user manual

MegaBACE 500 and 1000

Instrument New Features Guide
Version 3.1

um 375-746

Amersham
Biosciences

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June 2002

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Preface

Preface

About this guide

This *MegaBACE Instrument New Features Guide version 3.1* provides an overview of the new and revised features to be found in the MegaBACE™ Instrument Control Manager v3.1 software.

This document assumes that you are familiar with the operation of Instrument Control Manager v2.4 or v2.5. Please see the related publications that are provided with the MegaBACE 1000 and MegaBACE 500 instruments.

Related publications

This document supplements the following user documents that are provided with the MegaBACE 1000 and MegaBACE 500 instruments:

- *MegaBACE Instrument Operator's Guide v2.4*
- *MegaBACE Instrument Administrator's Guide v2.4*
- *MegaBACE Instrument Maintenance and Troubleshooting Guide v2.4*
- *MegaBACE SNP Genotyping Instrument Operator's Guide v2.5*
- *MegaBACE SNP Genotyping Instrument Administrator's Guide v2.5*

The Help, which is available from within the MegaBACE Instrument Control Manager v3.1 software, provides a detailed description of how to use the software. To display the Help, click **Help Topics** on the Help menu.

The MegaManual, which is posted in the MegaBACE User Zone pages of the following Web site, provides detailed troubleshooting guidelines for the sequencing application:

http://www.amershambiosciences.com

You can click the MegaBACE link to access the MegaBACE pages. From the MegaBACE pages, you can obtain a password for the User Zone pages. You need the serial number of your instrument to obtain a password.

Special notices

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

Caution **Indicates that loss of data or invalid data could occur if you fail to comply with the advice given.**

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

Note: Identifies items of general interest.

Assumptions

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

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.

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 instrument. 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

1 Overview of new features

This section provides an overview of the new and improved features in the MegaBACE Instrument Control Manager v3.1 software.

1.1 New features

The new features in Instrument Control Manager v3.1 include—

- **(Sequencing only) Ability to change the base-called data storage location—**See section 2.1 for details.
- **Automatic disk-space verification for each run—**Before starting a run, the software verifies that enough free disk space exists at the specified data storage location. The software calculates the disk-space requirements based on the specified run length and the selected base calling and file export options. If there is not enough disk space, a message tells you how much additional disk space is required. The software repeats the disk-space verification if you change the base calling options or the run length during the run.
- **An automatic Focus Capillaries protocol—**See section 5 for details.

1.2 Improvements in the graphical user interface

Although the following items function in the same way as the previous software version, the graphical user interface has a new look and feel:

- File storage feature for the raw data files (section 2.2)
- Instrument Control window (section 3)
- Current Monitor window (section 4)

1.3 File structure improvements (Administrator only)

The MegaBACE folder contains a new Instrument Control folder that stores all the system files, initialization files, and templates that control the instrument operation and plate setup. The MegaBACE folder contains a new Base Calling folder that stores the files related to the automatic base calling feature (section 6).

2 Changing the data file storage location

You can change the file storage location for the sample data files the system generates. For example, you can store the files on another hard drive on the instrument control computer if the computer is running out of space on the current drive. You can change the storage location for the—

- Base-called data files (section 2.1)—This is a new feature.
- Raw data files (section 2.2)—This is an updated feature.

2.1 Changing the storage location for base-called data files (Sequencing only)

Important **You cannot use the data storage feature to select a storage location on a remote computer.**

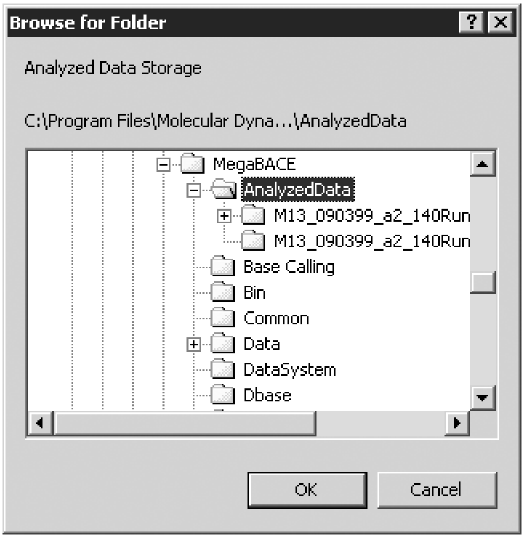
Changing the storage location affects only future files that are created on the data collection computer by the Instrument Control Manager. Changes that you make to the storage location using the Instrument Control Manager do not affect the MegaBACE Sequence Analyzer storage location setting.

The Instrument Control Manager stores all the base-called data folders in a single AnalyzedData folder.

Unless you specify a different location, the software stores the base-called data in the MegaBACE\AnalyzedData folder (default). Before the software performs automatic base calling for a run, you can specify a different storage location for the base-called files on the instrument control computer.

To change the base-called file storage location—

1. Make sure the Inject Samples and Run protocol is not running.
2. Choose **Analyzed Data Storage** from the Options menu. The Browse for Folder window appears (figure 1).



Analyzed Data Storage

C:\Program Files\Molecular Dyna...\AnalyzedData

Figure 1. The Browse for Folder window for changing the storage location of the base-called data files.

3. Select the local hard drive and folder you want to use and click **OK.** The next time the software performs automatic base calling, the software creates a folder for the base-called sample data and stores the base-called data folder in the storage location that you selected (for example, MegaBACE/AnalyzedData folder).

2.2 Changing the storage location for the raw sample data files

Important **You cannot use the data storage feature to select a storage location on a remote computer.**

Changing the storage location affects only future files that are created on the data collection computer by the Instrument Control Manager.

When you change the raw data storage location, the change affects only the selected application (for example, Sequencing).

After you run a plate, the Instrument Control Manager stores the .rsd files in a raw run folder in the default location or the folder you specified. Unless you specify a different location, the software stores the run data in the MegaBACE\Data folder. Before running a plate, you can specify a different storage location for the .rsd files on the instrument control computer.

To change the raw data file storage location—

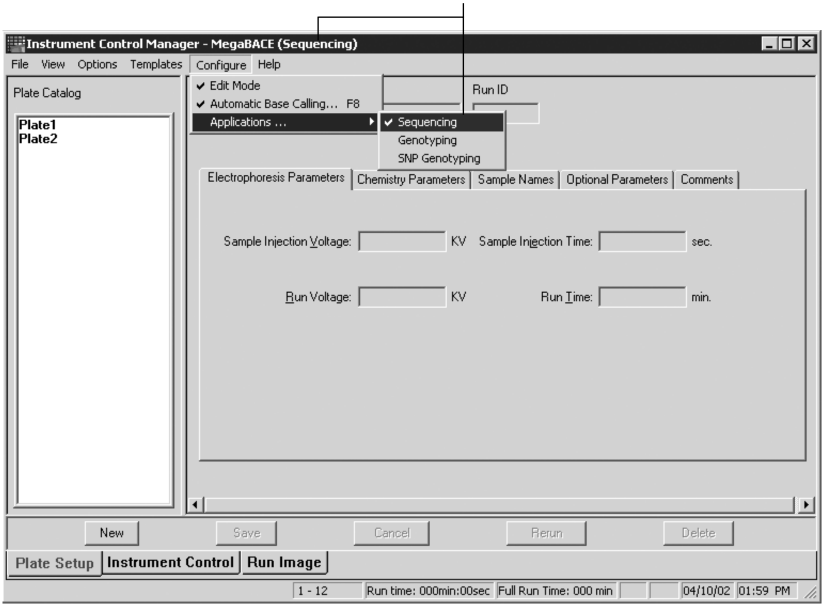
1. Make sure the Inject Samples and Run protocol is not running.
2. Make sure the application is selected for which you want to specify the storage location. Check the title bar of the Plate Setup window (figure 2a) or the Instrument Control window. The name of the application appears in the title bar next to the window name.

   If you need to change the application, on the Configure menu, point to **Applications,** and click the application name (for example, Sequencing).
3. Choose **Raw Data Storage** from the Options menu. The Browse for Folder window appears (figure 2b).
4. Select the local hard drive and folder you want to use and click **OK.** The window closes.

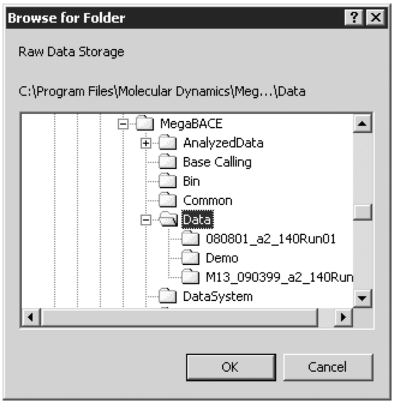
The next time you perform a run for the application selected in step 2, the software automatically stores the collected data in an application-specific data folder.

(a) The Plate Setup window

Make sure the application is selected for which you want to specify the storage location.



(b) The Browse for Folder window



Raw Data Storage

C:\Program Files\Molecular Dynamics\Meg...\Data

Figure 2. Changing the raw data storage location: (a) the Plate Setup window and (b) the Browse for Folder window.

3 The Instrument Control window

The Instrument Control window contains two new tabs that display the instrument parameters (figure 3):

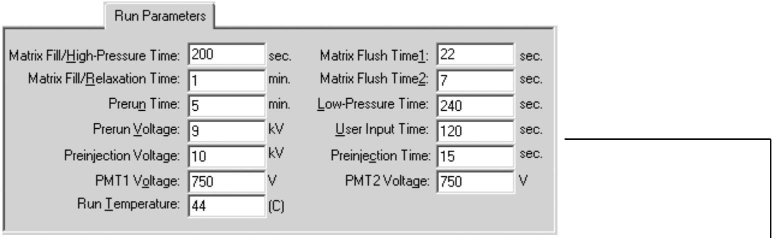
- **Run Parameters tab—**Contains the parameters that control the operation of the instrument during running of the protocols (figure 3a).

  Note: The value range of the PMT voltages is now 500–950 V.

- **Sleep Parameters tab—**Contains the field for selecting the Sleep After This Run option and allows you to specify the sleep duration and sleep temperature (figure 3b).

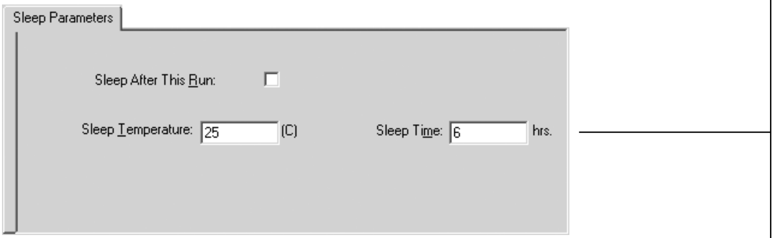
See the Help available within the Instrument Control Manager, or refer to the *MegaBACE Instrument Operator's Guide v2.4* for a description of the instrument parameters.

(a) The Run Parameters tab



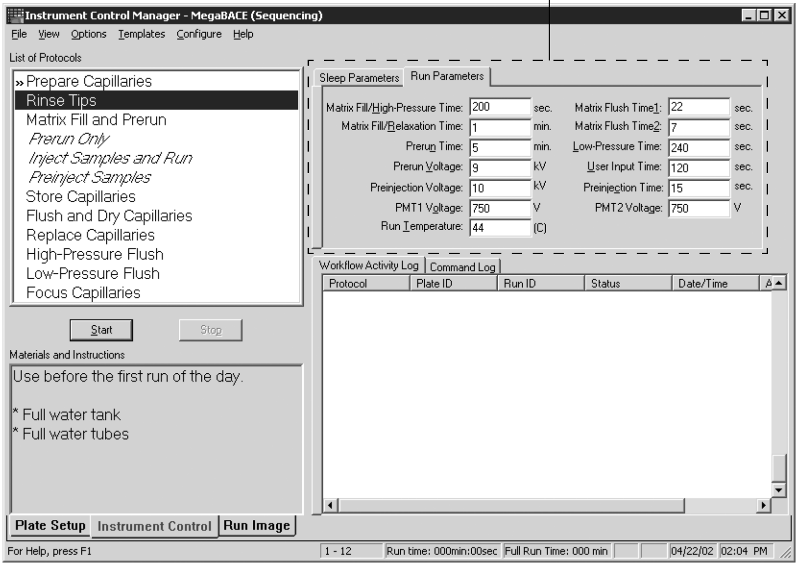
Matrix Fill/High-Pressure Time: 200 sec.
Matrix Fill/Relaxation Time: 1 min.
Prerun Time: 5 min.
Prerun Voltage: 9 kV
Preinjection Voltage: 10 kV
PMT1 Voltage: 750 V
Run Temperature: 44 (C)
Matrix Flush Time1: 22 sec.
Matrix Flush Time2: 7 sec.
Low-Pressure Time: 240 sec.
User Input Time: 120 sec.
Preinjection Time: 15 sec.
PMT2 Voltage: 750 V

(b) The Sleep Parameters tab



Sleep After This Run: ☐
Sleep Temperature: 25 (C)
Sleep Time: 6 hrs.

(c) The Instrument Control window



» Prepare Capillaries
Rinse Tips
Matrix Fill and Prerun
Prerun Only
Inject Samples and Run
Preinject Samples
Store Capillaries
Flush and Dry Capillaries
Replace Capillaries
High-Pressure Flush
Low-Pressure Flush
Focus Capillaries

Matrix Fill/High-Pressure Time: 200 sec.
Matrix Fill/Relaxation Time: 1 min.
Prerun Time: 5 min.
Prerun Voltage: 9 kV
Preinjection Voltage: 10 kV
PMT1 Voltage: 750 V
Run Temperature: 44 (C)
Matrix Flush Time1: 22 sec.
Matrix Flush Time2: 7 sec.
Low-Pressure Time: 240 sec.
User Input Time: 120 sec.
Preinjection Time: 15 sec.
PMT2 Voltage: 750 V

Use before the first run of the day.

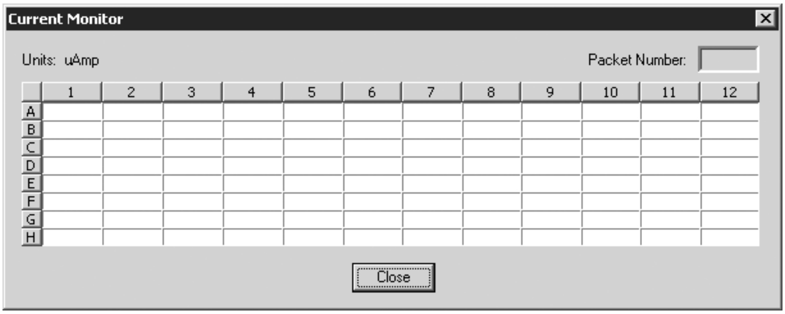
* Full water tank
* Full water tubes

For Help, press F1 | 1 - 12 | Run time: 000min:00sec | Full Run Time: 000 min | 04/22/02 | 02:04 PM

Figure 3. The Run Parameters tab (a) and the Sleep Parameters tab (b) in the Instrument Control window (c).

4 The Current Monitor window

The Current Monitor window (figure 4) allows you to check the electrical current in the capillaries during a run. Although the window has a new look, the functionality remains the same. See the Help available within the Instrument Control Manager, or refer to the *MegaBACE Instrument Operator's Guide v2.4* for a detailed description of how to use the Current Monitor window.



Units: uAmp

Figure 4. The Current Monitor window.

5 Focusing the capillaries

The Instrument Control Manager now allows you to focus the capillaries automatically or manually.

5.1 Materials required for the Focus Capillaries protocol

After replacing the capillary arrays, you must focus the capillaries using the Focus Capillaries protocol before you can use the new capillaries. For either the automatic or manual version of the Focus Capillaries protocol, you need the following materials (figure 5):

- A full tank, containing deionized filtered water.
- One tube for each installed array, each containing deionized filtered water (approximately 1.8-ml per tube). For example, you need six tubes if the instrument has six capillary arrays.
- One tube for each installed array, each containing LPA matrix (approximately 1.8-ml per tube).

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

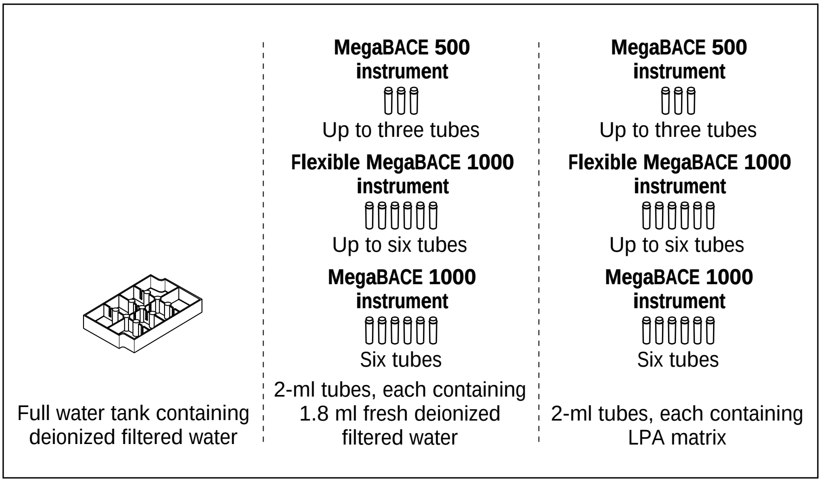


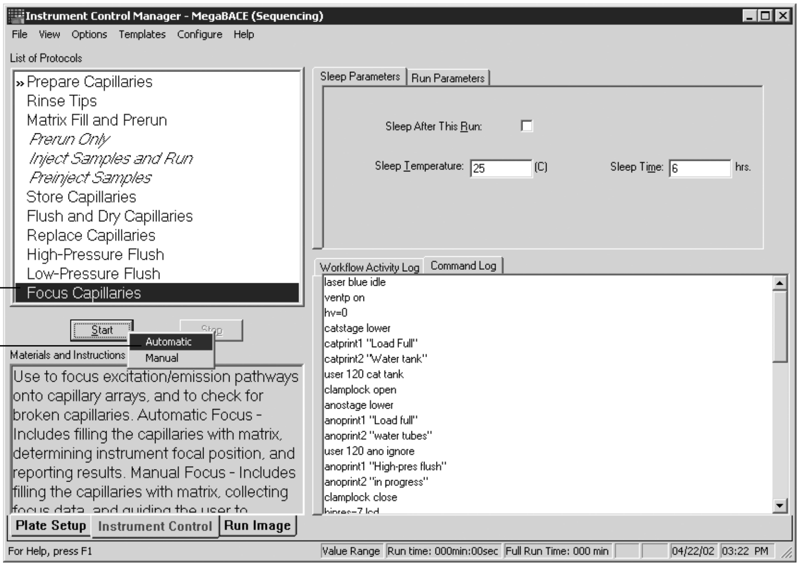
Figure 5. The materials required for the Focus Capillaries protocol.

5.2 Starting the Focus Capillaries protocol

To start the Focus Capillaries protocol—

1. In the Instrument Control window (figure 6), make sure the **Focus Capillaries** protocol is selected in the List of Protocols, and then click **Start.**

   Note: After the Replace Capillaries protocol, the software automatically selects Focus Capillaries as the next protocol.



Instrument Control Manager - MegaBACE (Sequencing)

List of Protocols

- » Prepare Capillaries
- Rinse Tips
- Matrix Fill and Prerun
- *Prerun Only*
- *Inject Samples and Run*
- *Preinject Samples*
- Store Capillaries
- Flush and Dry Capillaries
- Replace Capillaries
- High-Pressure Flush
- Low-Pressure Flush
- Focus Capillaries

Sleep After This Run: ☐

Sleep Temperature: 25 (C)

Sleep Time: 6 hrs.

Materials and Instructions

Use to focus excitation/emission pathways onto capillary arrays, and to check for broken capillaries. Automatic Focus - Includes filling the capillaries with matrix, determining instrument focal position, and reporting results. Manual Focus - Includes filling the capillaries with matrix, collecting focus data, and guiding the user to

```
laser blue idle
ventp on
hv=0
catstage lower
catprint1 "Load Full"
catprint2 "Water tank"
user 120 cat tank
clamplock open
anostage lower
anoprint1 "Load full"
anoprint2 "water tubes"
user 120 ano ignore
anoprint1 "High-pres flush"
anoprint2 "in progress"
clamplock close
hipres=7 lcd
```

For Help, press F1 | Value Range | Run time: 000min:00sec | Full Run Time: 000 min | 04/22/02 | 03:22 PM

1. Select Focus Capillaries.
2. Click Start, and then select Automatic or Manual.

Figure 6. Starting the Focus Capillaries protocol in the Instrument Control window.

2. In the pop-up window that appears, select either—
   - **Automatic—**Automatically calculates the optimal focus point and then focuses the capillaries.
   - **Manual—**Requires you to manually calculate the focusing value before the software can complete the Focus Capillaries protocol.
3. When instructed by the instrument displays, load the **full water tank** in the left side of the instrument. Then load the **tubes containing water** in the right side of the instrument. The instrument displays tell you that a high-pressure flush is in progress.

4. When instructed by the instrument displays, load the **matrix tubes** in the left side of the instrument. The instrument displays tell you that the capillaries are being filled.

   Next, the instrument displays tell you to **Inspect capillaries for leaks.** A Confirm to continue window appears and tells you to open the service door and inspect for leaks.

5. Open the electrophoresis compartment lid (service door), and look for signs of liquid in the electrophoresis compartment. Liquid in the compartment indicates that a capillary is broken.

   - If you do not detect a leak, click **Continue,** and proceed to step 6.
   - If you find a leak, click **Stop.** Refer to the *MegaBACE Instrument Maintenance and Troubleshooting Guide v2.4* for instructions on how to replace the leaking capillary array.

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

6. When the Confirm to continue window reappears and tells you to close the service door, close the electrophoresis compartment lid. Wait until the compartment comes up to the set run temperature, and then click **Continue.**

   - If you selected automatic focus, the instrument displays tell you that capillary focusing is in progress.
   - If you selected manual focus, a message appears, **Run Raw2Gel on [Current Storage Folder]\focus\cap.dat and analyze sep2.gel or sep4.gel using the ImageQuant software.** Then another message appears, **Specify scan line offset in Focus window.** See the *MegaBACE Instrument Maintenance and Troubleshooting Guide v2.4* for instructions on how to calculate the focusing value and complete the Focus Capillaries protocol.

The instrument displays tell you that capillary focusing is complete and show the time elapsed since completion.

6 Changes in the file structure (Administrator only)

The MegaBACE software folder contains two new folders: an Instrument Control folder and a Base Calling folder.

6.1 The new MegaBACE instrument control file path

During the Instrument Control Manager v3.1 software installation, the installer creates a MegaBACE\Instrument Control folder on the computer. The Instrument Control folder contains all the system files related to controlling the instrument. The Instrument Control folder includes the following folders:

- **DataSystem folder—**Contains the system initialization files, such as the MegaBACE.ini and Chemistry.ini.
- **Psd folder—**Contains the plate setup data files (.psd) that you create.
- **Templates folder—**Contains the plate setup and plate set setup templates (.tpl) and the instrument control parameters templates (.icp).

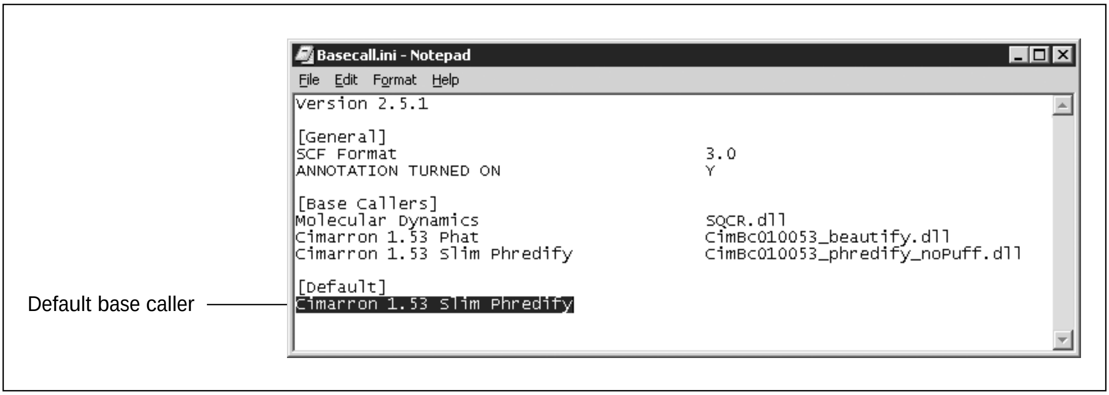
6.2 The new base calling file path (Sequencing only)

All the files that control the automated base calling feature are stored in the MegaBACE\Base Calling folder. The files in this folder are used by Sequence Analyzer v3.0 and later versions, as well as Instrument Control Manager v3.1.

6.3 Specifying the base calling options (Sequencing only)

The software includes an initialization file for the automated base calling feature (Basecall.ini). Instead of specifying a default base caller in the MegaBACE.ini file, you can set the default base caller in the Basecall.ini file (figure 7). The Basecall.ini file is in a two-column, tab-separated format. Table 1 provides a description of each section and field in the Basecall.ini file.

Important **For information on upgrading your software to use the most recent base callers, contact your MegaBACE representative. See Assistance in the preface for contact information.**



```
Version 2.5.1

[General]
SCF Format                              3.0
ANNOTATION TURNED ON                    Y

[Base Callers]
Molecular Dynamics                      SQCR.dll
Cimarron 1.53 Phat                      CimBc010053_beautify.dll
Cimarron 1.53 Slim Phredify             CimBc010053_phredify_noPuff.dll

[Default]
Cimarron 1.53 Slim Phredify
```

Figure 7. The Basecall.ini file allows you to specify a default base caller for automatic base calling.

Table 1. The sections and fields in the Basecall.ini file

Section and field name	Description	Setting type	Default
[General]			
SCF FORMAT	Used only if Cimarron base caller 3.12 or later is installed. Determines the SCF file format version.	2.0 or 3.0	3.0
ANNOTATION TURNED ON	Used only if Cimarron base caller 3.12 or later is installed. Determines whether the base caller produces annotation information, which is needed by the Sequencing Diagnostics software. **Note:** The base calling is slower when the setting is Y. However, you can set this field to N, and then use Sequence Analyzer to perform base calling on a run for which you need to use the Sequencing Diagnostics software.	Y or N	Y
[Base Callers]	Lists the available base callers. The first column lists the base caller names, and the second column lists the corresponding file names of the base callers.	–	–
[Default]	Specifies the default base caller for the laboratory.	–	Name of the most recent base caller