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user manual

MegaBACE

Instrument Maintenance and Troubleshooting Guide
Version 2.4

um 375-702

Amersham Biosciences

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

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

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

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Preface

About this guide

The *MegaBACE Instrument Maintenance and Troubleshooting Guide* provides instructions on maintaining the MegaBACE™ instrument and information on troubleshooting. It also describes how to replace and focus the capillary arrays.

Related publications

In addition to the *MegaBACE Instrument Maintenance and Troubleshooting Guide*, the following publications are available for the MegaBACE system:

- *MegaBACE Instrument Operator's Guide* describes how to use the MegaBACE DNA Analysis System to automatically set up plate definitions and perform runs.
- *MegaBACE Instrument Administrator's Guide* provides information on how the instrument works and how to manually set up plate definitions, create plate setup and instrument parameter templates, and use the configuration files.
- *MegaBACE Planning Guide* provides instructions for setting up the installation site for the MegaBACE instrument. Reading the planning guide is a prerequisite for the installation of the MegaBACE system.
- MegaBACE analysis software user's guides provide instructions on how to use the software to analyze the data collected from the MegaBACE instrument. **Note**: The *MegaBACE Genetic Profiler User's Guide* also contains troubleshooting guidelines.
- The MegaManual provides detailed troubleshooting guidelines for the sequencing application. The MegaManual is posted in the MegaBACE User Zone at the following Web site:

  www.amershambiosciences.com

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

Electronic versions of most of the documents listed above are available on the software CD provided with your MebaBACE system.

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Safety

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

Special safety text

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

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

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

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

Note: Identifies items of general interest.

Trained operator

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

Assumptions

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

Safety standards

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

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MegaBACE system site requirements

Electrical requirements

MegaBACE instrument

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

Power supply fan module

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

Environmental conditions

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

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Assistance

When calling for assistance, be prepared to supply the serial number of your instrument. The serial number is located on the lower right side of the MegaBACE instrument (figure 2-2). For contact by phone or fax, please use one of the numbers below.

Asia Pacific
Tel: +852 2811 8693
Fax: +852 2811 5251

Australasia
Tel: +61 2 9899 0999
Fax: +61 2 9899 7511

Austria
Tel: 01 576 0616 22
Fax: 01 576 0616 27

Belgium
Tel: 0800 73 888
Fax: 03 272 1637

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

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

Denmark
Tel: 45 16 2400
Fax: 45 16 2424

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

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

Germany
Tel: 0761 4903 291
Fax: 0761 4903 405

Italy
Tel: 02 27322 1
Fax: 02 27302 212

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

Latin America
Tel: +55 11 3667 5700
Fax: +55 11 3667 87 99

Middle East and Africa
Tel: +30 (1) 96 00 687
Fax: +30 (1) 96 00 693

Netherlands
Tel: 0165 580 410
Fax: 0165 580 401

Norway
Tel: 2318 5800
Fax: 2318 6800

Portugal
Tel: 21 417 70 35
Fax: 21 417 31 84

Russia & other C.I.S. & N.I.S.
Tel: +7 (095) 232 0250, 956 1137
Fax: +7 (095) 230 6377

Southeast Asia
Tel: +60 3 8024 2080
Fax: +60 3 8024 2090

Spain
Tel: 93 594 49 50
Fax: 93 594 49 55

Sweden
Tel: 018 612 1900
Fax: 018 612 1910

Switzerland
Tel: 01 802 81 50
Fax: 01 802 81 51

UK
Tel: 0800 616928
Fax: 0800 616927

USA
Tel: +1 800 526 3593
Fax: +1 877 295 8102

Web site

http://www.amershambiosciences.com

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Chapter 1 Introduction to the MegaBACE system

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

This chapter describes—

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

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

Table 1-1. Available models of the MegaBACE instrument

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

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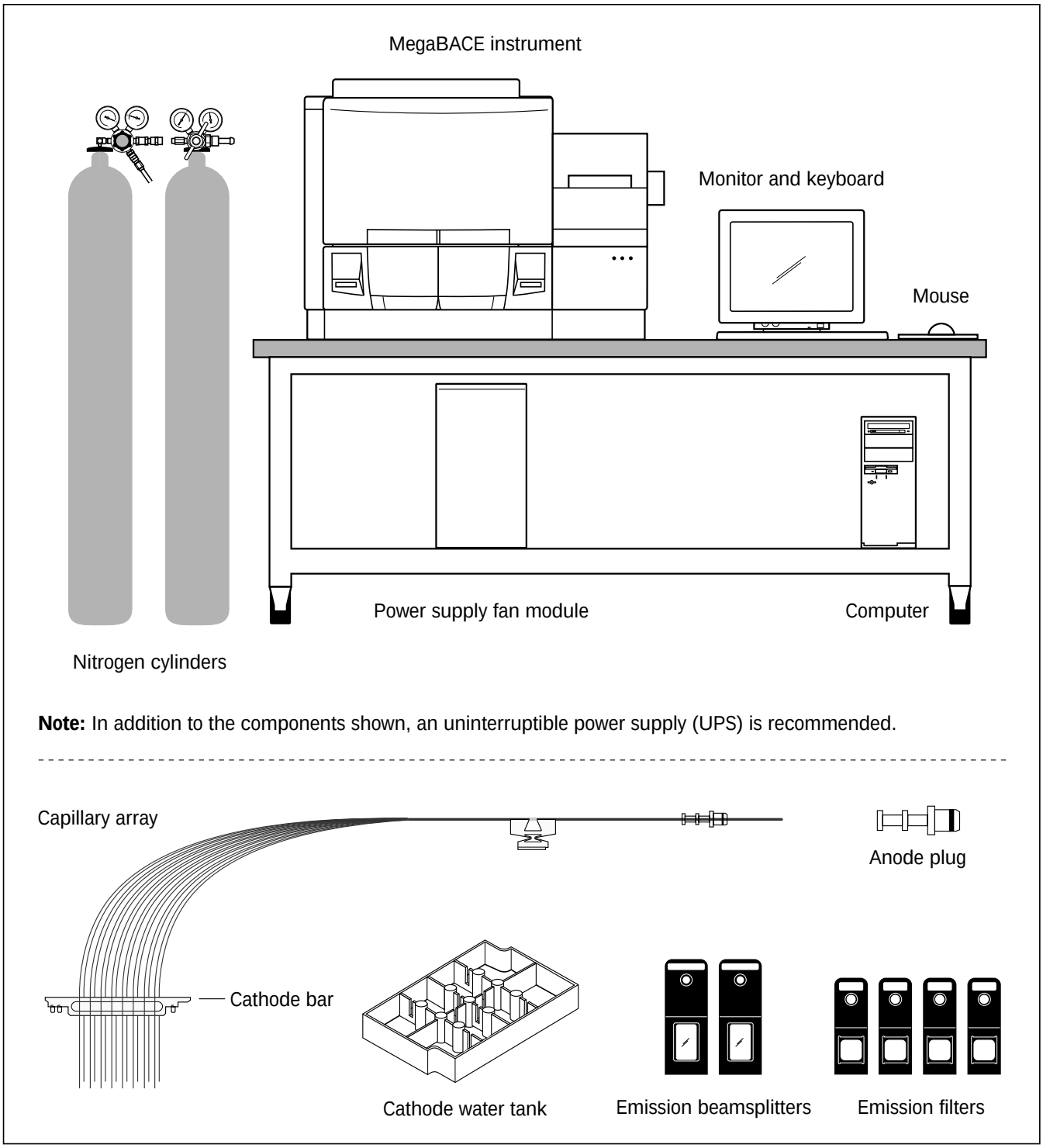
1.1 System hardware components

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

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

The system uses a **nitrogen pressure source** (cylinder with regulators or multiunit manifold). For more information on the nitrogen pressure source, see chapter 3.

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

Figure 1-1. The MegaBACE system and components.

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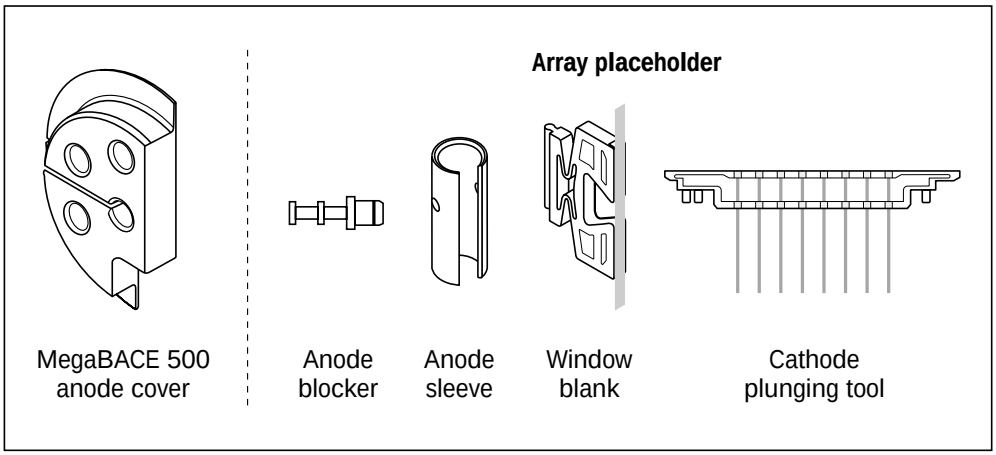


Figure 1-2. Additional components (MegaBACE 500 and flexible MegaBACE 1000 instruments only).

1.2 System functions

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

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

In addition, the MegaBACE software allows you to—

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

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

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1.3 Overview of instrument operation

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

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

1.4 Introduction to the Instrument Control Manager software

This section introduces the Instrument Control Manager software, which automates the process of plate setup, data collection, and data analysis.

The Instrument Control Manager allows you to—

- Set up plate definitions and import plate setup parameters and sample names automatically (instrument operator's guide).
- Collect data.
- (Sequencing only) Automatically call bases and export the data to other file formats after base calling (instrument operator's guide).
- Use the Replace Capillaries protocol to replace the capillary arrays when capillaries become clogged or broken (chapter 4).
- Use the Focus Capillaries protocol to focus the capillaries (chapter 4).

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1.5 Before you begin

Before performing any of the maintenance tasks on the MegaBACE instrument, become familiar with—

- Chapter 2: Safety precautions
- Chapter 3: Maintaining the instrument
- Chapter 4: Replacing the capillary arrays

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Chapter 2 Safety precautions

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

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

2.1 General safety precautions

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

Warnings



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

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

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

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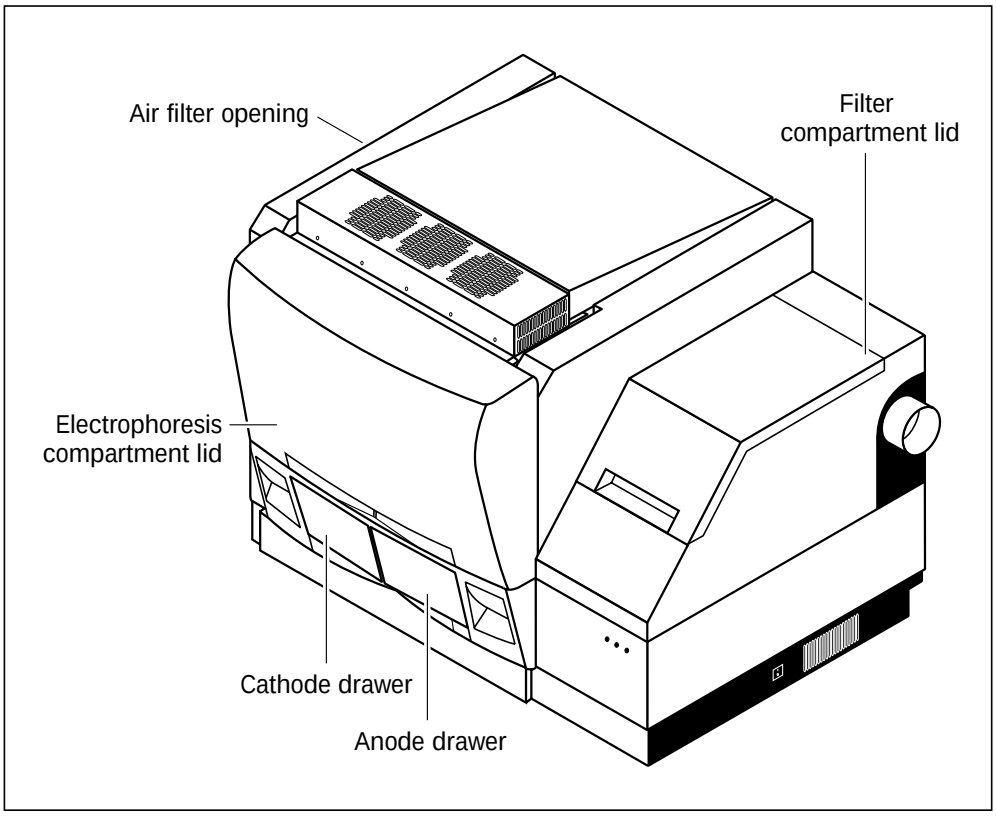


Figure 2-1. Locations of the drawers and lids used during routine operation of the MegaBACE system. The air filter opening is used infrequently.

Warnings

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

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

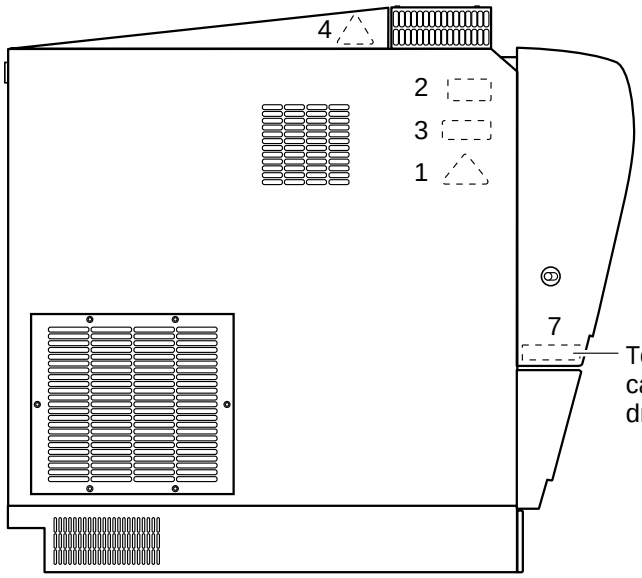
Do not attempt to lift the instrument. The MegaBACE instrument weighs approximately 272 kg (600 lb). Lifting the instrument can cause severe or fatal injury.

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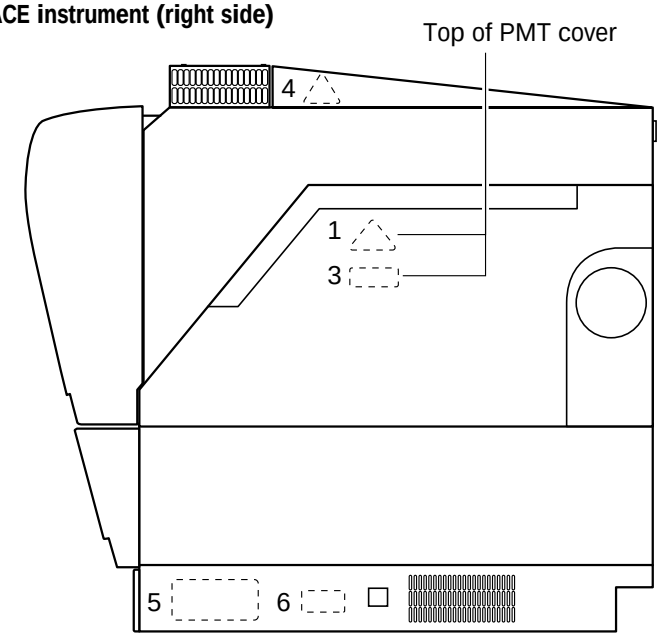
2.2 Locations of important labels

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

MegaBACE instrument (left side)



MegaBACE instrument (right side)



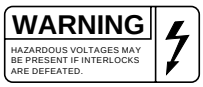
1. Laser light warning



2. Interlock defeat danger



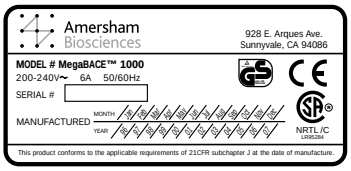
3. High-voltage warning



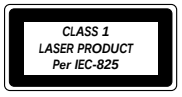
4. Pinching hazard



5. Serial number certification



6. Class 1 Laser product



7. Liquid spillage caution

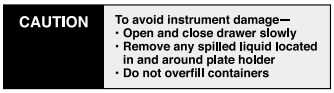
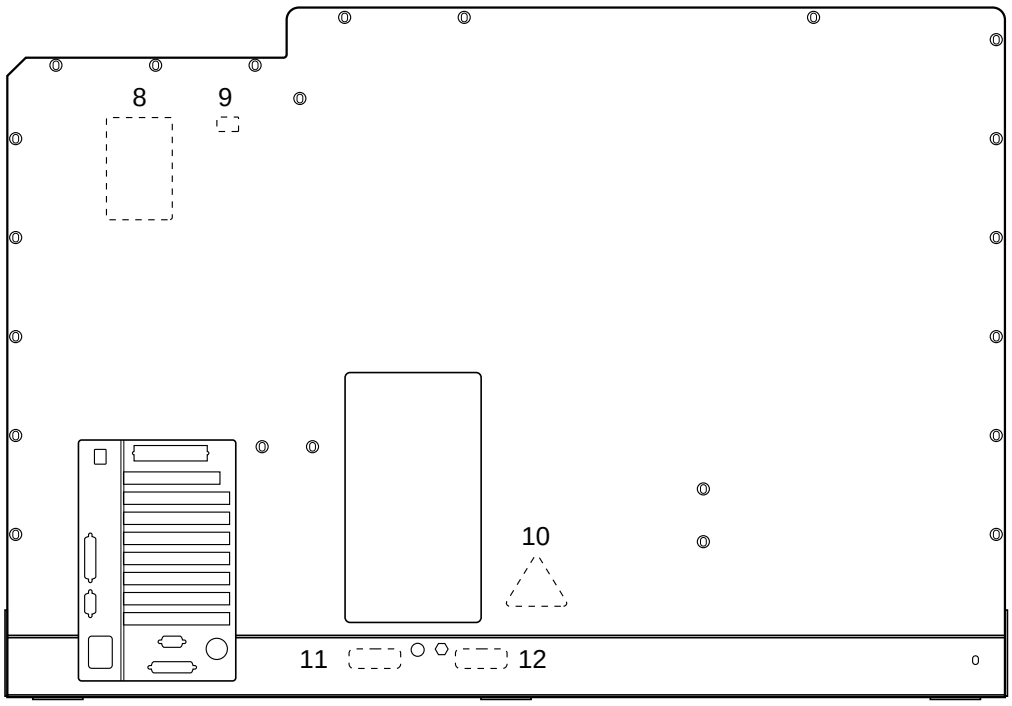


Figure 2-2. Locations of important labels on the MegaBACE instrument (side views).

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MegaBACE instrument (back view)

8. License agreement

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

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

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

AMERSHAM IS A LICENSED VENDOR FOR AUTHORIZED REAGENTS

9. Made in U.S.A.

10. Nitrogen pressure general hazard

11. Low-pressure nitrogen

100 PSI
(6.89 X 10^2 kPa)

12. High-pressure nitrogen

1000 PSI
(6.89 X 10^3 kPa)

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

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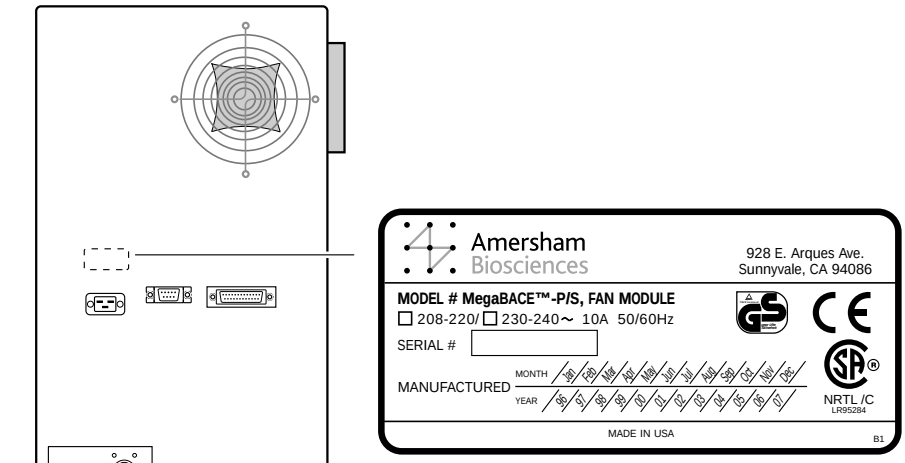


Figure 2-4. Location of the serial number certification label on the power supply fan module.

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

2.3 Cathode and anode compartments and instrument displays

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

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

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

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

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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 displayed on the instrument display. The drawer locks, and the system raises the stage. The system automatically moves to the next step.



Figure 2-5. The liquid spillage caution.

2.4 Electrophoresis compartment

You may occasionally need to open the electrophoresis compartment lid.

Warning ⚠

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

Achtung

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

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



Figure 2-6. The pinching hazard label.

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



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

Warnings

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

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

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

Cautions

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

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

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

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

Note: The capillaries become warm during electrophoresis.

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

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

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

2.5 Filter compartment

To make sure data is recorded properly, you should check that the appropriate filters and beamsplitters are installed before starting an electrophoresis run. (For details on changing filters and beamsplitters, see section 3.4.3.)

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

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

2.6 Internal electronics

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

Warning 

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

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Note: You can, however, remove the air filter panel on the left side of the instrument to clean the air filter (section 3.5.2).

2.7 Chemicals

Warning

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

2.8 Nitrogen cylinders and pressure regulators

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

2.8.1 Handling high-pressure cylinders and tubing

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

Warning

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

Achtung

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

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



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

Warning

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

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2.8.2 Regulating the nitrogen pressure

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

Warnings

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

The nitrogen pressure in the high-pressure line must not exceed 6.89×10^3 kPa (1 000 psi) of pressure. Never apply high pressure to the low-pressure line. This can damage the instrument or the low-pressure line and can cause injury.

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

Warning

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

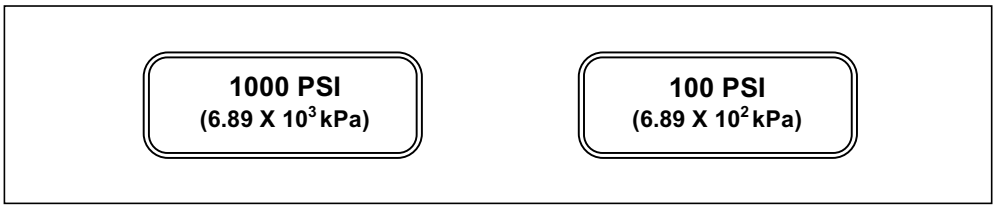


Figure 2-9. The high- and low-pressure nitrogen labels.

2.9 Lasers

Warning

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

2.9.1 Class 1 Laser Product label

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

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

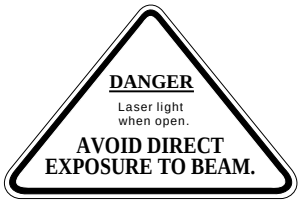
2.9.2 Laser light warning label

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

Warning

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

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



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

Figure 2-11. The laser light warning label.

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2.9.3 Safety interlock danger label

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

Warning

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

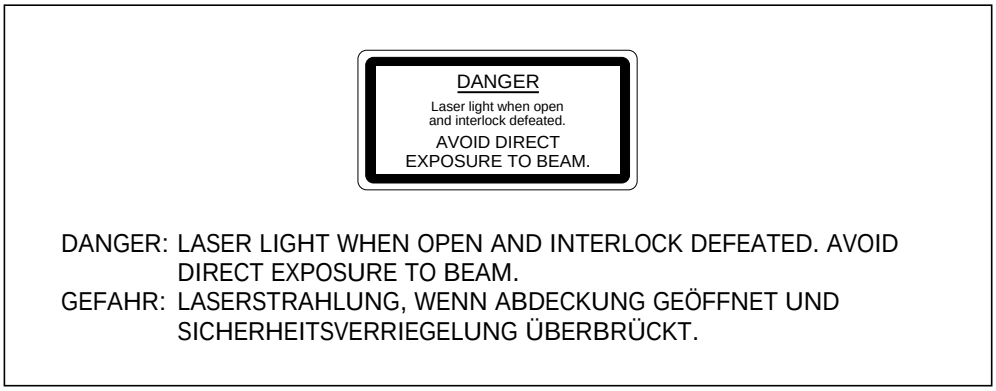


Figure 2-12. The interlock defeat danger label.

2.9.4 Light leaks

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

Caution

Ambient light can damage electrical components in the MegaBACE instrument, such as the PMT. 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.

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2.11 Power supply fan module, computer, and monitor

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

Warning

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

2.12 System electrical connections

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

Important

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

Warning

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

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

Cautions

In the event of a power failure (see the *MegaBACE Instrument Operator's Guide*), 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.

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2.13 Serial number labels

2.13.1 Instrument serial number label

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

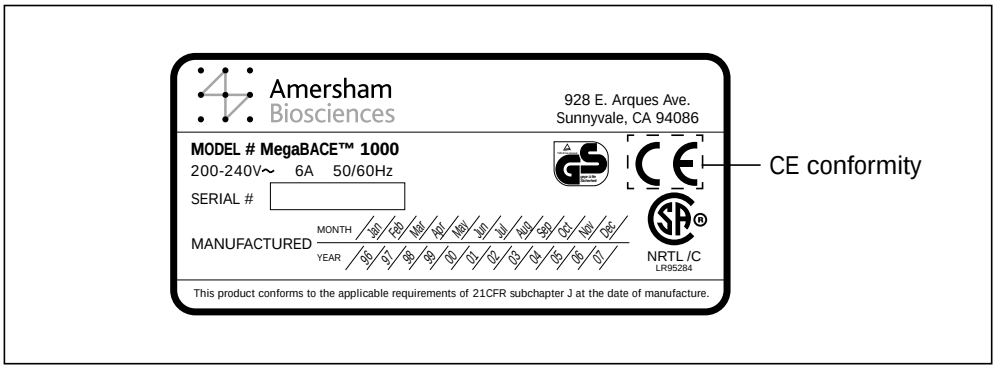


Figure 2-13. The MegaBACE instrument serial number certification label.

2.13.2 Power supply fan module serial number label

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

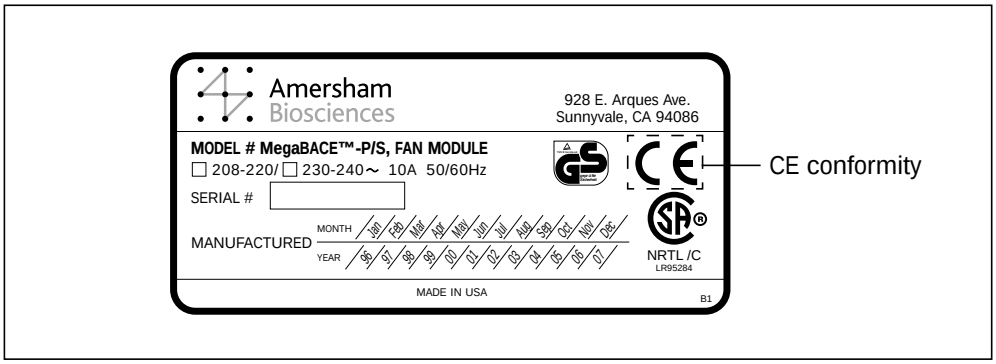


Figure 2-14. The power supply fan module serial number certification label.

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2.14 Service for the MegaBACE instrument

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

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

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Chapter 3 Maintaining the instrument

This chapter provides information on routine maintenance tasks and on protecting the MegaBACE instrument from damage.

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

The topics in this chapter are—

- Replacing nitrogen cylinders and setting the pressure regulators (section 3.1)
- Using the cathode and anode drawers (section 3.2)
- Cleaning the cathode plate holder and slider (section 3.3)
- Caring for the emission beamsplitters and filters (section 3.4)
- Maintaining the cooling system (section 3.5)

In addition, for information on—

- Safety, see chapter 2.
- Replacing capillary arrays or increasing and decreasing the number of capillary arrays, see chapter 4.
- Replacing power cords or cables or moving the instrument to a new location, see chapter 5 and the *MegaBACE Planning Guide*, and then contact MegaBACE System Technical Support.

3.1 Replacing nitrogen cylinders and setting the pressure regulators

You can use a centralized nitrogen source or provide a separate nitrogen cylinder for each instrument. This section describes the requirements for locally installed nitrogen cylinders, but the principles apply similarly to remote sources. You should use 5.0 ultrahigh-grade nitrogen. (See the *MegaBACE Planning Guide* for nitrogen specifications.)

Warning 

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

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3.1.1 Two possible regulator configurations

Depending on the needs of your lab, you may have either a single nitrogen source for both high and low pressure or a separate source for each. Both configurations supply—

- 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)

Warnings



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

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

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

3.1.2 Requirements for the high-pressure nitrogen system

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

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

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3.1.3 Requirements for the low-pressure nitrogen system

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

- **Standard low-pressure input—**6.89×10^2 kPa (100 psi)
- **Pressure sensing—**The instrument is equipped with a pressure switch to indicate low pressure.

  The pressure switch monitors low pressure in the range of 5.65×10^2 kPa to 6.07×10^2 kPa (82–88 psi) if the pressure falls below 6.21×10^2 kPa (90 psi). The pressure must be set above 6.07×10^2 kPa (88 psi) to satisfy the pressure switch, or the system will identify a low-pressure condition.
- **Pneumatic module pressure regulation—**12 psi
- **Volume used (estimated):**
  - **Single run—**0.013 m^3 (0.046 ft^3)
  - **Five runs per day—**0.065 m^3 (2.3 ft^3)

3.1.4 Checking the available nitrogen pressure

Check the available nitrogen pressure on a regular basis. You will need to determine when to replace the cylinders to prevent pressure loss during a run.

3.1.5 Replacing a nitrogen cylinder

You can replace a nitrogen cylinder when the instrument is off or between protocols when the instrument is on. To replace the cylinder, make sure you follow your local safety code requirements for the placement and mounting of the cylinder.

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

After you install the new cylinder—

- Make sure the valve area and the passageway are free of dust or dirt.
- Make sure you check the new connections for leaks.
- Set the gauges to the proper pressure:
  - High pressure: 6.89×10^3 kPa (1 000 psi)
  - Low pressure: 6.89×10^2 kPa (100 psi)

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Important **The instrument is equipped with a pressure switch to indicate low pressure. The pressure switch monitors low pressure in the range of 5.65×10^2 kPa to 6.07×10^2 kPa (82–88 psi) if the pressure falls below 6.07×10^2 kPa (88 psi). The pressure must be set above 6.07×10^2 kPa (88 psi) to satisfy the pressure switch, or the system will identify a low-pressure condition.**

3.2 Using the cathode and anode drawers

The cathode (left) and anode (right) drawers unlock during specific steps in the instrument workflow. 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 materials on the stage.

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.**

Each display on the instrument provides instructions for the adjacent drawer. When you are instructed to add a plate, water tank, or tubes to the cathode or anode side—

1. Grasp the bottom of the drawer and gently pull toward you horizontally. Pull the drawer out until it stops, so that you have access to the plate holder or reservoir holder that rests on the sliders.

Caution **Use care when you pull the drawer out so that you do not twist or bend the sliders or spill water on the cathode stage, which can cause damage to the electronics.**

2. Remove the plate, water tank, or tubes currently in position.
3. Place the new plate, water tank, or tubes in position.
4. Gently slide the drawer in until it stops. The drawer locks, the stage rises, and the software proceeds to the next step. The following message appears on the instrument display: **Thank you. Please wait.**

   If you follow the instructions but the displayed message does not change to the next step, carefully open and reclose the drawer.

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3.3 Cleaning the cathode plate holder and slider

When you replace the capillaries, you should inspect the cathode plate holder and slider to determine if they need cleaning.

To clean the cathode plate holder and slider, you remove the slider from the left side of the instrument.

3.3.1 Removing the cathode slider from the left side of the instrument

To remove the cathode slider—

1. Pull the left drawer all the way open and remove the plate or tray.
2. Using a screwdriver, reach under the slider and pull down on the ring hanging below the stage (figure 3-1). The ring is approximately 10 cm (4 inches) from the drawer.

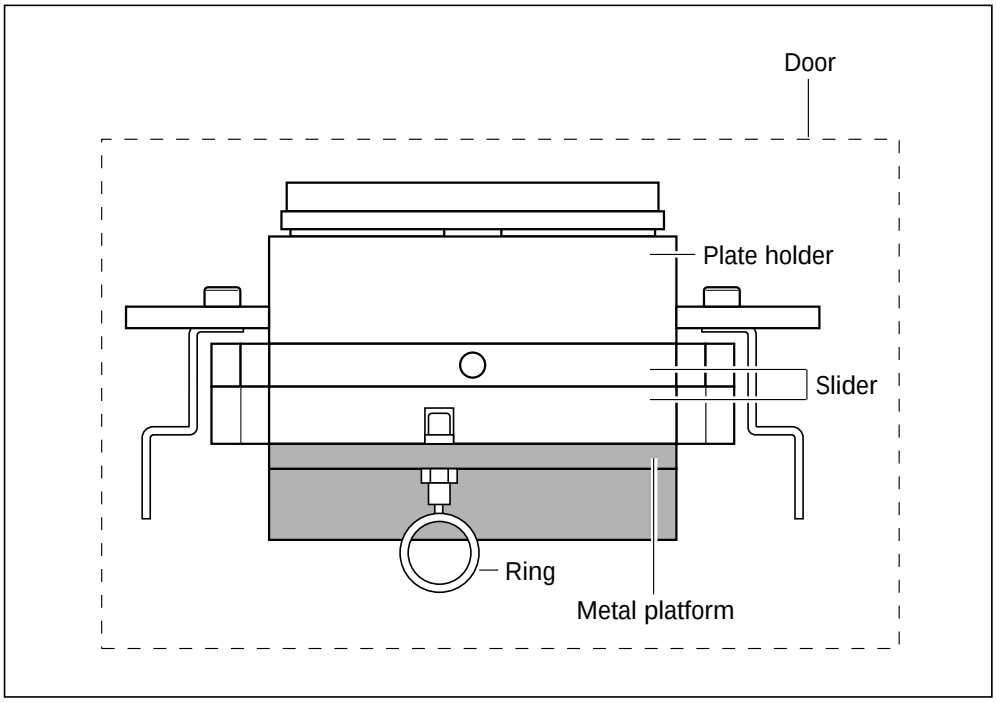


Figure 3-1. The cathode slider, front view.

3. While holding the ring down, grasp the center of the slider and pull the slider out until it stops. Then lift up to separate the front end of the slider from the platform.

Caution **Do not raise the slider too high. Raising the slider too high can damage the electrodes.**

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The slider is now tilted slightly from front to back. Release the ring.

Note: If you cannot lift the front end of the slider, pull the slider farther out and try again.

Caution **Protect the attachment between the drawer and the slider from damage. Do not hold onto the drawer or press on the drawer while removing the slider from the instrument.**

4. Using both hands, lift up on the slider so that the back of the slider releases and the slider is horizontal. Then continue to support the slider and slowly pull it out of the instrument.
5. To separate the two pieces of the slider—
   - Place the slider on the lab bench with the drawer hanging over the side of the bench.
   - Pull the ring on the upper side of the slider and at the same time slide the upper and lower sliders to lengthen the slider assembly to its full extension.
   - Then lift the upper slider off the lower slider (figure 3-2).
6. Using a clean cloth moistened with deionized filtered water, clean the plate holder and slider.

Important **Do not use any other solvents. Make sure that all parts are dry before reassembling the sliders as described in the next section.**

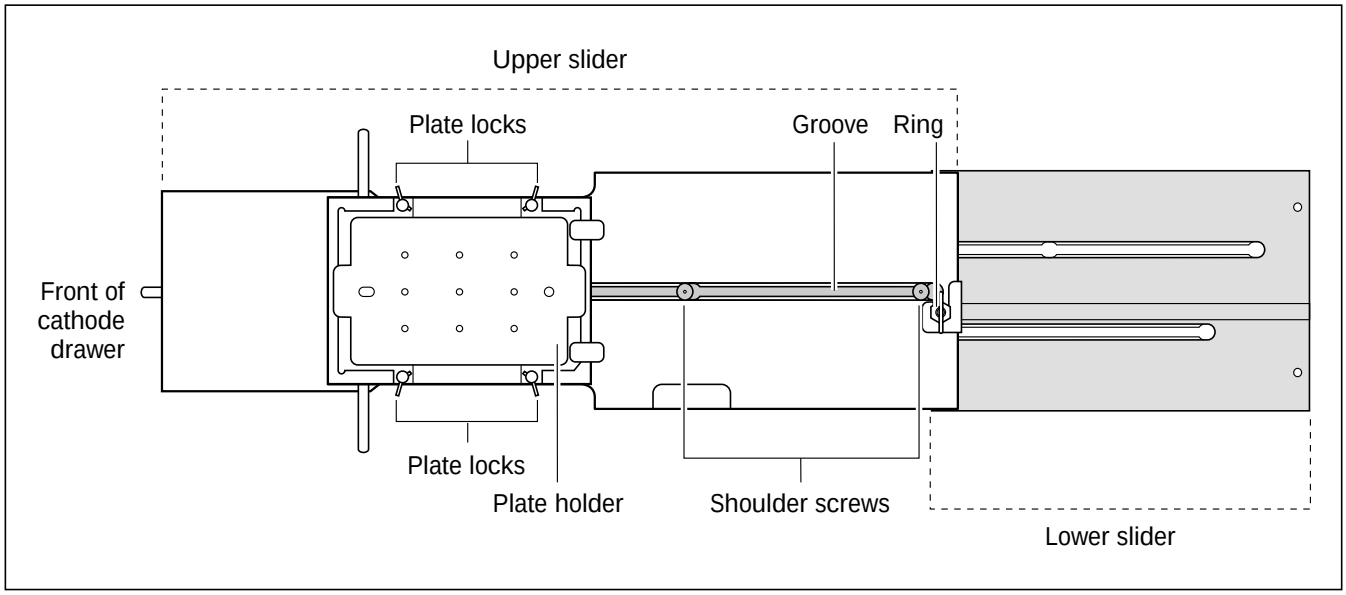


Figure 3-2. The upper and lower cathode sliders for the cathode assembly, top view.

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3.3.2 Reinstalling the cathode slider in the left side of the instrument

Caution **To prevent damage to the capillary and electrode tips, keep the lower slider below the level of the guide rails (figure 3-3).**

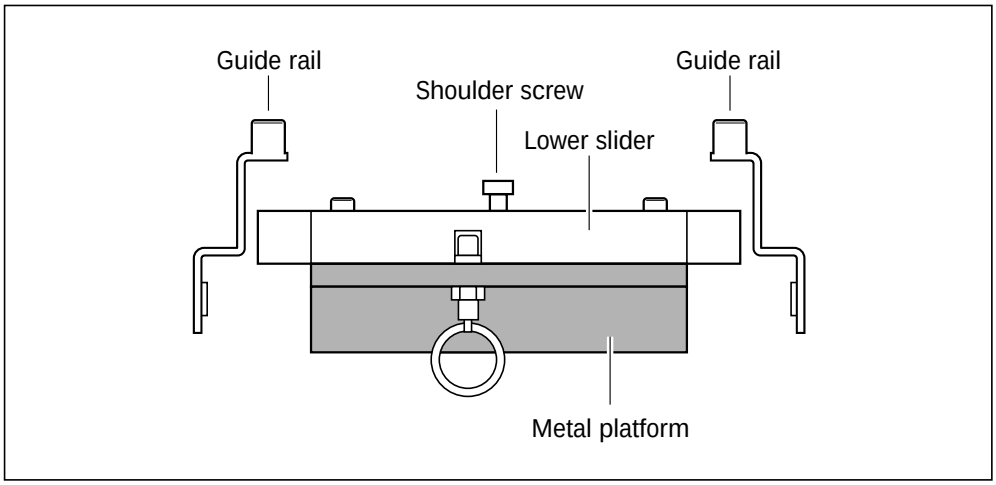


Figure 3-3. The support platform for the lower cathode slider. The platform has three shoulder screws that fit into the section to align and secure the platform.

Reinstalling the cathode slider requires installing the lower slider first and then installing the upper slider.

Installing the lower slider

To install the lower slider—

1. Hold the lower slider (the piece without the plate holder, figure 3-2) so that the shoulder screws face up and the wide end is toward the instrument.
2. Place the slider between the guide rails and metal platform (figure 3-3) and slide it in until the three wide slots (screw holes) in the two long slits in the slider align with the corresponding three shoulder screws in the metal platform.

   Note: If you have difficulty aligning the three shoulder screws, look into the cathode drawer opening and make sure that the metal platform is straight and not twisted to the side.
3. Lower the slider into position on the shoulder screws and then push the section in (approximately 0.5 cm, 0.25 inch) until you hear a click. The lower slider is locked in place.
4. Pull the slider out as far as it will go to make installation of the upper slider easier.

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Installing the upper slider

To install the upper slider—

1. Hold the slider so that the plate holder faces up and the drawer is toward you.
2. Position the screw holes in the upper slider over the shoulder screws of the lower slider.
3. Push the two sliders together and hold the lower slider to keep it from moving. At the same time, slide the upper slider forward over the lower slider until you hear a click. The two pieces are locked together.
4. Slide the drawer closed.

Caution **Always leave the drawer closed to keep dust out of the interior of the instrument.**

3.4 Caring for the emission beamsplitters and filters

This section describes the optical filter compartment and how to handle, store, and clean the emission beamsplitters and filters. For additional information about emission beamsplitters and filters used in the MegaBACE instrument, see the *MegaBACE Instrument Administrator's Guide*. For step-by-step instructions on loading beamsplitters and filters, see section 3.4.3.

3.4.1 About the filter compartment and PMTs

Caution **Opening the filter compartment lid during an electrophoresis run interrupts data recording. If you need to open the lid during a run, stop the run first to protect the data you have already collected.**

Open the lid of the filter compartment only when the instrument is idle (has completed a protocol).

Caution **Opening the filter lid breaks a safety interlock. The instrument blocks the laser light and turns off and prevents further application of high voltage to the capillaries and the PMTs.**

Warning ⚠ **The instrument has interlocks that protect you from moving parts, high pressure, hazardous voltage, and laser light. Do not defeat the safety interlocks. You may be exposed to injury from the moving beamsplitter and filter holders and from laser light. The PMTs may be damaged if exposed to external light while connected to the high voltage.**

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3.4.2 Caring for the beamsplitters and filters

Cautions

Handle the emission beamsplitters and filters by the frame only. Do not touch the glass (figure 3-4).

Always wear powder-free, washed gloves. Powdered gloves can leave residue on the lenses, and the powder used in the gloves can fluoresce, causing unreliable data collection.

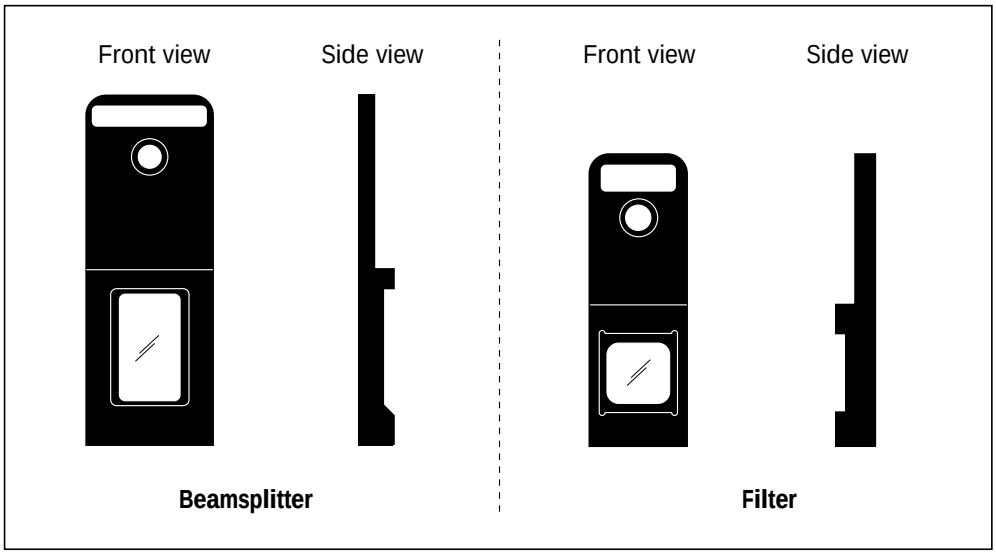


Figure 3-4. Emission beamsplitter and filter.

Cautions

To keep the glass clean and free from damage, protect your unused beamsplitters and filters by storing them in a clean, dry, lint-free box.

Fingerprints, oil, or water spots should be cleaned only by Amersham Biosciences service personnel. See Assistance in the preface for contact information.

Clean the surface of the beamsplitters and filters only when necessary.

Static electricity can bind dust very tightly to the glass so that special cleaning is needed. If you need to clean a beamsplitter or filter—

1. Use puffs of filtered dry nitrogen blown through an antistatic nozzle of a dust-free blower to remove most of the dust contamination. Alternatively, you can use a very clean bulb blower. (Do not use house compressed air, which can contain oil droplets.)
2. Remove any remaining particles with a soft cloth using light pressure.

Important

If a cleaned beamsplitter or filter produces a sample signal that is lower or a background signal that is higher than expected, clean the glass again. Take more time and be very careful to remove all surface-adhering contaminants.

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3.4.3 Changing the beamsplitters and filters

Important

Do not open the filter compartment lid or insert a filter during a scan. The light from the open lid can cause errors in your data and damage the PMT.

You may occasionally need to change emission beamsplitters or filters, or check that the appropriate ones are loaded. You should do this before starting an electrophoresis run.

To remove a filter or beamsplitter—

1. Open the filter compartment lid. Figure 3-5 shows the locations of the filters and beamsplitters.
2. Grasp the handle of the filter or beamsplitter and press back slightly. The filter or beamsplitter is spring-loaded, and pressing back releases it from the holder.
3. Pull upward to remove the filter or beamsplitter from the slot.

To insert a filter or beamsplitter—

1. Holding the new filter or beamsplitter by the handle (the labeled tab), position it in the slot and slide it downward.
2. Pull forward on the filter or beamsplitter slightly to seat it in the filter holder.

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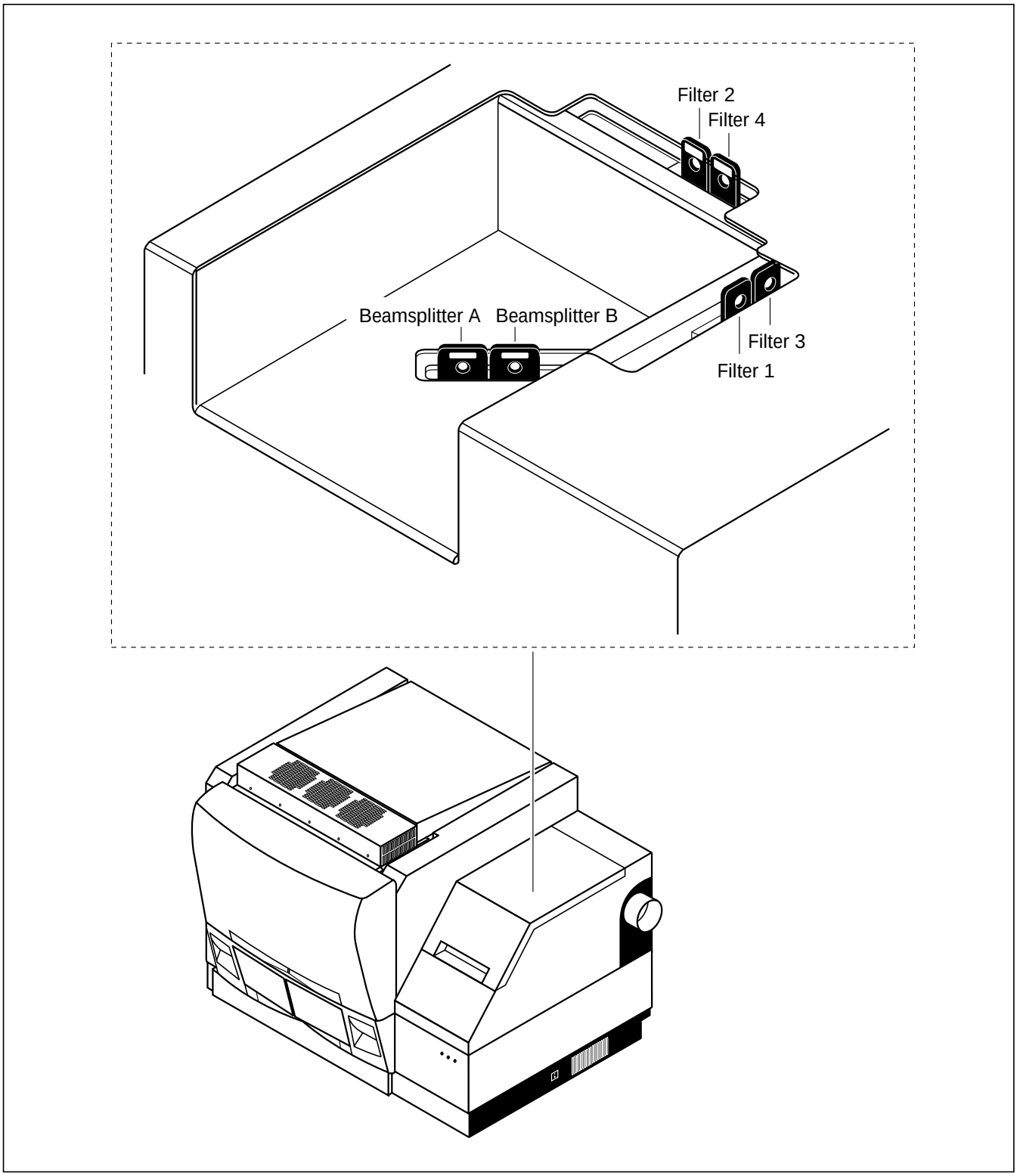


Figure 3-5. Locations of the filters and beamsplitters in the filter compartment.

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3.5 Maintaining the cooling system

This section provides information on the air vent locations and how to clean the air filter on the MegaBACE instrument.

3.5.1 Air vents on the MegaBACE instrument and the power supply fan module

Air vents for cooling are on the top and sides of the MegaBACE instrument and on the back of the power supply fan module (figure 3-6).

Caution **Do not place anything on top of the MegaBACE 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.**

To protect the blue laser, check that the cooling air hoses are firmly attached and free of holes. Make sure that the air vent on the back of the power supply fan module is free of obstruction.

If the laser ventilation fails, a thermal switch shuts down the blue laser before damage to the laser occurs.

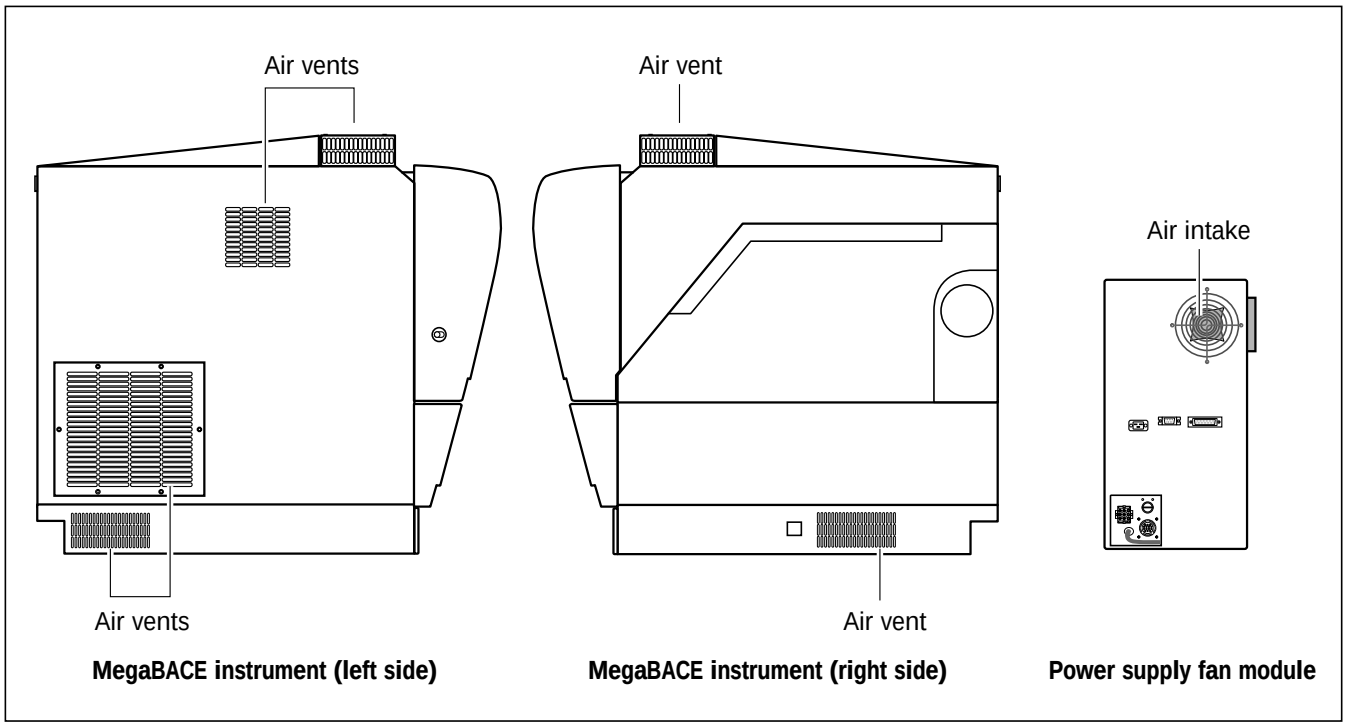


Figure 3-6. Air vents on the MegaBACE instrument and the power supply fan module.

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3.5.2 Cleaning the air filter in the MegaBACE instrument

You should inspect the air filter on the left side of the MegaBACE instrument to determine if the filter needs cleaning. The air filter needs to be cleaned periodically to keep dust out of the optics and to maintain an adequate cooling air flow.

Caution **If the air filter becomes frayed, clogged, or otherwise damaged, call MegaBACE System Technical Support to order a replacement part. See Assistance in the preface for contact information.**

To clean the air filter—

1. Turn off the MegaBACE instrument using the power switch on the right side of the instrument. It is not necessary to close the Instrument Control Manager and turn off the computer.
2. On the left side of the MegaBACE instrument, use a Phillips screwdriver to turn the six screws on the filter plate one-quarter turn counterclockwise (figure 3-7) and remove the filter plate.

Important **When you remove the filter, the MegaBACE instrument fuses are exposed. Do not change any of the fuses. If you need to replace a fuse, call MegaBACE System Technical Support. See Assistance in the preface for contact information.**

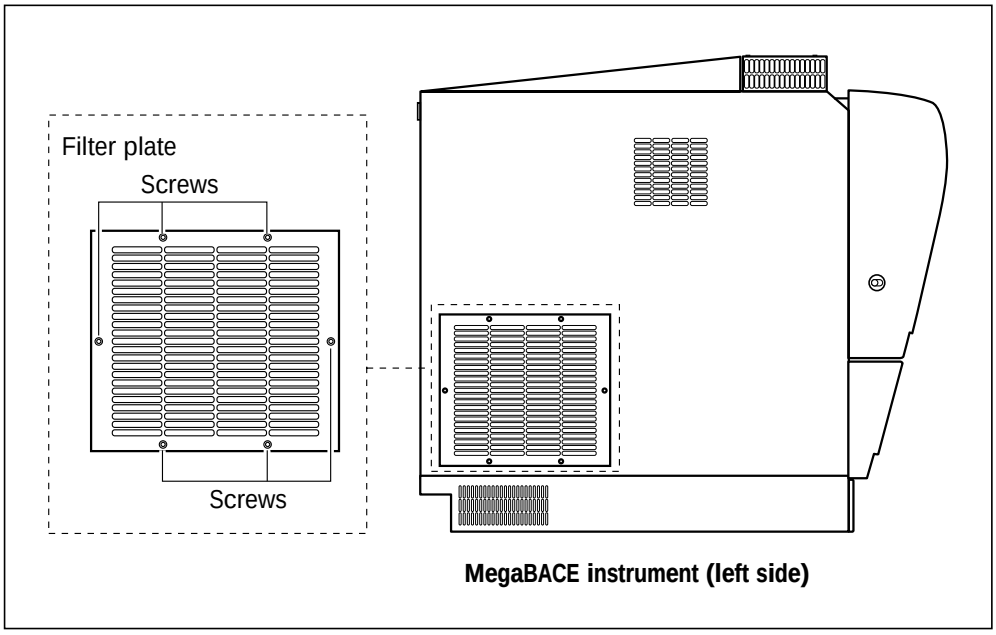


Figure 3-7. Air filter on the left side of the MegaBACE instrument.

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3. Remove the filter and rinse it in cold running water to remove accumulated dust particles.

   Note: If necessary, wash the filter with soapy water and then rinse thoroughly with clean running water.

4. Allow the filter to air dry. Make sure that the filter is completely dry before proceeding.

5. Place the filter in position on the left side of the MegaBACE instrument and then replace the ribbed metal filter plate.

6. Tighten each of the six attached screws by one-quarter turn clockwise to secure the filter plate in position.

7. Turn on the instrument.

   Note: If you turned off the computer, you must first turn on the instrument and then turn on the computer.

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Chapter 4 Replacing the capillary arrays

This chapter describes how to replace the capillary arrays. This chapter also describes how to install the array placeholders for empty array positions in the instruments with fewer than six capillary arrays. The topics are—

- About changing the number of arrays (MegaBACE 500 and flexible MegaBACE 1000 instruments only) (section 4.1)
- Locating the capillary arrays (section 4.2)
- Using the Replace Capillaries protocol (section 4.3)
- Releasing the capillary array locks (section 4.4)
- Removing the capillary arrays (section 4.5)
- Cleaning the capillary windows (section 4.6)
- Installing the new capillary arrays (section 4.7)
- Increasing or decreasing the number of capillary arrays (section 4.8)
- Locking the capillary arrays in position (section 4.9)
- Focusing the capillaries (section 4.10)

4.1 About changing the number of arrays (MegaBACE 500 and flexible MegaBACE 1000 instruments only)

You can change the number of capillary arrays installed in the instrument. Depending on your instrument model, you can have—

- **MegaBACE 500 instrument—**1, 2, or 3 arrays.
- **Flexible MegaBACE 1000 instrument—**1, 2, 3, 4, 5, or 6 arrays.

An array placeholder is used for each array position that does not contain capillaries (empty array position). An array placeholder (figure 4-1) consists of a—

- **Window blank—**Installed in the window platform in place of the missing capillary window. For proper operation, the instrument optical system requires a window blank or a capillary window at each of the six array positions in the window platform.
- **Anode blocker—**Installed in place of the capillary anode plug in the anode cover.

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- **Anode sleeve—**Installed in place of a tube in the anode reservoir to prevent you from loading a water tube or matrix tube for this array position.
- **Cathode plunging tool—**Installed in the cathode array stand to fill the wells in the plate for the missing capillaries.

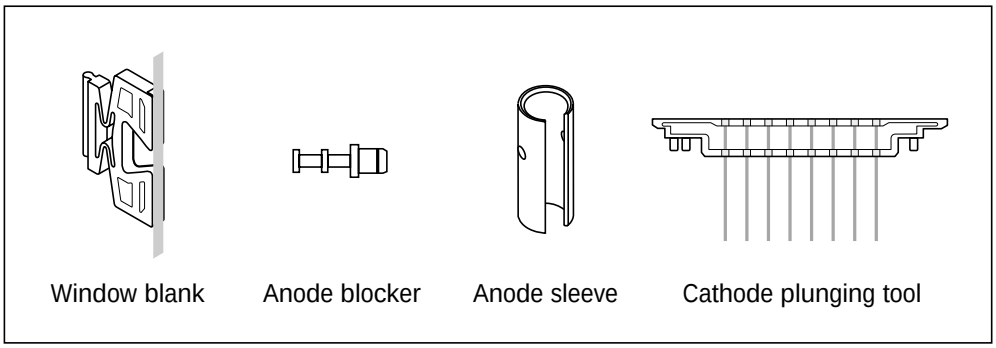


Figure 4-1. An array placeholder (MegaBACE 500 and flexible MegaBACE 1000 instruments only).

Tables 4-1 and 4-2 provide the workflow for increasing or decreasing the number of capillary arrays installed in the instrument.

Table 4-1. Workflow for increasing the number of capillary arrays

Step		Reference
1.	Perform the Replace Capillaries protocol to get access to the electrophoresis compartment and reset the array value.	Section 4.3
2.	Release the capillary array locks.	Section 4.4
3.	Remove the array placeholder, which includes an anode blocker, an anode sleeve, a window blank, and a cathode plunging tool.	Section 4.8.1
4.	Clean the capillary array window.	Section 4.6
5.	Install the capillary array.	Section 4.7
6.	Lock the capillary array in position.	Section 4.9
7.	Focus the capillaries.	Section 4.10

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Table 4-2. Workflow for decreasing the number of capillary arrays

Step	Reference
1. Perform the Replace Capillaries protocol to get access to the electrophoresis compartment and reset the array value.	Section 4.3
2. Release the capillary array locks.	Section 4.4
3. Remove the capillary array.	Section 4.5
4. Clean the window blank.	Section 4.6
5. Install the array placeholder, which includes an anode blocker, an anode sleeve, a window blank, and a cathode plunging tool.	Section 4.8.2
6. Lock the capillary array in position.	Section 4.9
7. Focus the capillaries.	Section 4.10

4.2 Locating the capillary arrays

The capillary arrays are contained within the electrophoresis compartment (figure 4-2).

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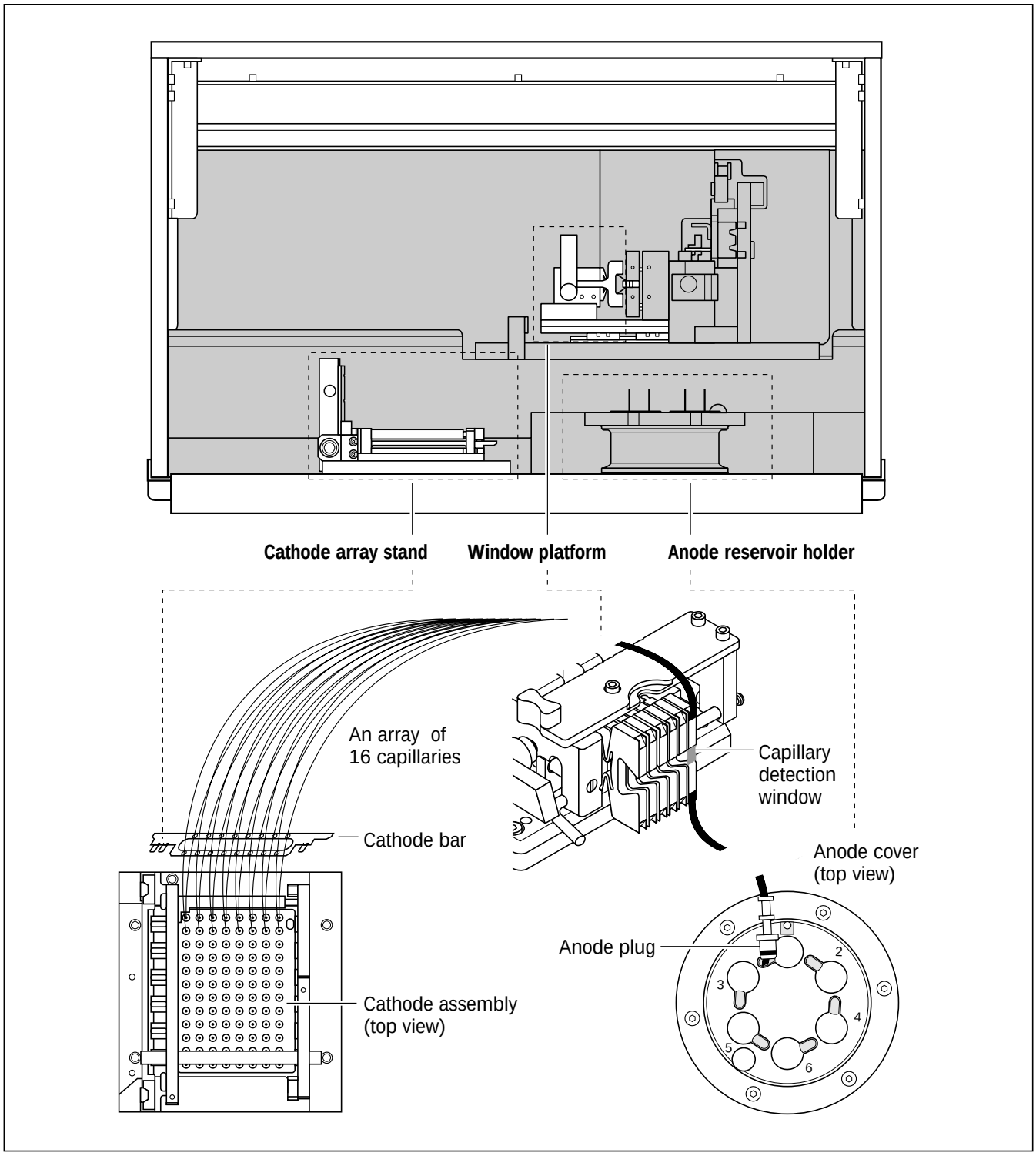


Figure 4-2. A capillary array in the electrophoresis compartment.

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4.3 Using the Replace Capillaries protocol

You use the Replace Capillaries protocol in the Instrument Control Manager to rinse the capillary tips and to unlock the electrophoresis compartment lid so that you can access the capillaries.

Important **Always use the Replace Capillaries protocol before you remove the capillary arrays or array placeholders.**

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 6.2).

4.3.1 Materials required

For the Replace Capillaries protocol, you need (figure 4-3)—

- A tank containing deionized filtered water
- One 2-ml tube for each array, containing deionized filtered water (about 1.8 ml per tube for each array)
- Arrays of capillaries

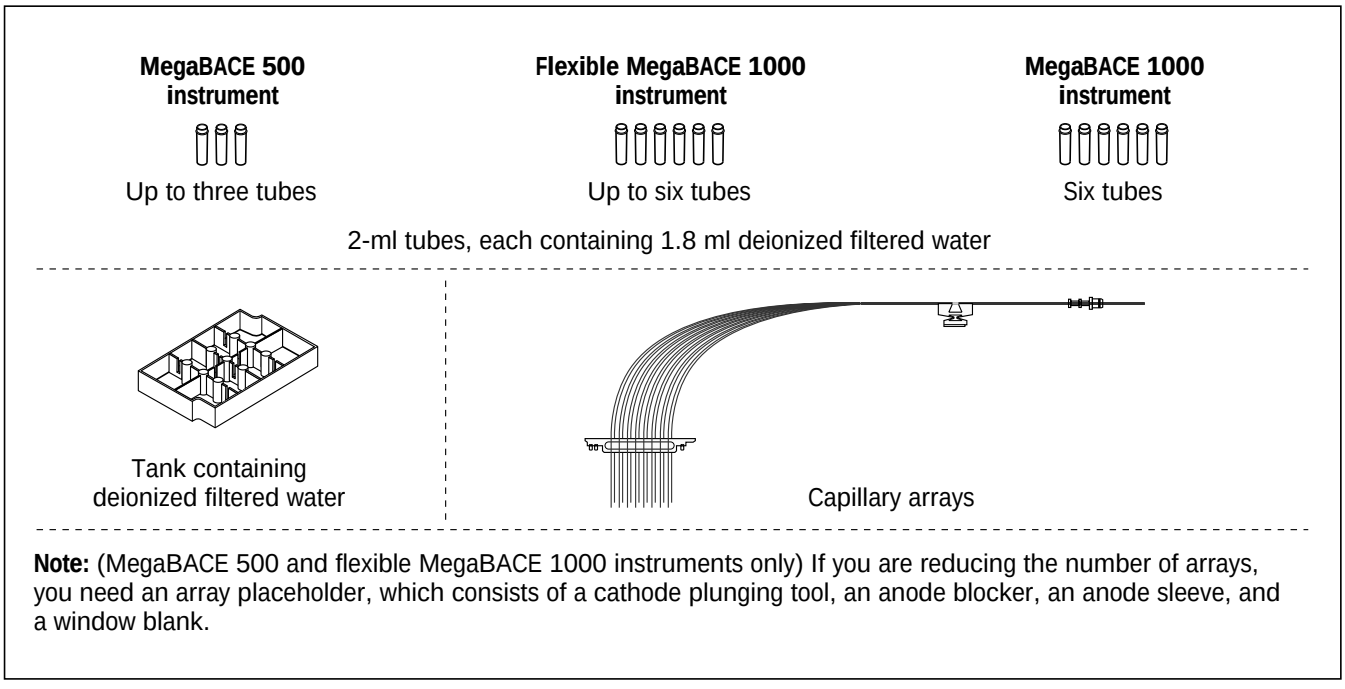


Figure 4-3. The materials for the Replace Capillaries protocol.

Important **Before you begin, set up or select the instrument parameters in the Instrument Control window (figure 4-4). The Focus Capillaries protocol requires high-pressure flush time parameters and PMT voltages.**

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4.3.2 The Replace Capillaries protocol

To use the Replace Capillaries protocol—

1. In the Instrument Control window (figure 4-4), click the **Replace Capillaries** protocol, and then click **Start.**

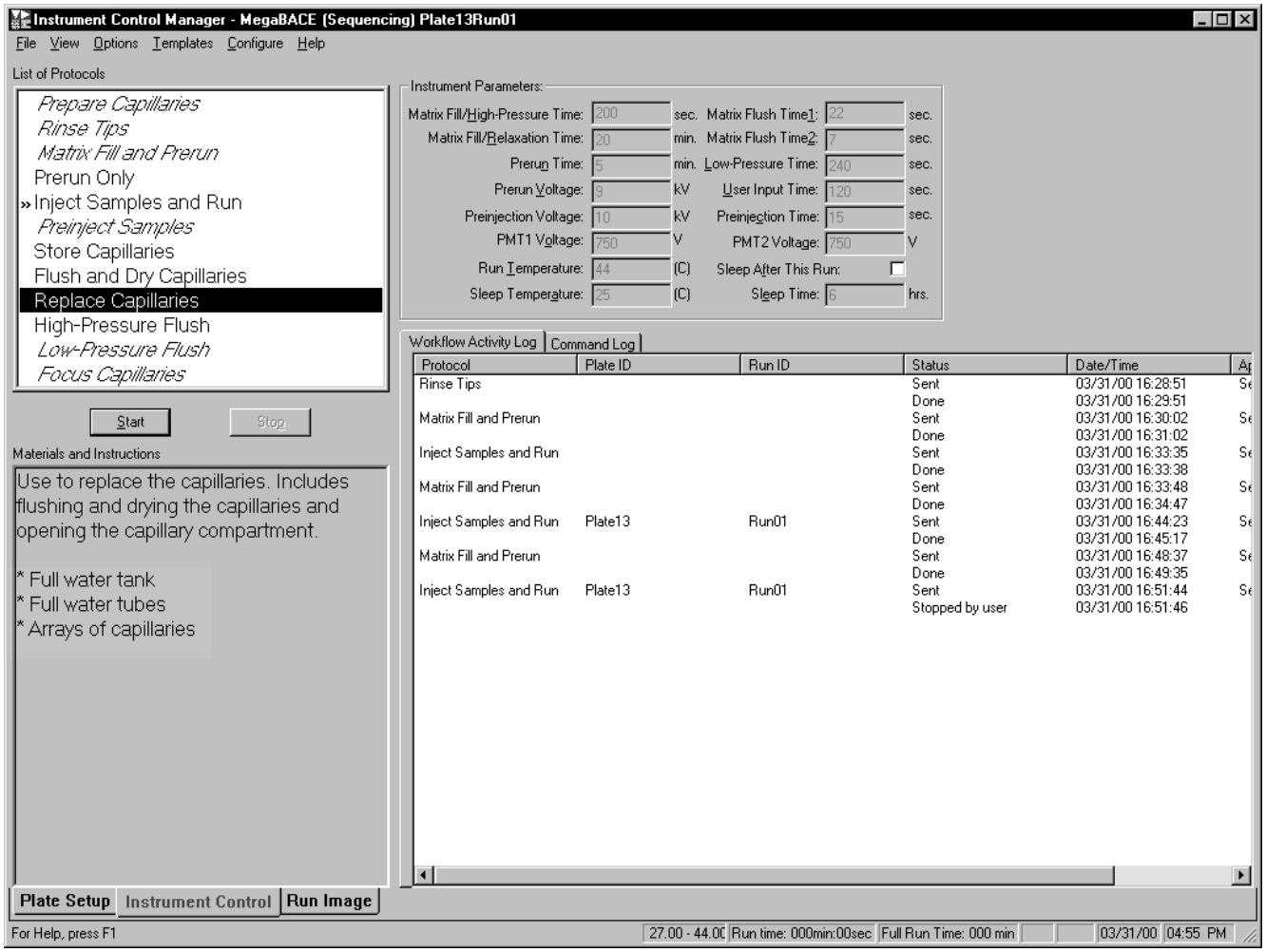


Figure 4-4. The Instrument Control window.

2. When the software asks you to enter the number of arrays, type the **value** for the number of arrays you want to use and then click **OK**. The choices are 1, 2, 3 or 1, 2, 3, 4, 5, or 6, depending on your instrument model.

   A message appears and asks you to confirm the number of arrays you entered. Click **OK**.

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Caution **The number of installed arrays must match the value you entered in step 2. Valid data will not be generated from extra arrays. If the value entered is not correct, click Stop. Then go back to step 1 and restart the Replace Capillaries protocol.**

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

3. When instructed by the left instrument display, remove the **buffer plate** or **water tank** from the left side of the instrument and replace it with a **full water tank.**

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

4. When instructed by the right instrument display, load the **tubes containing deionized filtered water** 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.

   When the protocol is finished, the instrument displays give you a message, **Ready to replace capillaries**. The software unlocks the electrophoresis compartment lid. The Confirm to continue message appears and asks you to open the service door.

5. Open the electrophoresis compartment lid. Follow the procedures to replace the capillaries:

   - Release the capillary array locks (section 4.4).
   - Remove the capillary arrays (section 4.5).

     (MegaBACE 500 and flexible MegaBACE 1000 instruments only) Remove the window blanks.
   - Clean the capillary windows (section 4.6).
   - Install the new capillary arrays (section 4.7) and lock them in position (section 4.9).

     (MegaBACE 500 and flexible MegaBACE 1000 instruments only) Install the window blanks.
   - Increase or decrease the number of arrays, if necessary (section 4.8).

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4.4 Releasing the capillary array locks

Depending on your model, you can use up to six arrays containing 16 capillaries each. The structure of a capillary array is shown in figure 4-5.

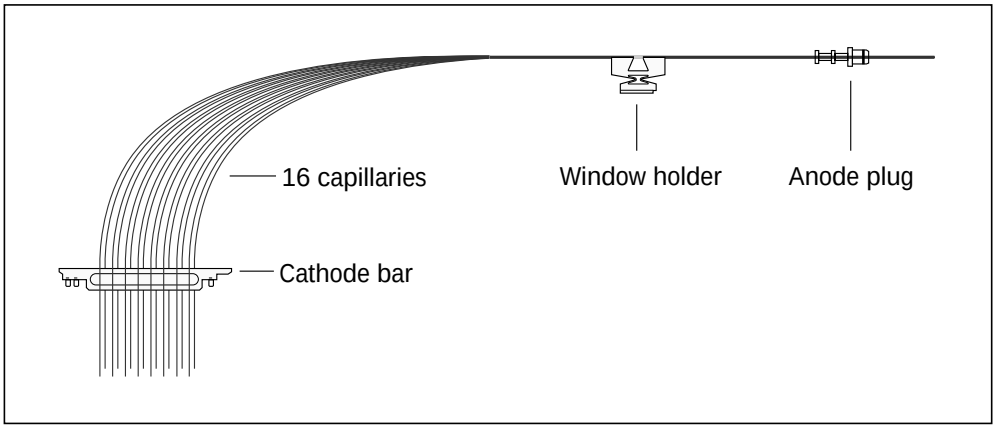


Figure 4-5. A capillary array.

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.

Three locks hold the arrays in place. 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 4-6a).
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 4-6b).

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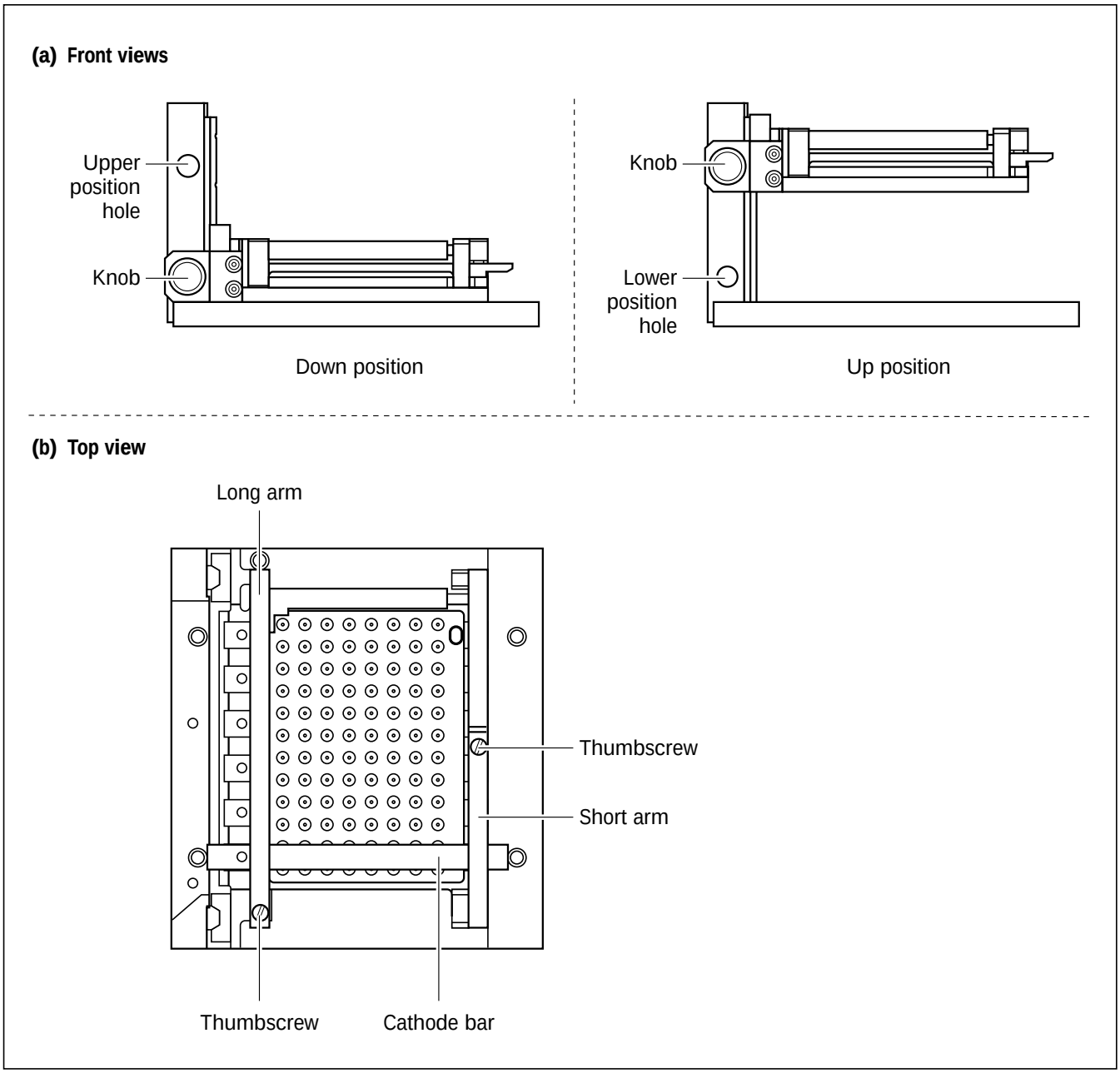


Figure 4-6. The cathode array stand: (a) front views and (b) top view.

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3. Rotate the handle at the front of the window platform counterclockwise to the open position (figure 4-7). The window platform moves to the left, away from the scanning face.

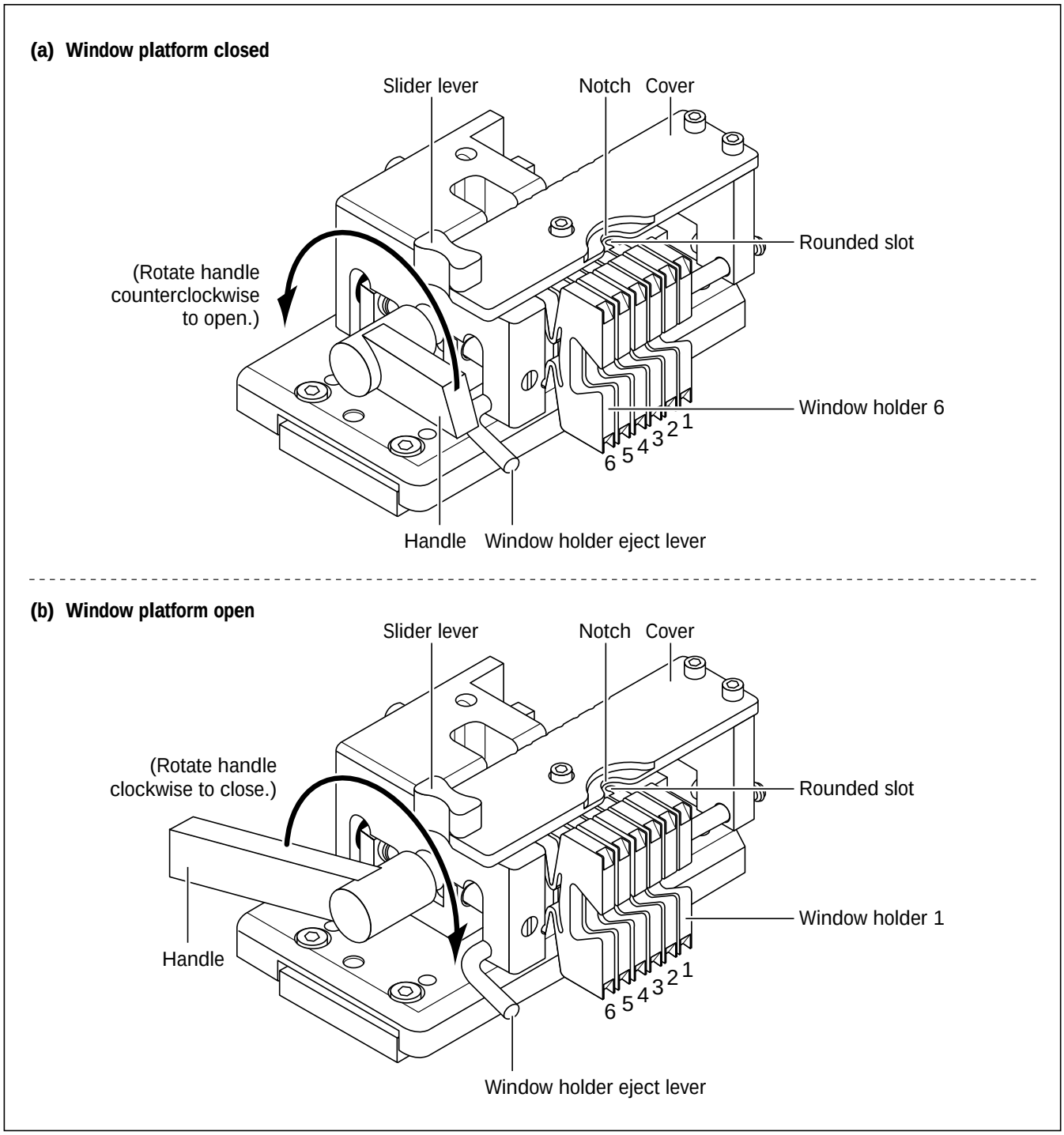


Figure 4-7. The window platform: (a) closed position and (b) open position.

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4. Pull up the knob on the anode cover and rotate the cover counterclockwise (figure 4-8a). 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 4-8b).

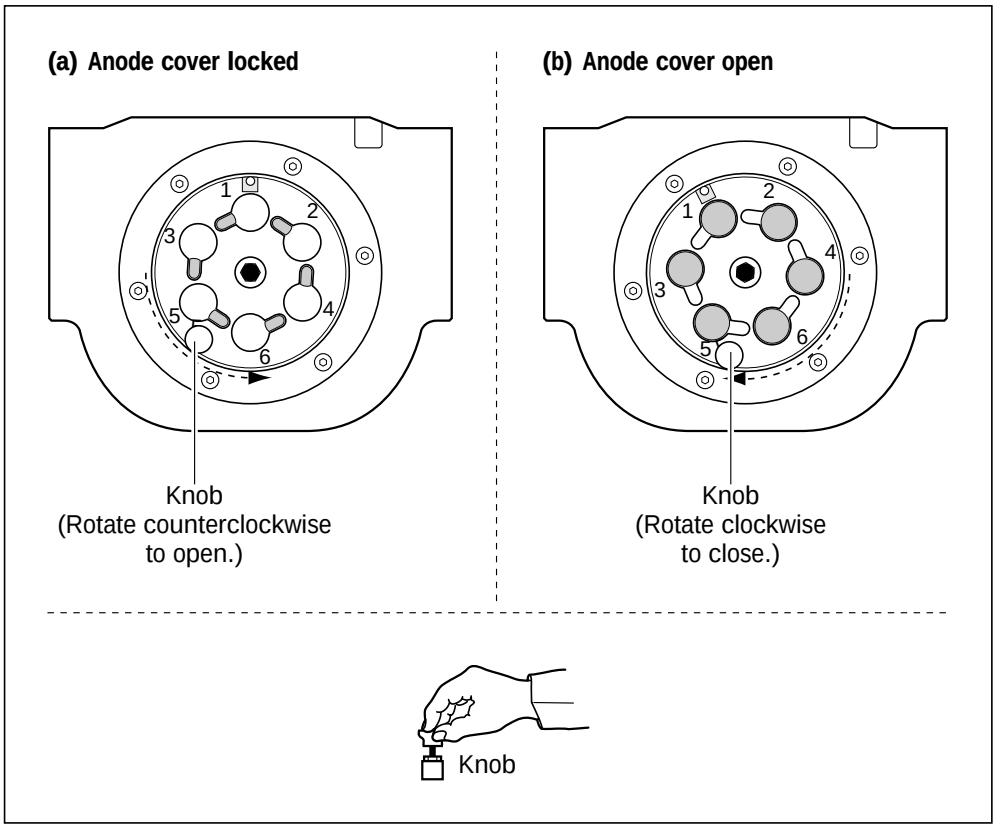


Figure 4-8. The anode cover (top view): (a) cover locked and (b) cover open.

4.5 Removing the capillary arrays

Caution **(MegaBACE 500 and flexible MegaBACE 1000 instruments only) For proper focusing of the capillaries, the instrument optical system requires a window blank or a capillary window at each of the six array positions in the window platform. The elastic properties of the plastic window holders change over time, which can cause the window blanks to be out of focus with newly installed capillaries. Make sure you replace all window blanks when you replace the set of capillary arrays to ensure optimum instrument performance.**

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.

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Warning

Do not pull on the capillaries to release the cathode bar (figure 4-2). The capillaries are fine glass tubes that can break, leaving sharp ends or fragments, which can cause injury.

To remove the capillary arrays—

(MegaBACE 500 and flexible MegaBACE 1000 instruments only) To remove the window blanks, see section 4.8.1).

1. At the window platform (figure 4-7b), 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.
2. Rotate the window holder eject lever counterclockwise to eject the number 6 window holder.
3. Grasp the number 6 window holder and the handle of the corresponding anode plug (see numbering in figure 4-8). 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 may pop out suddenly.
4. Start with the front array labeled 6. Gently lift the left and right ends of the front cathode bar (figure 4-6) to release the bar from the cathode stand.
5. The array is now released from all three locks. Remove the array from the instrument.
6. Repeat steps 1 through 5 to remove each of the remaining capillary arrays in order, starting from position 5.

Warning

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

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4.6 Cleaning the capillary windows

You should clean the capillary windows before installing the arrays in the instrument. 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™ (see the *MegaBACE Planning Guide* for the part number), 5% solution
- Micro Absorbond™ swabs (see the *MegaBACE Planning Guide* for the part number)
- Laboratory wipes
- Squirt bottle containing 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 4.5) or from the box by grasping the cathode bar (figure 4-5).
2. Wet a swab with the 5% solution Liqui-Nox.
3. Carefully grasp the capillary array by the window holder with one hand (figure 4-9), and with the other hand, use the wet swab to clean the entire window area (front and back).

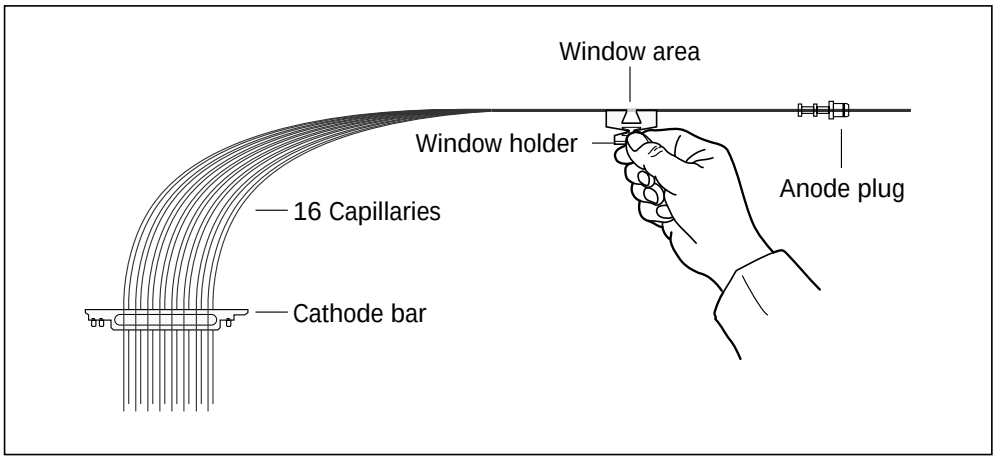


Figure 4-9. Cleaning the capillary windows.

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Caution **Move the swab from the center of the window to the outer edge 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.**

4.7 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.

(MegaBACE 500 and flexible MegaBACE 1000 instruments only) To install the window blanks, see section 4.8.2.

To install the capillary arrays—

1. Using care to avoid touching the new capillaries with the grease, lubricate the O-rings of the anode plugs with a small dab of Dow Corning™ high-vacuum silicone grease. See the *MegaBACE Planning Guide* for the part number and a suggested vendor.

Caution **Do not use water to lubricate the O-ring of the anode plug. The water will dry out the O-ring, and you will find it difficult to remove the anode plug.**

2. Make sure that the cathode stand is in the up position, and the three locks on the cathode stand, window platform, and anode cover are in the open position (section 4.4).
3. Remove the capillary array from its box by grasping the cathode bar (figure 4-5). Hold onto the cathode bar and let the rest of the array hang down in a straight line.

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

4. Start at the back position, which is labeled 1 on the cathode stand (figure 4-6b). 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.

5. 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.

6. 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.

7. Slide the cover of the window platform away from you until the notch is over the back window holder position, which is labeled 1.

8. On the anode cover, locate the back-left position labeled 1 (figure 4-8).

9. On the window holder platform, locate the round slots that you will slide the rounded portion of the window holder into.

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.**

10. 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 4-7).

11. Lower the anode plug into position on the anode reservoir. You may feel some resistance when you insert the plug.

12. Make sure that the anode plug is correctly seated and pressed in all the way.

13. Repeat steps 3 through 12 to install each of the remaining capillary arrays in order, from position 2 through position 6.

14. Lock the capillary arrays in position (section 4.9).

(MegaBACE 500 and flexible MegaBACE 1000 instruments only) Repeat steps 6, 8, and 9 for any window blanks that you need to install.

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4.8 Increasing or decreasing the number of capillary arrays

Cautions

The number of installed arrays must match the value you entered during the Replace Capillaries protocol. Valid data will not be generated from extra arrays. If the value entered is not correct, click Stop and restart the Replace Capillaries protocol (section 4.3).

The elastic properties of the plastic window holders change over time, which can cause the window blanks to be out of focus with newly installed capillaries. Make sure you replace the entire set of capillary arrays and window blanks to ensure optimum instrument performance.

To increase the number of capillary arrays, you remove the array placeholder for each additional array you want to install (section 4.8.1).

To decrease the number of capillary arrays, you install an array placeholder for any empty array position (section 4.8.2).

4.8.1 Removing the array placeholders (MegaBACE 500 and flexible MegaBACE 1000 Instruments only)

To remove an array placeholder, you remove the window blank, the anode plug, the anode sleeve, and the cathode plunging tool.

Removing the window blank

To remove the window blank—

1. At the window platform (figure 4-7b), grasp the slider lever between your thumb and forefinger, and then 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 desired position (figure 4-10).
2. At the position containing the window blank, rotate the window holder eject lever counterclockwise to eject the window blank.
3. Grasp the window blank by the holder and pull up to free the window blank from the window platform.

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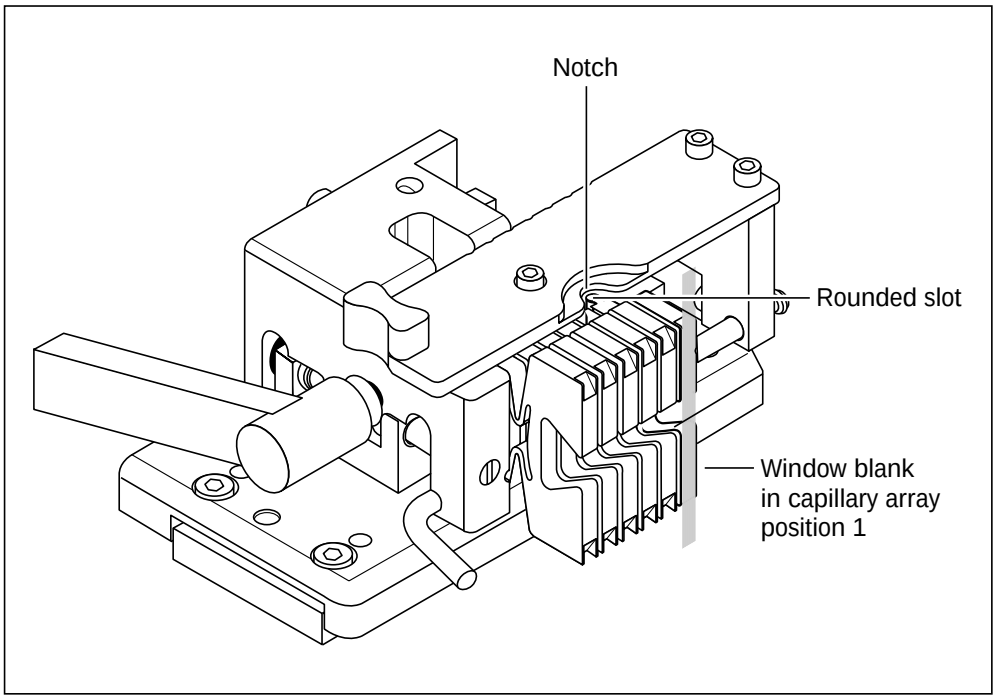


Figure 4-10. Removing or installing the window blank (MegaBACE 500 and flexible MegaBACE 1000 instruments only).

Removing the anode blocker

To remove the anode blocker—

1. At the anode cover (figure 4-8), locate the anode blocker that you want to remove.
2. Grasp the anode blocker and pull it up and out of the cover.

Removing the anode sleeve

To remove the anode sleeve—

1. Open the anode door and gently pull the drawer toward you until it stops.
2. At the anode reservoir holder (figure 4-11), locate the reservoir containing the anode sleeve you want to remove.
3. Grasp the anode sleeve and pull it up and out of the reservoir (figure 4-11).

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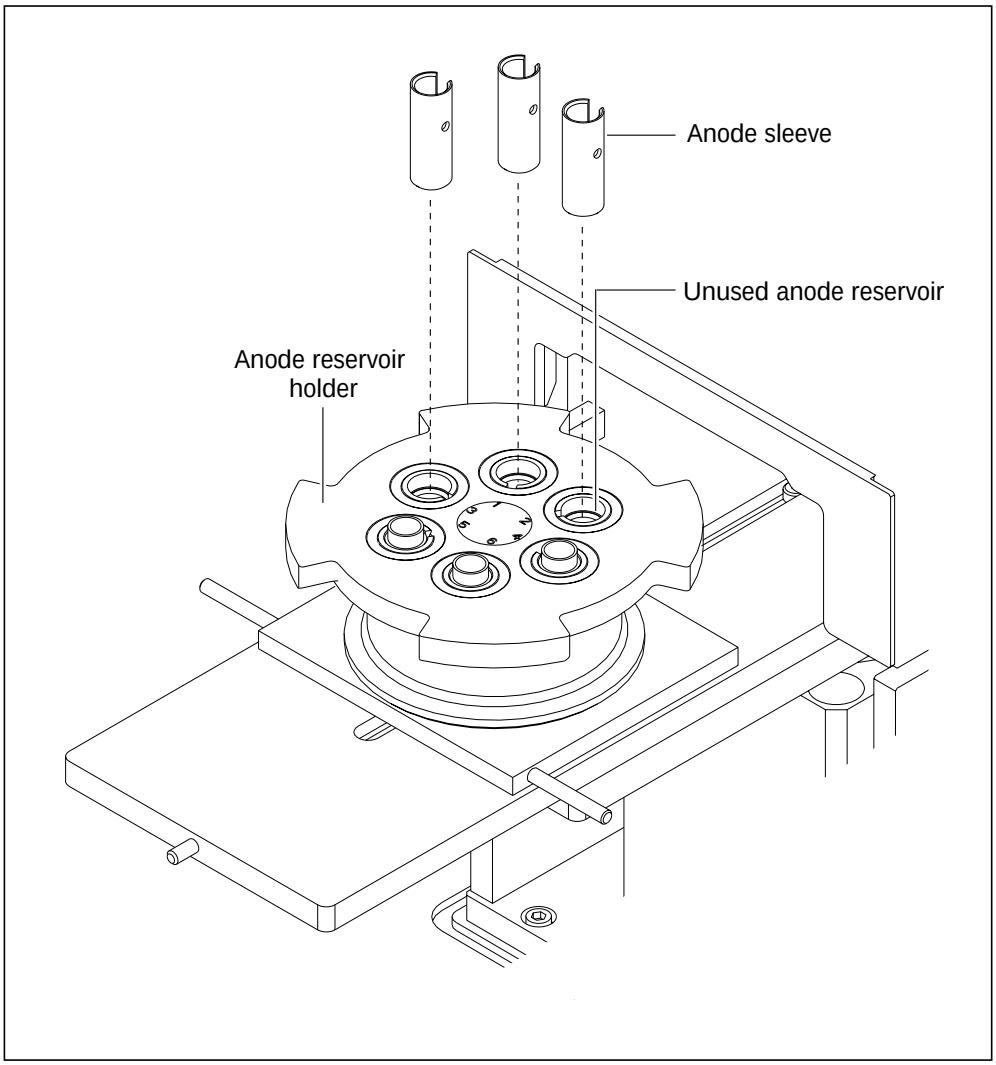


Figure 4-11. Removing or installing an anode sleeve (MegaBACE 500 and flexible MegaBACE 1000 instruments only).

Removing the cathode plunging tool

To remove the cathode plunging tool—

1. Make sure the locks of the cathode array stand are released (section 4.4).
2. Gently lift the left and right ends of the bar of the cathode plunging tool to release it from the cathode array stand. Lift the cathode plunging tool out of the array stand (figure 4-12).

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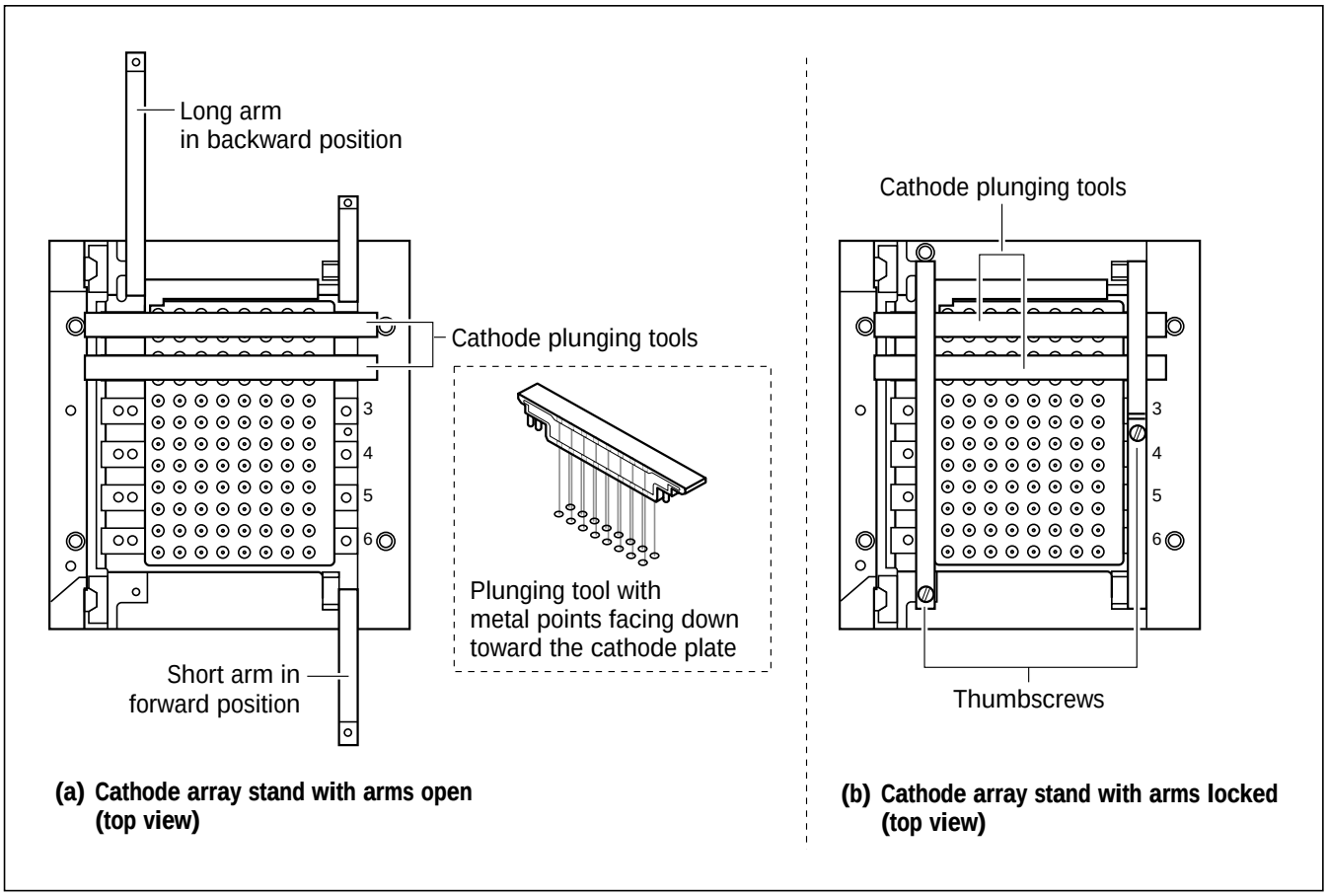


Figure 4-12. Installing or removing the cathode plunging tool (MegaBACE 500 and flexible MegaBACE 1000 instruments only).

4.8.2 Installing a new array placeholder (MegaBACE 500 and flexible MegaBACE 1000 instruments only)

To install an array placeholder, you install the window blank, the anode plug, the anode sleeve, and the cathode plunging tool.

Make sure the cathode array stand is in the up position. Make sure the three locks on the cathode array stand, window platform, and anode cover are in the open position (section 4.4).

Installing the cathode plunging tool

To install the cathode plunging tool—

1. In the cathode array stand, locate the empty position where you want to install the cathode plunging tool (figure 4-12).

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2. Place the cathode plunging tool in the cathode plate with the metal points facing down toward the cathode plate.

3. Make sure you 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 bar of the cathode plunging tool are correctly seated and pressed in all the way.

Installing the anode blocker

To install the anode blocker—

1. At the anode cover (figure 4-8), locate the empty reservoir where you want to install the anode blocker.

2. Place the anode blocker into the reservoir.

3. For all unused anodes, make sure that an anode blocker is seated and pressed in all the way.

Installing the anode sleeve

To install the anode sleeve—

1. Open the anode door and gently pull the drawer toward you until it stops.

2. At the anode reservoir holder (figure 4-11), locate the empty reservoir where you want to install the anode sleeve.

3. Pinch the anode sleeve between your thumb and forefinger and place it into the empty reservoir.

Installing the window blank

Important **Make sure you clean each window blank before you install it. You can use the same procedure that you use for cleaning the capillary windows (section 4.6).**

To install the window blank—

1. At the window platform (figure 4-10), locate the empty slot where you want to install the window blank. Locate the round slot that you will slide the rounded portion of the window holder into.

Caution **Make sure that you fit the rounded portion of the window holder into the round slot on the window holder platform.**

2. Holding the window blank by the plastic window holder, gently lower the window holder into the slot, making sure that the rounded portion of the window holder fits into the round slot.

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4.9 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. Make sure that the anode plugs are correctly seated and pressed in all the way. Then pull up the knob on the anode cover and rotate the cover clockwise (figure 4-8b). Make sure that the pin is seated in the stop hole.

   Note: If you cannot rotate the cover, check that all the plugs are pressed in all the way and then try again.

2. Slide the cover of the window platform all the way back until the notch in the cover is in back of the last window holder, and all the window holders are locked in the down position (figure 4-7a).

3. Rotate the handle at the front of the window platform clockwise to the closed position (figure 4-7b).

   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 4-6b).

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 4-6a). 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. Close the lid of the electrophoresis compartment and press it shut until the lock engages.

8. In the Instrument Control Manager, click **Continue** in the Confirm to continue window (figure 4-5). The Confirm to continue window reappears and tells you the capillaries are replaced and the service door is closed.

9. 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 Focus Capillaries protocol in the List of Protocols on the Instrument Control window (figure 4-4), The Focus Capillaries protocol (section 4.10) is the protocol you perform after replacing the capillary arrays.

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4.10 Focusing the capillaries

After replacing the capillary arrays, you focus the capillaries using the Focus Capillaries protocol. The software automatically selects the Focus Capillaries protocol as the next protocol. The protocol has five parts:

- Flushing the capillaries with water (section 4.10.2)
- Checking for leaks (section 4.10.3)
- Filling the capillaries with fluorescent dye and scanning (section 4.10.4)
- Obtaining the scan line offset value (section 4.10.5)
- Focusing the capillaries and flushing the dye from the capillaries (section 4.10.6)

4.10.1 Materials required

For the Focus Capillaries protocol, you need (figure 4-13)—

- An empty water tank
- One tube for each array, containing deionized filtered water (approximately 1.8 ml per tube)
- One tube for each array, containing fluorescent dye

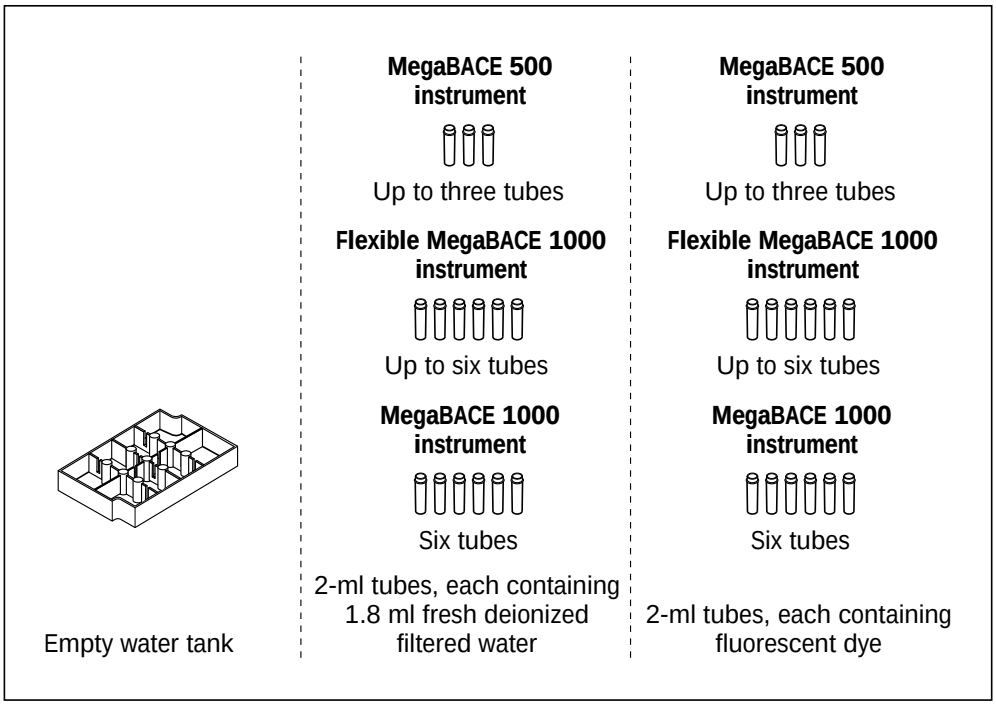


Figure 4-13. Materials for the Focus Capillaries protocol.

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4.10.2 Flushing the capillaries with water

Important **Deionized water bottles should be sterilized on a regular basis to prevent microbial growth in the bottle. Always use fresh deionized filtered water for flushing the capillaries to avoid contamination that can cause damage to the capillaries.**

To flush the capillaries with water—

1. With the Focus Capillaries protocol selected, click **Start.**
2. When instructed by the instrument displays, load an **empty water tank** into the left side of the instrument, and load the **tubes of fresh deionized filtered water** into the right side of the instrument. The instrument displays tell you that high-pressure flush 1 is in progress.
3. When instructed by the instrument displays, refill the tubes with fresh deionized filtered water. The instrument displays tell you that high-pressure flush 2 is in progress and immediately the message, **Inspect capillaries for leaks,** appears.

Important **Be sure to listen for an audible click to indicate that the software has unlocked the lid to the electrophoresis compartment so that you can check the capillaries for leaks (section 4.10.3).**

4.10.3 Checking for leaks

After installing a new capillary array and flushing the capillaries with water, you should check for leaks that would indicate a broken or chipped capillary. To do this—

1. When the message, **Inspect capillaries for leaks,** appears, quickly open the electrophoresis compartment lid (service door).
2. Raise the lid and look for signs of liquid in the compartment. Liquid in the compartment indicates that a capillary is broken, and you must replace the capillary array.
3. If you detect a leak, continue with step 4; otherwise, proceed directly to step 6.
4. If you detect a leak, visually inspect each capillary array to determine which array contains the broken capillary. If you cannot determine which array has the broken capillary, you should call MegaBACE System Technical Support. See Assistance in the preface for contact information.
5. Replace the array(s) following the instructions in sections 4.3 through 4.9, and then run the Focus Capillaries protocol (section 4.10.2).

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6. If there are no leaks, close the lid and then allow the electrophoresis compartment to come up to the set run temperature and continue with the procedure in section 4.10.4.

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 (table 4-3). Insufficient temperature can cause unreliable capillary focusing.**

Table 4-3 provides examples of warmup times you should allow before focusing the capillaries. If the compartment has not stabilized to the set run temperature, you cannot properly focus the capillaries.

Table 4-3. Examples of warmup times for the electrophoresis compartment for a set temperature of 44 °C (111.2 °F)

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

4.10.4 Filling the capillaries with fluorescent dye and scanning

Important **Make sure the temperature in the electrophoresis chamber has reached the default setting of 44 °C (111.2 °F).**

To fill the capillaries with fluorescent dye—

1. When instructed by the instrument displays, load the **tubes containing the fluorescent dye** in the right side of the instrument. The instrument displays tell you that the capillaries are being filled with dye and that capillary focusing is in progress. The instrument performs a scan.

   After scanning is finished, a message appears, **Run Raw2Gel on [Current Storage Folder]\focus\cap.dat and analyze sep2.gel or sep4.gel using the ImageQuant software** (section 4.10.5).

   Then another message appears, **Specify scan line offset in Focus window.**

2. Click **Continue.** The Focus window appears (figure 4-14).

   Note: To obtain the scan line focus offset, use the procedure in section 4.10.5.

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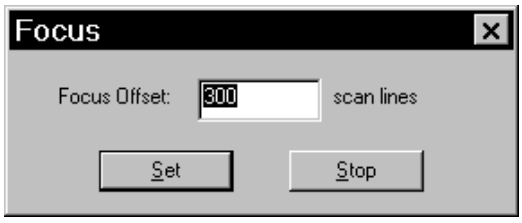


Figure 4-14. The Focus window.

3. At this point, leave the Instrument Control Manager running with the Focus window open and start the Raw-to-Gel Conversion software (section 4.10.5).

4.10.5 Obtaining the scan line offset value

To obtain the scan line focus offset value, you need to perform a series of steps that require the use of the Raw-to-Gel Conversion software and the ImageQuant™ software, which you can access by double-clicking the appropriate icon in the MegaBACE folder on the desktop.

Converting the cap.dat file using Raw-to-Gel Conversion

The cap.dat file is stored in the [Current Storage Folder]\focus folder. You need to convert the cap.dat file to an image file (.gel) that can be read by the ImageQuant software.

To convert the .dat file to a .gel file—

1. In the MegaBACE folder on the desktop, double-click the **Raw-to-Gel Conversion** icon. The Raw-to-Gel window opens and displays a list of folders (figure 4-15).

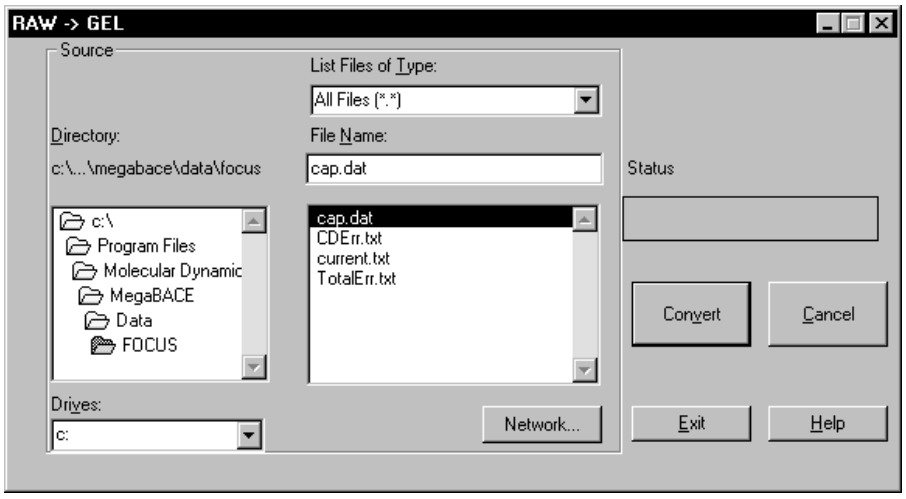


Figure 4-15. The Raw-to-Gel window for converting a .dat file to a .gel file.

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2. In the [Current Storage Folder]\focus folder, double-click the **cap.dat** file. The file is converted to an image file with the .gel extension.

Using ImageQuant to obtain the scan line focus offset value

The ImageQuant software allows you to analyze an image file. See the ImageQuant Help for more detailed instructions on using the features described below.

To obtain the focus offset value, you must determine the area of greatest fluorescence intensity within a capillary. To do this—

1. Double-click the **ImageQuant** icon in the MegaBACE folder. The ImageQuant window appears.
2. Choose **Open** from the File menu and select the .gel file (sep2.gel) you just converted. The image file appears in the ImageQuant window.
3. Choose **Actual Size** from the View menu.
4. Choose Gray/Color Adjust from the View menu to enhance the appearance of the capillaries within the image file. Depending on the Gray/Color Adjust settings you used, the image should look similar to the image in figure 4-16.

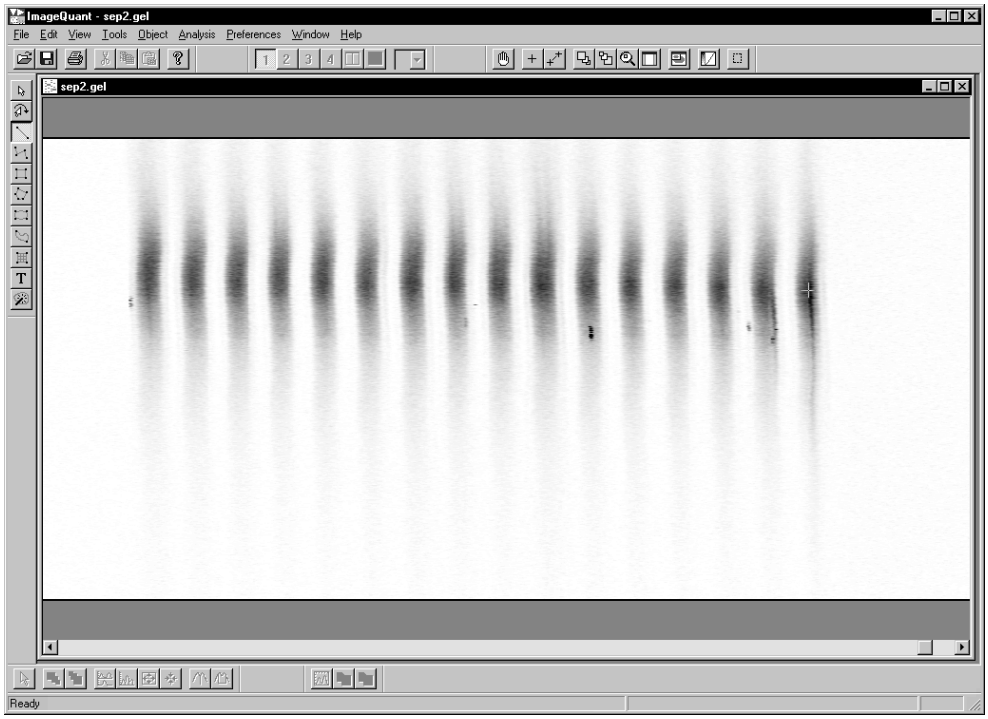


Figure 4-16. A converted image file displayed in ImageQuant.

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5. Using the **Line** ( ) button on the toolbar, draw a vertical line down the center of a capillary that shows good fluorescence intensity.
6. Choose **Object Attributes** from the Object menu.
7. Select the line on the capillary and type **10** in the **Width Each Side** box to average the signal for 10 pixels on either side of the line. Click **Set.**
8. Copy the line, paste the line, and drag it to one capillary in the next array. Repeat for each of the remaining arrays. To obtain a more accurate average of the fluorescent intensity, use more capillaries (10 is optimum).

Important **(MegaBACE 500 and flexible MegaBACE 1000 instruments only) Make sure you do not use any portion of the image created by the window blanks.**

9. To get a better view of the capillaries, choose **Magnification** from the View menu and choose 200%. The image changes accordingly.
10. If necessary, move the lines to better positions on the capillaries until the lines appear to be in the center of the capillaries (figure 4-17).

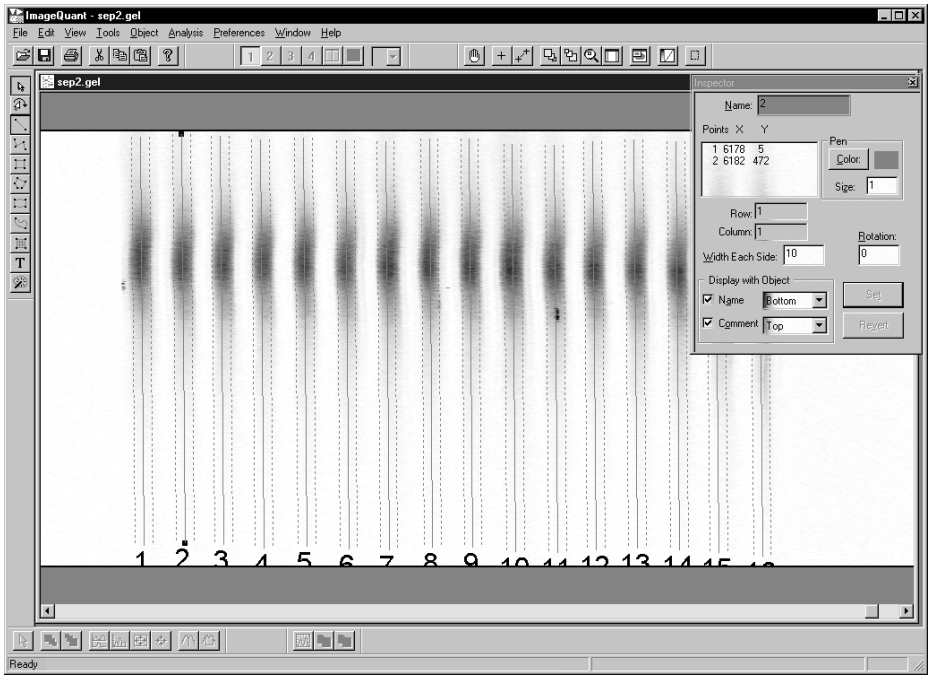


Figure 4-17. Image file with vertical lines drawn on capillaries.

11. Select one of the capillaries that has a vertical line.
12. Click the **Create Graph** ( ) button on the toolbar to create a line graph for the selected capillary. The line graph appears (figure 4-18).

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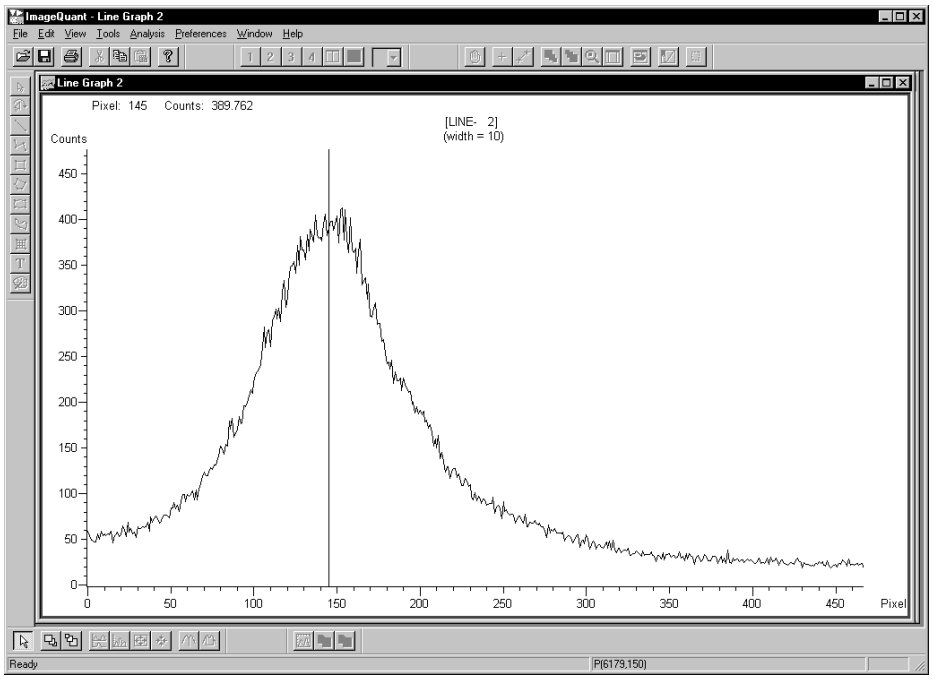


Figure 4-18. The line graph in ImageQuant.

13. Move the pointer to the middle of the curve (this may not always be the highest point of the peak). The x- and y-axis values appear in the status bar as: P(x,y), where x = 6179 and y = 150 (figure 4-18).
14. Make a note of the y-axis value (counts).
15. Repeat steps 11 through 14 until you obtain the y-axis values for all the capillaries you marked with vertical lines.
16. Average the y-axis values. The average of the values is the focus offset value you type in the Focus window in the Instrument Control Manager.
17. Close the ImageQuant and Raw-to-Gel Conversion software and return to the Instrument Control Manager.

4.10.6 Focusing the capillaries and flushing the dye from the capillaries

To focus the capillaries and flush the dye from the capillaries—

1. Type the focus offset value in the Focus window and click **Set.** The protocol focuses the capillaries. The instrument displays tell you that focusing is in progress.
2. When instructed by the instrument displays, replace tubes with **tubes containing fresh deionized filtered water.** The instrument displays tell you that flushing the dye is in progress.

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3. When instructed by the instrument displays, load a **full water tank** in the left side of the instrument and refill the water tubes. The instrument displays tell you that capillary focus is complete, and the double arrows (>>) appear to the left of the Prepare Capillaries protocol indicating that it is the next protocol.

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Chapter 5 Moving and reinstalling the instrument

This chapter describes—

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

An Amersham Biosciences representative installs your MegaBACE instrument after delivery. If you need to move the instrument, contact MegaBACE System Technical Support. See Assistance in the preface for contact information.

5.1 Moving the instrument

Warning ⚠

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

Caution

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

If you need to move your MegaBACE instrument, review the *MegaBACE Planning 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.

5.2 Required parts for reinstalling the instrument

The following parts are required for installing the MegaBACE system.

5.2.1 Major components and parts

The MegaBACE system includes—

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

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5.2.2 Power cords

The MegaBACE system includes power cords for—

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

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

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

5.3 Connecting a new computer

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

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

To connect your MegaBACE instrument to a new computer, first turn off the MegaBACE instrument using the instructions in the *MegaBACE Instrument Operator's Guide.*

Make sure that a PCI SCSI (small computer systems interface) adapter card is installed in the computer. The MegaBACE software requires Windows NT or Windows 2000 at a minimum. If you are using Windows NT, make sure the device drivers are loaded. See the instructions provided with the adapter card and in the Windows manuals. For information about SCSI and network connections, call MegaBACE System Technical Support. See Assistance in the preface for contact information. See the *MegaBACE Planning Guide* for networking configurations.

Install the system and analysis software using the instructions provided with the software.

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Chapter 6 Troubleshooting

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.

Note: For sequencing troubleshooting guidelines, see the MegaManual. For genotyping troubleshooting guidelines, see the *MegaBACE Genetic Profiler User's Guide.*

The topics in this chapter are—

- On-screen error messages (section 6.1)
- Power and communication (section 6.2)
- Electrical current (section 6.3)
- Run (section 6.4)
- Cathode and anode stages (section 6.5)
- Fan (section 6.6)

The problems in bold print are followed by possible causes and solutions.

6.1 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 may require a field service call (section 6.1.1)
- Hardware-related error messages that you can resolve (section 6.1.2)
- Software-related error messages that you can resolve (section 6.1.3)

6.1.1 Hardware-related error messages that may require a field service call

In general, if you receive hardware error messages such as the following, you should call MegaBACE System Technical Support for assistance. Before you call, be sure to write down the error message and the condition that caused it. In addition, you will need the serial number of your MegaBACE instrument.

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Examples of hardware-related error messages that may require a field service call are—

A current read in switch (mux) has failed. Fatal error.

Blue laser power is low.

Blue laser did not turn on.

Check the laser power supply and the vent/exhaust hoses.

DANGER! Input voltage to HV power supply is on when it should be off. Call Service.

EPHV PCA Error.

Error: Instrument is down.

Restart the system.

Green laser did not turn on.

HV board self-test failure—+10v onboard reference.

Temperature board self-test failure—A/D ground input test.

Restart the system.

6.1.2 Hardware-related error messages that you can resolve

You can address some hardware-related error messages before calling for assistance. If the solution does not correct the error, call MegaBACE System Technical Support. See Assistance in the preface for contact information. Some examples are—

Abnormal instrument status: High pressure too low.

Check the high-pressure nitrogen regulator. Make sure the nitrogen pressure is set at 6.89×10^3 kPa (1 000 psi).

Cathode door not closed within timeout period.

Try opening and closing the door.

Cathode stage not in. Sensor 1 or Sensor 2.

Open and then fully close the cathode drawer.

Check the nitrogen pressure.

Make sure the high pressure is set at 6.89×10^3 kPa (1 000 psi) and the low pressure is set at 6.89×10^2 kPa (100 psi).

The electrophoresis compartment door is open.

Close the door.

No tank on cathode stage. Stage cannot be raised.

Place a water tank on the stage.

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The temperature is not at setpoint.

- Close the electrophoresis compartment lid.
- Allow the instrument to warm up according to the warmup times (section 4.10.3).

Timer expired while trying to raise the anode stage.

Close the anode drawer and retry.

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.

This message means the container you have placed on the stage is not the one the protocol requires. Change the container and try again.

6.1.3 Software-related error messages that you can resolve

Some common software-related error messages that you can address without assistance are—

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 the instrument operator's guide 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.

The Post-Prerun time expired.

More than 15 minutes have passed since you used the Inject Matrix and Prerun protocol. The software selects the Prerun Only protocol as the next protocol you should use before injecting samples.

You must first select a plate that has no run ID to run the samples.

Click the Plate Setup window and select from the Plate Catalog a plate ID that has no run ID. Then click the Instrument Control window and start the Inject Samples and Run protocol.

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6.2 Power and communication

You can resolve many of the power and communication problems. The following are some common problems and solutions you can try before calling MegaBACE System Technical Support for assistance. See Assistance in the preface for contact information.

The power light on the MegaBACE instrument will not turn on.

- Check that the instrument is turned on and that the power cord is plugged in.
- The wall outlet may 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 may be faulty. Test the outlet or try another outlet.

The computer is not communicating with the MegaBACE instrument.

- Check that the instrument power is turned on. Be sure to wait 45 seconds before turning on the computer.
- Turn off the instrument and turn off the computer. Turn on the instrument and wait 45 seconds before turning on the computer.
- The SCSI cable may have come loose or may be damaged. Check all SCSI cables and connections.
- The wall outlet may be faulty. Test the outlet or try another outlet that supplies the same voltage.
- The Host Scan Controller (HSC) may not be running. Start the HSC software (see the instrument operator's guide).

The power has gone off, and you cannot open the lid of 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, into the access hole (figure 6-1).

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The tool hole is recessed approximately 4 cm (1.5 in). If necessary, look through the access hole to guide the tool into position.

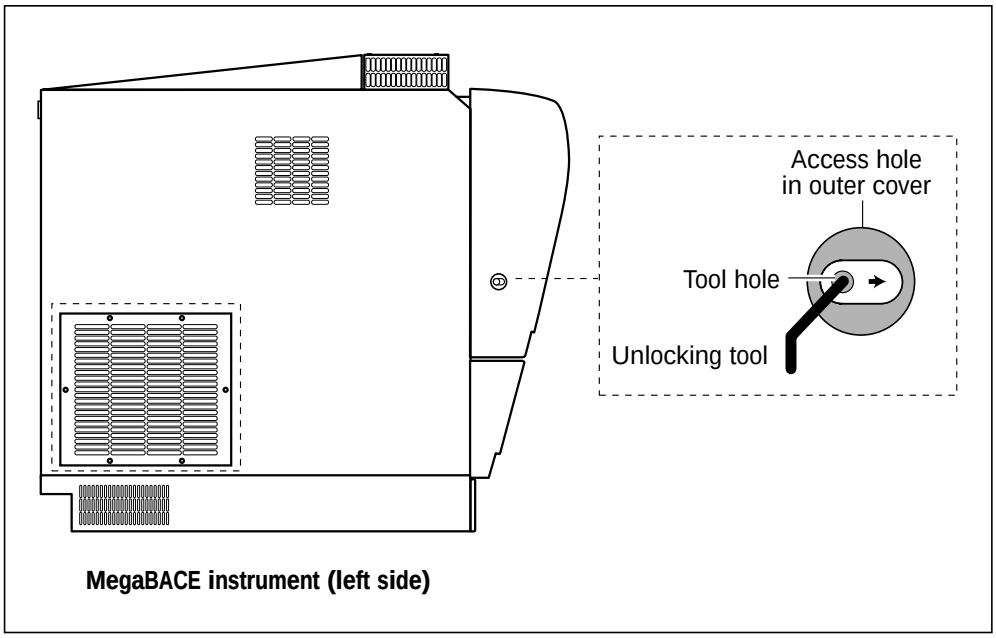


Figure 6-1. The unlocking tool and the access hole on the left side of the electrophoresis compartment lid.

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.

6.3 Electrical current

The Current Monitor window allows you to monitor the current in each of the 96 capillaries. Some common problems and solutions are—

High current fluctuations are displayed in individual capillaries during the prerun or electrophoresis run.

- Dust or other impurities may be present in the capillary. Make sure the system is free of excess dust. Flush the capillaries and reinject fresh matrix.
- Some of the capillaries are clogged. Flush the capillaries and reinject fresh matrix.
- The tips of the capillaries at the cathode end are not cleaved correctly and are jammed into the wells. Replace the capillary array and refocus the capillaries using the Replace Capillaries and Focus Capillaries protocols (chapter 4).

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The current drops to a value near 0 or to 0 in one or more capillaries during the prerun or electrophoresis run.

- Dust or other impurities may be present in the capillary. Make sure the system is free of excess dust. Flush the capillaries and reinject fresh matrix.
- You have overinjected the sample DNA (template), which can affect the current during the run. Lower the injection time and/or voltage. Call MegaBACE System Technical Support for assistance. See Assistance in the preface for contact information.
- The DNA (template) may contain very large strands of SS-DNA (thousands of base pairs).
- An electrode on the cathode side of the capillary may be bent or broken. Call MegaBACE System Technical Support for assistance.
- A capillary is broken. (This can be diagnosed by observing matrix leaking from the capillary.) Replace the capillary array and refocus the capillaries using the Replace Capillaries and Focus Capillaries protocols (chapter 4).
- Air bubbles may be present in the capillaries. Always degas or centrifuge reagents before starting a run.

6.4 Run

Some problems and solutions you may encounter during the run are—

When running samples, the computer crashes if the screen saver is on and you touch the mouse or keyboard.

If you have less than 128 MB RAM, do not run a screen saver or any other CPU-intensive applications on the data collection workstation during a run. You could interrupt communications between the computer and the MegaBACE instrument. This could result in a crash and cause you to lose data from the run in progress.

If you must use a screen saver, use the Blank Screen screen saver. Do not use any of the 3D OpenGL screen savers.

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Individual capillaries have no data (signal) after the run.

- DNA samples were not properly injected, possibly because of bubbles in a sample well. Reload the samples into the wells and make sure there are no bubbles in the samples. Then start a new run by injecting fresh matrix and injecting the samples.
- The current dropped or was nonexistent during the injection and/or electrophoresis run. See section 6.3.
- There is an insufficient volume of DNA sample in the well. Make sure that the sample volume in the well is at least 5 µl.

None of the capillaries has data (signal) after the run.

- The capillaries were not properly focused prior to the start of the run. Refocus the capillaries using the Focus Capillaries protocol (chapter 4). If the problem is not solved, call MegaBACE System Technical Support for assistance. See Assistance in the preface for contact information.
- No filters or incorrect filters are installed in the filter holders. Install the correct filters.
- The laser malfunctioned or failed. Contact MegaBACE System Technical Support for assistance if the laser is nonoperational.
- The optics are out of alignment. Contact MegaBACE System Technical Support for assistance.

After the run, the quality of the data is poor, resulting in unreadable data or very low signal.

- If the instrument is used for multiple applications (sequencing and genotyping)—
  - Make sure the correct application is selected for the plate you are running.
  - Make sure the filters and beamsplitters in the instrument are appropriate for the selected application (sequencing or genotyping). Also, make sure the filters are installed in the correct positions.
- The DNA sample is contaminated. Make sure the sample is clean by removing contaminants, such as excess salts (buffer) or dye-labeled fragments.
- The matrix has degraded. Use fresh matrix if you suspect that the LPA polymer has been stored at room temperature for extended periods, has been stored below 0 °C, or has been contaminated in any way.

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- The capillary array coating has reached its performance lifetime and is degraded. Replace the capillary array(s) and refocus the capillaries (chapter 4).
- Replace the capillary array(s) and refocus the capillaries (chapter 4) if the quality is poor, if the resolution is poor, or the signal has a lot of noise.
- Check to see if the filters or beamsplitters are dirty. Clean the filters or beamsplitters (chapter 3).
- The data show excessive current fluctuations. See section 6.3.
- Check the signal level in each spectral channel. If one or two spectral channels show poor signal, optimize the samples.

6.5 Cathode and anode stages

The cathode or anode stage will not move up or down.

A variety of factors may cause the cathode or anode stages not to move up or down. Call MegaBACE System Technical Support for assistance.

6.6 Fan

The fan in the power supply fan module is on, but the software displays a message that the airflow is low.

- The exhaust grill on the back of the power supply fan module may be blocked. Remove any obstruction.
- The air hose between the MegaBACE instrument and the power supply fan module may be loose or have a hole in it. Check that the connections are tight and the hose is in good condition.
- The exhaust fan may not be turned on. This is an external fan that is sometimes in the ceiling and operated by a switch on the wall. Check that the switch is turned on.
- If the air path is free and the system is cool but the problem persists, call MegaBACE System Technical Support for assistance. See Assistance in the preface for contact information.

Important **Always leave the key on the front of the power supply fan module in the horizontal or on position (figure 6-2).**

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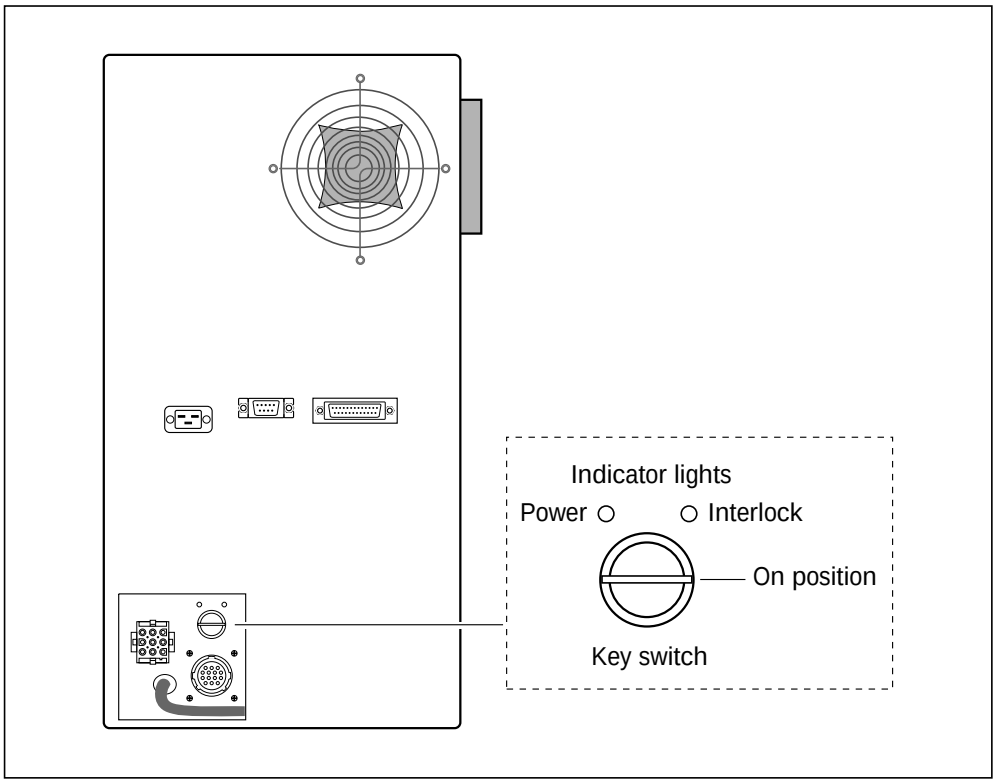


Figure 6-2. The key on the power supply fan module.

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Glossary

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

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

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

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

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

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

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

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

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

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

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

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

calibration run—see spectral calibration run.

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

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

channels—see spectral channel.

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

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

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

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

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

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

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

LPA (linear polyacrylamide)—see sieving matrix.

matrix—see sieving matrix or spectral overlap matrix.

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

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

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

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

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

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

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

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

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

sample files—see .rsd files. For sequencing, see also .abd files, .esd files, .seq files, and .scf files.

s**ample names—**the designation you provide for given wells in the sample names tab or in a plate setup template or .psd file.

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

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

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

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

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

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

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

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

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

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

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