time. In addition, the software contains instrument protocols enhanced for use with the MegaBACE 4500 system.
- **M13 dye terminator standard—**The standard provides a control that you can use to track the performance of the MegaBACE 4500 system.
- **ReadCheck Pro software—**The software contains a MegaBACE system test that allows you to check whether the MegaBACE 4500 system is performing to specification. In addition, ReadCheck Pro provides general sequence alignment capabilities.

See the *MegaBACE 4500 Site Preparation Guide* for capillary array and reagent ordering information.

1.2.1 Hardware components

The MegaBACE 4500 system (figure 1-1) consists of the following hardware components:

- **MegaBACE instrument—**Electrophoresis components and temperature regulation system, solid-state laser and light-collection system, and scanner electronics
- **Computer—**Computer, monitor, keyboard, and mouse
- **Emission beamsplitters and filters—**Installed, but changeable
- **MegaBACE 4500 capillary arrays v2**
- **Accessory kit—**Cathode buffer tank and water tank, cables, fittings, anode plugs, sample plate adapter, and deep-well plate adapter
- **Other components—**Documents and software CDs

In addition, Amersham Biosciences recommends the use of an uninterruptible power supply (UPS) with battery storage.

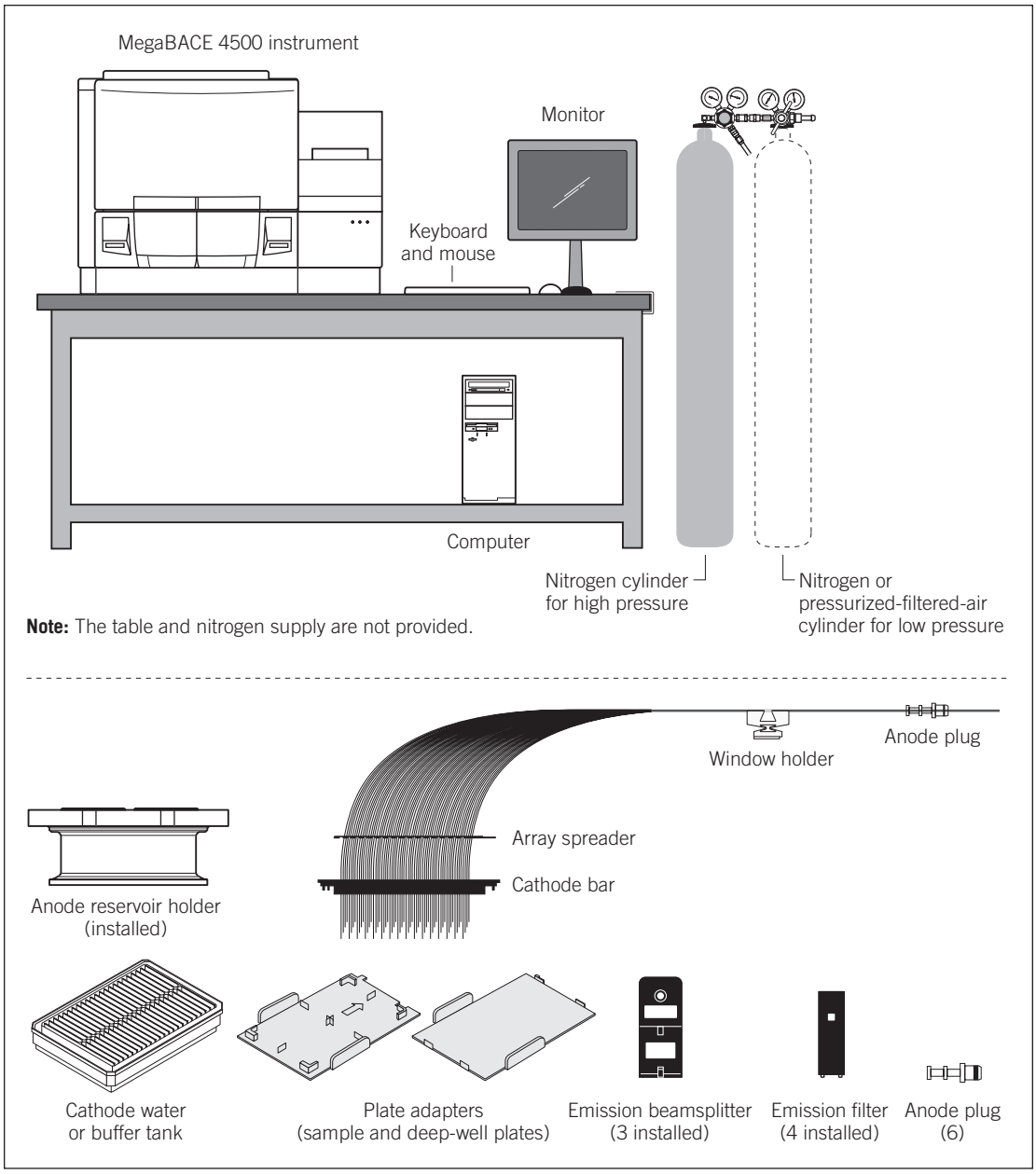

Figure 1-1. The MegaBACE 4500 system and components.

The system uses a regulated nitrogen pressure source for high-pressure functions. The nitrogen source can be either a cylinder with regulators or a multiunit manifold. The set point for high pressure is 6.89×10^3 kPa (1000 psi). For low-pressure functions, the system uses either pressurized nitrogen or pressurized, filtered air. The set point for low pressure is 6.89×10^2 kPa (100 psi). The *MegaBACE 4500 Site Preparation Guide* provides the specification for the nitrogen system. For more information on the nitrogen pressure source, see the *MegaBACE 4000 Instrument User's Guide*.

1.2.2 Software components

The MegaBACE 4500 software consists of the—

- **Instrument Control Manager—**Contains the graphical interface that allows you to control the instrument (section 1.5).
- **Host Scan Controller—**Provides the communication between the MegaBACE 4500 instrument and the Instrument Control Manager.
- **ReadCheck Pro—**Provides a MegaBACE system test that allows you to check whether the MegaBACE 4500 system is performing to specification, and provides sequence alignment capabilities. For details see the Help available within the software.
- **Sequencing Analysis software—**Includes the MegaBACE Sequence Analyzer software and the Automatic Base Calling feature in the Instrument Control Manager. For details on setting up the Automatic Base Calling feature, see the *MegaBACE 4000 Instrument User's Guide v3.2*, chapter 5. For information on using MegaBACE Sequence Analyzer, see the Help within the software or your *MegaBACE Sequence Analyzer User's Guide*.

  Note: Only the sequencing application has been validated with the MegaBACE 4500 system.
- **MegaBACE Header Editor—**Allows you to view and edit the data in the extended header of the raw sample data files (.rsd) and analyzed sample data files (.esd). For details see the Help available within the software.

Caution **The MegaBACE instrument control workstation is shipped free of computer viruses. However, the manufacturer does not test the performance compatibility of the MegaBACE system with virus protection software. The use of virus protection software with the MegaBACE system might cause problems with the system performance.**

1.3 Sequencing workflow overview

Figure 1-2 provides an overview of the system workflow for sequencing.

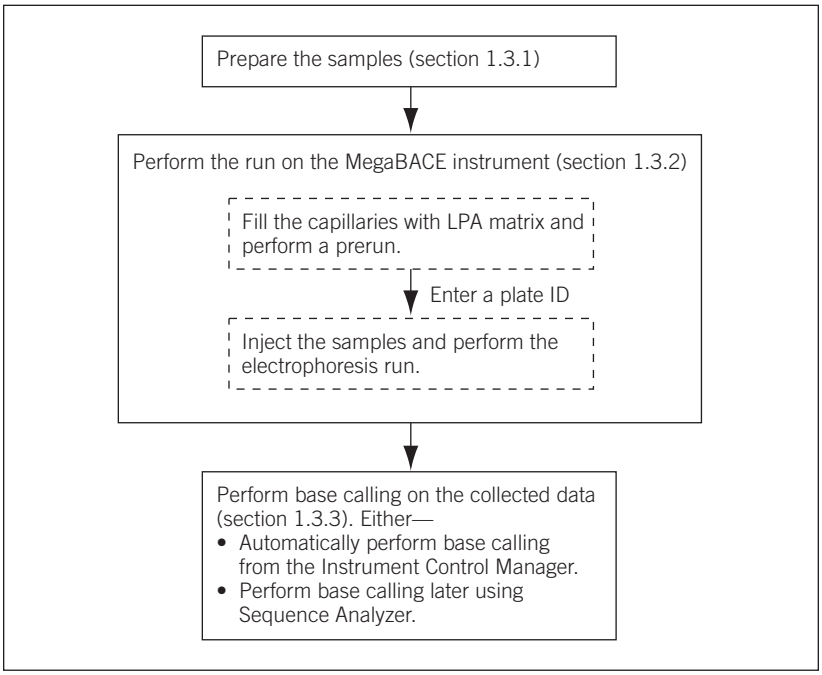

Figure 1-2. Workflow overview for sequencing.

1.3.1 About sample preparation for sequencing

You prepare the samples according to the appropriate reagent protocol. See the *MegaBACE 4500 Site Preparation Guide* for information on chemistry requirements and for ordering the recommended reagents.

1.3.2 About performing a sequencing run

The Instrument Control Manager prompts you for the appropriate steps in each instrument protocol, including rinsing the capillaries, filling the capillaries with sieving matrix, performing a prerun, and performing the sample injection for the electrophoresis run.

For each run, the Instrument Control Manager requires a plate definition. A plate definition consists of—

- **Plate ID—**The name for the plate you inject in a run. You can provide the plate ID using a .psd file or you can type the plate ID in the Plate Setup window. The software uses the plate ID to name the raw run folder that stores the data from the run.
- **Plate setup parameters—**The electrophoresis parameters, the chemistry parameters, the sample names, plate comments, and other parameters. You can use a .psd file or a plate setup template to provide the values for these parameters, or you can manually enter the values in the Plate Setup window. A .psd file allows you to define additional parameters that do not appear in the Plate Setup window. See section 3.9 for a description of the plate setup parameters.

The Instrument Control Manager allows you to provide the information in various ways. The workflow that your laboratory uses can vary depending on how your administrator has configured the Instrument Control Manager and whether your laboratory uses plate setup data files (.psd). Figure 1-3 shows the workflows.

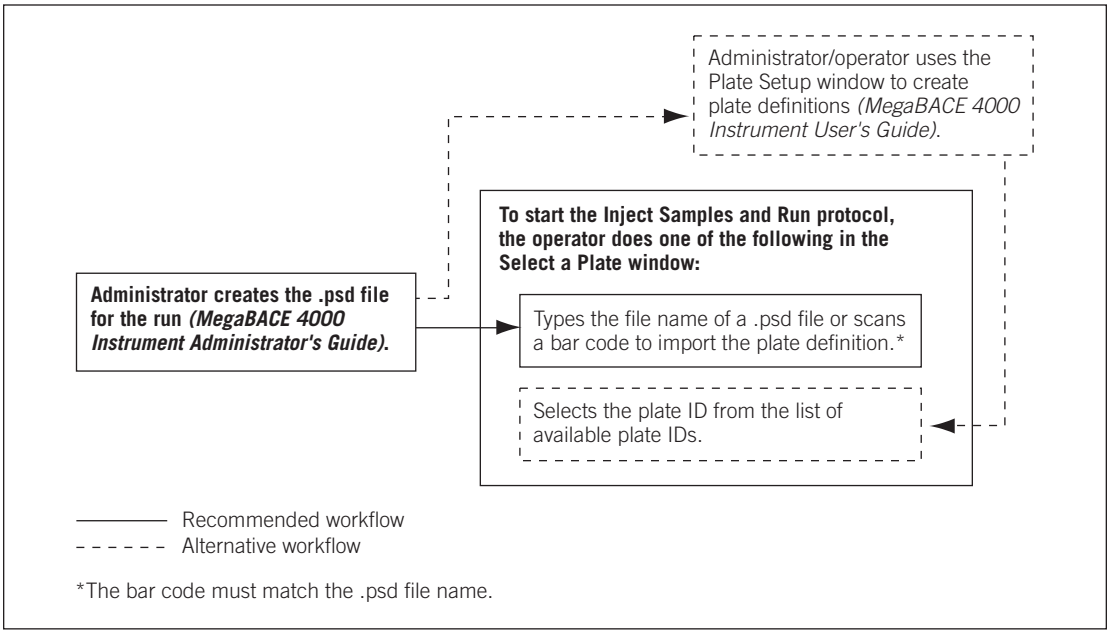

Figure 1-3. Typical sequencing workflows for providing the plate definition.

The basic workflows are—

- **Automated workflow—**For the most automated workflow, you can import a .psd file containing the plate definition when you start the Inject Samples and Run protocol. Your administrator creates the .psd files for you to use *(MegaBACE 4000 Instrument Administrator's Guide)*.
- **Alternative plate setup workflow—**You can use the Plate Setup window to create the plate definition before you perform each run, or you can create multiple plate definitions and then perform the runs later. The software allows you to select the precreated plate ID when you start the Inject Samples and Run protocol. See the *MegaBACE 4000 Instrument User's Guide* for instructions on how to create a plate definition in the Plate Setup window.

For detailed information on configuring the Instrument Control Manager software, see your administrator *(MegaBACE 4000 Instrument Administrator's Guide)*.

1.3.3 About performing base calling automatically after a run

The Instrument Control Manager software creates the raw sample data files (.rsd) from each run. You can use the Instrument Control Manager to automatically call bases from the raw data after each plate has been run. The software can be configured to automatically export the base-called sample data files (.esd) to other file formats (ABD, FASTA, SCF, and ASCII text).

For a description of how to use the automatic base calling feature in the Instrument Control Manager, see the *MegaBACE 4000 Instrument User's Guide*. You can use the Automatic Base Calling feature only if your administrator has enabled this feature (*MegaBACE 4000 Instrument Administrator's Guide)*.

Important **Base calling requires a large amount of computer processing time. Do not use the Instrument Control software to perform other tasks while automated base calling is in process.**

Alternatively, you can use the Sequence Analyzer software to perform base calling and to view the electropherograms.

1.4 Sequencing task overview

Table 1-2 provides the sequencing task overview for the MegaBACE 4500 system. The tasks can vary depending on whether you have administrator responsibilities in addition to operating the MegaBACE 4500 instrument. The administrator is responsible for configuring the Instrument Control Manager software initialization files and creating the plate setup data files (.psd).

Table 1-2. Sequencing task overview for the MegaBACE 4500 system

Task	Frequency	Reference
(Optional) The administrator can tailor the software settings to match your laboratory's workflow.	When you first receive the MegaBACE 4500 system	*MegaBACE 4000 Instrument Administrator's Guide v3.2*
The operator starts the MegaBACE 4500 system.	After any system shutdown	Section 3.1
(Optional) The administrator or operator sets up the automatic base calling feature.	When you first receive the MegaBACE 4500 system software and any time you want to modify your settings	*MegaBACE 4000 Instrument User's Guide v3.2*, chapter 5 and *MegaBACE 4000 Instrument Administrator's Guide v3.2*
The administrator or operator creates the plate definition for the run using the MegaBACE 4500 plate setup parameter values.	Every run	Section 3.9
To create the plate definition use one of the following workflows: • The administrator creates the .psd files that allow the operator to import the plate definition at the start of the Inject Samples and Run protocol. • (Alternative) The administrator or the operator uses the Plate Setup window to create plate definition before starting a run. The operator selects the precreated plate definition at the start of the Inject Samples and Run protocol.	– –	*MegaBACE 4000 Instrument Administrator's Guide v3.2* *MegaBACE 4000 Instrument User's Guide v3.2,* chapter 6
(Optional) The administrator or operator specifies the storage location for the raw run data.	When you first receive the MegaBACE 4500 system software and any time you want to change the location	Section 3.10
For each run, the operator uses the— • Matrix Fill and Prerun protocol • Inject Samples and Run protocol	Every run	Chapter 4

Table 1-2. Sequencing task overview for the MegaBACE 4500 system (continued)

Task	Frequency	Reference
(Optional) The operator monitors the run.	Every run	*MegaBACE 4000 Instrument User's Guide v3.2,* chapter 16
The operator stores the capillaries when not in use:		
• For up to 16 hours, use the Sleep After This Run option.	After the last run of the day	Section 4.4
• For up to 7 days, store the capillaries wet. Use the Store Capillaries protocol. This protocol turns off the heater and turns off the laser.	–	Chapter 5
• For more than 7 days, store the capillaries dry. Use the Flush and Dry Capillaries protocol.	–	Chapter 5
The operator performs routine maintenance on the instrument.	Periodically, depending on the throughput of your laboratory	Chapter 6

1.5 Overview of the Instrument Control Manager software

The Instrument Control Manager allows you to—

- Import the plate definition from a .psd file at the start of the Inject Samples and Run protocol. Alternatively, you can use the Plate Setup window to create a plate definition before the run, and then select the plate ID when you start the Inject Samples and Run protocol.
- Use a template to specify the plate setup values. A template (default) containing the recommended parameters is included with the software.
- Automatically define the instrument control parameters for a run by using a template to specify the values. A template (default) containing the recommended parameters is included with the software.
- Use predefined instrument protocols to fill the capillaries with matrix and perform a prerun, and then inject the samples and perform a run.
- Automatically store the collected data in an application-specific data folder.
- Automatically perform base calling on the collected data as soon as the sequencing run is complete.

1.6 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 three 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. Four PMTs detect the filtered light and convert the light into an electrical current, which is digitized to produce an electropherogram for each capillary.

Figure 1-4 shows the spectral channels of detection in the instrument for the DYEnamic™ ET dye terminator chemistry.

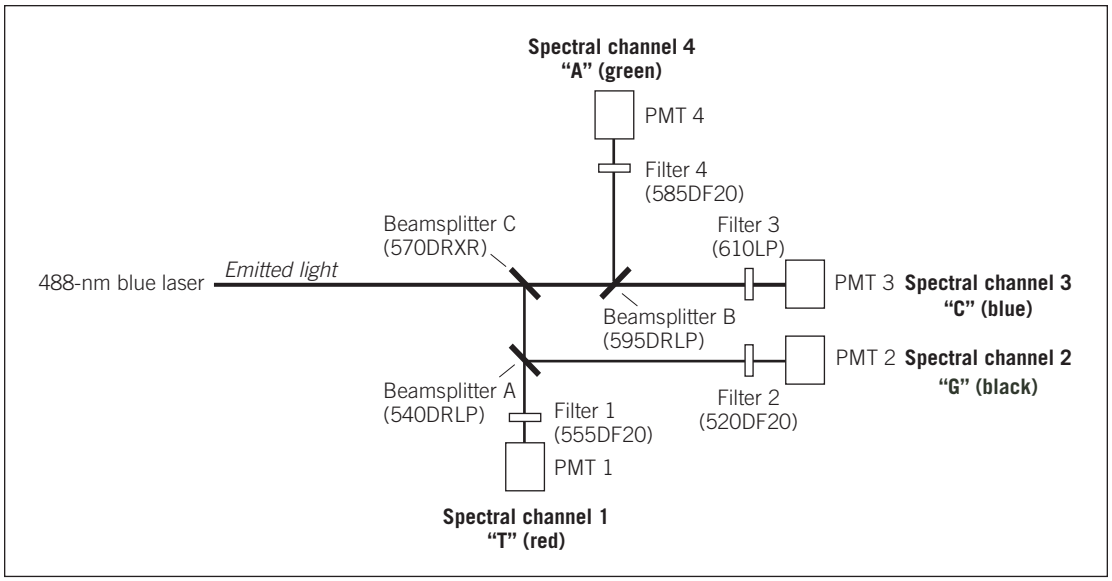

Figure 1-4. The MegaBACE spectral channels and the DYEnamic ET dye terminators.

1.7 Before you begin

Before using the MegaBACE 4500 system, you should become familiar with—

- Chapter 2: Safety precautions
- Chapter 3: Getting started

Chapter 2 Safety precautions

The MegaBACE 4500 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 (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)
- Laser (section 2.9)
- PMTs (section 2.10)
- Computer and monitor (section 2.11)
- Electrical connections (section 2.12)
- Serial number label (section 2.13)
- Moving the instrument (section 2.14)
- Service for the MegaBACE 4500 instrument (section 2.15)

2.1 General safety precautions

While using the MegaBACE 4500 instrument, you should follow the laboratory procedures appropriate for the experiments you are performing.

Warnings

The operator of the MegaBACE 4500 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 might 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.

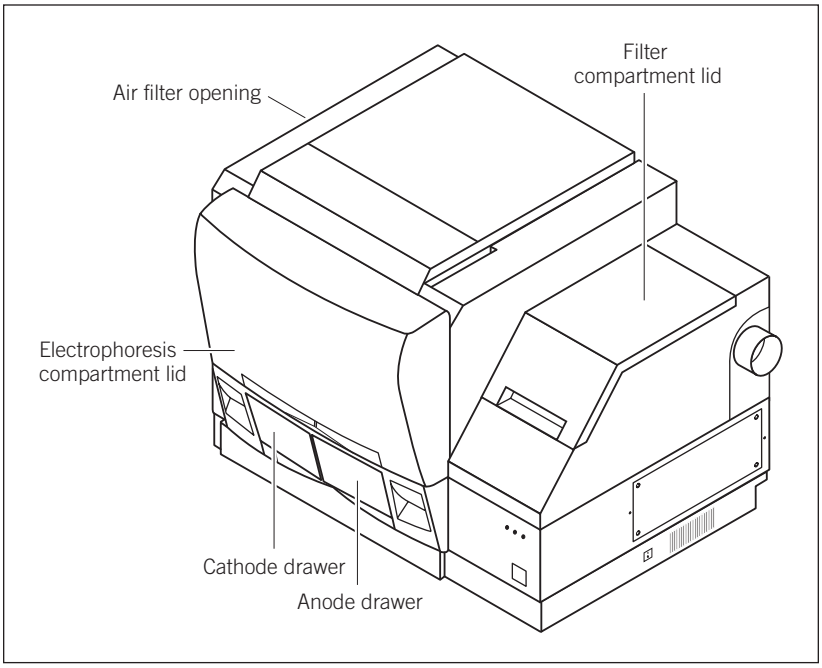

Figure 2-1. Locations of the drawers and lids used during routine operation of the MegaBACE system. The air filter opening is used infrequently.

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 4500 instrument weighs approximately 230 kg (508 lb). Lifting the instrument can cause severe or fatal injury.

2.2 Locations of important labels

The locations of important labels on the MegaBACE 4500 instrument are shown in figures 2-2 and 2-3.

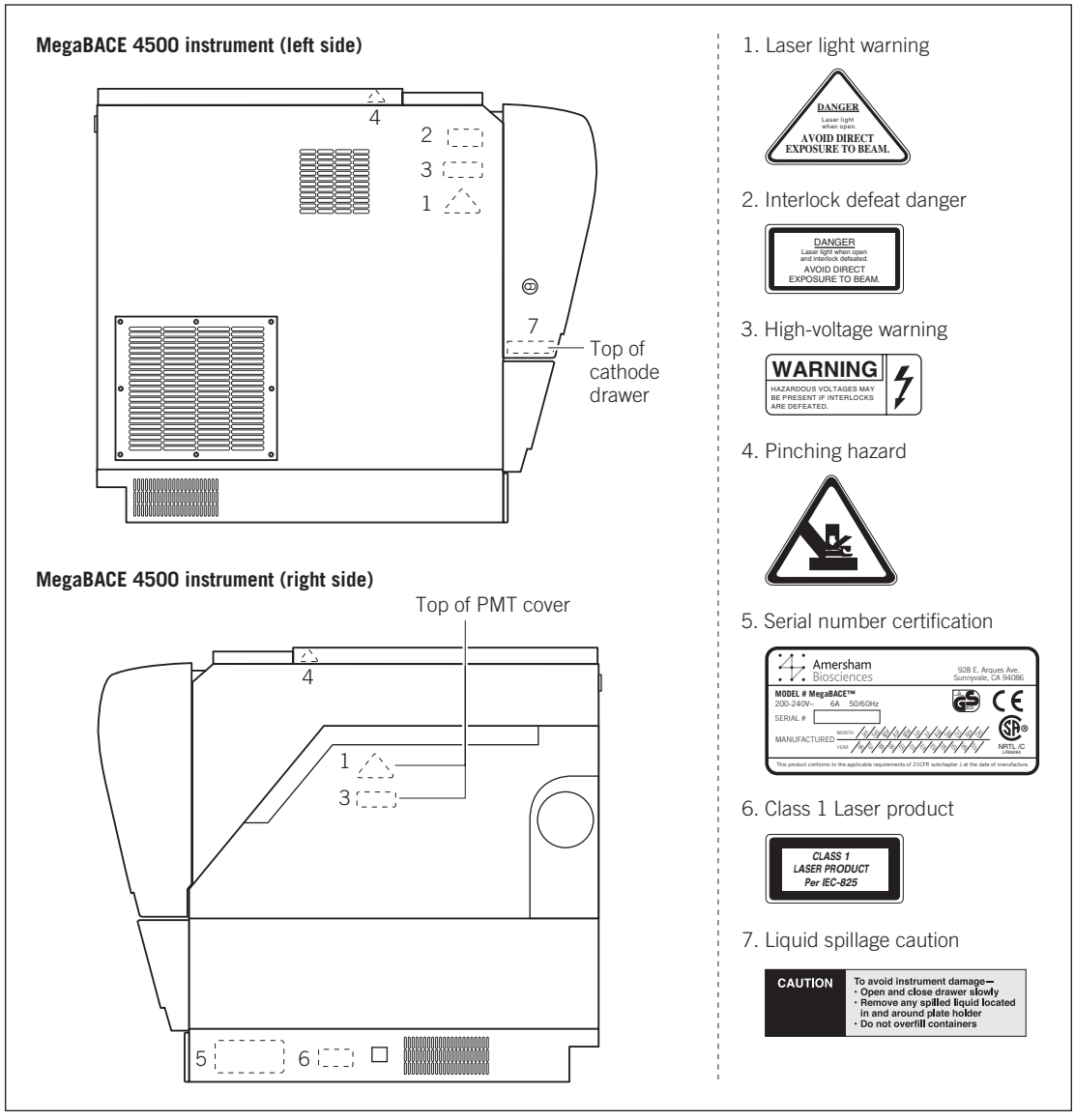

Figure 2-2. Locations of important labels on the MegaBACE 4500 instrument (side views).

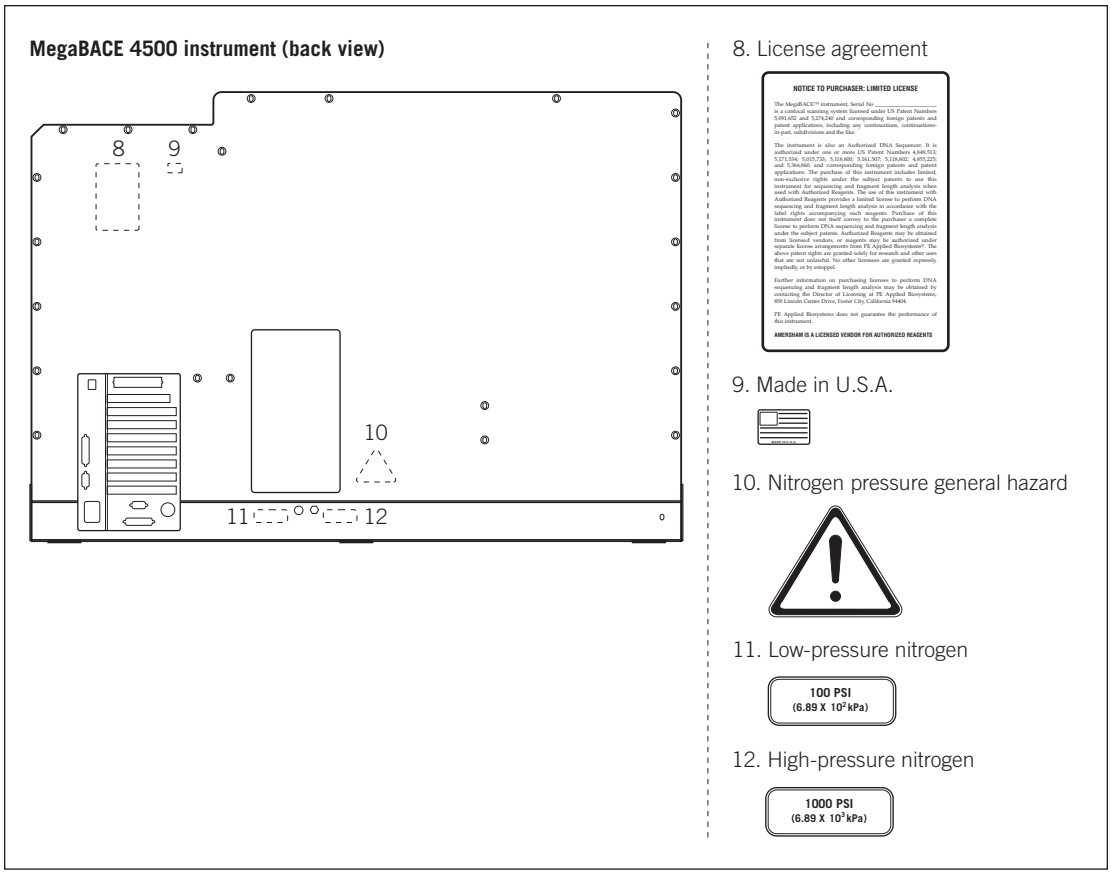

Figure 2-3. Locations of important labels on the MegaBACE 4500 instrument (back view).

If a label becomes illegible for any reason, 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

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 2-4 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.

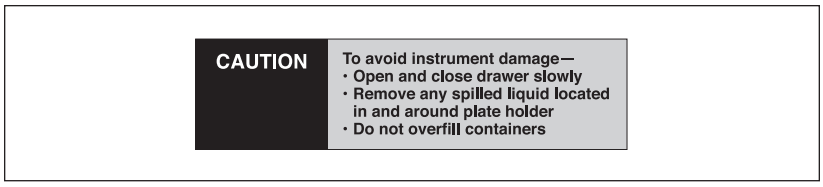

Figure 2-4. The liquid spillage caution label.

2.4 Electrophoresis compartment

You might 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.

The label shown in figure 2-5 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 2-5. The pinching hazard label.

Under normal operating conditions, you are protected from high voltage. Nevertheless, during the prerun and sample electrophoresis, voltages up to 15 kV are present in the electrophoresis compartment. The label in figure 2-6 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 2-6. 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.

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.

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

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. (For details on changing filters and beamsplitters, see the *MegaBACE 4000 Instrument User's Guide*.)

2.6 Internal electronics

Under normal operating conditions, you are protected from high voltage within the instrument electronics. Nevertheless, voltages up to 15 kV can be present in the instrument during a scan. The label in figure 2-6 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 4000 Instrument User's Guide* for the procedure.

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.

The label in figure 2-7 warns of this danger. Figure 2-3 shows the location of the label on the back of the instrument.

Figure 2-7. 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 and filtered-air pressure

The regulators on the external nitrogen and filtered-air cylinder(s) control the amount of 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 2-8 shows the labels that are placed on the back of the instrument next to the high- and low-pressure nitrogen or filtered-air 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.

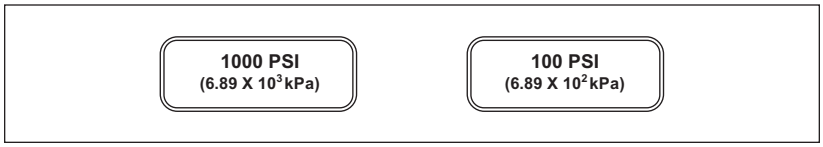

Figure 2-8. The high-pressure nitrogen label and the low-pressure nitrogen or pressurized-filtered-air label.

2.9 Laser

Warning

Changing controls, making adjustments, or performing procedures other than those specified in the MegaBACE 4500 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 2-9 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 2-9. 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 solid-state laser with power up to 100 mW at 488 nm with <1.2-mrad divergence.

Warning

Do not remove any of the inner covers of the MegaBACE 4500 instrument. The laser power specified in the paragraph above could be accessible if you remove the inner covers.

The label in figure 2-10 warns of laser light danger. The label is located on the PMT cover and in the electrophoresis compartment of the instrument.

Figure 2-10. The laser light warning label.

2.9.3 Safety interlock danger label

The label in figure 2-11 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.

Warning

Do not defeat the interlocks or try to gain access to the interior of the MegaBACE 4500 instrument through any other opening. Exposure to laser light can cause injury.

Figure 2-11. The interlock defeat danger label.

2.9.4 Light leaks

If a panel becomes damaged and the MegaBACE 4500 instrument is no longer light-tight, do not continue to use the instrument.

Caution

Ambient light can damage electrical components in the MegaBACE 4500 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 Computer and monitor

Voltages are exposed inside the computer and monitor. See the computer manufacturer documentation for the high-voltage hazard warning. Make sure you follow the manufacturer's instructions for the safe operation of the computer.

Warning

Do not open the monitor. The internal components can carry a voltage, which can cause injury if you touch them. If you must open the computer, follow the computer manufacturer's instructions.

2.12 Electrical connections

The MegaBACE 4500 system includes three components that require electrical power: the instrument, the computer, and the monitor. An electrical power cord is supplied with each component. See your *MegaBACE 4500 Site Preparation 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.

If you use an uninterruptible power supply (UPS), it should be 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, 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 label

You can find the serial number and model number of your MegaBACE instrument on the serial number label (figure 2-12). 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.

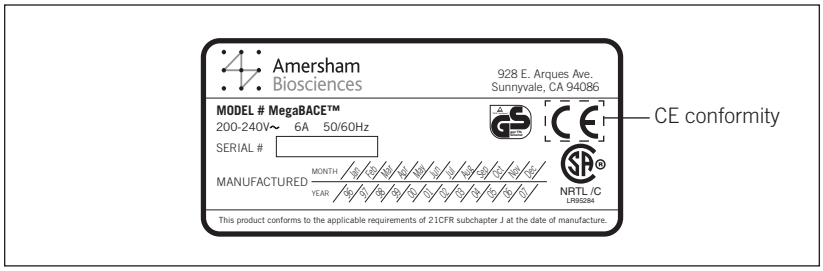

Figure 2-12. The MegaBACE instrument serial number certification label.

2.14 Moving the instrument

Warning

The MegaBACE 4500 instrument weighs approximately 230 kg (508 lb). The instrument requires adequate physical support. Never attempt to lift the instrument without using proper equipment and trained personnel. Lifting the instrument without proper support can cause severe or fatal injury.

Caution

Do not attempt to move your MegaBACE 4500 instrument. Doing so will void your warranty. Instead, contact MegaBACE System Technical Support to set up an appointment. See Assistance in the preface for contact information.

If you need to move your MegaBACE instrument, review the *MegaBACE 4500 Site Preparation Guide* for information on selecting the new location, as well as the nitrogen pressure and electrical power requirements for your instrument. Make sure you have all the required parts for reinstallation.

2.15 Service for the MegaBACE 4500 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. You can find the serial number on the serial number certification label (figures 2-2 and 2-12).

Chapter 3 Getting started

This chapter describes the required startup procedures to prepare the instrument for operation, to warm up the laser and electrophoresis compartment, and to prepare the capillaries for matrix fill and sample injection. The topics are—

- Before you turn on the instrument (section 3.1)
- Starting the system (section 3.2)
- Warming up the instrument (section 3.3)
- Using the Instrument Control Manager (section 3.4)
- Setting the instrument control parameters (section 3.5)
- About the MegaBACE 4500 protocols (section 3.6)
- Using the instrument cathode and anode drawers (section 3.7)
- Preparing the capillaries (section 3.8)
- Specifying the plate setup parameters (section 3.9)
- Changing the data file storage location (section 3.10)
- Guidelines for adjusting the run conditions (section 3.11)

Important **Before you turn on the MegaBACE 4500 instrument, see chapter 2 for important safety information.**

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 7 days. For information on leaving the instrument idle or shutting down the system, see chapter 5 in this guide.

3.1 Before you turn on the instrument

Before you turn on the MegaBACE 4500 instrument, check the following:

- **High-pressure and low-pressure nitrogen system—**The nitrogen cylinders (if applicable), 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 laboratory).
  - High pressure: 6.89×10^3 kPa (1 000 psi)
  - Low pressure: 6.89×10^2 kPa (100 psi)

    Note: For low pressure, the system can use a regulated nitrogen source or a pressurized-filtered-air source.

- **Instrument cooling—**Nothing is blocking free air access to the air vents on the sides and top of the MegaBACE instrument (figure 3-1).
- **Power connections—**The MegaBACE instrument, computer, and monitor are plugged in, and the power cords are in good condition. If your site uses a universal power supply (UPS) for these components, make sure that the UPS is plugged in and turned on.

Caution **Do not place anything on top of the MegaBACE 4500 instrument. Large objects can block the air flow. Small objects, such as pens and tubes, can fall into the interior of the instrument and cause damage. Allow free air access to both sides of the instrument and keep the air vents free of obstructions.**

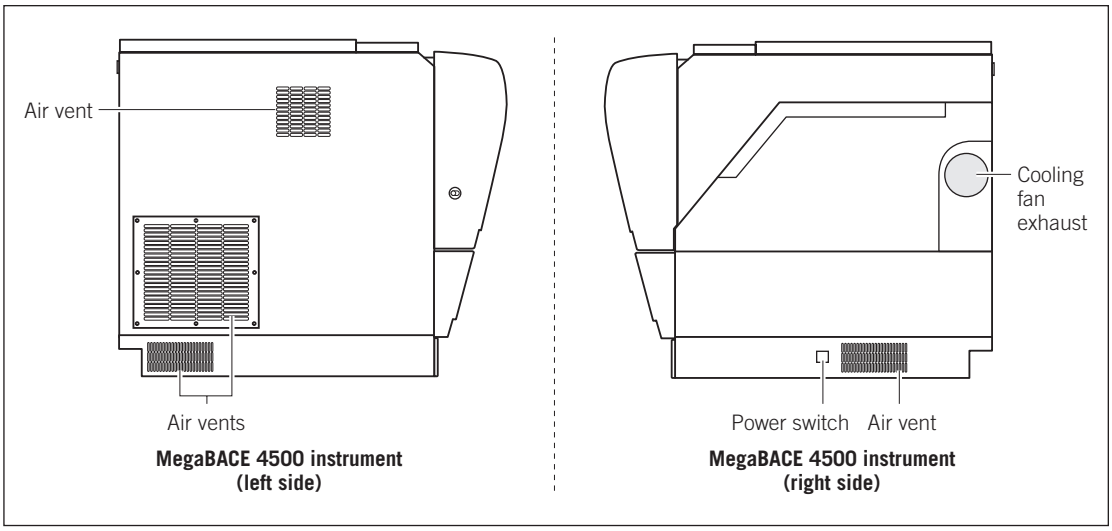

Figure 3-1. Make sure the airflow openings on the MegaBACE 4500 instrument are free of obstructions.

3.2 Starting the system

To start the MegaBACE 4500 system—

1. Make sure the nitrogen source is turned on and that the high- and low-pressure is set per the specifications (section 3.1).
2. 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.
   - After the internal diagnostics are complete, a MegaBACE message appears in the displays on the front of the instrument.
3. Turn on the computer and the monitor.
4. Start the Host Scan Controller software. To do this, double-click the **Host Scan Controller** shortcut icon in the **MegaBACE** folder on the Windows desktop (figure 3-2). Alternatively, you can start the Host Scan Controller using the Start menu on the Windows desktop.

Figure 3-2. The MegaBACE shortcut folder on the Windows desktop.

5. (Optional) Minimize the Host Scan Controller window.

   Note: 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 software, you can use the Command Log tab on the Instrument Control window to monitor the system status during a run.

Important **The Host Scan Controller must be running before you start the Instrument Control Manager.**

6. Start the Instrument Control Manager. To do this, double-click the **Instrument Control Manager** shortcut icon in the **MegaBACE** folder on the Windows desktop (figure 3-2). Alternatively, you can start the Instrument Control Manager using the Start menu.

Important **For first-time operation, use the Prepare Capillaries protocol (section 3.8) before running a plate of samples.**

Caution **If you are turning off the instrument for a period of more than 7 days, be sure you store the capillaries dry. See chapter 5 for instructions on storing the capillaries dry and shutting down the system. See section 3.8 for instructions on rehydrating the capillaries after they have been stored dry.**

3.3 Warming up the instrument

When you start up the instrument, allow approximately 3 hours for the components in the electrophoresis compartment to stabilize at the set run temperature.

Cautions **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.**

If you change the run temperature, you must refocus the capillaries for the new run temperature. Insufficient temperature can cause unreliable capillary focusing.

If you open the electrophoresis compartment before you perform a plate run, you need to allow for additional warmup time in the compartment. Table 3-1 provides examples of the warmup times. 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 3-1. Examples of warmup times after opening the electrophoresis compartment for a set run temperature of 55 °C (131 °F)

Compartment open time	Warmup time
1 minute	10 minute
1–10 minutes	60 minutes
> 10 minutes	90 minutes

3.4 Using the Instrument Control Manager

The Instrument Control Manager provides the various protocols that step you through using the instrument. The Instrument Control Manager has the following three windows:

- **Instrument Control window—**Displays the instrument control parameters (section 3.5), allows you to select from a list of protocols, displays the materials required for the selected protocol, and contains a Command Log tab that displays the system status.
- **Plate Setup window—**Displays the plate setup parameters (section 3.9). Depending on the laboratory workflow, you might use the Plate Setup window to create plate definitions, or you might use plate setup data files (.psd) to import plate definitions containing the plate setup parameters.
- **Run Image window—**Displays a simulated gel view of the samples during the run, and allows you to display the electropherogram of a selected capillary. See the *MegaBACE 4000 Instrument User's Guide* for details on using the Run Image window to monitor a run.

3.5 Setting the instrument control parameters

The Instrument Control window (figure 3-3) in the Instrument Control Manager displays the instrument control parameters. To display the Instrument Control window, click the **Instrument Control** tab. The instrument control parameters include the run parameters and the sleep parameters.

Important **You can edit the instrument control parameters in the Instrument Control window 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 the *MegaBACE 4000 Instrument Administrator's Guide* for details.**

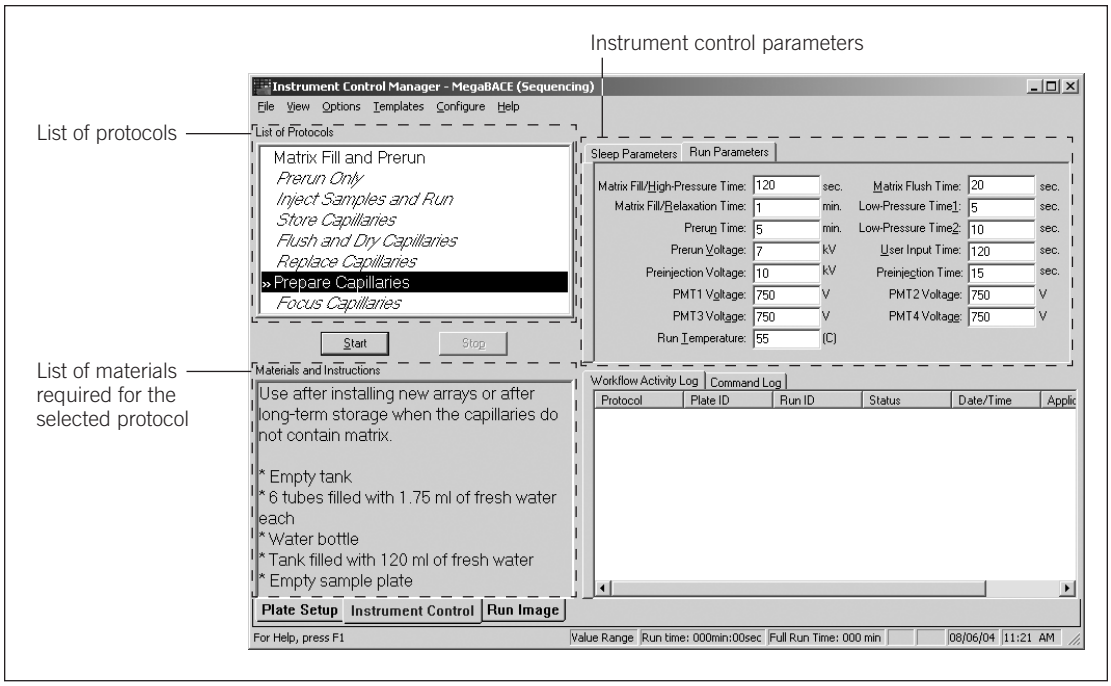

Figure 3-3. The Instrument Control window.

Table 3-2 lists the recommended values for the MegaBACE 4500 instrument control parameters. You can use an instrument control parameters template (.icp file) to set the parameter values. For details on how to create new instrument control templates, see the *MegaBACE 4000 Instrument Administrator's Guide*.

To set the recommended values for the MegaBACE 4500 instrument using a template—

1. Click the **Instrument Control** tab to display the Instrument Control window.
2. On the **Templates** menu in the Instrument Control window, point to **Instrument Templates,** and click **Select Template.** The Select Template window appears and displays the available instrument control templates.
3. In the Select Template window, choose **normal.icp,** and then click **Open.** The Instrument Control window displays the parameters for the selected template.

Table 3-2. Instrument control run parameters and the recommended values

Field name	Description (value range)	Recommended value
Run Parameters tab		
Matrix Fill/High-Pressure Time	Time duration (seconds) for applying high pressure to fill the capillaries with matrix (integer)	120
Matrix Fill/Relaxation Time	Time duration (minutes) to allow the matrix to equilibrate (integer)	1
Prerun Time	Time duration (minutes) for the electrophoresis prerun (integer)	5
Prerun Voltage	Voltage (kV) for the prerun (integer or fraction)	7
Preinjection Voltage*	Placeholder only (integer or fraction)	10
PMT1–PMT4 Voltage	Voltage (V) for each of the four PMTs (integer or fraction)	750
Run Temperature	Temperature (°C) of the electrophoresis compartment during the run (integer) **Caution: If you change the run temperature, you must refocus the capillaries for the new run temperature. Insufficient temperature can cause unreliable capillary focusing.**	55 °C
Matrix Flush Time	Time duration (seconds) for the first matrix flush (integer)	20
Low-Pressure Time1	Time duration (seconds) for the nitrogen pressure to flush water through the capillaries (integer)	5
Low-Pressure Time2	Time duration (seconds) for the low-pressure matrix flush (integer)	10
User Input Time	Time duration (seconds) you need to open and close the anode and cathode drawers after the display on the instrument gives the instruction to load the plate, tank, or tubes in either the anode or the cathode side of the instrument (integer)	120
Preinjection Time*	Time duration (seconds) for preinjecting samples (integer)	15
Sleep Parameters tab		
Sleep Time	Time duration (hours) for the Sleep After This Run protocol (integer)	8
Sleep Temperature	Temperature (°C) for the Sleep After This Run protocol (integer) **Note:** This number is a placeholder only. The protocol turns off the heater in the electrophoresis compartment.	25

* The Preinject Samples protocol is not used.

3.6 About the MegaBACE 4500 protocols

Table 3-3 lists the protocols that are available in the Instrument Control Manager and specifies the appropriate reference for the MegaBACE 4500 procedure.

Table 3-3. The protocols in the Instrument Control Manager*

Protocol	Description	MegaBACE 4500 reference
Prepare Capillaries	Use before starting a run after long-term storage or before focusing the capillaries after installing new arrays. Use only if there is no matrix in the capillaries.	Section 3.8
Matrix Fill and Prerun	Use to fill the capillaries with matrix, to equilibrate the matrix, and to perform a prerun.	Section 4.2
Prerun Only	Use to perform a prerun when the capillaries already contain fresh matrix. Also, use to repeat the prerun if the post-prerun time has expired.	–
Inject Samples and Run	Use to inject the samples and begin electrophoresis.	Section 4.3
Sleep After This Run	Use to store the capillaries between runs, for less than 16 hours.	Section 4.4
Store Capillaries	Use to store the capillaries overnight or for up to 7 days. Once completed, the instrument is brought up to the set run temperature.	Section 5.1
Flush and Dry Capillaries	Use to flush the matrix out with water and dry the capillaries for storage longer than 7 days. Also use when temporarily removing arrays from the system.	Section 5.3
Replace Capillaries	Use to replace the capillary arrays. The protocol includes rinsing the capillary tips and opening the electrophoresis compartment.	Chapter 7
Focus Capillaries	You focus the MegaBACE 4500 capillaries for the run temperature of the instrument. If you change the run temperature, you need to refocus the capillaries for the new run temperature.	Chapter 7

* The Sleep After This Run option is available from the Sleep Parameters tab of the Instrument Control window. All the other instrument protocols appear in the List of Protocols area of the Instrument Control window. The Rinse Tips and Preinject Samples protocols are not used.

3.7 Using the instrument cathode and anode drawers

The cathode (left) and anode (right) drawers unlock during specific steps in the instrument workflow (figure 3-4). When a step requires access to the cathode or anode stage, the stage lowers, and then the drawer unlocks. Next, a message on the display at the front of the instrument instructs you to place a specific vessel on the stage.

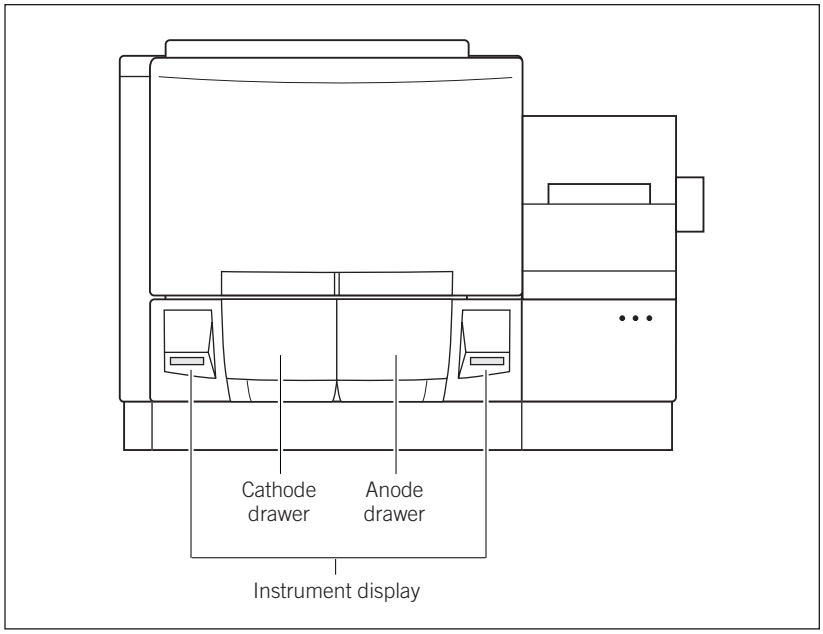

Figure 3-4. The instrument displays.

Important **After you open the drawer and then close it, the software assumes you have performed the required step. The drawer locks, the stage rises, and the system goes to the next step. If you follow the instructions but the displayed message does not change to the next step, carefully open and reclose the drawer.**

Caution **Use care when you pull the drawers out and push them in so that you do not twist or bend the sliders. Use care when you load and unload the tanks and plates in the cathode drawer to avoid liquid spills on the cathode stage, which can contaminate the cathode assembly and cause damage to the electronics.**

3.8 Preparing the capillaries

You use the Prepare Capillaries protocol after replacing the capillary arrays or after an extended shutdown during which the capillaries were stored dry. The Prepare Capillaries protocol rinses the capillaries with water at low pressure to hydrate the capillaries and prepare them for matrix and sample injection.

3.8.1 Materials required

For the Prepare Capillaries protocol, you need (figure 3-5)—

- An empty water tank
- A clean tank containing 120-ml fresh deionized, filtered water
- Six 2-ml tubes, each containing 1.75-ml fresh deionized, filtered water
- A squirt bottle filled with fresh deionized, filtered water

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 contaminate the cathode assembly and damage the electrodes in the cathode stage.**

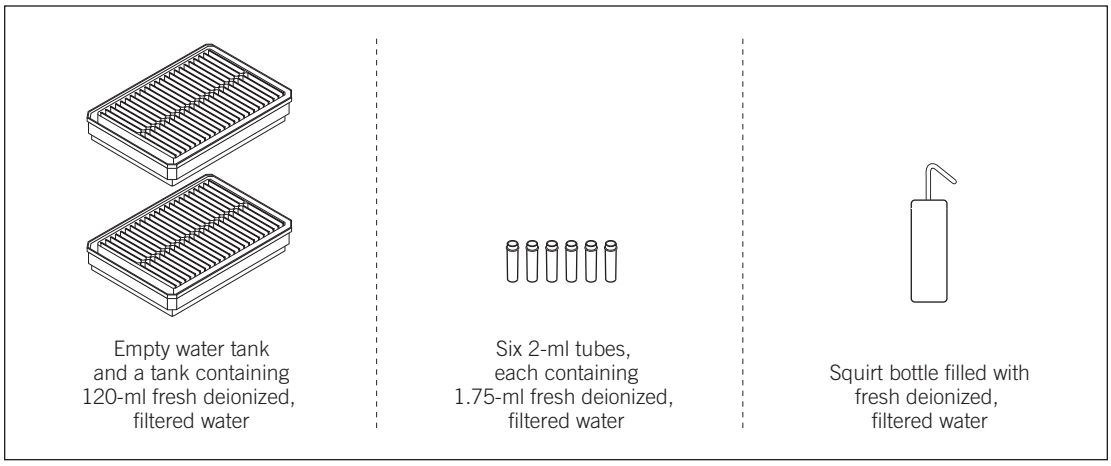

Figure 3-5. The materials for the MegaBACE 4500 Prepare Capillaries protocol.

3.8.2 The MegaBACE 4500 Prepare Capillaries protocol

After you start a protocol, observe the two displays on the front of the instrument (figure 3-4). The displays provide instructions for your interactions with the instrument while you are using the protocols.

To prepare the capillaries—

1. In the Instrument Control window (figure 3-3), 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 complete the following low-pressure flushes:

   - Load the empty water tank into the left side of the instrument and the filled water tubes into the right side of the instrument. The instrument displays tell you that low-pressure flush 1 is in progress.
   - Refill the water tubes in the right side of the instrument. The instrument displays tell you that low-pressure flush 2 is in progress.
   - Refill the water tubes in the right side of the instrument. The instrument displays tell you that low-pressure flush 3 is in progress.

3. Follow the instructions to refill the water tubes and load the full water tank. The Workflow Activity Log in the Instrument Control window lists the end time for the Prepare Capillaries protocol and state that the capillaries are ready for focusing.

The Prepare Capillaries protocol prepares the capillaries for either the—

- **Matrix Fill and Prerun protocol—**If the capillaries are already focused, the Matrix Fill and Prerun is the protocol that you should use next (section 4.2).
- **Focus Capillaries protocol—**If you need to focus the capillaries, see chapter 7 for instructions.

3.9 Specifying the plate setup parameters

Table 3-4 lists the recommended plate setup values for runs of the MegaBACE 4500 dye terminator standard. The Plate Setup window (figure 3-6) displays the plate setup parameters. Depending on the laboratory workflow, you might import the plate setup parameters from a plate setup data file (.psd) when you start a run instead of using the Plate Setup window. Alternatively, you might use a .psd file or a plate setup template (.tpl file) to import the plate setup values into the Plate Setup window.

Table 3-4. The plate setup parameters and the recommended values for the MegaBACE 4500 instrument

Field name	Description	Recommended value
Electrophoresis Parameters tab		
Injection Voltage	Voltage (kV) for the sample injection (integer or fraction)	3
Injection Time	Duration (s) of the sample injection (integer)	25
Run Voltage	Voltage (kV) during the run (integer or fraction)	7
Run Time	Duration (min) of the run (integer)	180
Chemistry	Name of the chemistry parameter set (string) **Note:** The name must match a name in the sequencing section of the Chemistry.ini file. See the *MegaBACE 4000 Instrument Administrator's Guide v3.2* for details.	ET Terminators
Sample Names tab (Well_ID, for example A01)	Sample name for each well ID (string)	
Optional Parameters tab **(Run Temperature, PMT Voltage1–PMT Voltage4, Base Caller)**	Parameter values for a special run The Run Temperature and PMT Voltage values are typically defined as instrument control parameters (table 3-2). The values can be defined here for a special run. If defined here, the values temporarily overwrite the same values in the Instrument Control window or instrument control parameters template. If used, the Base Caller field can specify a plate-specific base caller that overwrites the default base caller specified in the Automatic Base Calling window.	
Comments tab	Comments about the plate that you want to include (string)	

The *MegaBACE 4000 Instrument User's Guide* describes how to use the Plate Setup window. The *MegaBACE 4000 Instrument Administrator's Guide* describes how to create .psd files and templates.

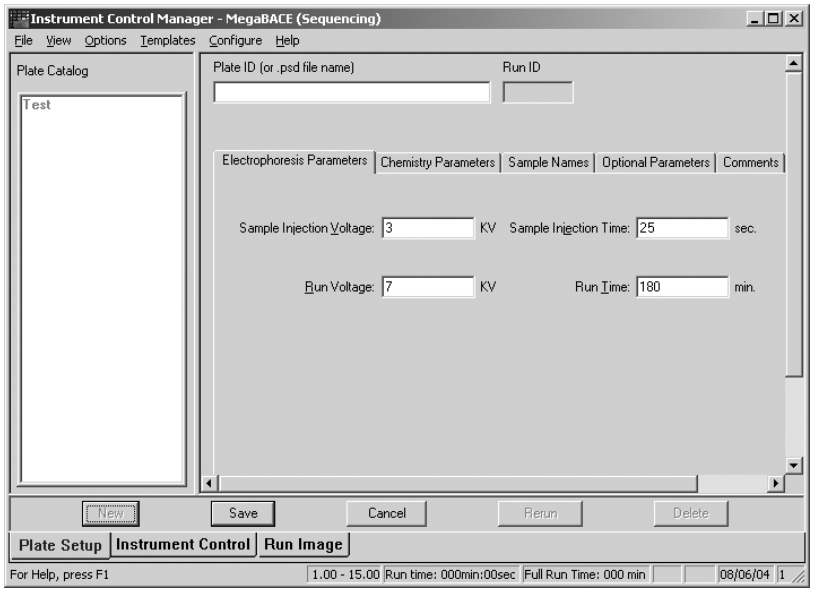

Figure 3-6. The Plate Setup window displaying the electrophoresis parameters.

To create a plate definition using a template to specify the plate setup parameter values listed in table 3-4—

1. Click the **Plate Setup** tab in the Instrument Control Manager, and then click **New.**
2. On the **Templates** menu in the Plate Setup window, point to **Plate Setup Templates,** and then click **Select Template.** The Select Template window appears.
3. In the Select Templates window, select **StdDyeTerminator.tpl,** and then click **Open.** The Plate Setup window displays the parameters for the selected template (figure 3-6).
4. In the **Plate ID** box, type the plate ID, and then click **Save.** The plate ID appears in the Plate Catalog.

   Note: The software uses the plate ID and the run ID to name the raw run folder that stores the raw sample files (.rsd) from the run. Thus, a plate ID of a manageable size is advisable.

3.10 Changing the data file storage location

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. Before running a plate, you can specify a different storage location for the .rsd files on the instrument control computer. For instance, you can store the files on another hard drive on the instrument control computer if the computer is running out of space on the current drive.

Important **Changing the storage location affects only the future files that the software creates on the instrument control computer.**

Note: You cannot use the Raw Data Storage feature to select a storage location on a remote workstation.

To change the file storage location—

1. On the **Options** menu, click **Raw Data Storage.** The Browse for Folder window appears.
2. Select the local hard drive and folder you want to use, and click **OK**. The window closes. After you run the samples, the software creates a folder with a name based on the plate ID and run ID and stores it in the location you specified.

3.11 Guidelines for adjusting the run conditions

Table 3-5 shows the run time vs available called bases and aligned bases for MegaBACE 4500 runs at different run voltages. (Tables 3-2 and 3-4 list the values for the other plate setup and run parameters.) The data are from runs performed at Amersham Biosciences using an M13 terminator standard. You can use the data as a guide for how to adjust the run conditions.

Important **Read length will vary with custom samples and is highly dependent on sample quality.**

The data include the potential available bases, where—

- Called bases are bases of any quality that are called by the Cimarron™ base caller.
- Aligned bases are bases in the called sequence that are locally aligned to a known consensus sequence.

Figures 3-7 through 3-9 provide the graphs of the same data. In the figures, you can see that the number of available aligned bases reaches a plateau much earlier than the available called bases. This is due to the signal-to-noise limitations of the sample.

Table 3-5. The run time vs available called bases and aligned bases for MegaBACE 4500 runs of an M13 terminator standard

	Run voltage (7 kV)		Run voltage (6 kV)		Run voltage (5 kV)	
Run time (min)	**Called base**	**Aligned base**	**Called base**	**Aligned base**	**Called base**	**Aligned base**
20	0	0	0	0	0	0
40	75	18	8	0	0	0
60	263	249	181	151	91	15
80	424	416	326	318	224	208
100	572	567	457	451	341	332
120	718	713	579	576	448	435
140	873	867	698	694	549	531
160	1059	981	818	811	646	639
180	1292	1005	946	935	742	728
200	1451	1007	1096	1024	838	834
220			1287	1039	937	924
240			1527	1039	1040	1018
260					1152	1109
280					1282	1083
300					1421	1112
320					1562	1102

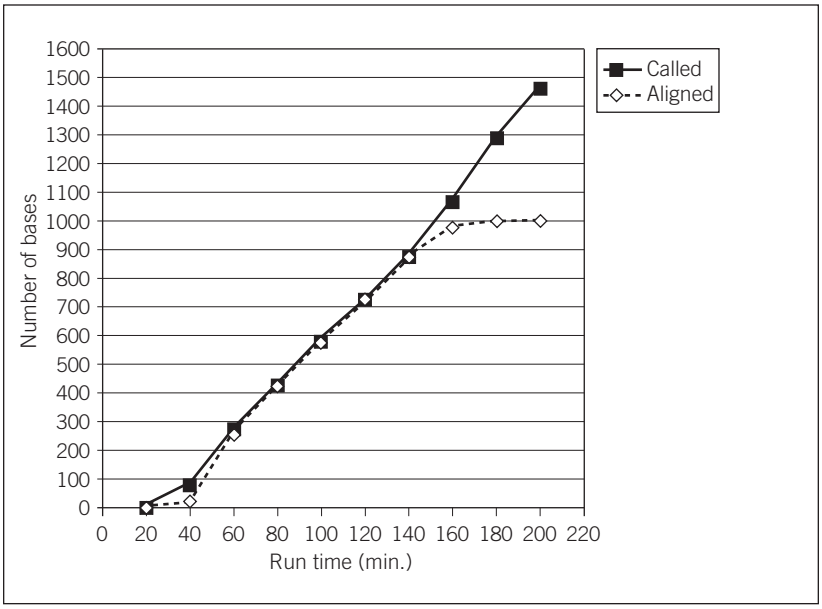

Figure 3-7. The run time vs the available called bases and aligned bases for an M13 terminator standard run at 7 kV.

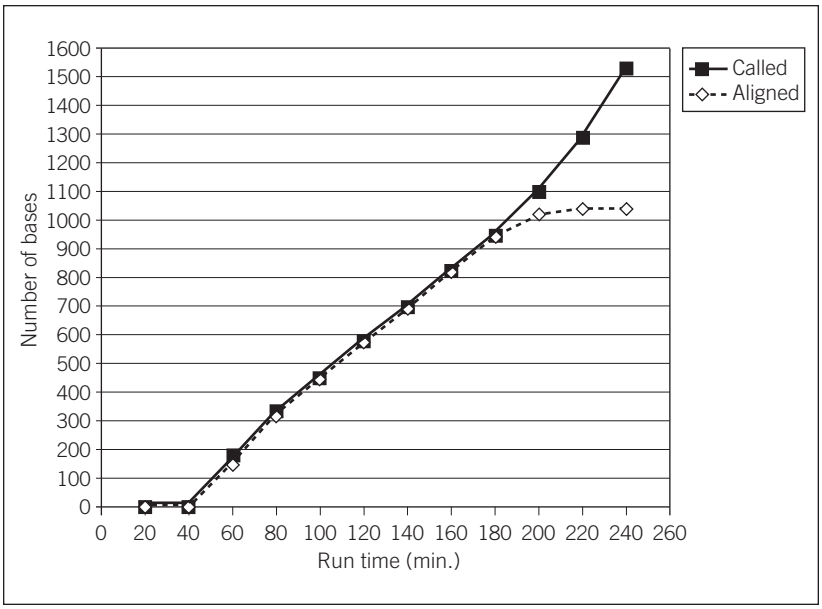

Figure 3-8. The run time vs the available called bases and aligned bases for an M13 terminator standard run at 6 kV.

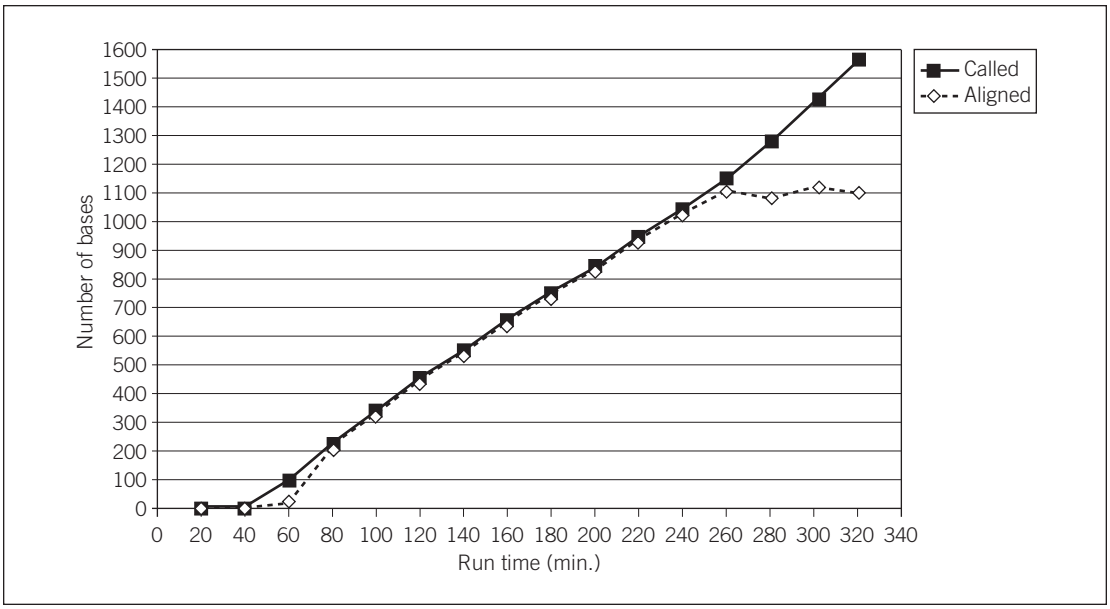

Figure 3-9. The run time vs the available called bases and aligned bases for an M13 terminator standard run at 5 kV.

Chapter 4 Performing sequencing runs

This chapter describes how you prepare for and perform a run on the MegaBACE 4500 instrument. The topics are—

- Workflow for performing a MegaBACE 4500 run (section 4.1)
- Filling the capillaries with matrix and performing a prerun (section 4.2)
- Injecting the samples and performing a sequencing run (section 4.3)
- Automatically storing the capillaries after a run (section 4.4)

4.1 Workflow for performing a MegaBACE 4500 run

A sequencing run on the MegaBACE 4500 instrument consists of the—

- Matrix Fill and Prerun protocol
- Inject Samples and Run protocol

For the MegaBACE 4500 plate setup parameters and instrument control parameters, see chapter 3. For information on setting up the automatic base calling feature, see the *MegaBACE 4000 Instrument User's Guide v3.2*, chapter 5.

Figure 4-1 shows an overview of the workflows for setting up plates and performing runs using the Instrument Control Manager. The automated plate setup workflow allows you to start the Inject Samples and Run protocol and then import the plate definition in the Select a Plate window. The alternative plate setup workflow requires the plate definition to be created in the Plate Setup window before starting the Inject Samples and Run protocol.

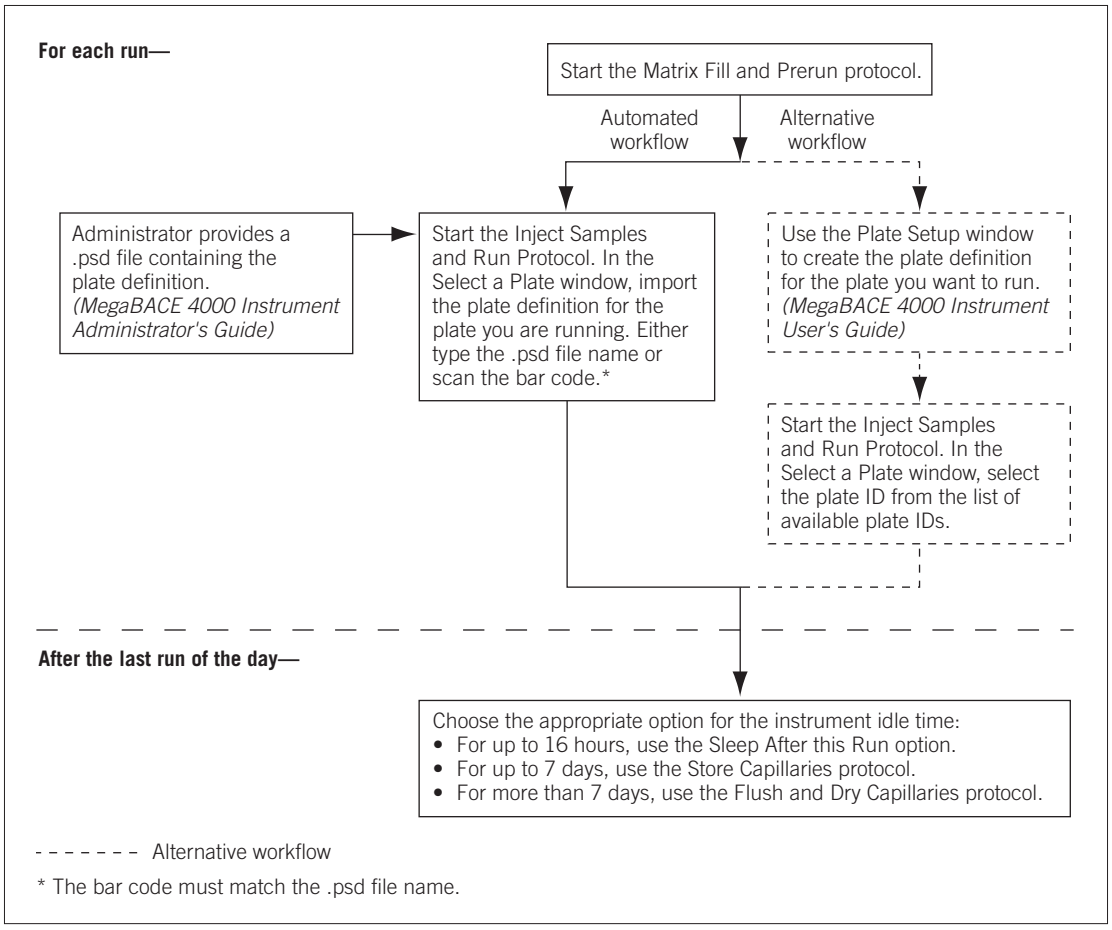

Figure 4-1. Workflow for performing sequencing runs on the MegaBACE instrument.

Table 4-1 describes the main tasks to prepare for and perform a run. Depending on how your software is configured, you can prepare for the run in two ways. You can import the plate definition for the run at the start of the Inject Samples and Run protocol (step 5). Alternatively, you can use the Plate Setup window to create plate definitions (step 3) and then select the pre-created plate definition when you start the Inject Samples and Run protocol.

Table 4-1. Workflow for performing a MegaBACE 4500 sequencing run

Task	Description	Reference
1. Check the instrument control parameters.	Click the Instrument Control tab to display the parameters.	Section 3.5
2. Fill the capillaries with MegaBACE 4500 long-read matrix v2 and perform a prerun.	Start the Matrix Fill and Prerun protocol and follow the instructions on the instrument displays to load the buffer tank and the matrix tubes into the instrument.	Section 4.2
3. (Alternative plate setup workflow only) Use the Plate Setup window to create the plate definition for the plate you are going to run.	In the Plate Setup window, click **New.** Type a plate ID or scan the bar code for each plate you will run. The software automatically imports the plate setup parameters from a plate setup data file (.psd), if it exists, or uses the default plate setup parameter template file.	*MegaBACE 4000 Instrument User's Guide v3.2,* chapter 6
4. Bring the materials to the instrument, and then start the Inject Samples and Run protocol.	Make sure you have the sample plate, the water tank, the deep-well buffer plate, and the buffer tubes available at the instrument. In the Instrument Control window, make sure the Inject Samples and Run protocol is selected, and then click **Start.**	Section 4.3.1
5. Enter a plate ID or bar code and import the plate definition for the plate you are going to run. (Alternative plate setup workflow only) If the plate definition has already been created in the Plate Setup window (step 3), select the Plate ID.	In the Select a Plate window, type a plate ID or scan a bar code. The plate definition is imported from the .psd file. Click **OK.** The Inject Samples and Run protocol continues. Follow the instructions on the displays.	Section 4.3.2
6. After the last run of the day, choose one of the following:		
- Store the capillaries up to 16 hours.	Click the **Sleep After This Run** check box in the Sleep Parameters area of the Instrument Control window before completing the last run of the day.	Section 4.4
- For up to 7 days, store the capillaries wet.	Use the Store Capillaries protocol. This protocol turns off the heater in the electrophoresis compartment and turns off the laser.	Chapter 5
- For storage of more than 7 days, store the capillaries dry.	Use the Flush and Dry Capillaries protocol if you are shutting down the instrument for more than 7 days.	Chapter 5

4.2 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.

4.2.1 Materials required

Cautions

Make sure you use the correct type of tank for the instrument. See the *MegaBACE 4500 Site Preparation Guide* for the qualified tank. Using the wrong tank can damage the instrument.

Be careful not to overfill the buffer tank. Open and close the cathode drawer slowly to prevent spilling liquid on the cathode stage. Spilled liquid can contaminate the cathode assembly and damage the electrodes in the cathode stage.

You need the following materials for the Matrix Fill and Prerun protocol (figure 4-2):

- A tank containing 120-ml buffer (diluted 1×)
- Six tubes MegaBACE 4500 long-read matrix v2

  Note: Centrifuge the matrix using the rpm and time listed in the package instructions.

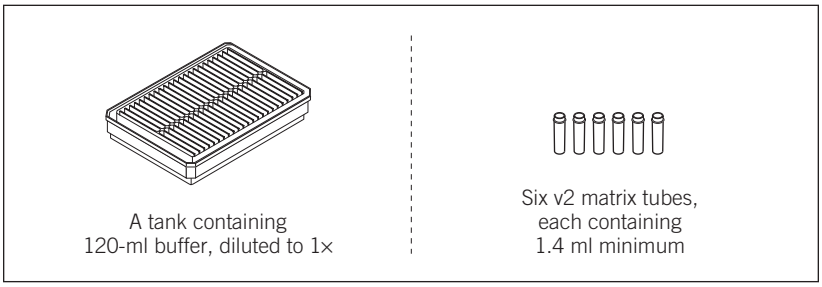

Figure 4-2. The materials for the Matrix Fill and Prerun protocol.

4.2.2 Performing the Matrix Fill and Prerun protocol for sequencing

To start the Matrix Fill and Prerun protocol—

1. In the Instrument Control window (figure 4-3), make sure the **Matrix Fill and Prerun** protocol is selected, and click **Start.**
2. Follow the instructions on the instrument displays to load the buffer tank into the cathode drawer (left side) of the instrument and the matrix tubes into the anode drawer (right side) of the instrument.

When the protocol is finished, the software selects the Inject Samples and Run protocol as the next protocol you should use. If more than 15 minutes elapse before you start the Inject Samples and Run protocol, you must perform another prerun before you inject the samples.

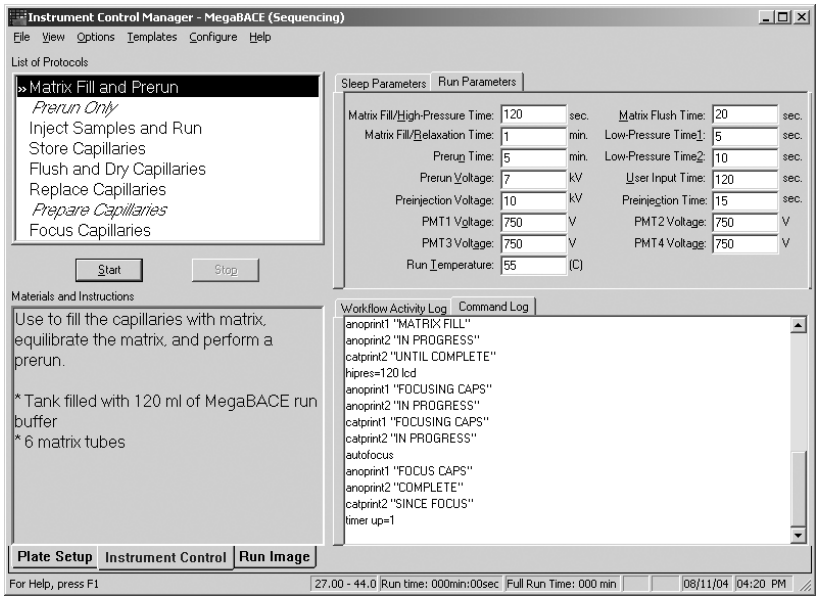

Figure 4-3. The Instrument Control window.

4.3 Injecting the samples and performing a sequencing run

Before you start the Inject Samples and Run protocol, make sure you have the required materials.

4.3.1 Materials required for the MegaBACE 4500 instrument

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 10 µl volume per well.

Make sure you use the correct type of plate for the instrument. See the *MegaBACE 4500 Site Preparation Guide* for a list of qualified plates. Using the wrong plate can damage the instrument.

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 contaminate the cathode assembly and damage the electrodes in the cathode stage.

You should have the following materials available at the instrument (figure 4-4):

- A clean tank containing 120-ml fresh deionized, filtered water
- A sample plate containing the samples prepared according to the applicable reagent protocol, 10 µl per well minimum
- A deep-well plate containing fresh buffer (diluted 1×), 85 µl per well
- Plate adapters for the sample plate and the buffer plate
- Six 2-ml tubes, each containing 1.75-ml buffer

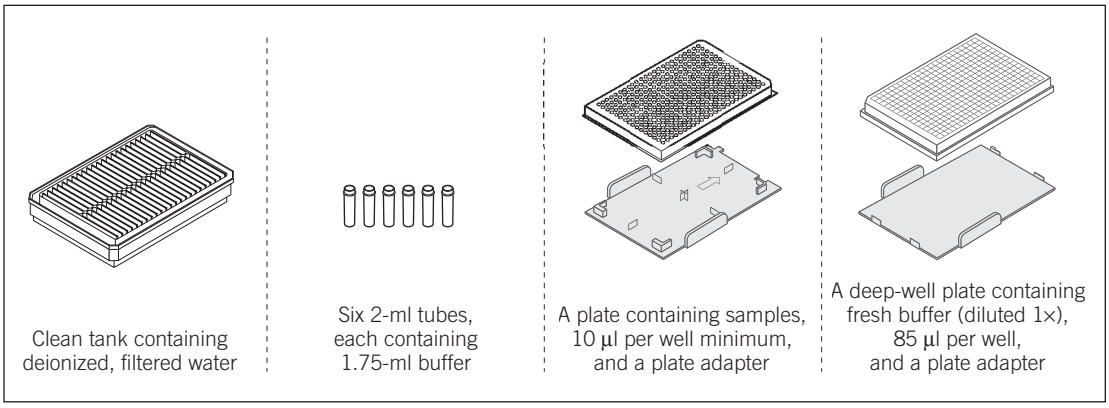

Figure 4-4. Materials for the MegaBACE 4500 Inject Samples and Run protocol.

4.3.2 Performing the MegaBACE 4500 Inject Samples and Run protocol for sequencing

Warning

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

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 4-5).

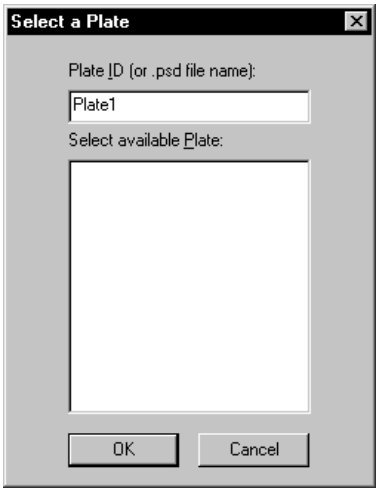

Figure 4-5. The Select a Plate window.

2. In the Select a Plate window, do one of the following:
   - In the **Plate ID** box, type the .psd file name or scan a bar code for the plate you want to run, and then click **OK.** The Instrument Control Manager selects the plate definition if it is already in the list, or the software imports the plate definition. The Inject Samples and Run protocol starts. **Note:** The bar code must match the .psd file name for the software to import the file.
   - In the **Select available Plate** list, select the plate ID, and then click **OK.** The Inject Samples and Run protocol starts.

3. Follow the instructions on the instrument displays to load the clean water tank. After the tip rinse, the Confirm to continue window appears (figure 4-6).

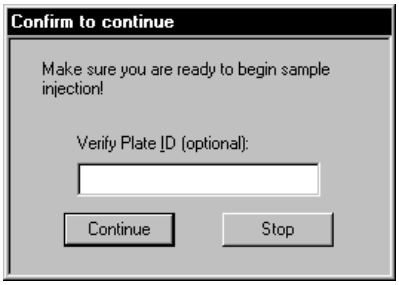

Figure 4-6. The Confirm to continue window with optional plate ID verification.

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** to add the samples, and then quickly load the sample plate in the cathode drawer.
6. After the instrument injects the samples, follow the instructions on the instrument displays to load the buffer plate and the buffer tubes. A message appears and tells you the sample run is in progress.

For information on monitoring the run, see the *MegaBACE 4000 Instrument User's Guide v3.2*.

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.

Note: If automatic base calling is selected, a window appears (figure 4-7) that shows the progress of the base calling.

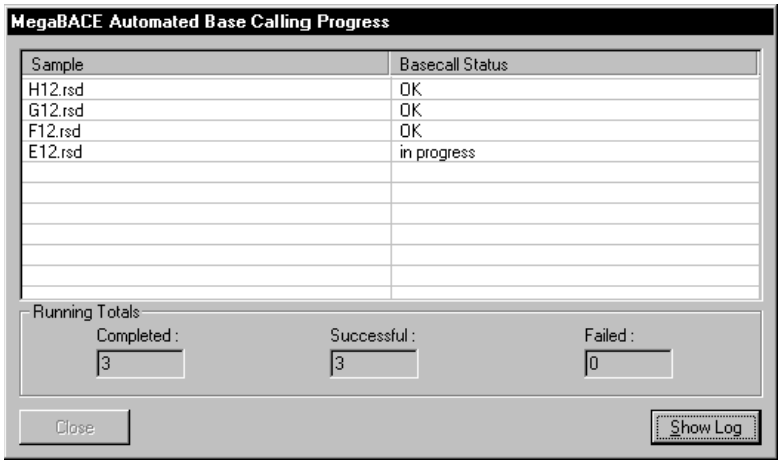

Figure 4-7. The Automated Base Calling Progress window.

4.4 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 Sleep Parameters tab of the Instrument Control window.

Caution **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 can select the Sleep After This Run check box anytime 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.

During the Sleep After This Run protocol, the Instrument Control Manager turns off the electrophoresis compartment heater and turns off the laser. When the sleep time has elapsed, the instrument starts warming up the electrophoresis compartment to the run temperature. The display on the instrument counts up the time since the temperature has reached the proper level.

To store the capillaries after a run—

1. In the **Sleep Parameters** tab of the Instrument Control window (figure 4-8), type the number of hours in the **Sleep Time** box.
2. Make sure the **Sleep Temperature** box contains a value.

Note: Although the Sleep Temperature box requires a value, the software turns off the electrophoresis compartment heater during the protocol.

3. Click the **Sleep After This Run** check box. A check mark appears.

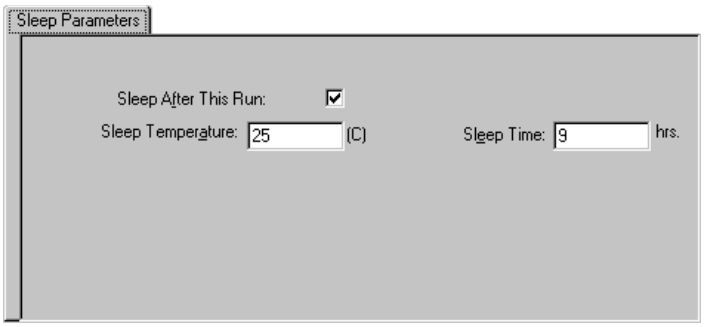

Figure 4-8. Sleep Parameters tab with Sleep After This Run selected.

Chapter 5 Leaving the system idle or shutting down

This chapter describes the requirements for leaving the instrument idle and for shutting down the system completely. The topics are—

- Leaving the MegaBACE 4500 instrument idle for up to 7 days (section 5.1)
- Shutting down the system for more than 7 days (section 5.2)
- Flushing and drying the MegaBACE 4500 capillaries (section 5.3)
- Shutting down the computer, the instrument, and the pressure source (section 5.4)
- Recovering from a power failure with a UPS (section 5.5)
- Recovering from a power failure without a UPS (section 5.6)

5.1 Leaving the MegaBACE 4500 instrument idle for up to 7 days

Caution **Do not store capillaries that are filled with deionized water or buffer. The MegaBACE 4500 capillaries have an internal, absorbed-surface coating that water can dissolve.**

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 MegaBACE 4500 instrument idle—

- **Overnight or for up to 16 hours—**Use the Sleep After This Run protocol to store the capillaries in MegaBACE 4500 matrix and buffer (section 4.4).
- **More than 16 hours, up to 7 days—**Use the Store Capillaries protocol to fill the capillaries with matrix and store the tips in water (section 5.1.1).

5.1.1 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 laser and the electrophoresis compartment temperature control. The protocol stores the stages in the up position.

5.1.2 Materials required

For the Store Capillaries protocol, you need (figure 5-1)—

- A clean tank containing 120-ml fresh deionized, filtered water
- Six 2-ml tubes, each containing 1.75-ml deionized filtered water

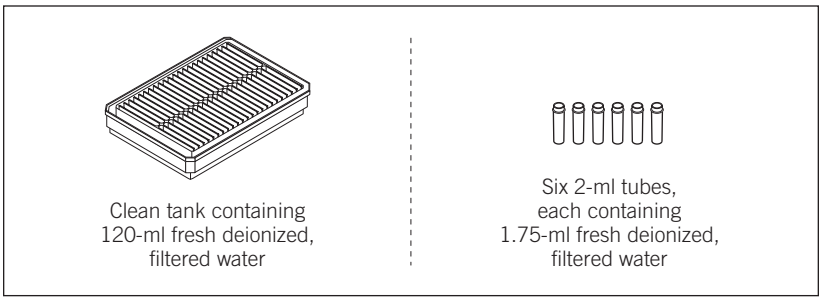

Figure 5-1. The materials for the Store Capillaries protocol.

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 contaminate the cathode assembly and damage the electrodes in the cathode stage. See the *MegaBACE 4000 Instrument User's Guide* for detailed instructions on using the cathode and anode drawers.**

5.1.3 Starting the Store Capillaries protocol

To start the Store Capillaries protocol—

1. Click the **Instrument Control** tab (figure 5-2) to display the Instrument Control window.
2. In the **Sleep Parameters tab,** type the length of time the instrument will be idle in the **Sleep Time** box. The time range is 1–168 hr, and the default time is 9 hr.
3. Make sure the **Sleep Temperature** box contains a value.

   Note: Although the Sleep Temperature box requires a value, the software turns off the electrophoresis compartment heater during the protocol.
4. In the **List of Protocols,** click **Store Capillaries,** and then click **Start.**
5. When instructed by the instrument displays, load a full water tank into the left side of the instrument and load the filled water tubes into the right side of the instrument.

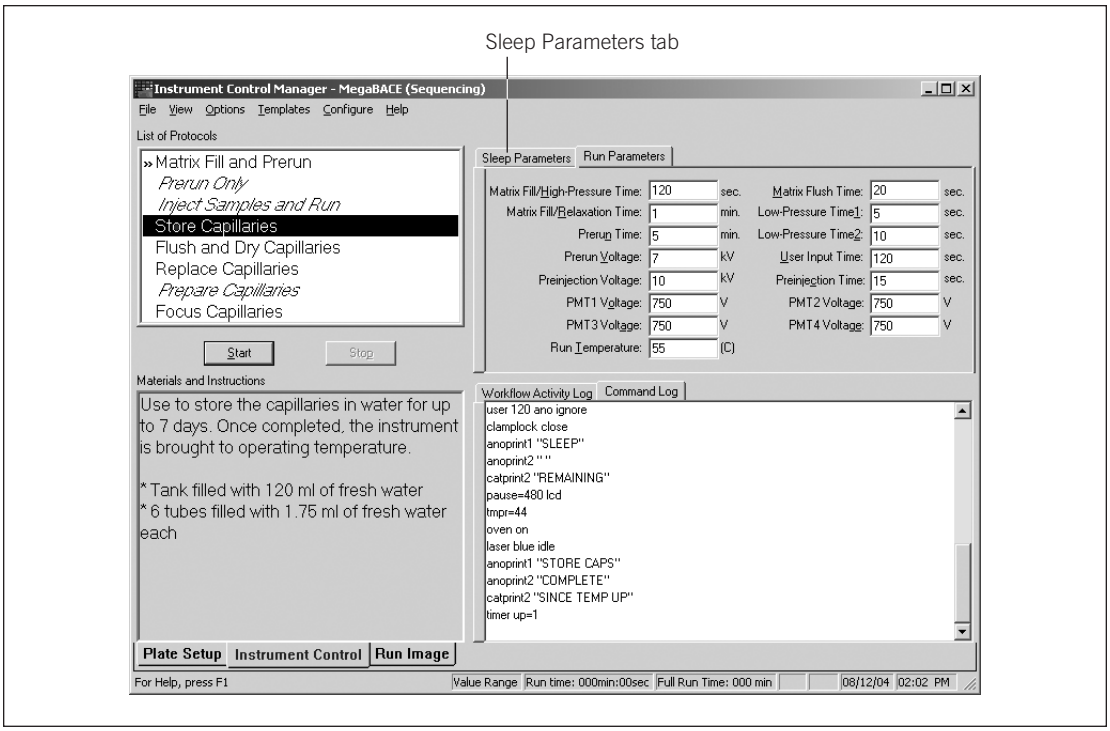

Figure 5-2. The Instrument Control window.

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 the electrophoresis compartment heater.

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

When the sleep time elapses, the instrument starts warming up the electrophoresis compartment to the set run temperature. 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 run temperature.**

5.2 Shutting down the system for more than 7 days

Caution **If you are shutting down the instrument for more than 7 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 need to be replaced.**

In general, shut down the system completely only when you will be leaving the instrument continuously unattended for more than 7 days. Before shutting down the system, you must use the Flush and Dry Capillaries protocol (section 5.3) to flush the matrix from the capillaries with water and store the capillaries dry.

Optionally, you can log off and shut down the computer, the turn off the instrument and turn off the high- and low-pressure systems (section 5.4).

5.3 Flushing and drying the MegaBACE 4500 capillaries

The Flush and Dry Capillaries protocol has the following parts:

- Starting the Flush and Dry Capillaries protocol (section 5.3.2)
- Opening the electrophoresis compartment lid and wiping the capillary anode tips (section 5.3.3)
- Flushing and drying the capillaries (section 5.3.4)

5.3.1 Materials required

For the Flush and Dry Capillaries protocol, you need (figure 5-3)—

- An empty water tank
- A clean tank containing 120-ml fresh deionized, filtered water
- 108 2-ml tubes, each containing 1.75-ml fresh deionized, filtered water
- Six empty 2-ml tubes
- Laboratory wipes

Cautions

Always use fresh deionized filtered water for flushing the capillaries to avoid contamination that can cause damage to the capillaries. Stale water can cause damage to the capillaries.

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 contaminate the cathode assembly and damage the electrodes in the cathode stage.

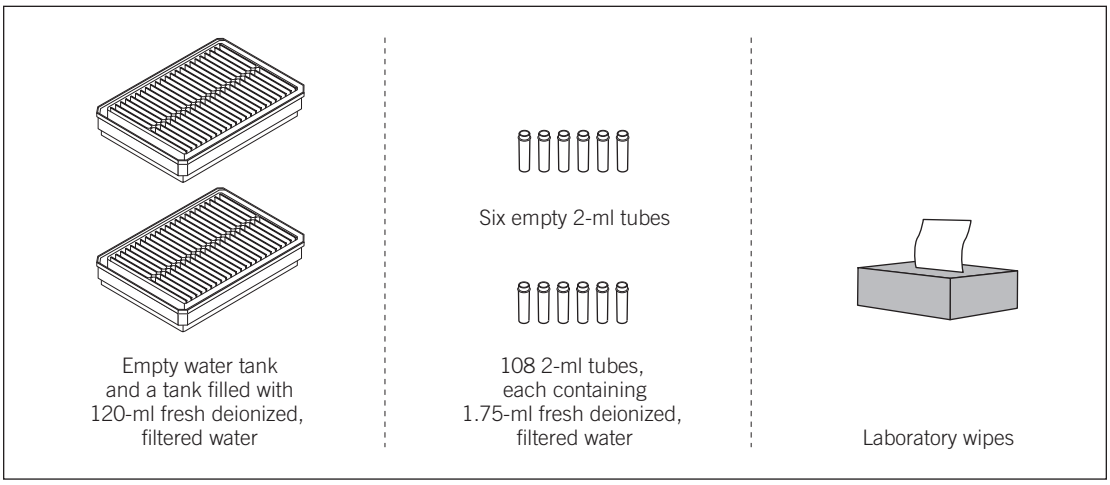

Figure 5-3. The materials for the Flush and Dry Capillaries protocol.

5.3.2 Starting the Flush and Dry Capillaries protocol

To start the Flush and Dry Capillaries protocol—

1. Click the **Instrument Control** tab (figure 5-4) 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, load the full water tank.
4. When instructed by the right instrument display, load the six filled water tubes into the right side of the instrument. The instrument rinses the capillary tips. The instrument displays tell you to open the service door and wipe the anode tips.

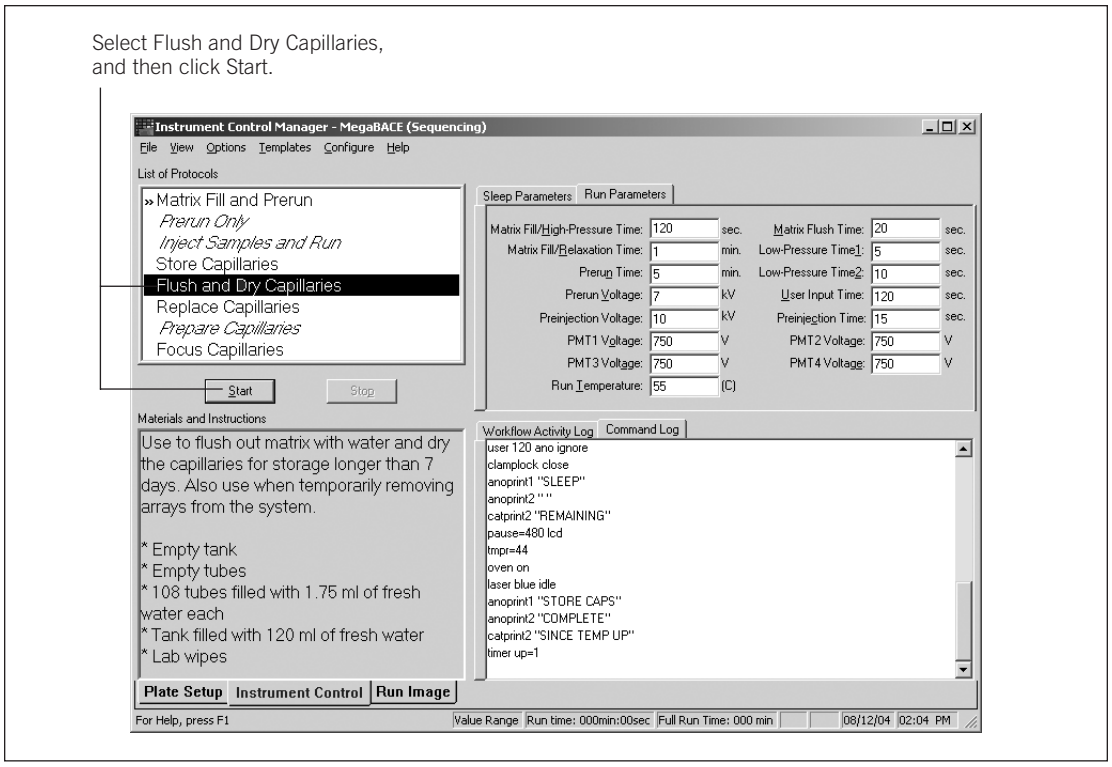

Figure 5-4. The Instrument Control window.

5.3.3 Opening the electrophoresis compartment lid and wiping the capillary anode tips

Caution **Protect the ends of the capillaries from dirt and chipping. Avoid touching or bumping the ends of the capillaries. Always wear powder-free gloves when handling the capillary arrays.**

1. When the Confirm to continue window appears and asks you to open the service door (electrophoresis compartment lid), click **Continue,** and then open the electrophoresis compartment lid.

   The Confirm to continue window reappears and asks you to wipe the anode bundles. Before clicking Continue, perform steps 2 through 8.

2. Unlock the anode cover. To do this, pull up the knob on the anode cover and rotate the cover counterclockwise (figure 5-5a). At the end of the rotation, make sure that the knob pin is seated in the stop hole and the capillary anode plugs are centered in the large holes in the anode cover (figure 5-5b).

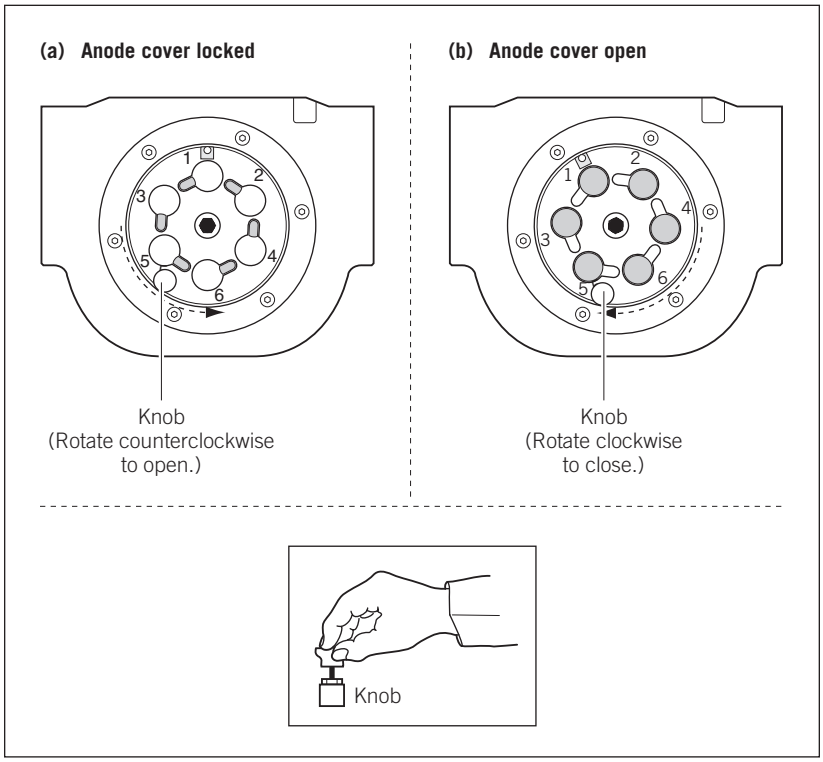

Figure 5-5. The anode cover (top view): (a) cover locked and (b) cover open.

3. Pull up on one anode plug to remove it from the anode cover.

Note: The anode plug has an O-ring to form the high-pressure seal. When you remove the plug, the seal attached to the plug might pop out suddenly.

Cautions

Handle the capillary anode bundles carefully. The capillaries are glass tubes coated on the outside with polyimide for strength. Avoid nicking the capillaries with jewelry.

Protect the tips of the capillaries from damage. Chips or cracks in the tips can interfere with accurate injection of the samples.

4. Using a laboratory wipe and starting just below the anode plug, gently wipe toward the anode tips of the capillaries to remove the matrix from between the capillaries in the anode bundle. See figures 5-6 and 5-7.

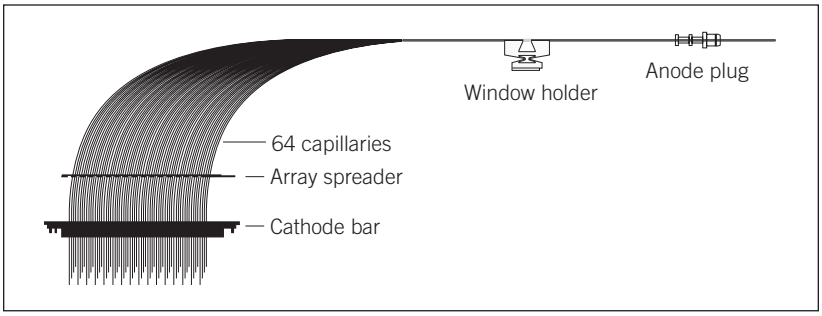

Figure 5-6. A capillary array.

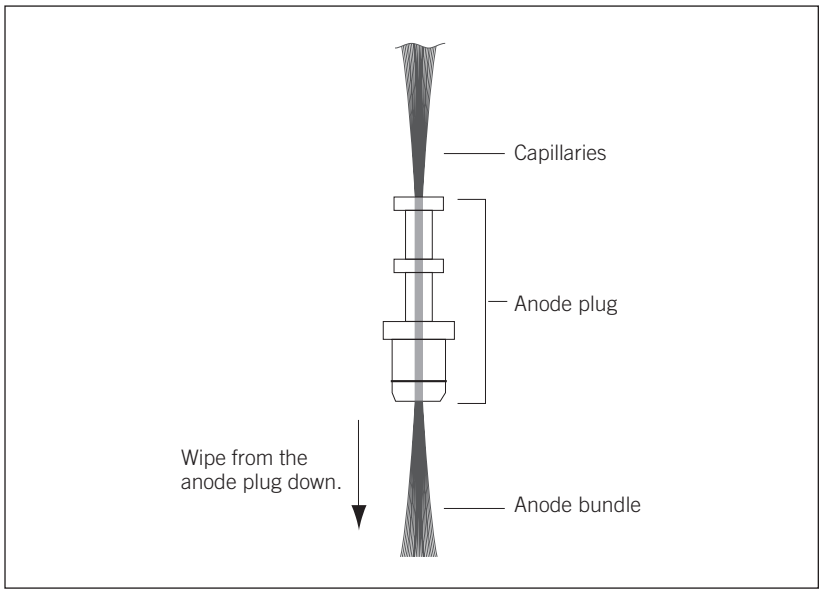

Figure 5-7. Removing the matrix from between the capillaries in the anode bundle.

5. Make sure the capillaries in the anode bundle are not spread out, and then lower the anode plug into position in the anode cover. **Note:** You might feel some resistance when you insert the plug. Do not force the bundle into the hole. Instead, gently work the plug into place.
6. Repeat steps 3 through 5 for each of the remaining anode plugs.

7. Make sure that each anode plug is correctly seated and pressed in all the way.
8. Lock the anode cover. To do this, pull up the knob on the anode cover and rotate the cover clockwise (figure 5-5b). Make sure that the pin is seated in the stop hole.
9. In the Confirm to continue window, click **Continue.** The Confirm to continue window reappears and tells you to lock the anode cover and close the service door.
10. Close the electrophoresis compartment lid, and then click **Continue** in the Confirm to continue window.

5.3.4 Flushing and drying the capillaries

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. Spilled water (or other material) can contaminate the cathode assembly and damage the electrodes in the cathode stage.**

1. When instructed by the instrument displays, load six new tubes filled with fresh water into the right side of the instrument (anode), and then load an empty tank into the left side of the instrument (cathode). The instrument displays tell you that a flush is in progress.
2. Follow the instructions on the instrument displays to complete the remaining high-pressure and low-pressure flushes.
3. When the Confirm to continue window appears and asks if you want to dry the capillaries, click **Continue.** Follow the instructions on the instrument displays to load an empty tank and empty tubes.
4. When the protocol is finished, the instrument displays tell you that capillary drying is complete and displays 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 5-4).

Important **Remember to run the Prepare Capillaries protocol (section 3.8) 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.**

5.4 Shutting down the computer, the instrument, and the pressure source

Caution **If you are leaving the instrument idle for up to 7 days, make sure you store the capillaries properly before you shut down the instrument (section 5.1). If you are leaving the instrument idle for more than 7 days, flush the matrix from the capillaries at the end of the last run (section 5.2).**

5.4.1 Logging off or shutting down the computer

You can leave the computer running and log off, 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.

5.4.2 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).

5.4.3 Turning off the high- and low-pressure systems

When storing the system for more than 7 days, you do not need to turn off the external nitrogen source or pressurized-filtered-air source. If you want to turn off the nitrogen source or pressurized-filtered-air source, follow the procedure established in your laboratory.

5.5 Recovering from a power failure with a UPS

5.5.1 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.

The cathode contains a vertical brake that holds the cathode plate in the up position in the event of a power failure or loss of low pressure. The instrument should continue its activities when the power is restored.

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

5.5.2 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 5.1.1) 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 5.1.1) to store the capillaries properly.

5.5.3 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 Store Capillaries protocol is running, allow the protocol to continue until it is complete.
   - If another protocol is running, click **Stop** to end the activity. Save the data collected thus far, and use the Store Capillaries protocol to safely store the capillaries.
2. Use one of the two following protocols, depending on how long you intend to leave the instrument idle:
   - **Short term (up to 7 days)—**Use the Store Capillaries protocol (section 5.1.1).
   - **Long term (more than 7 days)—**Use the Flush and Dry Capillaries protocol (section 5.3). 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 3.8). The Prepare Capillaries protocol hydrates the capillaries and prepares them for matrix and sample injection.**

5.6 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.**

If the anode and cathode stages were in the up position for the duration—

- **Brief power failure (less than 48 hours)—**Start with the Matrix Fill and Prerun protocol to prepare the capillaries for sample injection.
- **Extended power failure (more than 48 hours)—**As soon as the power comes on and you are able to begin normal operation, use the Flush and Dry Capillaries protocol (section 5.3), but do not dry the capillaries.

If the anode or cathode stage was in the down position for up to 1 hour, use the Prepare Capillaries protocol (section 3.8) and perform the MegaBACE system test (section 8.1) before proceeding.

If the anode or cathode stage was in the down position for 1–12 hours, flush the capillaries using the Flush and Dry Capillaries protocol (section 5.3), but do not dry the capillaries. Perform a system test run (section 8.1) to make sure the capillary arrays are still good. The capillary arrays might require several runs to recover.

Chapter 6 Maintaining the MegaBACE 4500 system

This chapter provides information on the routine maintenance of the MegaBACE 4500 system. The topics are—

- Routine maintenance tasks and schedule (section 6.1)
- Low- and high-pressure systems (section 6.2)
- Air vents and fan exhaust (section 6.3)

6.1 Routine maintenance tasks and schedule

Table 6-1 provides the schedule for routine maintenance of the MegaBACE 4500 system.

Table 6-1. MegaBACE 4500 instrument maintenance tasks and schedule

Task	Frequency	Reference
Check the available high and low pressure.	Periodically to prevent pressure loss during a run	Section 6.2
Make sure the air intake and exhaust vents are not blocked.	Periodically	Section 6.3
Clean the air filter to keep dust out of the optics and to maintain an adequate cooling air flow.	Periodically	*MegaBACE 4000 Instrument User's Guide v3.2,* chapter 18
Clean the cathode plate holder and slider	If a spill occurs in the cathode drawer or if the cathode drawer does not open and close easily	*MegaBACE 4000 Instrument User's Guide v3.2,* chapter 18
Clean the capillary windows	If you have trouble focusing the capillaries, a "cannot find capillaries" message appears, or there is a drop in the read length	Chapter 7
Focus the capillaries	Anytime you change the run temperature or replace the capillary arrays	Chapter 7
Replace the capillary arrays	If a capillary is broken or clogged	Chapter 7
Clean the emission beamsplitters or filters	If the data is unreadable or the signal is very low, and troubleshooting (chapter 8) indicates a problem with the emission filters	*MegaBACE 4000 Instrument User's Guide v3.2,* chapter 18

6.2 Low- and high-pressure systems

Periodically, check the low- and high-pressure sources to make sure the pressure is set properly:

- High pressure for injecting and removing the sieving matrix: 6.89×10^3 kPa (1 000 psi)
- Low pressure for rinsing the capillaries and for operating the cathode and anode stages: 6.89×10^2 kPa (100 psi)

Also, make sure that the tubing and nitrogen cylinder and regulators (if applicable) are connected correctly and in good condition. See the *MegaBACE 4000 Instrument User's Guide* for additional information about the low- and high-pressure systems.

6.3 Air vents and fan exhaust

Air vents for cooling are on the top and sides of the MegaBACE 4500 instrument (figure 6-1). A cooling fan exhaust for the laser compartment is located on the instrument right side.

Caution **Do not place anything on top of the MegaBACE 4500 instrument. Large objects can block the air flow. Small objects (pens, tubes) can fall into the interior of the instrument and cause damage. Allow free air access to both sides of the instrument and keep the air vents free of obstructions.**

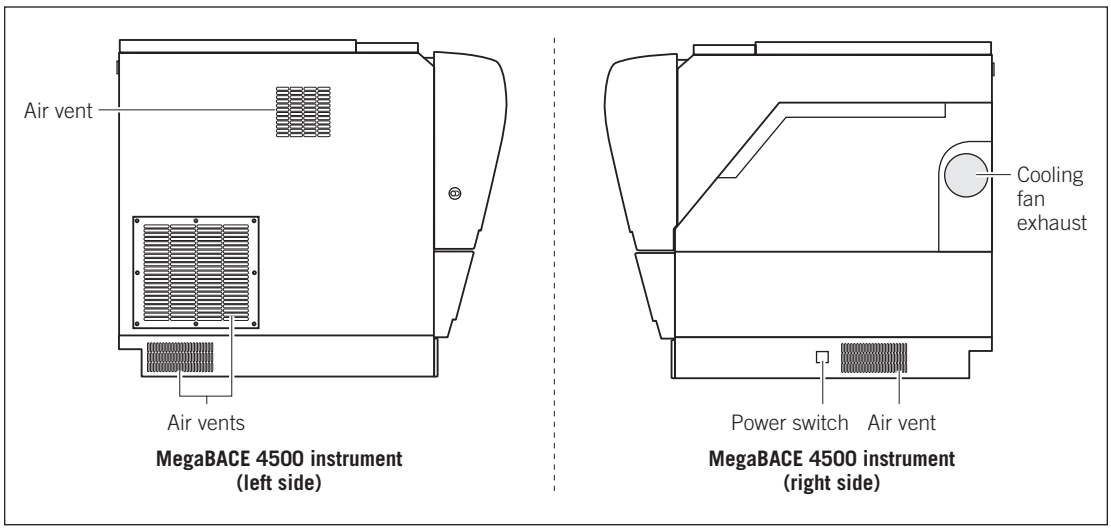

Figure 6-1. Air vents and fan exhaust on the MegaBACE 4500 instrument.

Chapter 7 Replacing the capillary arrays

This chapter describes how to replace the capillary arrays. The topics are—

- Workflow overview for capillary array replacement (section 7.1)
- Locating the capillary arrays (section 7.2)
- Using the Replace Capillaries protocol (section 7.3)
- Releasing the capillary array locks (section 7.4)
- Removing the capillary arrays (section 7.5)
- Cleaning the capillary windows (section 7.6)
- Installing the new capillary arrays (section 7.7)
- Locking the capillary arrays in position (section 7.8)
- Focusing the capillaries (section 7.9)

7.1 Workflow overview for capillary array replacement

Anytime you replace the capillary arrays—

1. Use the Replace Capillaries protocol (section 7.3) to unlock the lid of the electrophoresis compartment and gain access to the capillaries.
2. Use the Prepare Capillaries protocol (section 3.8) to hydrate the new capillaries.
3. Allow the instrument to warm up to the set run temperature (section 3.3).
4. Use the Focus Capillaries protocol (section 7.9) to focus the capillaries.
5. Perform the MegaBACE system test (section 8.1) to make sure the system is performing to specification. For new capillary arrays or capillary arrays that have been stored dry, you may need to perform up to three runs to reach the read length specification.

7.2 Locating the capillary arrays

The capillary arrays are contained within the electrophoresis compartment (figure 7-1).

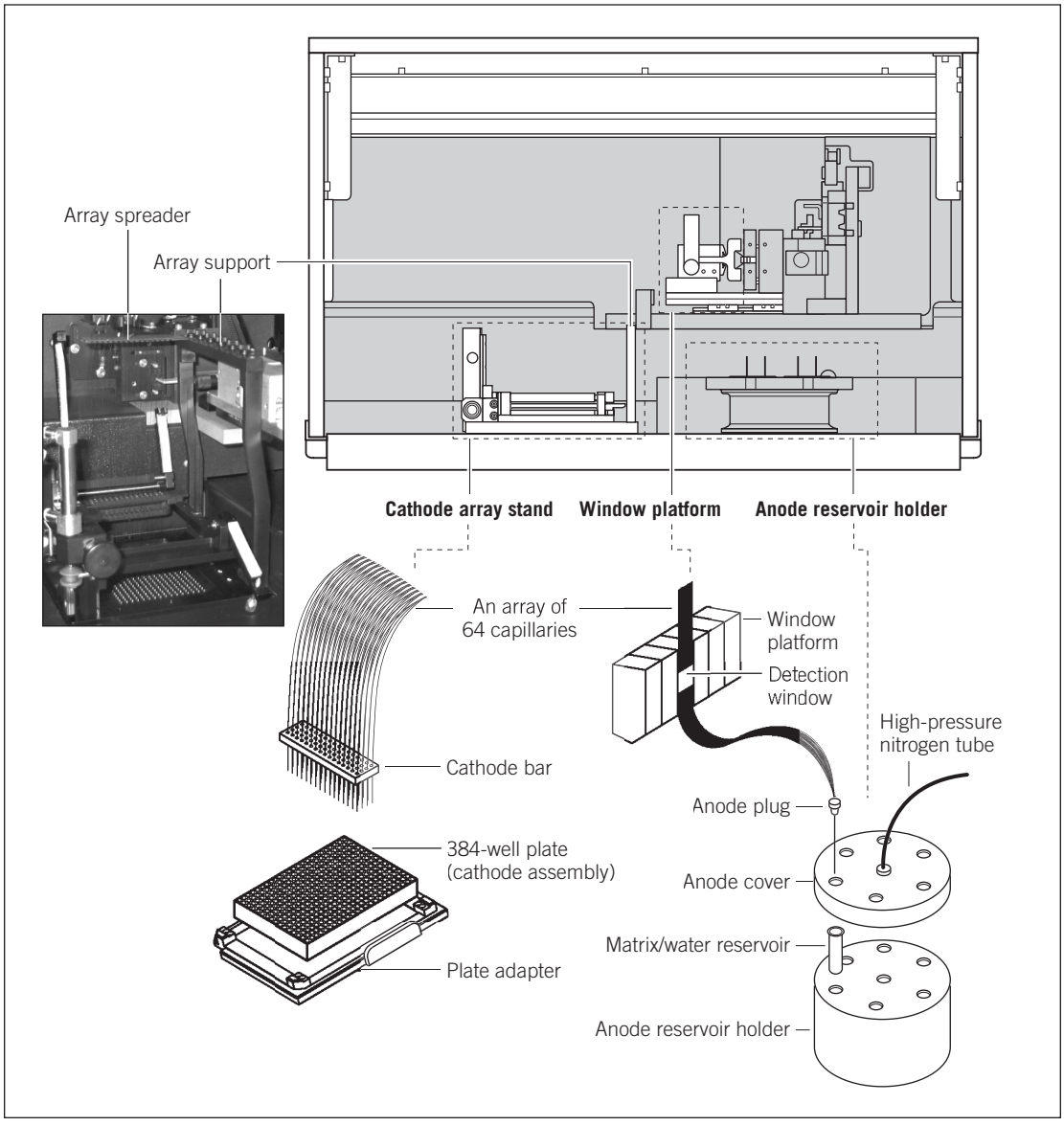

Figure 7-1. A capillary array in the electrophoresis compartment.

7.3 Using the Replace Capillaries protocol

You use the Replace Capillaries protocol in the Instrument Control Manager to replace damaged or old capillary arrays. The protocol rinses the capillary tips and unlocks the electrophoresis compartment lid so that you can access the capillaries.

Warning

The used capillary arrays might contain hazardous waste. Dispose of the used capillary arrays according to your local governmental regulations.

Cautions

Always use the Replace Capillaries protocol before you remove the capillary arrays.

The Replace Capillaries protocol does not adequately flush the capillaries for reuse.

During operation, the lid to the electrophoresis compartment is locked and cannot be opened until you use the Replace Capillaries protocol. In the event of a power failure, you can use an unlocking tool (section 8.5).

7.3.1 Materials required

For the Replace Capillaries protocol, you need (figure 7-2)—

- A tank containing 120-ml fresh deionized, filtered water
- Six 2-ml tubes, each containing 1.75-ml fresh deionized, filtered water
- MegaBACE 4500 capillary arrays v2

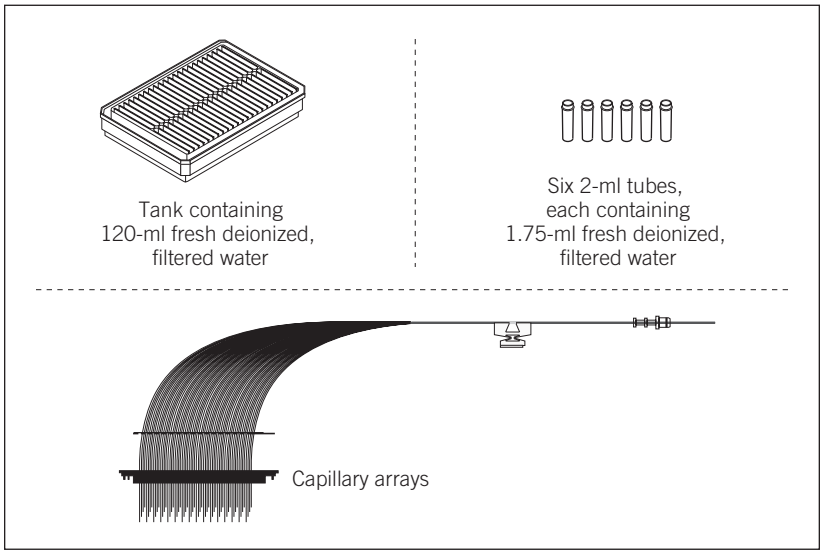

Figure 7-2. The materials for the Replace Capillaries protocol.

7.3.2 The Replace Capillaries protocol

To use the Replace Capillaries protocol—

1. In the Instrument Control window (figure 7-3), click the **Replace Capillaries** protocol, and then click **Start.**

   In the List of Protocols, the protocol name is highlighted to indicate it is running. The Workflow Activity Log records the start date and time for the protocol.

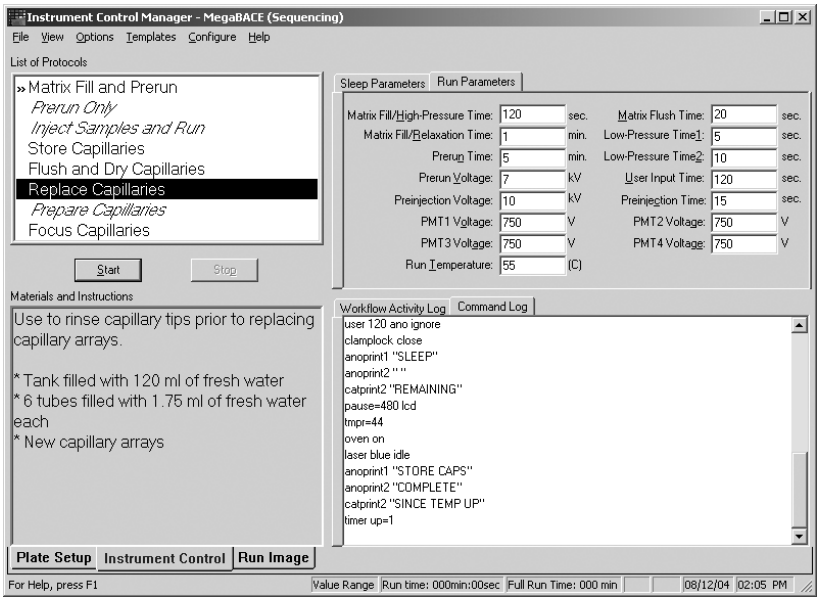

Figure 7-3. The Instrument Control window.

2. When instructed by the left instrument display, load a full water tank in the left side of the instrument.

Caution **Do not overfill the water tank. Close the cathode drawer slowly to prevent spilling the water on the cathode stage. Spilled water (or other material) can contaminate the cathode assembly and damage the cathode electrodes.**

3. When instructed by the right instrument display, load the filled water tubes in the right side of the instrument.

   The instrument rinses the capillary tips, and the instrument display tells you the tip rinse is in progress.

The message **Ready to replace capillaries** appears on the instrument displays. The software unlocks the electrophoresis compartment lid. The Confirm to continue message appears and asks you to open the service door.

4. Open the electrophoresis compartment lid. Use the following procedures to replace the capillaries:
   - Release the capillary array locks (section 7.4).
   - Remove the capillary arrays (section 7.5).
   - Clean the capillary windows on the new arrays (section 7.6).
   - Install the new capillary arrays (section 7.7) and lock them in position (section 7.8).

7.4 Releasing the capillary array locks

The structure of a capillary array is shown in figure 7-4.

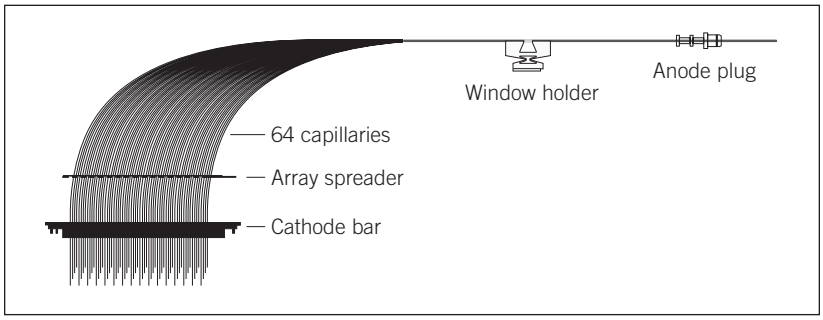

Figure 7-4. A capillary array.

Cautions

Protect the ends of the capillaries from dirt and chipping. Avoid touching or bumping the ends of the capillaries.

Handle the window holder carefully to prevent the glass from cracking. Be careful not to touch the clear window area of the capillaries. The protective polyimide coating has been removed from the capillaries in the clear window area to provide optical access for scanning.

Protect the glass, especially the clear window area of the capillaries, from dust, dirt, and skin oils. Always wear powder-free gloves when handling capillary arrays. Oils and salts from your skin could result in arcing between capillaries during high-voltage electrophoresis.

Four locks hold the capillary arrays in place (figure 7-5): cathode array stand, the window platform, the anode cover on anode reservoir, and the array spreader and support cross-bar.

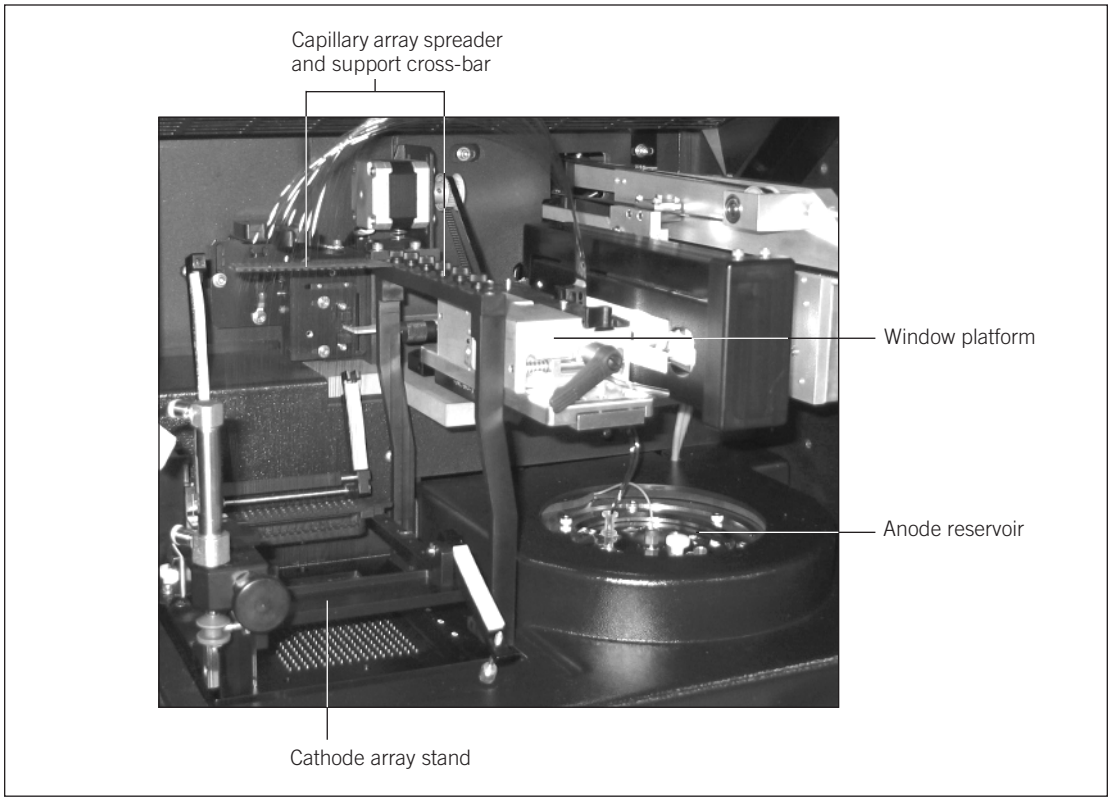

Figure 7-5. The electrophoresis compartment and the capillary array locks with one installed capillary array.

To release the locks—

1. Pull the knob on the cathode array stand forward and raise the stand to the up position. Release the knob gently and make sure that the knob pin enters the upper position hole (figure 7-6a).
2. Unscrew the two thumbscrews on the cathode array stand. Rotate the short (right) arm forward and then rotate the long (left) arm backward (figure 7-6b).

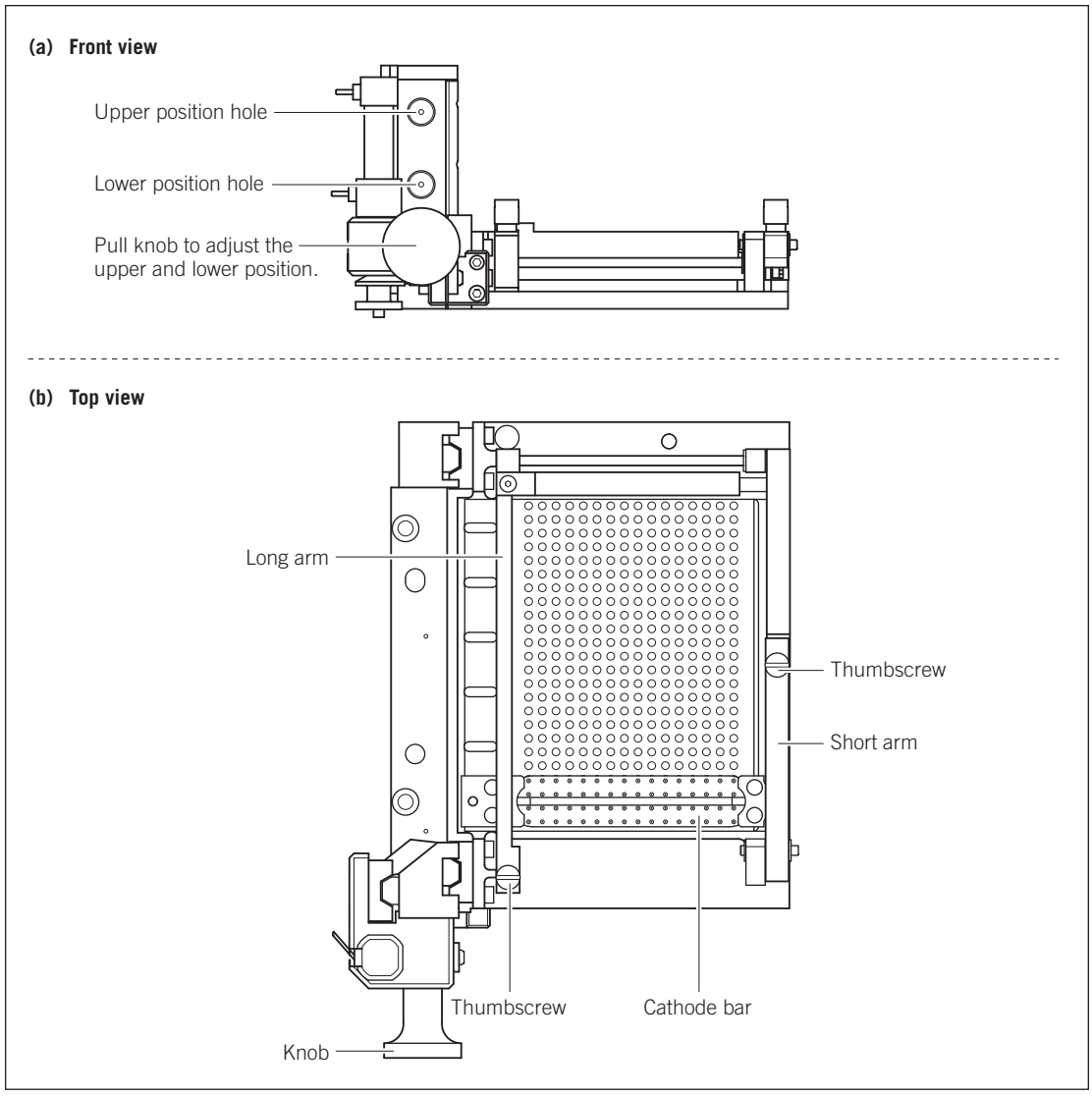

Figure 7-6. The cathode array stand: (a) front view and (b) top view.

3. Loosen the thumbscrew on the support cross-bar to release the array spreader on capillary array (figure 7-5).
4. Rotate the handle at the front of the window platform counterclockwise to the open position (figure 7-7). The window platform moves to the left, away from the scanning face.

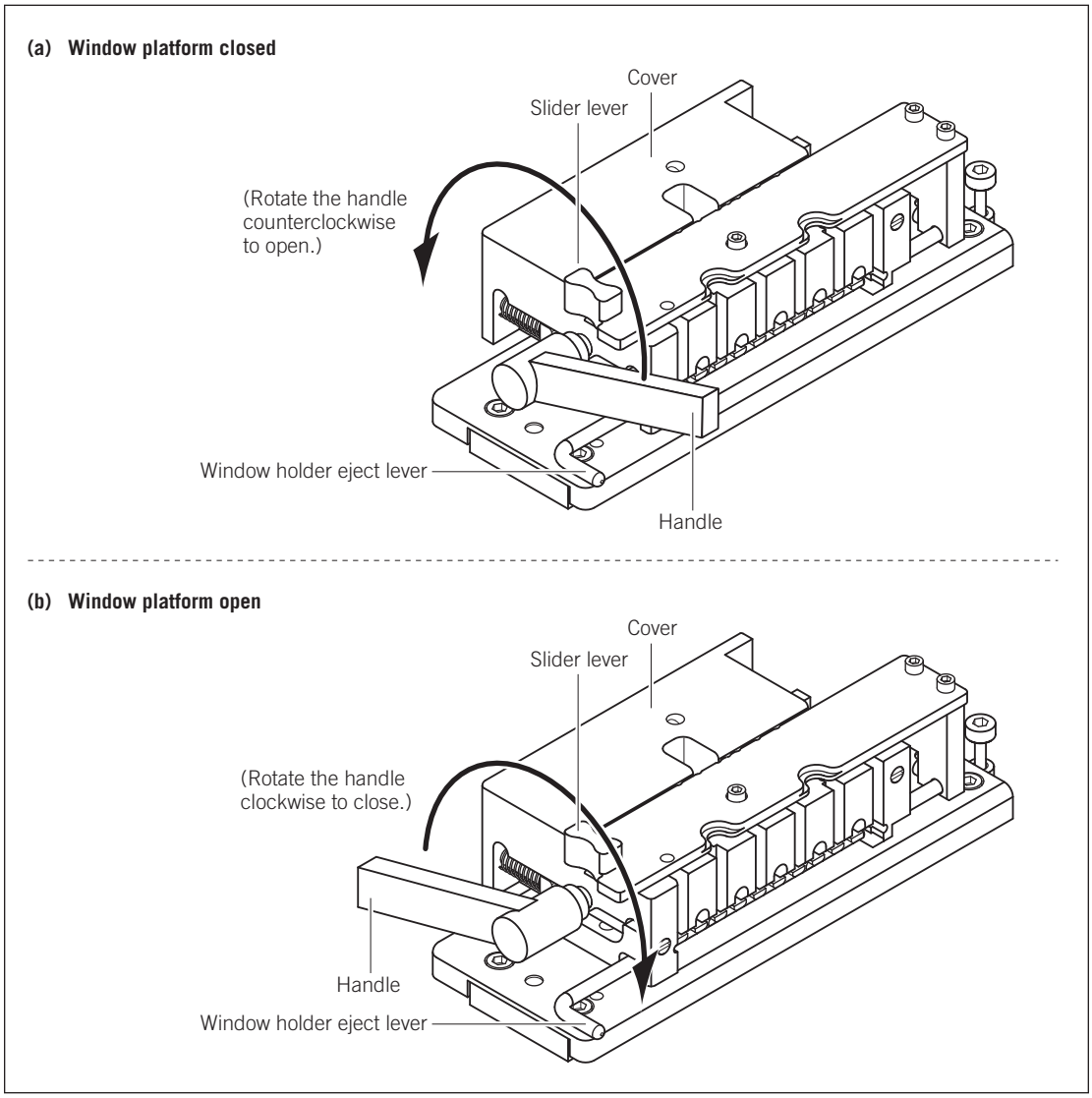

Figure 7-7. The window platform: (a) closed position and (b) open position.

5. Pull up the knob on the anode cover and rotate the cover counterclockwise (figure 7-8a). At the end of the rotation, make sure that the knob pin is seated in the stop hole and the capillary plugs are centered in the large holes in the anode cover (figure 7-8b).

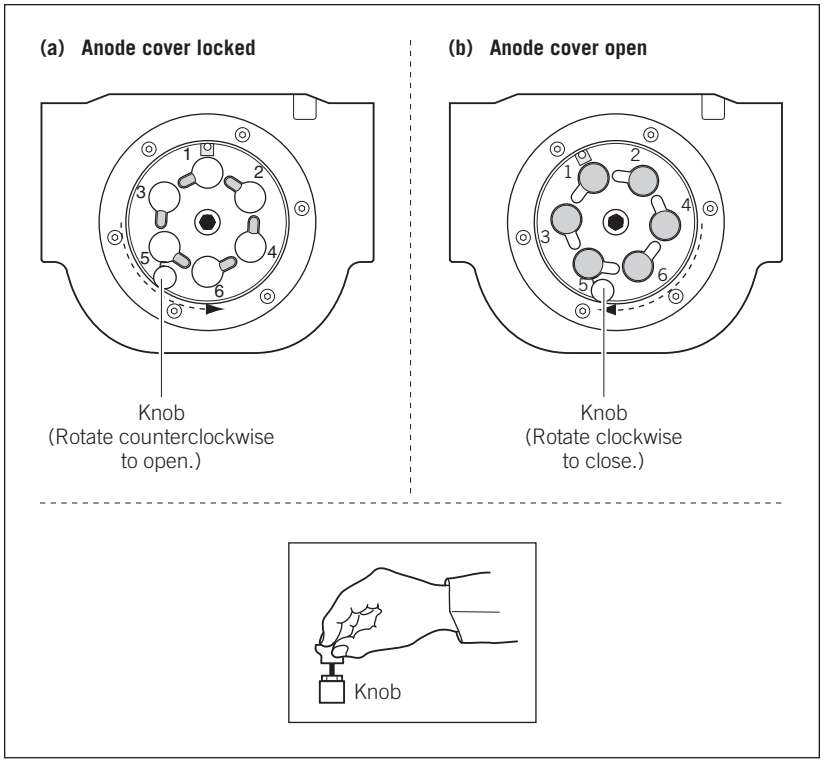

Figure 7-8. The anode cover (top view): (a) cover locked and (b) cover open.

7.5 Removing the capillary arrays

In general, replace the entire set of capillary arrays at the same time. However, if one or a few arrays contain broken or blocked capillaries and the remaining arrays have many runs remaining in their rated lifetime, you can replace only the problem arrays.

Warning ⚠

Do not pull on the capillaries to release the cathode bar (figure 7-1). The capillaries are fine glass tubes that can break, leaving sharp ends or fragments, which can cause injury.

To remove the capillary arrays—

1. Make sure the array spreader for position 6 is disengaged from the array support cross-bar (figure 7-5).
2. At the window platform (figure 7-7b), grasp the slider lever between your thumb and forefinger, and slide the cover of the window platform toward you until the notch in the right edge of the cover is over the front window holder. The cover clicks into place as the notch passes over each window holder, and the window holder eject lever moves forward to the number 6 position.
3. Rotate the window holder eject lever counterclockwise to eject the number 6 window holder.
4. Grasp the number 6 window holder and the handle of the corresponding anode plug (see numbering in figure 7-8). First pull up on the plug and then the window holder to free the array from the window holder platform and the anode cover.

   Note: The window holder comes out easily. However, the anode plug has an O-ring to form the high-pressure seal. When you remove the plug, the seal attached to the plug might pop out suddenly.
5. Start with the front array labeled 6. Gently lift the left and right ends of the front cathode bar (figure 7-6) to release the bar from the cathode stand.
6. The array is now released from all three locks. Remove the array from the instrument.
7. Repeat steps 1 through 6 to remove each of the remaining capillary arrays in order, starting from position 5.

Warning ⚠

The capillary arrays might contain hazardous waste. Dispose of the used capillary arrays according to your local and governmental regulations.

7.6 Cleaning the capillary windows

You should clean the capillary windows before installing the arrays in the instrument and periodically, if you have trouble focusing the capillaries. If the windows are dirty, you will collect noisy data or have capillary detection errors. Use the following procedure to clean the windows of the capillaries. These can be capillaries you have removed and intend to reuse or new capillaries.

You should use the following materials:

- Liqui-Nox™ 1% solution, Alconox Inc
- Micro Absorbond™ swabs, VWR Scientific TWTX762
- Laboratory wipes
- Squirt bottle containing fresh deionized-filtered water
- A small container to catch liquids during the wash procedure

Important **Never touch the window area with drying media or your fingers. If you touch the window area, you must reclean the window.**

To clean the capillary windows—

1. Remove a capillary array from the instrument (section 7.5) or from the box by grasping the cathode bar (figure 7-9).
2. Wet a swab with the 1% solution Liquinox.
3. Carefully grasp the capillary array by the window holder with one hand (figure 7-9), and with the other hand, use the wet swab to clean the entire window area (front and back).

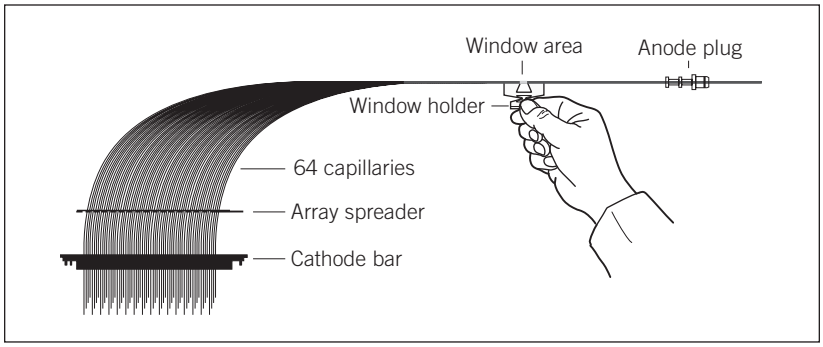

Figure 7-9. Cleaning the capillary windows.

Caution **Move the swab from the center of the window to the outer edges using minimum pressure. Pressing hard can break the window.**

4. Hold the capillary window area over a container. Using a steady stream of deionized filtered water from a squirt bottle, wet the front and back of the window area for 10 to 20 seconds.
5. Dry the body of the window holder using a laboratory wipe.
6. Touch the laboratory wipe next to the window to wick the water away from the window.

Important **Do not touch the capillary windows, or the windows can become scratched or dirty.**

7.7 Installing the new capillary arrays

Cautions **Handle the capillary arrays carefully. The capillaries are glass tubes coated on the outside with polyimide for strength. Avoid nicking the capillaries with jewelry. Handle the arrays by the plastic pieces only. Never touch the clear window area or the tips of the capillaries.**

Protect the tips of the capillaries from damage. Chips or cracks in the tips can interfere with accurate injection of the samples.

Protect the window holders from compression or stretching, which can break the unprotected glass of the window area.

To install the capillary arrays—

1. Make sure that the cathode stage is in the up position, and the three locks on the cathode stand, window platform, and anode cover are in the open position (section 7.4).
2. Remove the capillary array from its box by grasping the cathode bar (figure 7-4). Hold onto the cathode bar and let the rest of the array hang down in a straight line.

Caution **Protect the glass capillaries from nicks and scratches during the installation process.**

3. Start at the back position, which is labeled 1 on the cathode stand (figure 7-6b). Place the cathode bar of the array onto the stand.

   Note: To make it easier to place the cathode bar and capillary tips into position, lay the cathode bar on its side with the capillary tips toward you. Carefully rotate the bar forward so that the capillary tips are pointing downward.

4. Align the double pegs with the oval holes on the left side of the stand and the single peg with the round hole on the right. Make sure that both ends of the cathode bar are correctly seated and pressed in all the way.
5. Make sure that the capillary array is straight, not twisted.

   Between the cathode stand and the window platform, the capillaries must be fanned out and accessible to the air flow that regulates the temperature during a run.
6. Slide the cover of the window platform away from you until the notch is over the back window holder position, which is labeled 1.

Caution **Make sure that you fit the rounded portion of the window holder into the round slot on the window holder platform. An improperly seated window holder can damage the capillaries.**

7. Holding the plastic window holder in your left hand and the handle of the anode plug in your right hand, gently lower the window holder into the slot, making sure that the rounded portion of the window holder fits into the round slot (figure 7-7).
8. Lower the anode plug into position on the anode reservoir. You might feel some resistance when you insert the plug.
9. Make sure that the anode plug is correctly seated and pressed in all the way.
10. Slide the array spreader from the cathode bar up to the support cross-bar. Place the notched end of the array spreader in position under the thumbscrew on the support cross-bar. Tighten the thumbscrew to secure in place (figure 7-10).

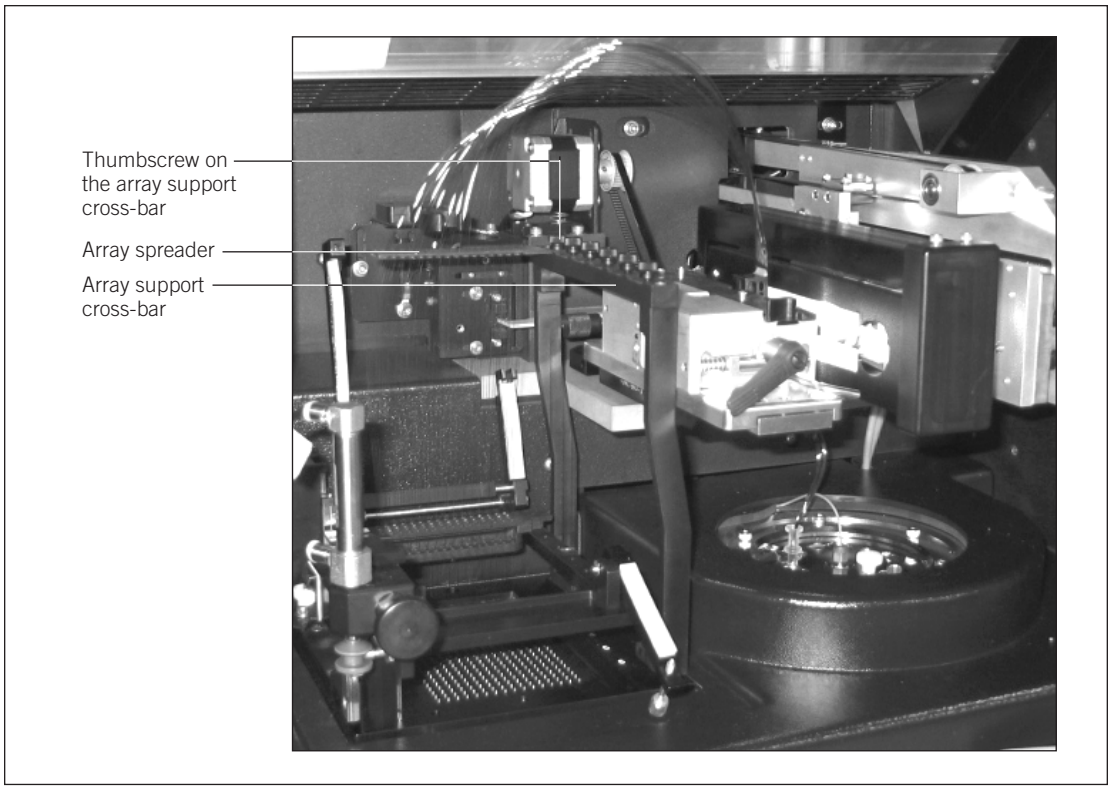

Figure 7-10. Securing the array spreader.

11. Repeat steps 2 through 10 to install each of the remaining capillary arrays in order, from position 2 through position 6.
12. Lock the capillary arrays in position (section 7.8).

7.8 Locking the capillary arrays in position

After replacing all the capillary arrays, use the following steps to lock the capillaries in position and close the electrophoresis compartment:

1. Pull up the knob on the anode cover and rotate the cover clockwise (figure 7-8b). Make sure that the pin is seated in the stop hole.
2. Slide the cover of the window platform halfway back until all the window holders are locked in the down position (figure 7-7a).
3. Rotate the handle at the front of the window platform clockwise to the closed position (figure 7-7b).

   Note: If you cannot rotate the handle past the cover of the window platform, push the cover farther back and try again.
4. On the cathode array stand, rotate the long (left) arm of the stand forward and down. Tighten the thumbscrew at the end of the long arm (figure 7-6b).
5. Rotate the short (right) arm of the stand backward and then tighten the thumbscrew at the end of the arm.
6. Pull the knob on the cathode array stand forward and gently lower the stand to the down position (figure 7-6a). Visually check that the capillary tips properly enter the holes in the top of the tray holder so that the tips are not bent. The bars and lockdown arms hold the capillaries in the correct position.
7. In the Instrument Control Manager, click **Continue** in the Confirm to continue window. The Confirm to continue window reappears and tells you to close the service door.
8. Close the lid of the electrophoresis compartment and press it shut until the lock engages.
9. In the Instrument Control Manager, click **Continue** again. 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 on the Instrument Control window.

You must first prepare the new capillaries using the Prepare Capillaries protocol (section 3.8) before you can focus the capillaries using the Focus Capillaries protocol (section 7.9).

7.9 Focusing the capillaries

7.9.1 Before you start the Focus Capillaries protocol

After replacing the capillary arrays or cleaning the capillary windows, you must do the following before you start the Focus Capillaries protocol:

- Prepare the capillaries for focusing using the Prepare Capillaries protocol (section 3.8).
- Allow the electrophoresis compartment to warm up to the set run temperature. If the compartment has not stabilized to the set run temperature, you cannot properly focus the capillaries. During the Replace Capillaries protocol, the temperature in the electrophoresis compartment drops when you open the compartment lid. See section 3.8 for guidance on the warmup times you should allow before focusing the capillaries.

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 capillary focusing.**

7.9.2 Materials required

For the Focus Capillaries protocol, you need (figure 7-11)—

- A tank filled with 120-ml fresh deionized, filtered water
- Six tubes MegaBACE 4500 long-read matrix v2

Caution **Be careful not to overfill the tank. Open and close the cathode drawer slowly to prevent spilling liquid on the cathode stage. Spilled liquid can contaminate the cathode assembly and damage the electrodes in the cathode stage.**

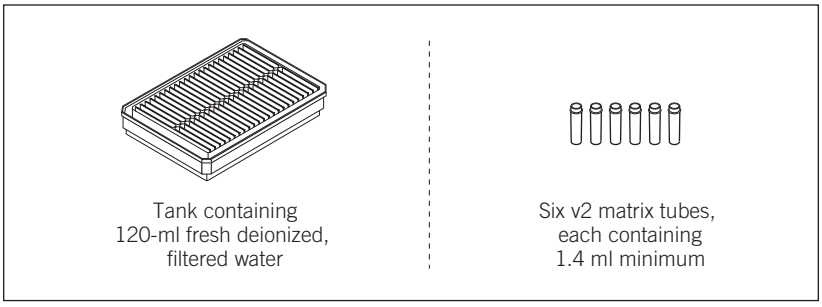

Figure 7-11. The materials for the Focus Capillaries protocol.

7.9.3 Starting the Focus Capillaries protocol

To start the Focus Capillaries protocol—

1. In the Instrument Control window, make sure the **Focus Capillaries** protocol is selected in the **List of Protocols,** and then click **Start.** In the pop-up window that appears, select either—

   - **Automatic—**Automatically calculates the optimal focus point and then focuses the capillaries.
   - **Manual—**Requires you to manually calculate the focusing value before the software can complete the Focus Capillaries protocol.

2. When instructed by the instrument displays, load the full water tank in the left side of the instrument. Then load the matrix tubes in the right side of the instrument. The instrument displays tell you that the capillaries are being filled and that capillary focusing is in progress.

   If you are using the automatic option, the instrument displays tell you that the capillary focusing is complete and show the time elapsed since completion.

   If you selected manual focus, follow the instructions in the *MegaBACE 4000 Instrument User's Guide* to complete the protocol.

Chapter 8 Monitoring and troubleshooting the system

This chapter provides guidelines for monitoring the performance of the MegaBACE 4500 system and for troubleshooting problems. The topics are—

- Monitoring the system performance (section 8.1)
- Troubleshooting a failed system test (section 8.2)
- On-screen error messages (section 8.3)
- Power and communication (section 8.4)
- Manually unlocking the electrophoresis compartment (section 8.5)
- Cathode and anode stages (section 8.6)

If you are having problems with your MegaBACE instrument, use the troubleshooting sections below to locate the description that matches your problem. If you cannot solve the problem, call MegaBACE System Technical Support for assistance. See Assistance in the preface for contact information. When calling for assistance, you will need the serial number of the MegaBACE instrument. The serial number is located on the right side of the instrument (figure 2-2).

8.1 Monitoring the system performance

As your first step in troubleshooting a problem, you should perform a MegaBACE system test run to make sure the system is performing to specification. Table 8-1 provides the workflow.

Table 8-1. MegaBACE 4500 system test workflow overview

Description	Reference
1. Prepare a sample plate of the MegaBACE M13 dye terminator standard. Make sure you follow the guidelines in the kit protocol booklet. (See the *MegaBACE 4500 Site Preparation Guide* for ordering information.)	MegaBACE M13 dye terminator standard kit protocol booklet
2. In the Instrument Control Manager, make sure you set the recommended values for the plate setup parameters and the instrument control parameters.	Sections 3.5 and 3.9
3. Perform a MegaBACE 4500 sequencing run.	Chapter 4
4. Use the MegaBACE system test in the ReadCheck Pro software to verify that the base-called run data is within specification. The MegaBACE system test measures several components, including the read length average per capillary array. The minimum read length average per capillary array is guaranteed to be within 3% of the plate average read length.	Help topics within ReadCheck Pro
5. If the run does not pass the MegaBACE system test in ReadCheck Pro— - Repeat the run using a fresh sample plate, and repeat the MegaBACE system test using ReadCheck Pro. For new capillary arrays or capillary arrays that have been stored dry, you may need to perform up to three system test runs to reach the read length specification. - Use the troubleshooting guidelines in section 8.2 to determine the problem. - If you cannot resolve the problem, contact MegaBACE System Technical Support	Section 8.2

8.2 Troubleshooting a failed system test

A run that fails to meet any one of the system specifications will fail the MegaBACE system test. If the run fails the MegaBACE system test, you should repeat the run and then repeat the MegaBACE system test using ReadCheck Pro. For new capillary arrays or capillary arrays that have been stored dry, you might need to perform up to three runs to reach the read length specification.

Table 8-2 provides additional guidelines for troubleshooting a failed MegaBACE system test. For troubleshooting the ReadCheck Pro error messages or problems with ReadCheck Pro, see the Help within the software.

If the problem persists after following the troubleshooting guidelines, contact MegaBACE System Technical Support.

Note: For additional troubleshooting guidelines, see the MegaManual.

Table 8-2. Failed MegaBACE system test results

Problem	Probable cause	Solution
Failed read length: The plate or an array failed the read length threshold.	The capillaries are new or were stored dry.	You might need to perform up to three runs to reach the read length specification.
	The capillary windows are dirty or were not properly focused for the run temperature.	Refocus the capillaries (section 7.9). If you have trouble focusing the capillaries, clean the capillary windows (section 7.6), prepare the capillaries for focusing (section 3.8), and refocus again.
	The MegaBACE long-read matrix v2 or the dye terminator standard was contaminated or was not stored properly.	Use fresh matrix and/or standard if you suspect contamination or improper storage (for example, stored at room temperature for extended periods, stored below 0 °C, or repeated freeze-thaw cycles).
	The capillary array coating has reached its performance lifetime and is degraded.	Replace the capillary arrays.
	The filters or beamsplitters are dirty.	Inspect the filters and beamsplitters and clean them if necessary. See the *MegaBACE 4000 Instrument User's Guide* for details.
	There were problems with the sample plate.	Verify the sample plate was prepared correctly and that the plate was injected only once.
Failed current: Some wells failed the Current Change or Current Quality threshold.	Dust or other impurities might be present in the capillaries or the capillaries might be clogged.	Use the Flush and Dry Capillaries protocol to flush the capillaries, but do not dry the capillaries. Reinject fresh matrix.
	Air bubbles might be present in the capillaries.	Make sure you follow the sample preparation guidelines packaged with the matrix and the standard. Degas or centrifuge reagents before starting a run.
	An electrode on the cathode side of the capillary might be bent or broken.	Check the electrodes. If necessary, replace the capillary arrays (chapter 7).
	A capillary or capillaries might be broken.	If the same capillary positions fail repeatedly, try replacing the capillary arrays (chapter 7).

Table 8-2. Failed MegaBACE system test results (continued)

Problem	Probable cause	Solution
Failed base calls: Individual capillaries have no data (signal).	The samples were not properly injected, possibly because of bubbles in a sample well.	Prepare a new sample plate, and make sure there are no bubbles in the samples. Then start a new run by injecting fresh matrix and injecting the samples.
		See Failed current.
	There was an insufficient volume of sample in the well.	Make sure that the sample volume in the well is at least 10 µl.
Failed base calls: None of the capillaries have data (signal) after the run.	The capillaries were dirty or were not properly focused prior to the start of the run.	Refocus the capillaries (section 7.9). If you have trouble focusing the capillaries, clean the capillary windows (section 7.6), prepare the capillaries for focusing (section 3.8), and refocus the capillaries again.
	The filters are not installed in the correct positions or the wrong filter set is installed in the instrument.	Check that the correct filters are installed in the correct locations. See the *MegaBACE 4000 Instrument User's Guide* for details.
	The optics are out of alignment.	Contact MegaBACE System Technical Support for assistance.
Signal-to-noise problems	The capillary windows are dirty or were not properly focused for the run temperature.	Clean the capillary windows (section 7.6), and refocus the capillaries (section 7.9).

8.3 On-screen error messages

The Instrument Control Manager displays on-screen error messages that are either hardware-related or software-related. These fall into three categories—

- Hardware-related error messages that might require a field service call (table 8-3)
- Hardware-related error messages that you can resolve (table 8-4)
- Software-related error messages that you can resolve (table 8-5)

The following tables provide solutions that you can try before calling for MegaBACE System Technical Support. Before you call, write down the error message and the condition that caused it. In addition, you will need the instrument serial number.

Table 8-3. Hardware-related error messages that might require a field service call

Error message	Solution
A current read in switch (mux) has failed. Fatal error. Blue laser power is low. Blue laser did not turn on. EPHV PCA Error. Error: Instrument is down.	Restart the system. If the error occurs again, call MegaBACE System Technical Support for assistance.

Table 8-4. Hardware-related error messages that you can resolve

Error message	Solution
Abnormal instrument status: High pressure too high or too low.	Check the high-pressure nitrogen regulator. Make sure the nitrogen pressure is set at 6.89×10^3 kPa (1 000 psi). The error message should not appear if the high pressure is within ± 25 psi.
Cathode door not closed within timeout period.	Click Retry, and then try opening and closing the drawer.
Cathode stage not in. Sensor 1 or Sensor 2.	Click Retry, and then open and fully close the cathode drawer.
Check the nitrogen pressure.	Make sure the pressure is set as follows: High pressure: 6.89×10^3 kPa (1 000 psi) Low pressure: 6.89×10^2 kPa (100 psi)
The electrophoresis compartment door is open.	Close the electrophoresis compartment lid.
No tank on cathode stage. Stage cannot be raised.	Place a tank on the stage.
The temperature is not at set point.	Close the electrophoresis compartment lid. Allow the instrument to warm up according to the warmup times (section 3.3).
Timer expired while trying to raise the anode stage.	Click Retry, and then close the anode drawer.
Won't power on. No POWER indicator light.	Check that the instrument is plugged into a power outlet. Ensure that the wall outlet has the correct voltage.
Wrong tank on cathode stage.	The container you have placed on the stage is not the one the protocol requires. Change the container and try again.

Table 8-5. Software-related error messages that you can resolve

Error message	Solution
The available free disk space (X MB) is less than the Y MB needed for the run. Are you sure you want to continue?	Stop the run and remove files from your hard disk to provide free disk space. See section 3.10 for instructions on how to change the storage location for the raw data files to a different hard drive.
Cannot delete the plate which is being used in the Instrument Control window.	The software will not allow you to delete a plate that is being run. You must wait until the run is finished or select another plate ID to delete.
You must first select a plate that has no run ID to run the samples.	If you want to rerun a plate, in the Plate Setup window, click Rerun. Alternatively, from the Plate Catalog, select a plate ID that has no run ID. Then click the Instrument Control window and start the Inject Samples and Run protocol.

8.4 Power and communication

Table 8-6 list some common problems and solutions you can try before calling MegaBACE System Technical Support for assistance.

Table 8-6. Troubleshooting power and communication problems

Problem	Solution
The power light on the instrument will not turn on.	Check that the instrument is turned on and that the power cord is plugged in. The wall outlet might be faulty. Test the outlet or try another one.
The displays on the front of the instrument will not turn on.	Check that the instrument is turned on and that the power cord is plugged in. The wall outlet might be faulty. Test the outlet or try another outlet.
The computer is not communicating with the MegaBACE instrument.	1. Turn off the instrument and shut down the computer. 2. Check all Ethernet™ cables and connections. 3. The wall outlet might be faulty. Test the outlet or try another outlet that supplies the same voltage. 4. Turn on the instrument and turn on the computer. 5. Start the Host Scan Controller software, and then start the Instrument Control Manager.
The power has gone off, and you cannot open the lid of the electrophoresis compartment.	During power outages, you can unlock the lid manually (section 8.5).

8.5 Manually unlocking the electrophoresis compartment

Generally, to unlock the lid of the electrophoresis compartment, you select the Replace Capillaries protocol, which unlocks the lid. During power outages, you can unlock the lid using the following procedure:

1. Remove the plastic plug that is inside the unlocking hole on the left side of the electrophoresis compartment lid.
2. Insert an unlocking tool, such as a hex wrench or screwdriver, to puncture the foam in the access hole (figure 8-1).

The tool hole is recessed approximately 4 cm (1.5 in). If necessary, look through the access hole to guide the tool through the foam. Slide the tool back and forth until you reach the tool hole.

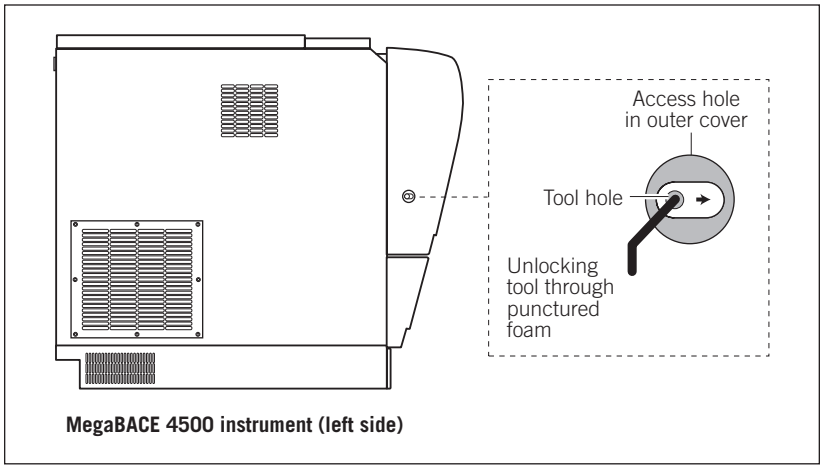

Figure 8-1. The unlocking tool and the access hole on the left side of the electrophoresis compartment lid.

3. Grasp the unlocking tool firmly and move the tool hole along the open slot, toward the front of the lid. Then pull the lid forward slightly. After the lid lock releases, you can remove the tool and open the lid all the way.

8.6 Cathode and anode stages

Table 8-7 lists problems that you might encounter with the cathode and anode stages.

Table 8-7. Troubleshooting problems with the cathode and anode stages

Problem	Solution
The cathode or anode stage will not move up or down.	A variety of factors might cause the cathode or anode stages not to move up or down. Call MegaBACE System Technical Support for assistance.
The cathode drawer does not open and close easily.	Make sure you have the correct plate type for the instrument, and use a plate adapter. See the *MegaBACE 4500 Site Preparation Guide* for a list of qualified plates. Using the wrong plate type can damage the instrument. The cathode slider might be dirty. Clean the cathode plate holder and slider. See the *MegaBACE 4000 Instrument User's Guide* for the procedure.

Index

C

D

E

F

H

I

L

M

U

V

W