user manual

MegaBACE

SNP Genotyping
Instrument Operator's Guide

um 375-718

Amersham Biosciences

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

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

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June 2002

Notice to purchaser: limited license

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

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

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

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

Amersham Biosciences is a licensed vendor for authorized reagents.

The purchase price of the MegaBACE SNuPe genotyping kit includes a limited, non-transferable license under US Patent Numbers 5,888,819; 6,004,744; and their foreign counterparts owned by Orchid BioSciences Inc of Princeton, New Jersey, to perform only the number of Genotypes listed on the packaging for this product (For purposes of this End User License, Genotyping means the detection or quantification of an individual SNP within a single sample.) solely for the detection and analysis of SNPs in samples for research and development purposes, either alone or in a bona fide collaborations with one or more third parties, only and only on an instrument used for gel electrophoretic separation for nucleotide analysis. This license specifically excludes performing services for a third party and any and all diagnostic or therapeutic uses. Information about purchasing licenses to practice primer extension technology covered by Orchid BioSciences, Inc patents for any other use may be obtained by contacting the Senior Director for Business Development at Orchid BioSciences Inc, Princeton, New Jersey, US, at (609) 750-2200.

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

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Preface

About this guide

The *MegaBACE SNP Genotyping Instrument Operator's Guide* describes how to perform multi-injection runs on the MegaBACE instrument for the SNP genotyping application. The guide also includes a troubleshooting overview for the SNP genotyping application. **Note:** This guide is a supplement to the *MegaBACE Instrument Operator's Guide.*

Depending on the laboratory, you might have administrator responsibilities for the MegaBACE system in addition to being an instrument operator. Administrators are responsible for configuring the MegaBACE system initialization files, for creating parameter templates, and for creating plate setup data files (.psd). Administrators should refer to the *MegaBACE SNP Genotyping Instrument Administrator's Guide* for additional guidelines.

Related publications

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

- Protocol booklets in the MegaBACE SNuPe™ genotyping kit and SNuPe multiple-injection marker kit describe how to prepare your SNP samples before running them on the MegaBACE instrument.
- *MegaBACE SNP Genotyping Instrument Administrator's Guide* describes how to configure the MegaBACE system software for SNP genotyping. The guide also provides details on how to set up .psd files for multi-injection runs on the MegaBACE. **Note:** This guide is a supplement to the *MegaBACE Instrument Administrator's Guide.*
- *MegaBACE SNP Profiler User's Guide* describes how to use the software to perform SNP genotyping on the data collected from a SNP genotyping run. This guide also provides detailed troubleshooting guidelines for the SNP genotyping application.
- MegaBACE Instrument Guides—
  - *MegaBACE Instrument Operator's Guide* describes how to use the MegaBACE DNA analysis system to perform runs and use the instrument protocols. This guide focuses on the requirements for the sequencing and microsatellite genotyping applications.

- *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, use the configuration files, and how to set up .psd files. This guide focuses on the requirements for the sequencing and microsatellite genotyping applications.
- *MegaBACE Instrument Maintenance and Troubleshooting Guide* provides instructions on maintaining the instrument and guidelines on troubleshooting.

• *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.

Electronic versions of the documents listed above are available on the corresponding software CD.

Safety

The safety chapter in the *MegaBACE Instrument Operator's 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™ 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.

MegaBACE system site requirements

Electrical requirements

MegaBACE instrument

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

Power supply fan module

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

Environmental conditions

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

Assistance

When calling for assistance, be prepared to supply the serial number of your instrument. The serial number is located on the lower right side of the MegaBACE instrument. 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

Chapter 1 MegaBACE SNP genotyping system overview

The MegaBACE SNP genotyping system provides the tools required to detect and genotype single-nucleotide polymorphisms (SNPs). The system uses a proprietary method of increasing SNP screening throughput by repeated, time-spaced injections of short DNA samples into the same set of capillaries on the MegaBACE instrument. This chapter provides an overview of the system. The topics are—

- SNP genotyping system components (section 1.1)
- Overview of the Instrument Control Manager software (section 1.2)
- SNP genotyping system workflow overview (section 1.3)
- About the plate set definition and the plate attributes (section 1.4)
- Task overview for the SNP genotyping system (section 1.5)
- How the system detects the SNuPe products (section 1.6)
- Before you begin (section 1.7)

You should use this guide as a supplement to the *MegaBACE Instrument Operator's Guide.*

1.1 SNP genotyping system components

The MegaBACE SNP genotyping system consists of the—

- **MegaBACE SNuPe genotyping kit—**Contains a premix of fluorescently labeled terminators and enzyme to assay your SNP samples. The kit uses single-nucleotide primer extension (SNuPe) technology.

  The kit also contains control primers, control template, and spectral matrix standards. See the kit protocol for a detailed description of the contents and recommended sample preparation.

- **MegaBACE SNuPe multiple-injection marker kit—**Required by the SNP Profiler software to distinguish the data in each injection of a multi-injection run. See the kit insert for a description of the contents and recommended sample preparation.

- **Instrument with sequencing filter set—**The blue laser mode is used to excite the dyes in the SNuPE products, and the MegaBACE sequencing filters and beamsplitters are required to detect the dye emissions.

- **MegaBACE SNP software—**
  - **Instrument Control Manager v2.5 software—**Allows you to perform multi-injection runs on the MegaBACE instrument.
  - **SNP Profiler v1.0 software—**Performs automated SNP genotyping of MegaBACE SNuPe data.

  Note: You can use Sequence Analyzer to help troubleshoot the instrument performance (section 7.2).

1.2 Overview of the Instrument Control Manager software

For the SNP genotyping application, the Instrument Control Manager makes a distinction between a plate and a set of plates (plate set). A plate set is a group of sample plates that are serially injected in a single run. The software allows you to—

- Import the plate set definition from a master plate setup data file (.psd) file at the start of the Inject Samples and Run protocol (section 6.5). Alternatively, you can use the Plate Set Setup window to create the plate set definition before the run (chapter 5), and then select the plate set when you start the Inject Samples and Run protocol.
- Automatically define the instrument control parameters for a SNP genotyping run by using a template to specify the values (section 6.2). A template (default) containing the recommended parameters is included with the software.
- Import the attributes for each plate (SNP marker names and sample names) from .psd files (section 6.5.1). Alternatively, you can manually specify the plate ID and SNP marker name for each injection when you inject the sample plates (section 6.5.2).
- Use predefined instrument protocols for the SNP genotyping application to fill the capillaries with matrix and perform a prerun, and then inject the samples and perform a run. The Inject Samples and Run protocol allows you to serially inject multiple sample plates in a single run (section 6.5).
- Automatically store the collected data in an application-specific data folder. For example, you can store all the SNP genotyping runs in a folder that is separate from your sequencing runs (section 4.3).

See the *MegaBACE Instrument Operator's Guide* for details on how to use the instrument protocols, other than the Matrix Fill and Prerun protocol and the Inject Samples and Run protocol for SNP genotyping.

1.3 SNP genotyping system workflow overview

Figure 1-1 provides an overview of the SNP genotyping system workflow.

1.3.1 About sample preparation

You should prepare the SNP samples using the SNuPe genotyping kit and the multi-injection marker kit. Make sure you follow the kit protocols for sample preparation. See chapter 2 for additional guidelines on sample preparation and how to design your multi-injection run.

1.3.2 About testing the SNP markers

You should test each SNP marker on DNA samples of known genotypes before you use the SNPs in your experiments. You test the SNP markers to make sure you obtain the expected results when using the SNuPe genotyping kit and the MegaBACE SNP genotyping system. See section 2.1 for details. **Note:** You can test your SNP markers and collect the system spectral calibration data during a single multi-injection run.

1.3.3 System spectral calibration

To ensure reliable SNP genotyping on the MegaBACE data, you—

- **Perform a spectral calibration run—**You use the spectral matrix standards from the SNuPe genotyping kit to prepare the sample plate for the run. The run consists of at least six injections of the plate that contains the spectral matrix standards. See chapter 3 for details.
- **Create a spectral overlap matrix—**You use SNP Profiler to create a spectral overlap matrix from the spectral calibration run data. SNP Profiler uses the spectral overlap matrix during analysis to perform spectral separation.

See section 1.6 for a description of the spectral channels and how the system detects the SNuPe products.

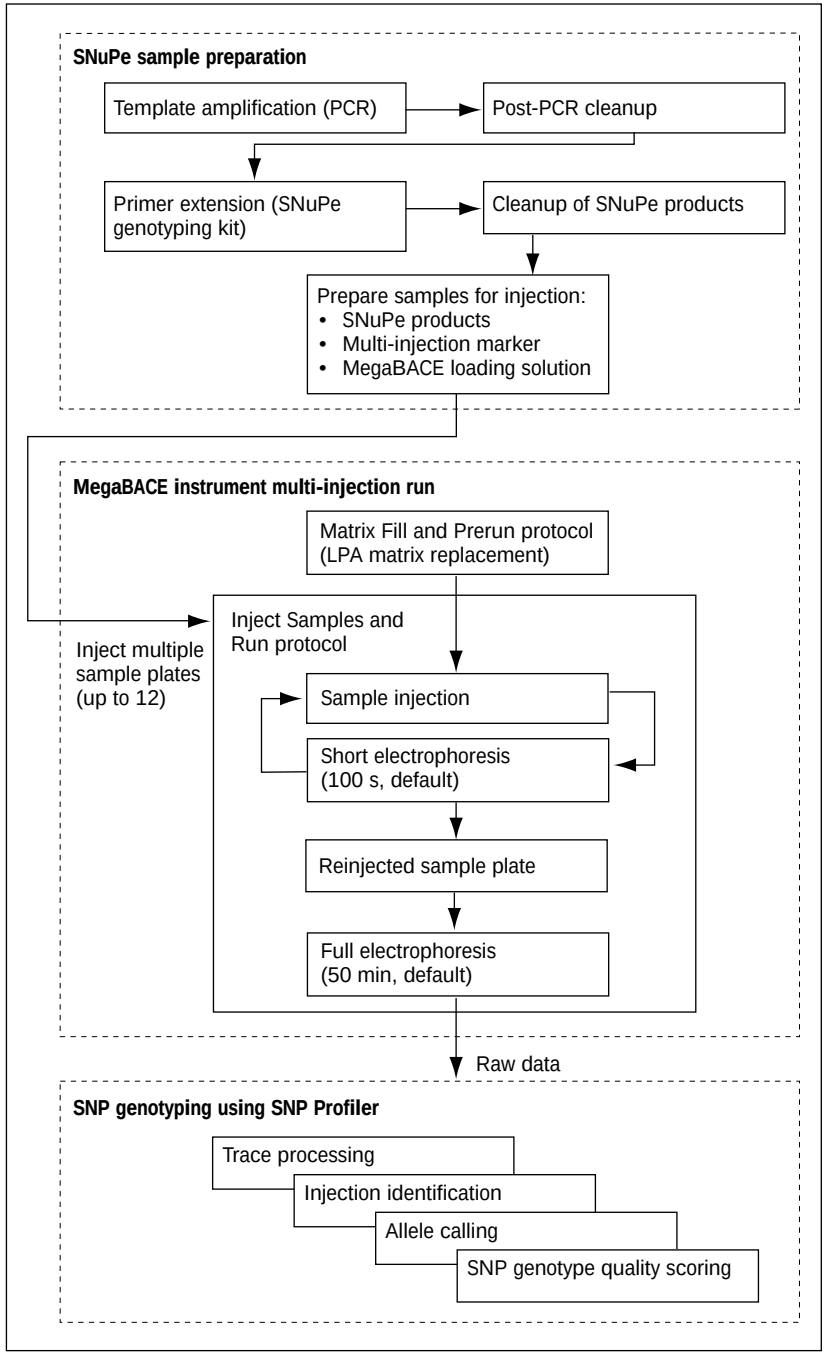


Figure 1-1. Workflow overview for the MegaBACE SNP genotyping system software.

1.3.4 About performing a multi-injection run

During a single run, you can serially inject multiple SNuPe sample plates into the same set of capillaries (multi-injection run). The Instrument Control Manager prompts you for the appropriate steps, including rinsing the capillaries, filling the capillaries with sieving matrix, performing a prerun, and performing the multiple sample injections for the electrophoresis run.

For each multi-injection run, you provide—

- A plate set definition (section 1.4.1)
- The plate-specific attributes for each injection (section 1.4.2)

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

- **Automated multi-injection workflow—**For the most automated workflow, you can import a master .psd file when you start the Inject Samples and Run protocol. The master .psd file contains the plate set definition and the plate-specific attributes for the first injection. For each additional injection, you import the plate-specific attributes from additional .psd files (section 6.5.1). Your administrator creates the .psd files for you to use *(MegaBACE SNP Genotyping Instrument Administrator's Guide).*
- **Alternative multi-injection workflow—**You can use the Plate Set Setup window to create the plate set definition (chapter 5). You can create a plate set definition before you perform each multi-injection run, or you can create multiple plate set definitions and then perform the runs later. The software allows you to select the precreated plate set ID when you start the Inject Samples and Run protocol. When you inject each plate for the multi-injection run, you can manually specify the plate ID and SNP marker name for the injection (section 6.5.2).

1.3.5 The multi-injection process

Important **All samples in a multi-injection run must be injected into the capillaries before the samples from the first injection reach the capillary detection windows.**

As figure 1-1 shows, each injection interval consists of a sample plate injection followed by a short electrophoresis interval. To complete the multi-injection process after you have injected the last sample plate, you must reinject one of the sample plates. By reinjecting a sample plate, you provide a multi-injection marker that flanks all the injections. SNP Profiler uses this final multi-injection marker to perform injection identification during the analysis.

After you complete the multi-injection process, the full electrophoresis begins.

1.4 About the plate set definition and the plate attributes

1.4.1 About the plate set definition

For each multi-injection run, you must provide a plate set definition. To provide the definition, you can either import a master .psd file when you start the Inject Samples and Run protocol, or you can use the Plate Set Setup window to create the definition before the run. A plate set definition consists of the—

- **Plate set ID—**The name for the set of plates you inject in a multi-injection run. You can provide the plate set ID using a .psd file, or you can type the plate set ID in the Plate Set Setup window. The software uses the plate set ID to name the raw run folder that stores the data from the run. **Note:** If you import the plate set definition from a master .psd file that is missing the plate set ID, the software uses the plate ID of the first plate (if included in the master .psd file) as the plate set ID. If the plate ID is also missing, the software uses the .psd file name.
- **Plate set setup parameters—**The electrophoresis parameters, the chemistry parameters, the file names, plate set comments, and other parameters shared by all the plates you inject in a single run. You can use a .psd file or a plate set setup template to provide the values for these parameters, or you can manually enter the values in the Plate Set Setup window.
- **(Optional) List of plates—**The list of bar codes for the plates contained in the plate set. You can specify the list of plates and the order in which to inject the plates only in the .psd file for the plate set.

1.4.2 The plate-specific attributes

The plate-specific attributes that you can provide for each plate in the multi-injection run can include—

- **(Optional) Plate ID—**You can provide a plate ID for each plate you inject. Depending on how your software is configured, you might import the information from a .psd file or manually type the plate ID at the time you inject the plate.
- **SNP marker names—**The Instrument Control Manager requires at least one SNP marker name for each injection. Depending on how your software is configured, you might import the information from a .psd file or manually type the SNP marker name at the time you inject the plate. To specify more than one SNP marker name per injection (one name per well), the software requires a .psd file for the injection.
- **(Optional) Sample names—**To specify the sample names for a plate, the Instrument Control Manager requires a .psd file for at least one plate in the multi-injection run. **Note:** If no sample names are provided during the run, SNP Profiler uses the well IDs as the sample names when you analyze the data.
- **(Optional) Plate comments—**To specify plate comments and other user-defined plate attributes, the software requires you to use a .psd file for the injection. **Note:** Any comments about the plate set should be included as part of the plate set setup parameters.

If you do not import the information for a given injection from a .psd file, you can specify only the plate ID and the SNP marker name.

1.5 Task overview for the SNP genotyping system

Table 1-1 provides a task overview for the MegaBACE SNP genotyping system. Your tasks can vary depending on whether you have administrator responsibilities in addition to operating the MegaBACE instrument. The administrator is responsible for the various files the Instrument Control Manager software uses.

Table 1-1. Task overview for the MegaBACE SNP genotyping system

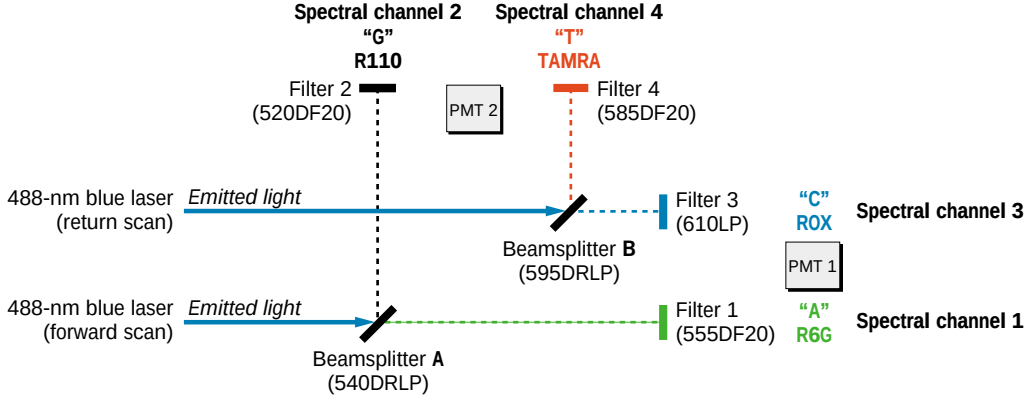
Task	Frequency	Reference
Although the Instrument Control Manager is already configured for SNP genotyping, your administrator can tailor the software settings to match your laboratory's workflow.	When you first receive the MegaBACE SNP genotyping system software	*MegaBACE SNP Genotyping Instrument Administrator's Guide*
Create .psd files to automate the definition of a plate set and specify the attributes of each plate in the set.	Recommended for every multi-injection run	*MegaBACE SNP Genotyping Instrument Administrator's Guide*
Start the MegaBACE system.	After any system shutdown	*MegaBACE Instrument Operator's Guide*
Test the SNP markers that you want to use.	Before using a new SNP marker	Section 2.1
Perform a spectral calibration run, and then create a spectral overlap matrix. SNP Profiler uses a spectral overlap matrix to perform reliable SNP genotyping.	For each instrument, periodically (monthly), depending on the throughput of your laboratory, and any time you— • Change your chemistry, run conditions, or protocols • Replace the capillary arrays • Move the instrument **Note:** If you are changing applications, you need to recalibrate only if there are changes to the run conditions, protocols, or chemistry within the SNP genotyping application.	Chapter 3
In the Instrument Control Manager, select the SNP genotyping application. Check that the correct filters are installed for detecting the SNuPe dye set.	Any time you change applications on the MegaBACE system. You might need to reselect the SNP genotyping application if you restart the Instrument Control Manager.	Chapter 4
Perform a SNP genotyping run.	–	Chapter 6
Use SNP Profiler to perform SNP genotyping on the collected data.	–	*MegaBACE SNP Profiler User's Guide*

1.6 How the system detects the SNuPe products

1.6.1 About the spectral channels

The MegaBACE system has four spectral channels that allow you to detect the emissions of four dyes per capillary (figure 1-2). A spectral channel is the combination of the beamsplitter, emission filter, and PMT that the system uses to detect the emissions from a specific dye.

For a typical four-color SNuPe run, the blue laser excites the dyes as the scan head moves forward and backward. Spectral channels 1 and 2 filter and detect the emissions from two dyes excited during the forward scan. Spectral channels 3 and 4 filter and detect the emissions from two other dyes excited during the return scan.



Notes:
Spectral channel 1—Detects emitted light from the forward scan through beamsplitter A and filter 1.
Spectral channel 2—Detects emitted light from the forward scan through beamsplitter A and filter 2.
Spectral channel 3—Detects emitted light from the return scan through beamsplitter B and filter 3.
Spectral channel 4—Detects emitted light from the return scan through beamsplitter B and filter 4.

Figure 1-2. The MegaBACE emission pathways. Each spectral channel detects the emission of a specific dye in the labeled sample. The example shows the spectral channels used to detect the dye terminators in the SNuPe genotyping kit.

1.6.2 The SNuPe dye set

A dye set is a collection of dyes that can be distinguished and detected efficiently in each capillary through the four MegaBACE spectral channels. Table 1-2 lists the dye terminators in the SNuPe genotyping kit, which have been validated for the MegaBACE SNP genotyping system.

Table 1-2. Dye terminators in the SNuPe genotyping kit

Dye	Excitation (nm)	Emission (nm)	Base (display color)	Channel
R6G	528	549	A (green)	1
R110	501	525	G (black)	2
ROX	587	607	C (blue)	3
TAMRA	560	582	T (red)	4

The channel-to-base mapping may change for different chemistries, but the display colors for the bases and the traces remain fixed. **Note:** The software displays the G trace in black for ease of viewing.

1.6.3 The SNuPe multi-injection marker

The SNuPe multi-injection marker is labeled with a fifth dye, which is detected in spectral channel 2. The Instrument Control Manager and SNP Profiler use the black trace to display both the SNuPe multi-injection marker and the G-type samples. The SNuPe multi-injection marker is easily distinguished from the sample peaks because of its characteristic double-peak pattern (figure 1-3).

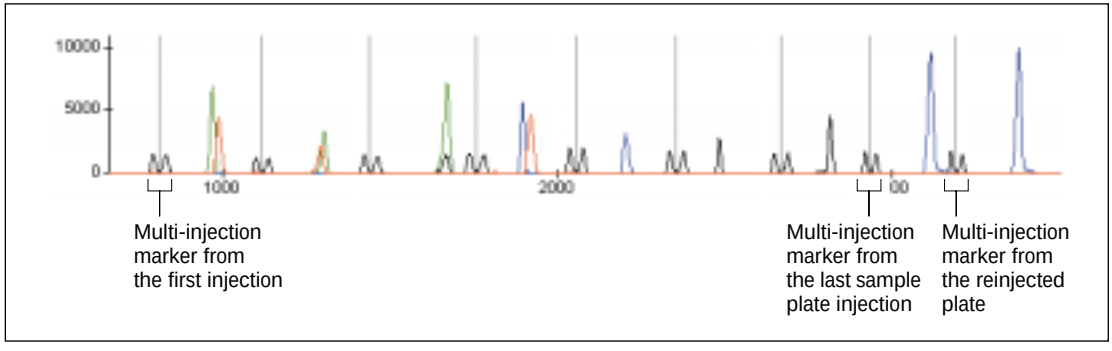


Figure 1-3. A trace-processed electropherogram from a MegaBACE SNuPe run.

1.7 Before you begin

Before using the MegaBACE SNP genotyping system, become familiar with the following topics in the *MegaBACE Instrument Operator's Guide:*

- Safety precautions
- Starting the MegaBACE system
- Instructions on how to use the instrument protocols

You should also become familiar with the following sections of this guide:

- Chapter 2: Sample preparation guidelines and how to design the run
- Chapter 3: The system spectral calibration requirements
- Chapter 4: How to change applications on the MegaBACE instrument

Chapter 2 Sample preparation and multi-injection run guidelines

This chapter provides guidelines for sample preparation and designing your MegaBACE runs. The topics are—

- Testing the SNP markers you want to use (section 2.1)
- Sample preparation workflow (section 2.2)
- Preparing the samples for loading (section 2.3)
- Designing the multi-injection run (section 2.4)

2.1 Testing the SNP markers you want to use

To make sure you obtain the expected results when using the SNuPe genotyping kit and the MegaBACE SNP genotyping system, you should test each SNP marker on DNA samples of known genotypes.

2.1.1 Workflow for testing the SNP markers

Table 2-1 provides a workflow overview for testing the SNP markers. Figure 2-1 illustrates the theory of the SNuPe chemistry.

Table 2-1. Workflow overview for testing the SNP markers

Task	Reference
1. Use the SNuPe genotyping kit to assay the SNP markers and the known DNA samples. Make sure you follow the protocols in the SNuPe genotyping kit and the multi-injection marker kit to prepare the samples.	Sections 2.2 and 2.3
2. Perform a multi-injection run:	Chapter 6
• If you have not already performed a spectral calibration run for your instrument and run conditions, you can combine the run to test the SNP markers and the spectral calibration run.	Chapter 3
• You can use plate setup data files (.psd) to specify different SNP markers, by well, for each injection.	*MegaBACE SNP Genotyping Instrument Administrator's Guide*

Table 2-1. Workflow overview for testing the SNP markers (continued)

Task	Reference
3. Use SNP Profiler to perform automated SNP genotyping on the data from the run.	*MegaBACE SNP Profiler User's Guide*
4. Make sure the SNP fits within the injection interval for the given set of interval conditions. Make sure you obtain the expected genotype results. To resolve unexpected results, see the troubleshooting guidelines in the *MegaBACE SNP Profiler User's Guide.* You can also try assaying the SNP marker by designing a new SNuPe primer on the opposite strand.	Section 2.1.2

2.1.2 Making sure the SNP allele peaks fit within the injection interval

When you test the SNP markers, make sure the MegaBACE system can detect the SNPs being assayed within the injection interval for the given set of interval conditions. If the SNuPe product migrates—

- Past the multi-injection marker of the subsequent injection, use a longer injection interval. See section 2.4.1 for guidelines on setting the interval time.
- Before the multi-injection marker for the given injection, the primer cannot be used. SNP Profiler cannot perform injection identification on the collected data. In this case, you should redesign the SNuPe primer to be longer or use the primer on the opposite strand.

For the highest throughput and most efficient turnaround time on the MegaBACE instrument, your SNuPe primers should be between 18 and 25 bases long. SNuPe primers of these lengths typically fall within the default injection interval parameters (100 s at 9 kV). However, some primers within the recommended size range (18–25 bases) might be unusable. Because the primers are such small pieces of DNA, primers of the same length can migrate very differently depending on their individual sequence.

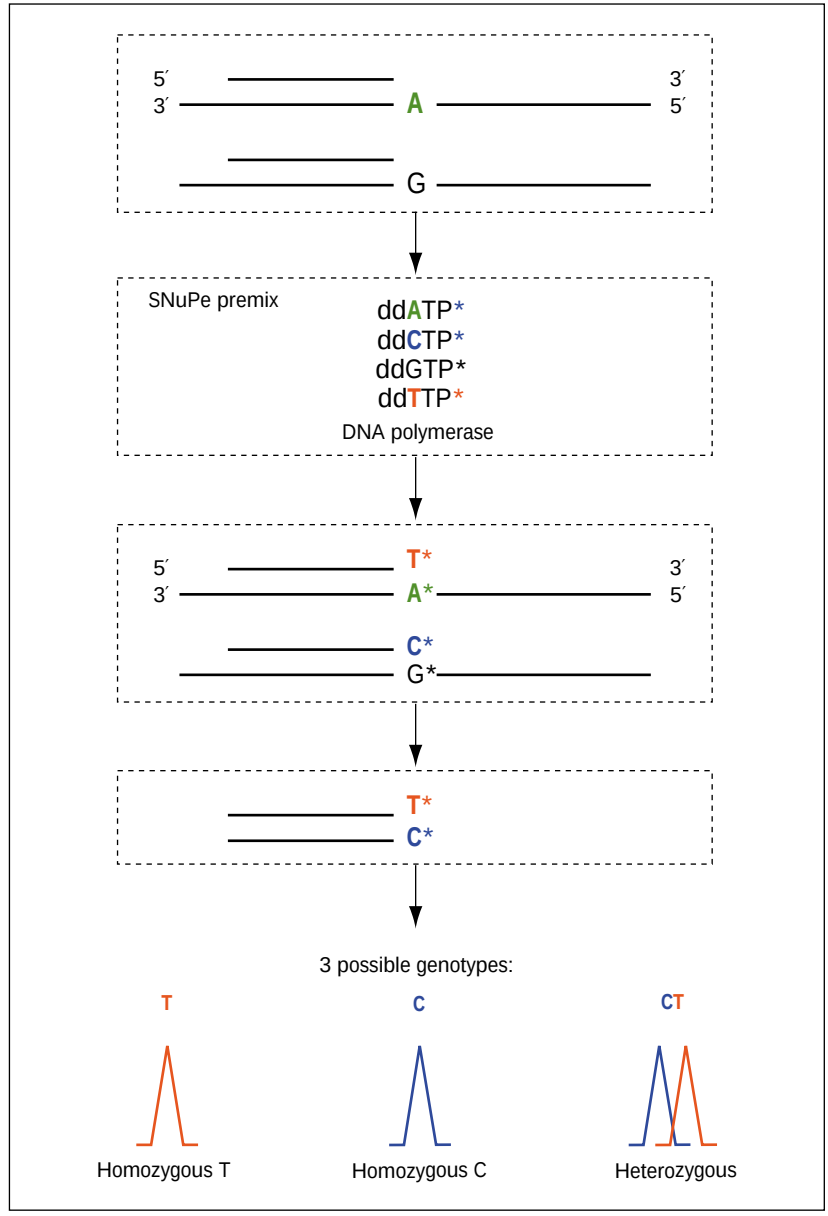


Figure 2-1. The SNuPe (Single Nucleotide Primer Extension) theory.

2.2 Sample preparation workflow

Figure 1-1 shows an overview of the SNuPe sample preparation workflow.

2.2.1 Template amplification (PCR) guidelines

To amplify the region of DNA that contains the SNP(s) of interest, use the following guidelines:

- Use PCR products that are >350 bp in length. If you must use short PCR products (<350 bp), see section 2.4.2 for additional guidelines.
- Make sure the PCR primer does not amplify in more than one region. To do this, conduct a BLAST (Basic Local Alignment Search Tool) search. For more information on BLAST, see the National Center for Biotechnology Information (NCBI) Web page at http://www.ncbi.nlm.nih.gov/BLAST/.
- You can design the PCR products to contain several SNPs.
- Make sure the PCR primers do not contain SNPs, as this will yield unexpected SNuPe results.

2.2.2 Post-PCR cleanup guidelines

To remove excess dNTPs and residual PCR primers, you must perform post-PCR cleanup. Amersham Biosciences recommends ExoI/SAP for post-PCR cleanup:

- ExoI (Exonuclease I) removes residual PCR primers.
- SAP (shrimp alkaline phosphatase) removes excess dNTPs.

For ExoI/SAP ordering information, see the Amersham Biosciences Web site at www.amershambiosciences.com.

(Optional) You can run the PCR products on an agarose gel to make sure the amplification is adequate and that no extra bands of DNA are present.

2.2.3 SNuPe primer design guidelines

To make sure that you have optimum results on the MegaBACE instrument, use the following guidelines for designing the SNuPe primers:

- Purify your SNuPe primers with high-performance liquid chromatography (HPLC) to make sure the primers yield clean results.
- For the highest throughput and most efficient turnaround time on the MegaBACE instrument, the length of the primer should be between 18 and 25 bases. See section 2.1 for additional guidelines on primer length.

- Use primer-design software to check for primers that form “hairpins.” If the SNuPe primer forms a hairpin, the primer might extend by using itself as the template. Using such a primer can result in poor quality genotypes.
- Using a G-base in the 3´-position of your SNP primer (for example, TCGGATCGTACAACTCTCG) might cause uneven incorporation of the SNP bases being assayed (figure 2-1). You can choose a primer without a G-base in that position by selecting the primer on the opposite strand.
- If there is a SNP in the region where you want to design your SNuPe primer, you can synthesize your SNuPe primer with a mixed base (wobble base) in the position that contains the additional SNP.

2.2.4 Cleanup of SNuPe products

Caution **To achieve efficient electrokinetic injection for SNP genotyping, you must desalt your samples. Failure to desalt the samples can result in data with low signal intensity or no signal.**

Prior to electrokinetic injection on the MegaBACE instrument, you must clean up the SNuPe products to—

- Remove excess terminators
- Desalt the sample

Cleaning up the SNuPe products can help you to achieve sufficient signal intensity from the samples.

To clean up the SNuPe products, you should filter the SNuPe products through a 96-well filtration plate. The filtration method yields SNuPe products with the best and most consistent signal intensity. Various cleanup methods exist, but other cleanup methods can result in poorer quality data.

Amersham Biosciences recommends that you use the AutoSeq™96 dye terminator clean-up kit. See the Web site at www.amershambiosciences.com for ordering information. Make sure you perform at least one additional wash with water before adding samples to the columns.

2.3 Preparing the samples for loading

This section describes how to prepare the SNP samples for loading in the MegaBACE instrument.

2.3.1 Materials required for sample loading

- "Clean" SNuPe products. See the SNuPe genotyping kit protocol for detailed instructions on how to prepare the SNuPe products.
- MegaBACE SNuPe multi-injection marker. See the multi-injection marker kit protocol for detailed instructions on how to prepare the multi-injection marker.
- MegaBACE loading solution.

2.3.2 Procedure for preparing the samples for loading

Warning

Use good laboratory practice 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.

For each 96-well plate to be loaded—

1. Combine the MegaBACE loading solution and the SNuPe multi-injection marker using the following proportions:

   497.5 µl loading solution

   2.5 µl multi-injection marker

   500.0 µl Total

2. Vortex the mixture prepared in step 1 and dispense 5 µl in each well on the plate.
3. Dispense 5 µl "clean" SNuPe product in each well on the plate. Make sure the total volume in each well is 10 µl. The plate can contain one SNP reaction per well.
4. Vortex the prepared samples thoroughly, and then spin down the sample plate in a tabletop centrifuge.

2.4 Designing the multi-injection run

2.4.1 Determining the injection interval time

You should select an injection interval time for the run based on the length of the SNuPe primers and the results from the initial testing of your SNP markers

(section 2.1). The interval time that you set for a given run applies to all the injections for the run. Therefore, you should—

- Determine the interval time based on the SNP product in the run that requires the longest interval.
- Try to run the SNPs requiring similar injection intervals in the same run.

Table 2-2 lists the maximum number of injections that can be performed with each interval time.

Table 2-2. The injection interval time and maximum number of injections

Interval time*	Interval voltage	Maximum number of sample injections†
80 s	9 kV	12
90 s	9 kV	10
100 s	9 kV	9
120 s	9 kV	8

* For injections with intervals less than 100 s at 9 kV, make sure you carefully optimize the SNPs.
† The number of sample injections does not include the final injection for which you reinject a sample plate.

2.4.2 Determining the order in which you inject the plates

If the PCR product is too short (<350 base pairs), an extra A peak might be added to the PCR product (+A effect). If the +A peak(s) overlap the peaks in subsequent injections, SNP Profiler might not be able to perform accurate allele calling. To make sure the potential +A peaks do not overlap with the allele peaks from other injections, you should load the samples made from short PCR products in the last few injections.

2.4.3 About the number of SNP markers

For each injection, the plate can contain one SNP reaction per well for the SNP Profiler analysis. You provide the SNP marker names during the run so that the collected data can be analyzed using SNP Profiler. The Instrument Control Manager requires at least one SNP marker name for each injection. You can specify a global SNP marker name for each plate you inject, or you can specify a SNP marker name for each well in the plate. To specify the SNP marker names for a run, you can—

- Import SNP marker names from a plate setup data file (.psd) during each injection. To specify more than one SNP marker per injection, you must use a .psd file. The .psd file for the injection can also specify the sample

names for the plate. Your administrator sets up the .psd files for the run *(MegaBACE SNP Genotyping Instrument Administrator's Guide).*

- Manually enter one SNP marker name per plate for each injection. If you do not import a .psd file containing the SNP marker names for a given injection, the software displays a window that allows you to specify one SNP marker name for the entire plate.

Chapter 3 Performing spectral calibration for SNP genotyping

This chapter describes the system spectral separation requirements and how to perform a spectral calibration run for SNP genotyping. The topics are—

- Why you perform spectral calibration runs for SNP genotyping (section 3.1)
- Frequency of spectral calibration (section 3.2)
- About preparing the plate for the spectral calibration run (section 3.3)
- Performing a spectral calibration run (section 3.4)

3.1 Why you perform spectral calibration runs for SNP genotyping

To ensure reliable SNP genotyping on the MegaBACE data, you must remove the spectral overlap from the raw data. A spectral calibration run measures the spectral overlap present in each spectral channel for a specific instrument and a given set of run conditions. For the spectral calibration run, you use a single plate that contains the spectral matrix standards for each dye in the SNuPe dye set.

You use the data from the spectral calibration run to create a spectral overlap matrix in SNP Profiler. SNP Profiler uses the spectral overlap matrix during automated analysis to perform spectral separation on the raw data for each electropherogram.

Each dye in a multicolor experiment emits fluorescent light that is filtered and detected through a spectral channel of the MegaBACE instrument (section 1.6). However, portions of the fluorescent emission from each dye usually occur within the emission range of the other dyes in the dye set and can be present in other spectral channels. The overlap in the fluorescent emissions across the spectral channels is called spectral overlap.

3.2 Frequency of spectral calibration

For each MegaBACE instrument, you perform a spectral calibration run and create a new spectral overlap matrix anytime you—

- Change your chemistry (reaction chemistry and dyes), run conditions (time and voltage for the injection, the interval, and the run), or protocols

  Note: If you are switching between applications (sequencing, microsatellite genotyping, SNP genotyping), you need to recalibrate only if there are changes within the application.

- Replace the capillary arrays
- Move the instrument

You should also recalibrate the system periodically (monthly), depending on the throughput of your laboratory.

3.3 About preparing the plate for the spectral calibration run

3.3.1 Materials required for spectral calibration

- SNuPe spectral matrix standards. In the SNuPe genotyping kit, you should receive the following four tubes for doing the spectral calibration run. Each SNuPe spectral matrix standard contains one peak labeled with the given dye.
  - A (R6G)
  - G (R110)
  - C (ROX)
  - T (TAMRA)
- MegaBACE loading solution

3.3.2 Designing the plate layout

See the SNuPe genotyping kit protocol for instructions on how to prepare the dye samples using the spectral matrix standards. To prepare the plate, you can either—

- Prepare a plate containing only the dye samples.
- Prepare a plate that contains dye samples in some wells and SNuPe samples in other wells. (For example, you can reserve some wells on the plate for

the SNP markers that you want to test.) You must have at least one well for each dye sample. However, you may want to use a pair of wells for each dye sample in case there is a problem with the signal from one of the wells.

Make sure the total volume in each well is 10 µl minimum.

3.4 Performing a spectral calibration run

To perform a spectral calibration run for SNP genotyping—

1. Check that the sequencing filter set is installed in the instrument and that the SNP genotyping application is selected in the Instrument Control Manager (section 4.2.1).
2. Perform a multi-injection run with at least six injections (chapter 6), using the same sample plate (section 3.3.2) for each injection. Because SNP Profiler requires at least six peaks in each color to create a valid spectral overlap matrix, you inject the plate at least six times.

   Note: Even though the plate might not contain SNP markers, the Instrument Control Manager requires you to provide at least one SNP marker name for each run. Instead of a SNP marker name, you can enter a descriptive name as a placeholder, such as dye_matrix. However, if the spectral calibration plate also contains SNuPe samples in some wells, you do not need to provide a placeholder for the SNP marker name.

After the spectral calibration run is complete, use SNP Profiler to create a spectral overlap matrix from the collected data. For details, see the *MegaBACE SNP Profiler User's Guide* or the Help available within SNP Profiler.

Chapter 4 Changing applications on the MegaBACE instrument

If you are using the MegaBACE instrument for multiple applications, you need to change the application settings each time you want to perform runs for a different application. This chapter describes how to change applications on the MegaBACE instrument. The topics are—

- Requirements for each application (section 4.1)
- Changing the application (section 4.2)
- Specifying application-specific data storage (section 4.3)

4.1 Requirements for each application

In addition to SNP genotyping, the Instrument Control Manager supports the sequencing and microsatellite genotyping applications. Before you can use the MegaBACE system for multiple applications, your instrument must be configured to support the appropriate applications. Table 4-1 lists the laser and filter configuration required for each application.

Table 4-1. Lasers and filters required for each application

Application	Laser mode	Filters and beamsplitters
SNP Genotyping	Blue	Sequencing filter set
Sequencing	Blue	Sequencing filter set
Genotyping	Green and blue	Genotyping filter set I or filter set II

In addition, your site must have the appropriate software license for the analysis software.

4.2 Changing the application

To change applications on the MegaBACE instrument, you—

1. Check that the correct filter set is installed (section 4.2.1).
2. Select the application and the associated parameter templates in the Instrument Control Manager (section 4.2.2).

4.2.1 Checking that the correct filter set is installed

Make sure the appropriate filter set is installed in the instrument for the application that you want to run. Make sure that each spectral channel contains the appropriate filter from the filter set. As table 4-2 shows, you use the sequencing filter set for both the SNP genotyping and sequencing applications. Table 4-3 shows the filter sets you can use for microsatellite genotyping. For instructions on how to change the filters installed in the instrument, see the *MegaBACE Instrument Maintenance and Troubleshooting Guide.*

Table 4-2. The sequencing filter set and the SNP genotyping and sequencing dyes

SNP genotyping SNuPe dye (base order)	Sequencing DYEnamic™ ET terminator dye (base order)	Sequencing ET primer dye (base order)	Spectral channel	Filter	Beamsplitter
R6G (A)	ET-R6G (T)	ET-R6G (A)	1	555DF20	A: 540DRLP
R110 (G)	ET-R110 (G)	ET-R110 (C)	2	520DF20	
ROX (C)	ET-ROX (C)	ET-ROX (T)	3	610LP	B: 595DRLP
TAMRA (T)	ET-TAMRA (A)	ET-TAMRA (G)	4	585DF20	

Table 4-3. The microsatellite genotyping filter sets and dyes

Dye	Color	Spectral channel	Filter	Beamsplitter
Dye set 2* and filter set 2				
ET-ROX	Red	1	610DF20	A: 540DRLP
FAM	Blue	2	520DF20	
NED	Yellow or black	3	580DF20	B: 570DRLP
HEX	Green	4	555DF20	
Dye set 1 and filter set 1 (alternative filter set)				
ET-ROX	Red	1	610DF20	A: 540DRLP
FAM	Blue	2	520DF20	
HEX	Yellow or black	3	565DF20	B: 555DRLP
TET	Green	4	545DF20	

* Alternatives for dye set 2 are ET-ROX, FAM, TAMRA, and HEX, or ET-ROX, FAM, TAMRA, and JOE.

Note: You can use the filter cards that are included with this user's guide to keep track of which filters are currently installed in the instrument. Each time you change the filter set, make sure the corresponding filter card is posted on the instrument.

4.2.2 Selecting the application and the associated parameter templates

When you change the selected application in the Instrument Control Manager, the change remains in place until you select a different application or restart the software. Each time you restart the Instrument Control Manager, the software automatically selects the default application. Your administrator can specify SNP genotyping as the default application *(MegaBACE SNP Genotyping Instrument Administrator's Guide).*

1. Make sure the Instrument Control Manager is open but that no protocols are running. From the Configure menu, point to **Applications** and then choose the **name of the application** you want to use. The options are Sequencing, Genotyping, or SNP Genotyping. A check mark appears in front of the selected application. The title bar of each of the Instrument Control Manager windows changes to display the name of the selected application.
2. If you selected—
   - **SNP Genotyping—**Click the **Plate Set Setup** tab at the bottom of the window to display the Plate Set Setup window (figure 4-1).
   - **Sequencing or Genotyping—**Click the **Plate Setup** tab to display the Plate Setup window.

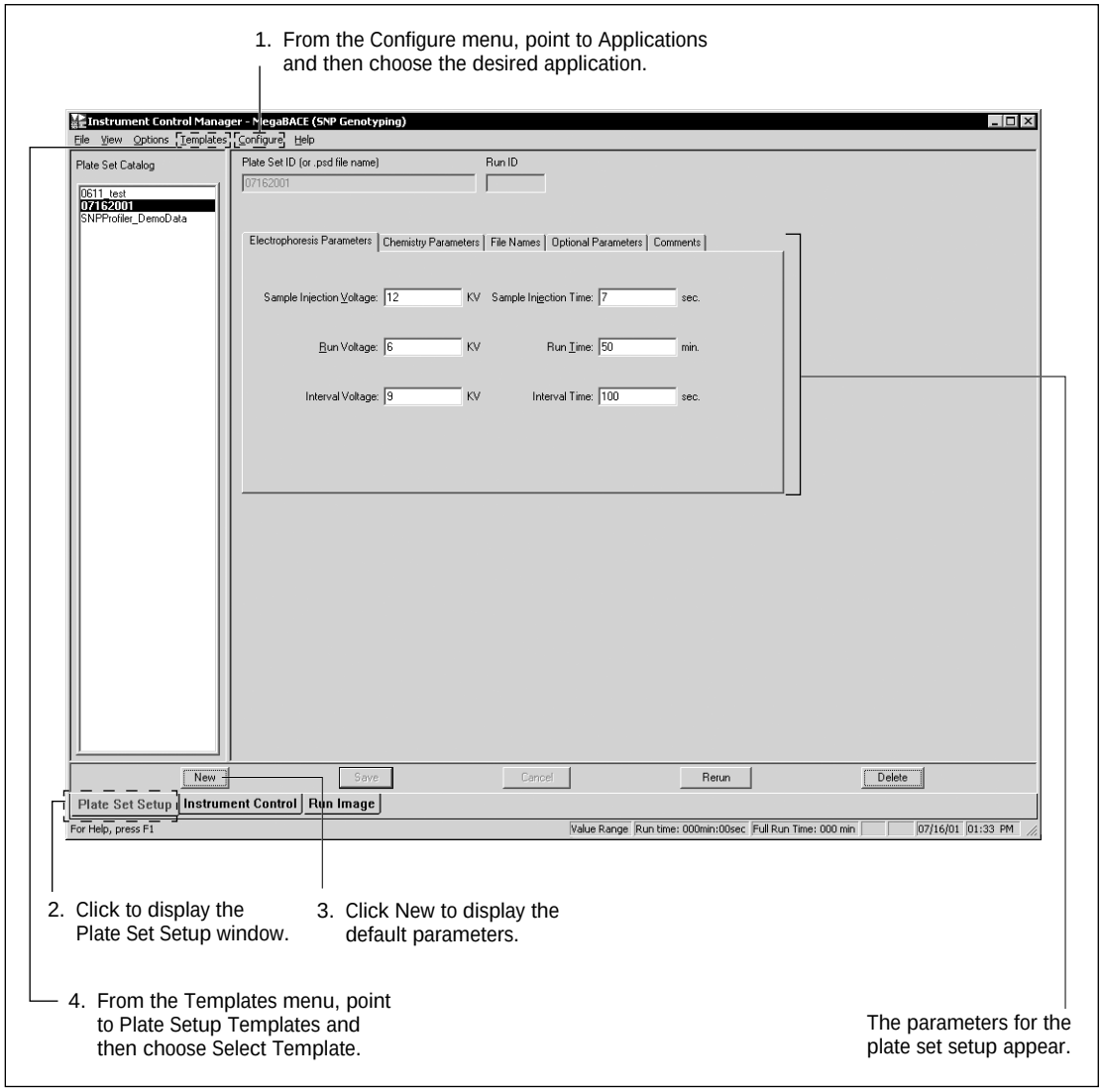


Figure 4-1. The Plate Set Setup window for the SNP genotyping application.

3. In the Plate Set Setup window (SNP genotyping) or Plate Setup window (sequencing or microsatellite genotyping), click **New.**

   - If your software is configured to use a default plate setup template for the selected application, the values for the parameters appear in the window. Only the applicable parameters for the selected application appear. Skip to step 7.
   - If your software has not been configured to use a default template, the parameter boxes in the tabs of the window are empty. To choose a template for the selected application, follow the instructions in steps 4 through 6.

Important **The edit mode must be turned on to allow you to select different templates. If the edit mode is not enabled, see your administrator *(MegaBACE Instrument Administrator's Guide).***

4. From the Templates menu, point to either—

   - **Plate Set Setup Templates** (SNP genotyping)
   - **Plate Setup Templates** (sequencing or microsatellite genotyping)

   Choose **Select Template.** The Select Template window appears and displays the available plate setup templates (figure 4-2).

5. In the Select Templates window, choose the appropriate **template (.tpl)** for your application. The following templates are included with the software, but you might have different choices depending on how your administrator has configured the software:

   - **SNP Genotyping—**The software includes the StdSNP.tpl for the SNuPe chemistry.
   - **Sequencing—**The software includes the StdDyePrimer.tpl, StdDyeTerminator.tpl, LongDyePrimer.tpl, and the LongDyeTerminator.tpl.
   - **Genotyping—**The software includes StdGenotyping.tpl for the dye set II chemistry.

6. Click **Open.** The Plate Set Setup window (SNP genotyping) or the Plate Setup window (sequencing or microsatellite genotyping) displays the parameters for the selected template (figure 4-1).

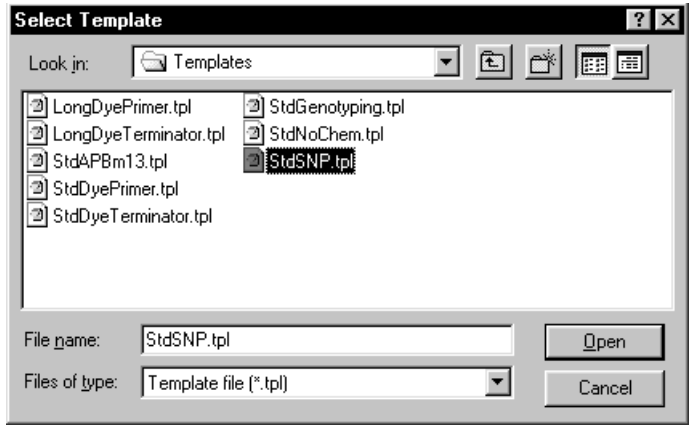


Figure 4-2. The Select Template window displaying the available plate setup templates.

7. Click the **Instrument Control** tab at the bottom of the window to display the Instrument Control window (figure 4-3).
   - If your software is configured to use a default instrument control template for the selected application, the values for the parameters appear in the Instrument Parameters area.
   - If your software has not been configured to use a default template, the Instrument Parameters area does not contain values. To choose an instrument control template for the selected application, follow the instructions in steps 8 through 10.
8. From the Templates menu, point to **Instrument Templates** and choose **Select Template.** The Select Template window appears (figure 4-4) and displays the available instrument control templates.
9. In the Select Template window, choose the appropriate **instrument control template (.icp)** for your application. The following templates are included with the software, but you might have different choices depending on how your administrator has configured the software:
   - **SNP genotyping—**The default template is SNP_Typing.icp.
   - **Sequencing—**The default template is Normal.icp.
   - **Genotyping—**The default template is Genotyping.icp.
10. Click **Open.** The Instrument Control window displays the parameters for the selected template (figure 4-3).

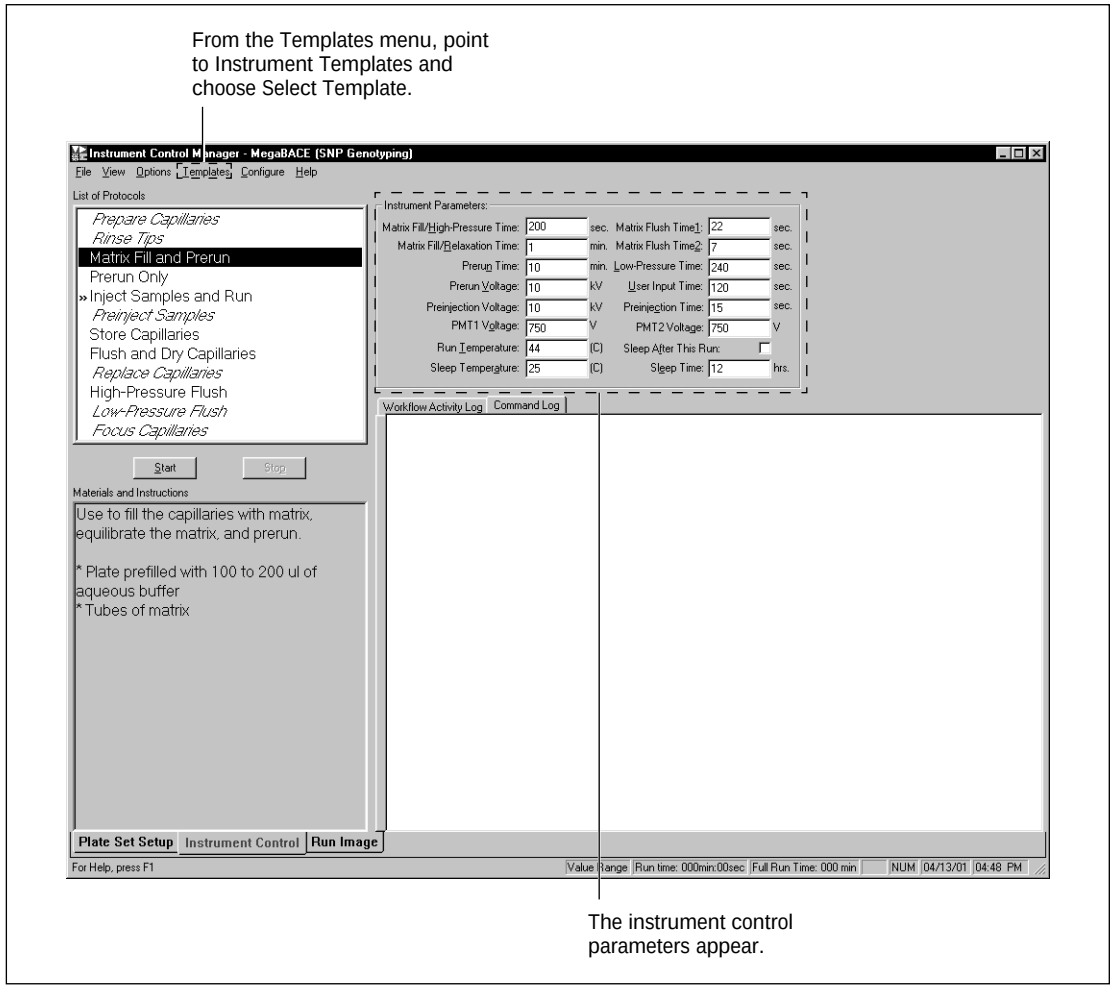


Figure 4-3. The Instrument Control window.

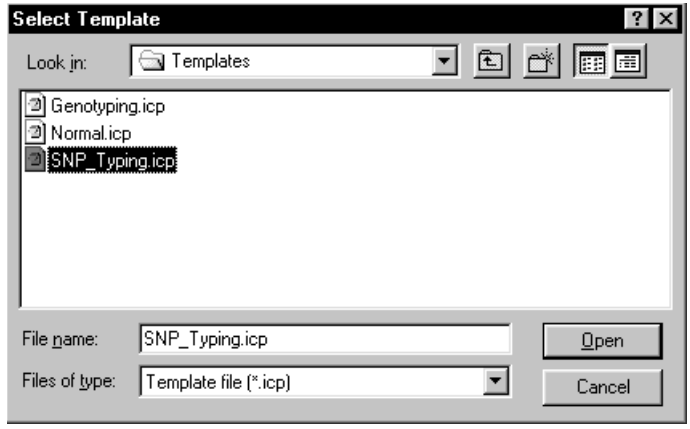


Figure 4-4. The Select Template window displaying the available instrument control templates.

4.3 Specifying application-specific data storage

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

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

After each run, the Instrument Control Manager stores the raw sample data files (.rsd) representing the collected data in a raw run folder. Unless you specify a different location, the software stores the run data for all applications (SNP genotyping, sequencing, and microsatellite genotyping) in the same folder …\MegaBACE\Data folder (default).

You can change the default data storage location so that the software automatically stores the raw run data for each type of application in distinct data folders. To specify the raw data storage location for an application—

1. Make sure the Instrument Control Manager is open but that no protocols are running. From the Configure menu, point to **Applications** and then choose the **name of the application** you want to use. The options are Sequencing, Genotyping, or SNP Genotyping. A check mark appears in front of the selected application. The title bar of each of the Instrument Control Manager windows changes to display the name of the selected application.
2. From the Options menu, choose **File Storage.** The File Storage window appears (figure 4-5).

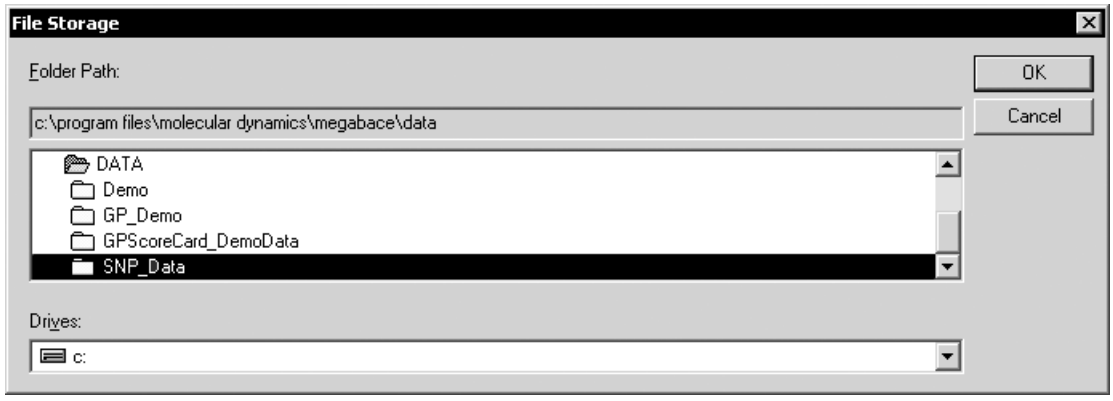


Figure 4-5. The File Storage window.

3. In the Drives box, make sure the desired **drive** appears. If you want to select a different drive, use the list scroll bar to display the available drives.
4. In the Folder Path box, choose the **folder** on this drive where you want to store the data for the selected application. Use the list scroll bar to display the available folders.
5. Click **OK.** The window closes.

The next time you perform a run for the application selected in step 1, the Instrument Control Manager stores the raw run data at the new application-specific location.

Chapter 5 Creating a plate set definition before the run

This chapter describes how to create plate set definitions before the run using the Plate Set Setup window. The topics in this chapter are—

- Alternative workflows for creating the plate set definitions (section 5.1)
- About the Plate Set Setup window and the plate set parameters (section 5.2)
- Creating the plate set definition automatically (section 5.3)
- Creating the plate set definition manually (section 5.4)

If your laboratory uses a plate setup data file (.psd) to import the plate set definition during the run, you can skip this chapter and proceed to chapter 6.
Note: The *MegaBACE SNP Genotyping Instrument Administrator's Guide* describes how to create .psd files.

5.1 Alternative workflows for creating the plate set definitions

You can create a plate set definition before you perform each multi-injection run, or you can create multiple plate set definitions and then perform the runs later. Before the run, you can create the plate set definition automatically using the Plate Set Setup window (figure 5-1) in the Instrument Control Manager. To do this, you enter a plate set ID and either—

- Import the plate set parameters from a master .psd file.
- Use a template to specify the plate set parameters. A template containing the recommended parameters (default) is provided with the software.

Alternatively, you can manually create the plate set definition if the edit mode is on.

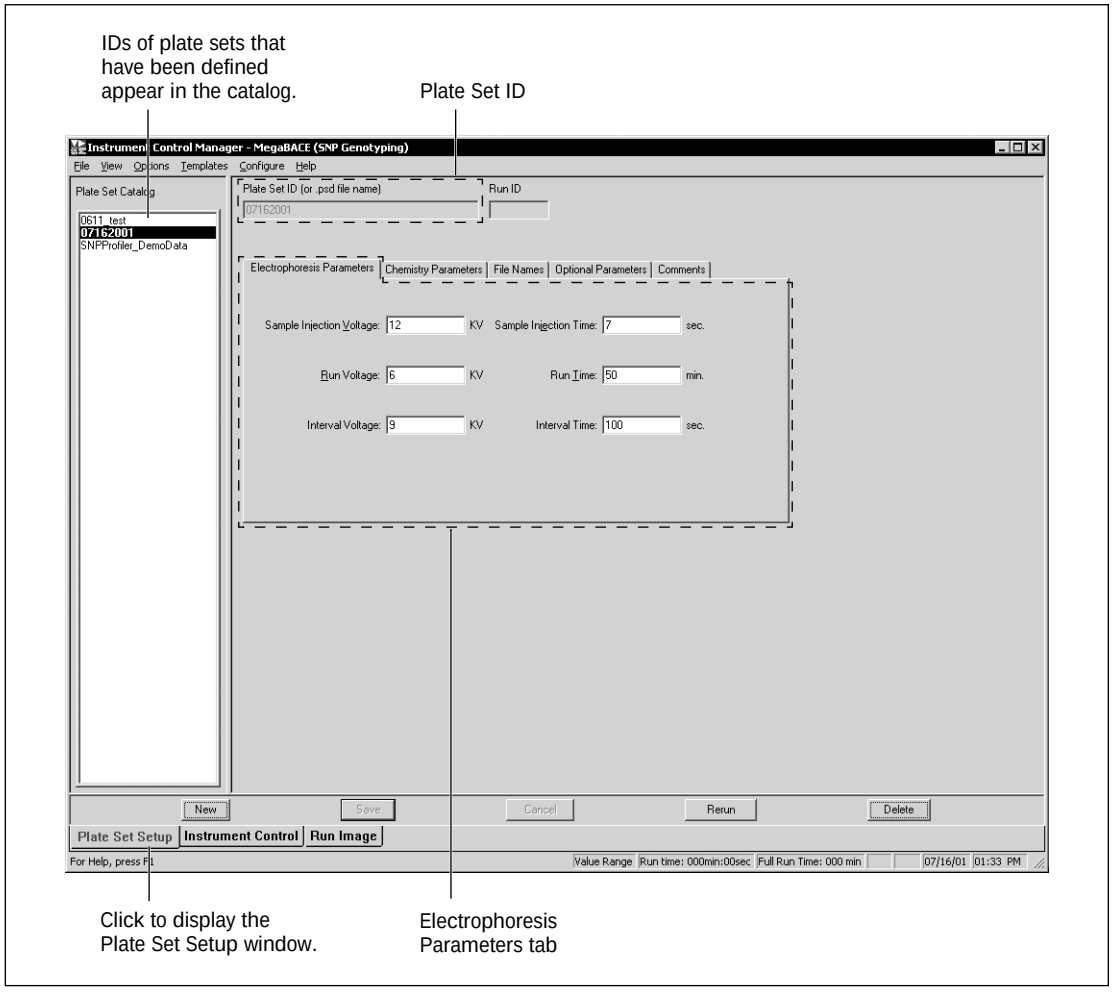


Figure 5-1. The Plate Set Setup window for SNP genotyping.

5.2 About the Plate Set Setup window and the plate set parameters

To display the Plate Set Setup window (figure 5-1), click the **Plate Set Setup** tab at the bottom of the Instrument Control Manager window. The Plate Set Setup window contains five tabs—

- Electrophoresis Parameters tab (figure 5-1)
- Chemistry Parameters tab (figure 5-2)
- File Names tab (figure 5-3)
- Optional Parameters tab (figure 5-4)
- Comments tab (figure 5-5)

Table 5-1 describes the parameters that can be specified in each tab.

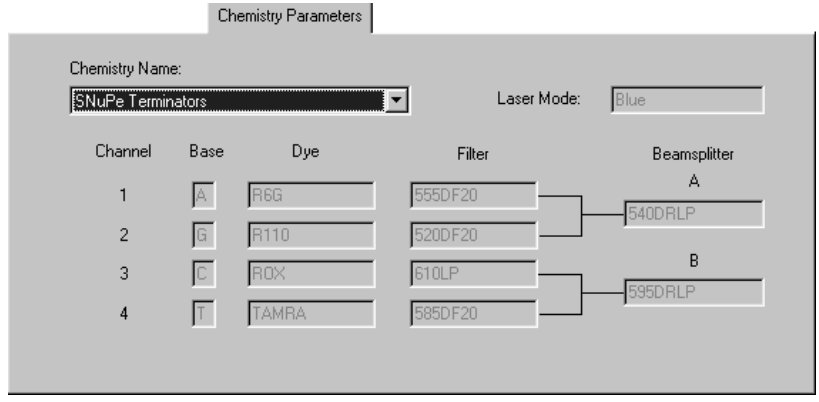


Figure 5-2. The Chemistry Parameters tab of the Plate Set Setup window, displaying the parameters for the SNuPe dye set.

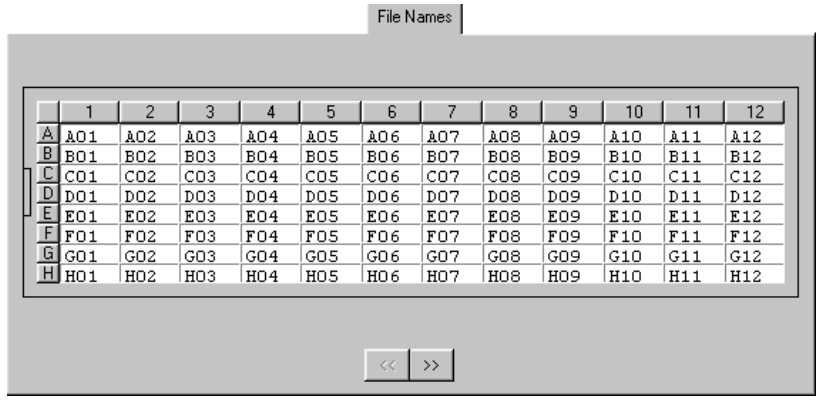


Figure 5-3. The File Names tab of the Plate Set Setup window, displaying the file name for each capillary. The software uses the well location as the default file names, but alternative file names can be specified in a plate setup template, a master .psd file for the plate set, or in the File Names tab.

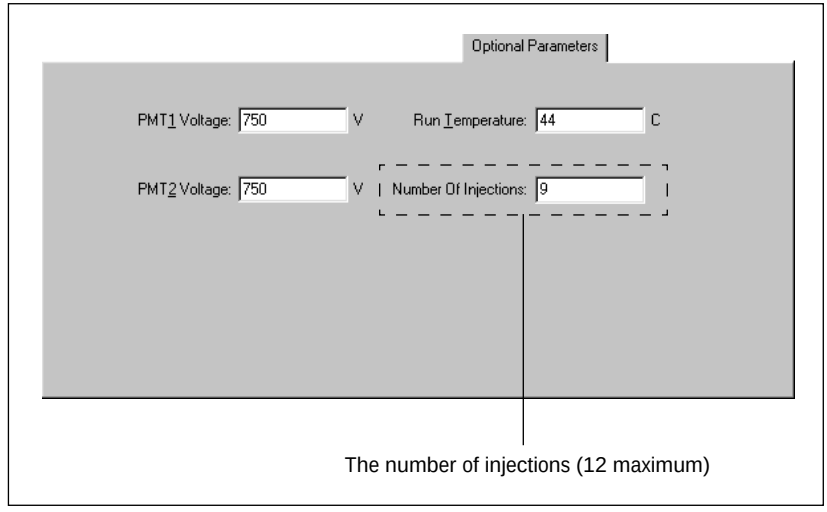


Figure 5-4. The Optional Parameters tab of the Plate Set Setup window, displaying the number of injections parameter.

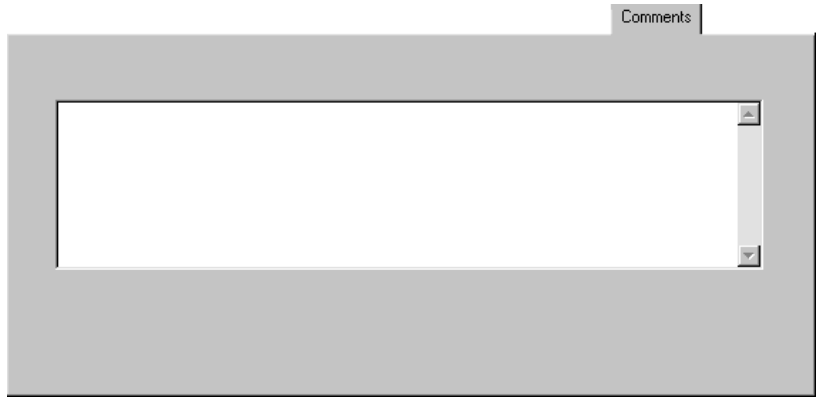


Figure 5-5. The Comments tab of the Plate Set Setup window.

Table 5-1. The plate set setup parameters

Tab in Plate Set Setup window	Parameters	SNP genotyping default values
Electrophoresis Parameters (figure 5-1)	The electrophoresis parameters are— • Sample injection voltage and time • Run voltage and time • Interval voltage and time All the fields must contain values.	The default values are— • 12 kV for 7 s • 6 kV for 50 m • 9 kV for 100 s
Chemistry Parameters (figure 5-2)	To specify the chemistry parameters, you specify or select a predefined **chemistry name.** Each chemistry name has an associated set of parameters, including laser mode, base order and dye names, the filter names, and the beamsplitters used for the run. The laser mode and base order are required fields for SNP genotyping.	SNuPe Terminators is the default chemistry name. The associated parameters specify the blue laser mode and the AGCT base order.
File Names (figure 5-3)	You can specify one file name for each capillary. **Note:** Unlike sequencing or microsatellite genotyping, the sample names for each SNuPe plate are injection-specific attributes and can be specified only in the .psd file for each plate.	Well IDs

Table 5-1. The plate set setup parameters (continued)

Tab in Plate Set Setup window	Parameters	SNP genotyping default values
Optional Parameters (figure 5-4)	The optional parameters are— • Parameters typically defined in the Instrument Control window that you want to override for a given plate set: PMT1 voltage, PMT2 voltage, and run temperature. **Important: The PMT voltages are instrument specific. Contact MegaBACE System Technical Support before you alter the PMT voltages.** • Number of injections (12 maximum). See section 2.4 for guidelines.	No defaults specified
Comments (figure 5-5)	You can specify any comments about the plate set. The software stores the plate set comments in the extended header of each raw sample data file (.rsd) from the run.	No defaults specified
Other user-defined plate set attributes	You can specify additional attributes in a master .psd file. See your administrator for details *(MegaBACE SNP Genotyping Instrument Administrator's Guide).*	

5.3 Creating the plate set definition automatically

To create a plate set definition automatically before the run, you can either use a template to specify the plate set setup parameters (section 5.3.1) or you can import the parameters from a master .psd file (section 5.3.2).

5.3.1 Using a template to specify the parameters

To create a plate set definition using a template—

1. In the Plate Set Setup window (figure 5-6), click **New.**
2. Check to see if values appear for the electrophoresis parameters in the Plate Set Setup window (figure 5-6). If values appear, your software has been configured to use a default plate setup template.

   If no values appear, or if you want to use a different template, see section 4.2.2 to choose a plate set setup template before continuing with this procedure.
3. In the Plate Set ID box of the Plate Set Setup window, type the **plate set ID** and click **Save** to save the plate set definition. The plate set ID appears in the Plate Set Catalog (figure 5-6).

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

5.3.2 Using a .psd file to specify the parameters

To create a plate set definition using a .psd file—

1. In the Plate Set Setup window (figure 5-6), click **New.**
2. In the Plate Set ID box, type the **.psd file name** or scan a **bar code.** The software automatically saves the new plate set definition, and the plate set ID appears in the Plate Set Catalog (figure 5-6).
3. Repeat steps 1 and 2 to create additional plate set definitions.

If you receive a message that the software cannot find the .psd file, see your administrator. The default path to the .psd file is …\MegaBACE\Psd.

Important **The text entry or bar code for the plate set ID must match the file name of the .psd file for the software to import the file.**

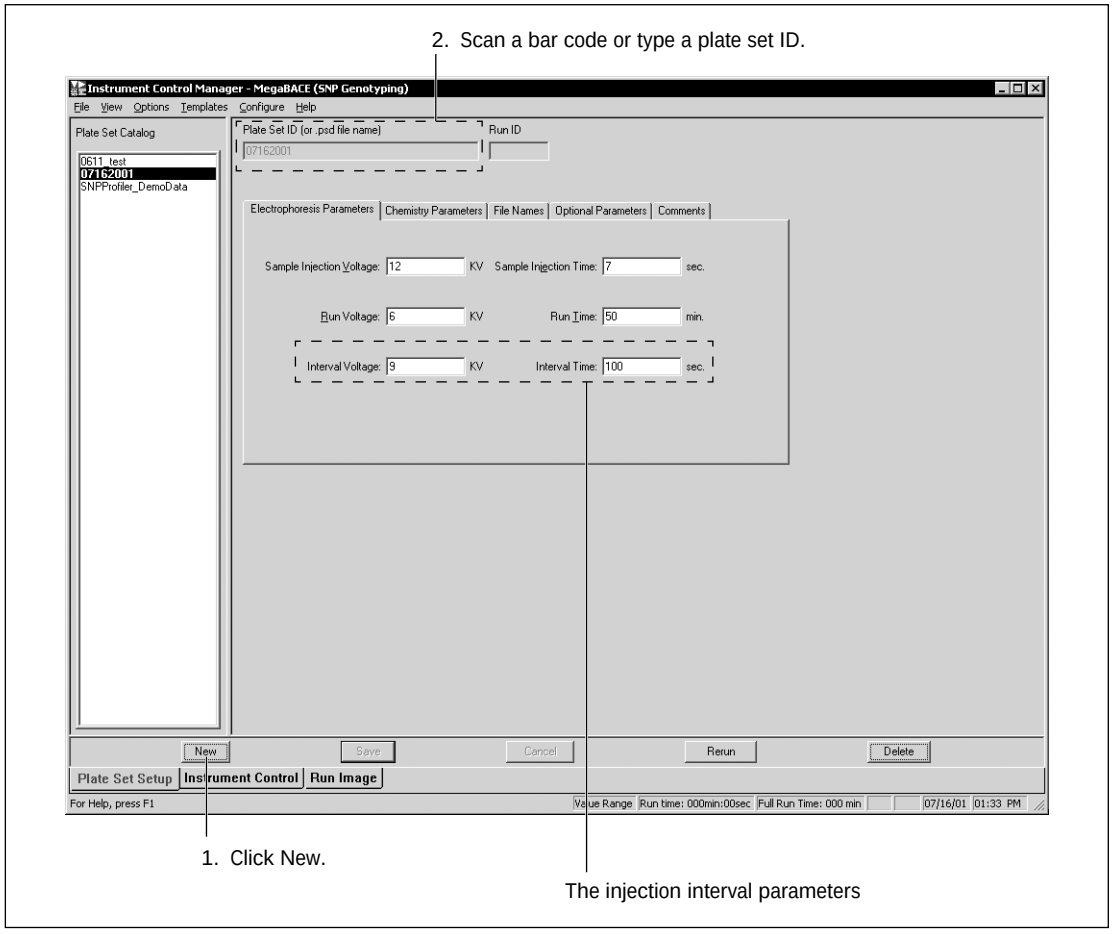


Figure 5-6. The Plate Set Setup window showing electrophoresis parameters for SNP genotyping.

5.4 Creating the plate set definition manually

Important

You can manually enter the parameters only if the edit mode is turned on. If the command is gray and cannot be selected, the edit mode is disabled. To enable the edit mode, see your administrator *(MegaBACE Instrument Administrator's Guide).*

1. Make sure the edit mode is turned on. To turn on the edit mode, choose **edit mode** from the Configure menu. A check mark appears in the Configure menu next to the edit mode command to indicate the edit mode is turned on.
2. In the Plate Set Setup window (figure 5-6), click **New.**
3. In the Plate Set ID box, type the **plate set ID**.

   Note: The software uses the plate set ID and the run ID to name the raw run folder that stores the raw sample files (.rsd) from the run. Thus, a plate set ID of a manageable size is advisable.
4. To enter the plate set parameters—
   - Enter the **electrophoresis parameters** in the Electrophoresis Parameters tab (figure 5-6).
   - Select a **Chemistry Name** in the Chemistry Parameters tab (figure 5-2).
   - Enter or edit the **file names** in the File Names tab (figure 5-3).
   - Enter or edit the **optional parameters** in the Optional parameters tab (figure 5-4).
   - Enter or edit **comments** about the plate set in the Comments tab (figure 5-5).

   See table 5-1 for an explanation of the parameters in each tab.
5. Click **Save** to save the plate set definition. The plate set ID appears in the Plate Set Catalog (figure 5-6).

Chapter 6 Performing a SNP genotyping run

This chapter describes how to perform a multi-injection SNP genotyping run. The topics in this chapter are—

- Workflow overview for SNP genotyping (section 6.1)
- Checking the instrument control parameters (section 6.2)
- Filling the capillaries with matrix and performing a prerun (section 6.3)
- Materials required for the Inject Samples and Run protocol (section 6.4)
- Starting the Inject Samples and Run protocol (section 6.5)
- About monitoring a multi-injection run (section 6.6)
- How the raw data files are stored (section 6.7)

6.1 Workflow overview for SNP genotyping

Figure 6-1 shows the protocol workflow for performing a SNP genotyping run. Table 6-1 provides a reference for each step in the workflow.

Caution **Failure to follow the described workflow for SNP genotyping can cause irregular and unexpected peak morphology and migration in the multi-injection marker and the SNuPe samples. Problems in the multi-injection marker peak morphology can cause SNP Profiler to have trouble correctly identifying the injections in the resulting run data.**

The SNP genotyping workflow for the following protocols is different than the workflow for a sequencing or microsatellite genotyping run:

- Matrix Fill and Prerun (section 6.3)
- Inject Samples and Run (sections 6.4 and 6.5)

For details on how to use the protocols that are not described in this chapter, see the *MegaBACE Instrument Operator's Guide.*

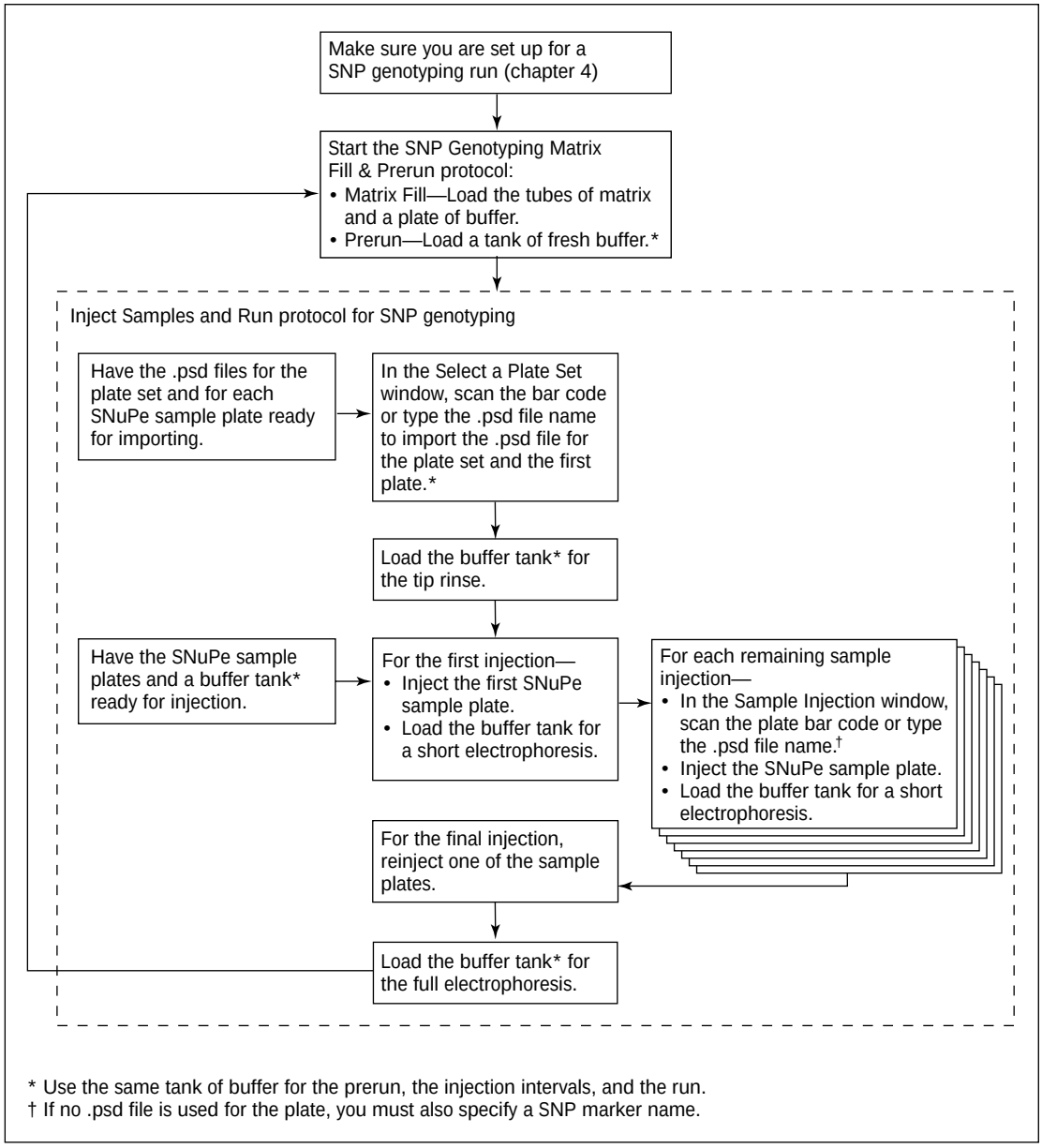


Figure 6-1. A typical workflow for a SNP genotyping run on the MegaBACE instrument.

Table 6-1. Typical protocol workflow for SNP genotyping on the MegaBACE instrument

Task	Description	Reference
1. Rinse the capillary tips.	Before the first run of the day, use the Rinse Tips protocol. Follow the instructions on the instrument displays to load a clean water tank and water tubes into the instrument.	*MegaBACE Instrument Operator's Guide*
2. If changing applications, make sure the instrument and the software are set up for SNP genotyping.	• Check that the sequencing filter set is installed in the instrument. • In the Instrument Control Manager, select the SNP genotyping application.	Chapter 4
3. Check the instrument control parameters.	Make sure the correct parameters appear in the Instrument Control window.	Section 6.2
4. For each multi-injection run, fill the capillaries with matrix and perform a prerun.	Follow the instructions on the instrument displays: • Matrix Fill—Use a buffer plate and matrix tubes. • Prerun—Use a fresh buffer tank.*	Section 6.3
5. Bring the materials for the Inject Samples and Run protocol to the instrument.	• Buffer tank (from the Prerun)* • SNuPe sample plates, prepared using the SNuPe genotyping kit and the multi-injection marker kit protocols.	Section 6.4
6. Inject the samples and perform a multi-injection run.	Start the Inject Samples and Run protocol. Follow the instructions on the displays to inject each SNuPe sample plate and then perform a run.	Section 6.5
7. After the last run of the day, store the capillaries up to 16 hours.	Click the Sleep After This Run check box in the Instrument Parameters area.	Section 6.2.2
For up to 3 days, store the capillaries wet.	Use the Store Capillaries protocol. This protocol reduces the temperature in the electrophoresis compartment to 25 °C (77 °F) and turns off the lasers.	*MegaBACE Instrument Operator's Guide*
For storage of more than 3 days, store the capillaries dry.	Use the Flush and Dry Capillaries protocol if you are shutting down the instrument for more than 3 days.	

* Make sure you use a plate of buffer for the Matrix Fill protocol and a tank of buffer for the Prerun. Then use the same tank of buffer for the Inject Samples and Run protocol.

6.2 Checking the instrument control parameters

Important **You can edit the instrument control parameters only if the edit mode is turned on. If the edit mode is turned on, the edit mode command on the Configure menu has a check mark in front of it. If the edit mode is turned off, the command is gray and cannot be selected. See your administrator for information about the edit mode.**

To view the instrument control parameters, click the **Instrument Control** tab. The Instrument Control window appears and displays the parameters in the Instrument Parameters area (figure 6-2). When you start the Inject Samples and Run protocol, the Instrument Control Manager uses the instrument parameters that are displayed.

6.2.1 About selecting an instrument control template

If no default template has been specified for the selected application, the parameters in the Instrument Parameters area are blank. See section 4.2.2 for a description of how to select an instrument parameters template.

Note: If the edit mode is turned off (no check mark appears in front of the edit mode command), the only instrument control parameters you can change are **Sleep After This Run, Sleep Time,** and **Sleep Temperature.**

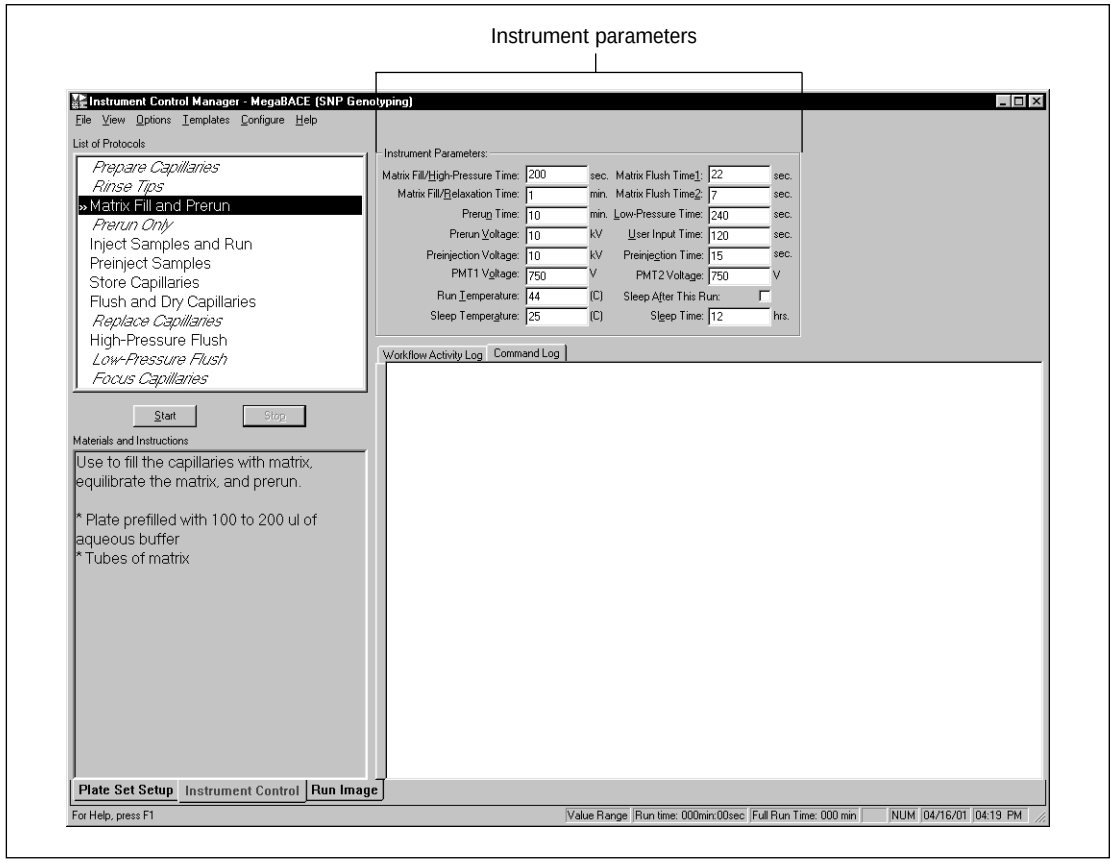


Figure 6-2. The Instrument Control window with the parameters displayed for a SNP genotyping run.

6.2.2 Selecting the Sleep After This Run option

You can select the Sleep After This Run check box so that the Instrument Control Manager automatically stores the capillaries in matrix and buffer after the current run has finished (figure 6-3).

Caution **If Sleep After This Run is selected and you exceed the time allowed for a given injection, the software starts the sleep protocol after the missed injection.**

If you select Sleep After This Run—

- Anytime before the Inject Samples and Run protocol has finished, the software stores the capillaries and lowers the instrument temperature after the current run.

- After the Inject Samples and Run protocol has finished, the software waits until after the next run and then stores the capillaries and lowers the instrument temperature.

Make sure you set the Sleep Time correctly before selecting the Sleep After This Run check box.

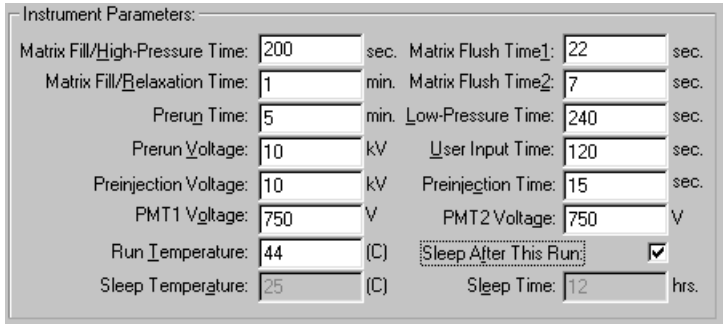


Figure 6-3. Instrument Parameters area with Sleep After This Run selected.

6.3 Filling the capillaries with matrix and performing a prerun

6.3.1 Materials required for the Matrix Fill and Prerun protocol

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

- A plate containing 100 µl buffer (diluted 1×) in each well.
- A tank containing 75 ml buffer (diluted 1×).
- Tubes, each containing 0.7 ml LPA matrix. Use one 2-ml tube for each array installed in the instrument. Centrifuge the matrix at the rpm and time listed in the LPA package instructions.

Caution **Do not overfill the buffer 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.**

6.3.2 Starting the Matrix Fill and Prerun protocol

Caution **Make sure you use the buffer plate for the Matrix Fill and the buffer tank for the Prerun as specified in the following procedure. Failure to follow the described workflow can cause irregular and unexpected peak morphology and migration in the multi-injection marker and the SNuPe samples. Problems in the multi-injection marker peak morphology can cause SNP Profiler to have trouble correctly identifying the injections in the resulting run data.**

To use the Matrix Fill and Prerun protocol—

1. In the Instrument Control window, with the Matrix Fill and Prerun protocol selected, click **Start.**
2. Follow the instructions on the instrument displays to load the **buffer plate** into the left side of the instrument and the **matrix tubes** into the right side of the instrument. The displays tell you that equilibration is in progress, and ask you to be ready to put in a fresh buffer tank.
3. Make sure you load the **tank filled with fresh buffer** when the display prompts you to do so. After you load the buffer tank, the prerun begins and progresses according to the time specified in the instrument parameters.

During the protocol, make sure you follow the instructions on the instrument displays. When the protocol is finished, the software selects the Inject Samples and Run protocol as the next protocol you should use.

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

6.4 Materials required for the Inject Samples and Run protocol

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

Make sure you use the same tank of buffer for the prerun, the injection intervals, and the run. Otherwise, SNP Profiler might have trouble correctly identifying the injections in the resulting run data.

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

For the Inject Samples and Run protocol, you should have the following materials available for each run (figure 6-4):

- Plates containing the samples prepared using the SNuPe genotyping kit and multi-injection marker kit protocols. Make sure you use a minimum 10 µl in each well.
- The tank of buffer from the prerun already in place in the cathode side of the instrument.
- The tubes of matrix already in place in the anode side of the instrument.

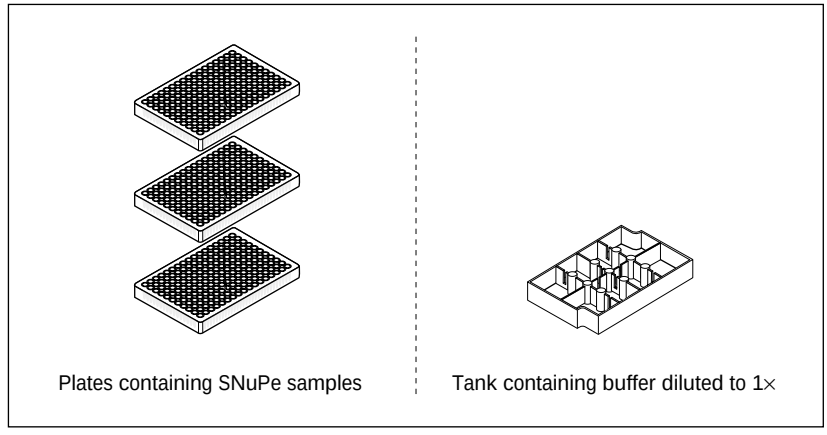


Figure 6-4. Materials for the Inject Samples and Run protocol.

6.5 Starting the Inject Samples and Run protocol

Warning

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

Cautions

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.

All samples in a multi-injection run must be injected into the capillaries before the samples from the first injection reach the capillary detection windows.

Before you begin a run, check the free disk space on the drive where your system is storing the raw sample data files (.rsd). Make sure you have at least 150 MB of free disk space for the run.

The procedure that you follow for the Inject Samples and Run protocol varies depending on how the administrator has configured the software—

- If your lab uses .psd files to import the plate set definition and the plate attributes for each injection, see section 6.5.1.
- If your lab uses the Plate Set Setup window to create plate set definitions (chapter 5) and manually enters the plate attributes for each injection, see section 6.5.2 for an alternative workflow.

6.5.1 Using .psd files during the Inject Samples and Run protocol

Important **Regardless of the method used to enter the plate set ID or plate ID, the text entry or bar code must match the file name of the .psd file for the software to import the file.**

To start a SNP genotyping run—

1. In the Instrument Control window, with the Inject Samples and Run protocol selected, click **Start.** The Select a Plate Set window appears (figure 6-5).

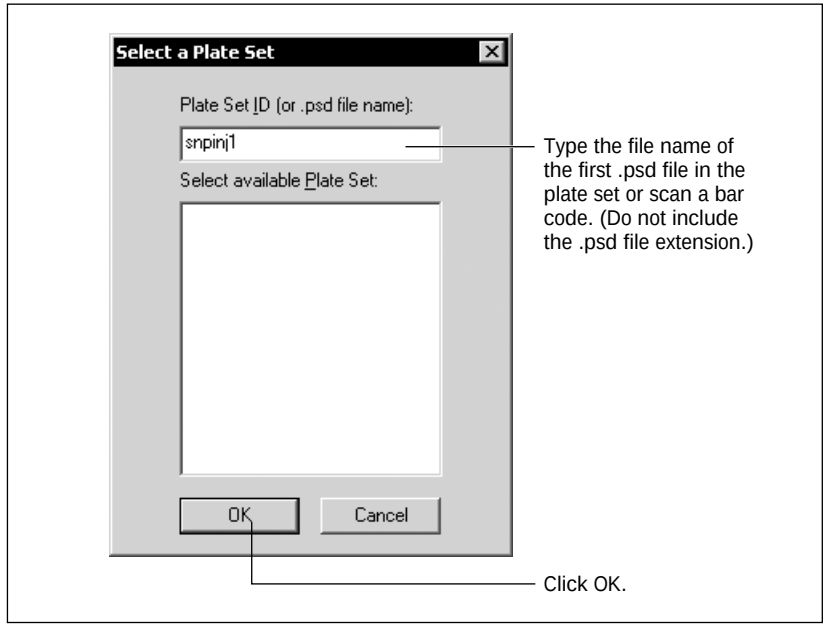


Figure 6-5. The Select a Plate Set window.

2. In the Select a Plate Set window, type the **file name** of the first .psd file in the plate set or scan a **bar code** in the Plate Set ID box, and click **OK.** (Do not include the .psd file extension.) The Instrument Control Manager imports the precreated—

   - Plate set definition
   - Attributes for the first plate

   If the Specify Marker Name/Plate ID window appears (figure 6-6), proceed to step 3. Otherwise, skip to step 4.

3. If the .psd file does not contain the SNP marker name or the plate ID of the first plate, the Specify Marker Name/Plate ID window appears (figure 6-6). Type the **SNP Marker Name** and/or **Plate ID** and then click **OK.**

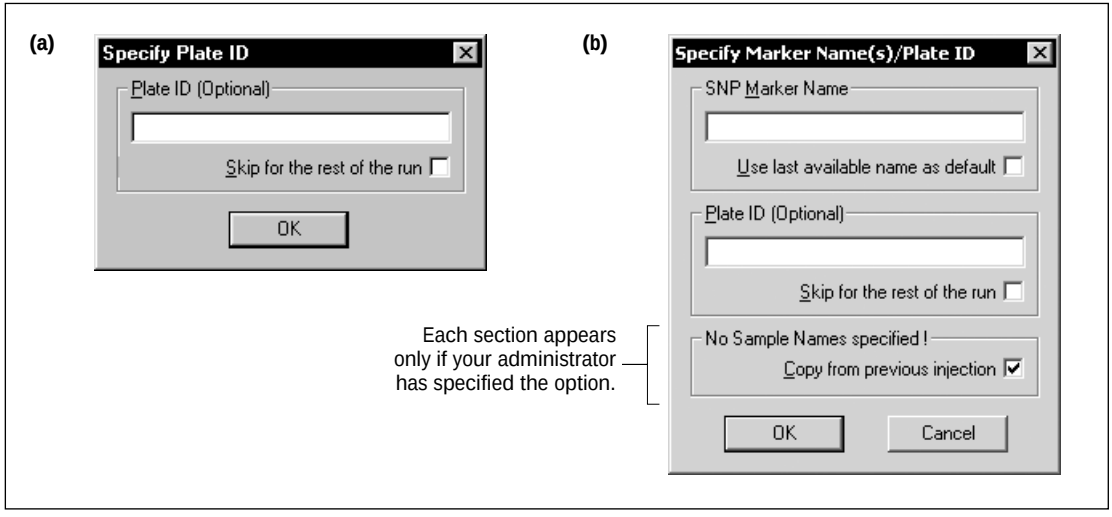


Figure 6-6. Examples of the Specify Marker Name/Plate ID window. The window changes dynamically to reflect which information is missing: (a) appears for a missing plate ID and (b) appears for missing SNP marker name, plate ID, and sample names.

4. When the instructions on the instrument displays ask you to load the buffer tank for the tip rinse, carefully open and close the **cathode door.** The buffer tank from the prerun should already be in place in the instrument. After the first tip rinse is finished, a Confirm to Continue window appears and asks if you are ready to inject the first plate.

5. To confirm the ID of the first plate, you can type the **plate ID** or scan the **bar code.** Then, click **Continue** and immediately load the **sample plate** in the instrument.

6. When the instructions on the instrument displays ask you to load a buffer tank, make sure you use the **same tank of buffer** that you used for the prerun.

Caution **When the Sample Injection window appears, make sure you load the sample plate as soon as possible. If you do not inject the plate before the time remaining for the injection elapses (15 minutes, default), the software aborts the Inject Samples and Run protocol. Also, if the Sleep After This Run option was selected, the instrument stores the capillaries and lowers the instrument temperature.**

7. When the Sample Injection window appears for the next injection (figure 6-7), note that the timer in the window begins counting down the time remaining in the injection timeout period. Do one of the following:

   - To inject another sample plate, type or scan the **bar code** for the plate and click **Inject.** Proceed to step 8. **Note:** The bar code must match the name of the .psd file for the plate.
   - If you want to do fewer injections then specified or if you did not specify the number of injections and you want to proceed with the final injection (reinjected plate) and the actual run, click **Run**. Then skip to step 12. **Note:** The title bar of the Instrument Control window displays the injection number and the total number of injections expected.
   - Alternatively, to abort the protocol, click **Stop.**

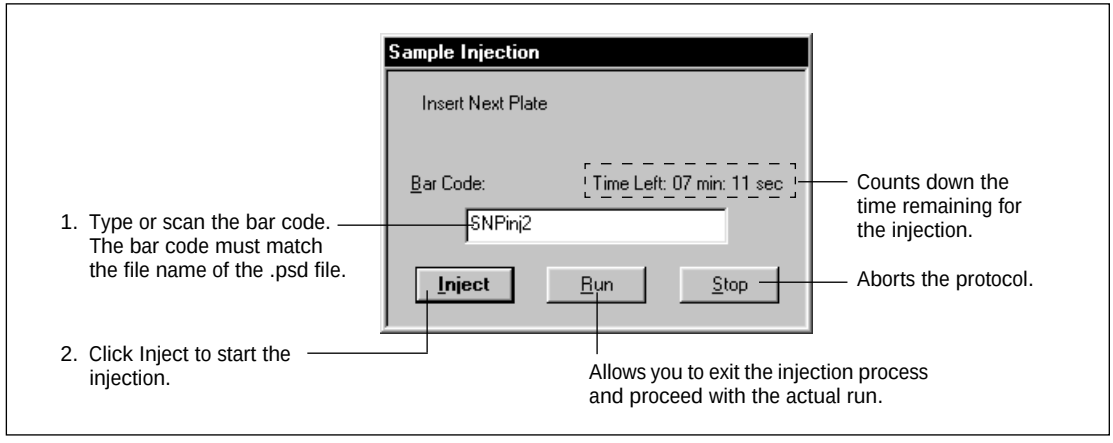


Figure 6-7. The Sample Injection window displaying the time remaining for the injection.

8. If a version of the Specify Marker Name/Plate ID window or Copy Sample Names window appears (figure 6-6), you can provide the missing information for the plate and click **OK.**

9. Immediately, load the **sample plate** in the instrument. You should load the sample plate within about 1–2 minutes of when the Sample Injection window appears.

10. When the instructions on the instrument displays ask you to load a buffer tank, make sure you use the **same tank of buffer** that you used for the prerun.

11. Repeat steps 7 through 10 for all remaining sample injections.

    After the last sample plate injection is finished, a Confirm to Continue window appears and asks you to inject the final injection plate to conclude the multi-injection process.

12. Click **Continue** and then reinject one of the **sample plates. Note:** The repeated injection provides a multi-injection marker at the end of the last injection. SNP Profiler requires this last multi-injection marker to perform the injection identification during automated SNP genotyping.

13. After the final injection, reload the **buffer tank** for the final electrophoresis. Make sure you use the same tank of buffer that you used for the prerun. A message appears and tells you the sample run is in progress.

After the Inject Samples and Run protocol finishes, the software selects the Matrix Fill and Prerun protocol as the next protocol to use, unless you selected the Sleep After This Run check box in the Instrument Parameters area of the Instrument Control window (figure 6-3).

6.5.2 Alternative workflow for the Inject Samples and Run protocol

If you use the Plate Set Setup window to create plate set definitions (chapter 5) and specify the attributes of each plate manually, you use the alternative workflow for the Inject Samples and Run protocol.

To start a SNP genotyping run—

1. In the Instrument Control window, with the Inject Samples and Run protocol selected, click **Start.** The Select a Plate Set window appears (figure 6-8).

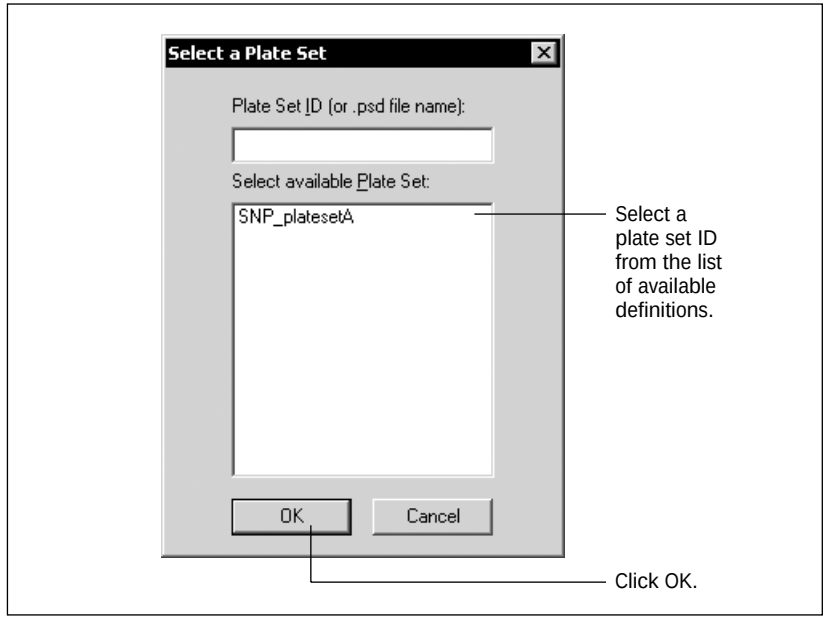


Figure 6-8. Choosing an available or previously defined plate set ID in the Select a Plate Set window.

2. In the Select a Plate Set window, select the **plate set ID** from the Select available Plate Set box, and click **OK.** The Specify Marker Name/Plate ID window appears (figure 6-9).

Figure 6-9. The Specify Marker Name/Plate ID window.

3. In the Specify Marker Name/Plate ID window (figure 6-9), enter the applicable information for the plate to be injected—

   - **SNP Marker Name—**Type the **SNP marker name.** You must provide at least one SNP marker name for each injection.

     Depending on how your administrator configured the software, you might be able to use the same SNP marker name for subsequent injections in the run that are missing this attribute. To do this, select the **Use last available name as default** check box. A check mark appears and indicates this option is selected.

   - **Plate ID—**Type the **plate ID.**

     Depending on how your administrator configured the software, you might not need to provide the plate IDs. If you are prompted for a plate ID and you want to omit the plate IDs, select the **Skip for the rest of the run** check box. A check mark appears and indicates this option is selected. If you select the check box, the Specify Plate ID prompt does not reappear and the software proceeds without plate IDs for the remaining injections.

   Click **OK.**

4. When the instructions on the instrument displays ask you to load the buffer tank for the tip rinse, carefully open and close the **cathode door.** The tank of buffer from the prerun should already be in place in the instrument. After the first tip rinse is finished, a Confirm to Continue window appears and asks if you are ready to inject the first plate.

5. To confirm the ID of the first plate, you can type the **plate ID** or scan the **bar code.** Then, click **Continue** and immediately load the **sample plate** in the instrument.

6. When the instructions on the instrument displays ask you to load a buffer tank, make sure you use the **same tank of buffer** that you used for the prerun.

Caution **When the Sample Injection window appears, make sure you load the sample plate as soon as possible. If you do not inject the plate before the time remaining for the injection elapses (15 minutes, default), the software aborts the Inject Samples and Run protocol. Also, if the Sleep After This Run option was selected, the instrument stores the capillaries and lowers the instrument temperature.**

7. When the Sample Injection window appears for the next injection (figure 6-10), note the amount of time left to inject the plate. The timer in the window begins counting down the time remaining in the injection timeout period. Do one of the following:

   - To inject another sample plate, click **Inject**. The Specify Marker Name/Plate ID window appears (figure 6-9). Proceed to step 8. **Note:** Make sure you leave the Bar Code box blank unless you have a .psd file for the plate. If you enter a name in the Bar Code box, the software searches for a .psd file that has the same file name as the bar code.
   - If you want to do fewer injections then specified or if you did not specify the number of injections and you want to proceed with the final injection (reinjected plate) and the actual run, click **Run**. Then, skip to step 11. **Note:** The title bar of the Instrument Control window displays the injection number and the total number of injections expected.
   - Alternatively, to abort the protocol, click **Stop.**

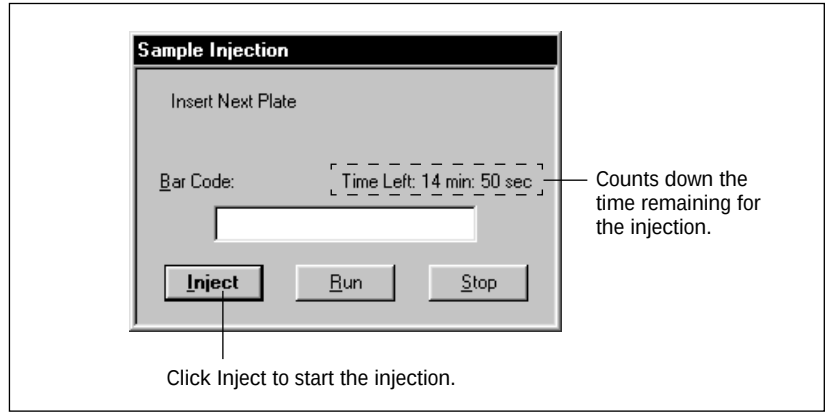


Figure 6-10. The Sample Injection window with no entry in the Bar Code box.

8. In the Specify Marker Name/Plate ID window, you can type the **SNP marker name** and the **plate ID.** Click **OK** and immediately load the **sample plate** in the instrument.
9. When the instructions on the instrument displays ask you to load a buffer tank, make sure you use the **same tank of buffer** that you used for the prerun.

10. Repeat steps 7 through 9 for all remaining sample injections.

    After the last sample plate injection is finished, a Confirm to Continue window appears and asks you to inject the final injection plate to conclude the multi-injection process.

11. Click **Continue** and then reinject one of the **sample plates**. **Note:** The repeated injection provides a multi-injection marker at the end of the last injection. SNP Profiler requires this last multi-injection marker to perform the injection identification during automated SNP genotyping.

12. After the final injection, reload the **buffer tank** for the final electrophoresis. Make sure you use the same tank of buffer that you used for the prerun. A message appears and tells you the sample run is in progress.

After the Inject Samples and Run protocol finishes, the software selects the Matrix Fill and Prerun protocol as the next protocol to use, unless you selected the Sleep After This Run check box in the Instrument Parameters area of the Instrument Control window (figure 6-3).

6.6 About monitoring a multi-injection run

See the *MegaBACE Instrument Operator's Guide* for instructions on how to use the Run Image window to monitor the run.

When you use a buffer tank during the run, the Current Monitor window displays almost the same value for all the capillaries. The value that appears for each capillary represents a portion of the total electrical current for all the capillaries. That is, if there are 96 capillaries in the instrument, each value represents 1/96 portion of the total electrical current.

6.7 How the raw data files are stored

During data collection, the Instrument Control Manager creates raw sample data files (.rsd) for the data collected from each capillary and stores them in a raw run folder. The raw run folders are stored in the …\MegaBACE\Data folder (default) or the location you specified (section 4.3).

For a multi-injection run, the software uses the following conventions:

- **Raw run folder name—**The software names the folder by appending the run ID to the plate set ID. **Note:** If you use a master .psd file to provide the plate set definition but the plate set ID is missing, the software uses the plate ID of the first plate if provided in the master .psd file. If the plate ID is also missing from the .psd file, the software uses the .psd file name.

- **Raw sample data file (.rsd) names—**The software uses the well locations to name the .rsd files (default). Alternatively, file names can be specified in a plate set setup template, the master .psd file for the plate set, or the Plate Set Setup window.

See section 4.3 for details on how to change the storage location of the raw run folders. See the *MegaBACE SNP Genotyping Instrument Administrator's Guide* for a detailed description of the file storage.

Chapter 7 Troubleshooting the instrument

This chapter provides a reference for troubleshooting the MegaBACE SNP genotyping instrument. The topics are—

- Where to find the troubleshooting guidelines (section 7.1)
- Verifying the SNP genotyping instrument (section 7.2)
- Verifying the multi-injection run workflow (section 7.3)

If the problem persists after you follow the appropriate troubleshooting guidelines, contact MegaBACE Technical Support. See Assistance in the preface for contact information.

7.1 Where to find the troubleshooting guidelines

Table 7-1 provides a reference for where to find troubleshooting guidelines for the MegaBACE SNP genotyping system.

Table 7-1. Where to find troubleshooting guidelines for the SNP genotyping system

Troubleshooting topic	Reference
Instrument problems: • On-screen error messages • Power and communication • Cathode and anode stages • Fan **Note:** Depending on the throughput of your laboratory, you should periodically clean the cathode plate holder and slider.	*MegaBACE Instrument Maintenance and Troubleshooting Guide*
SNP genotyping instrument verification	Section 7.2
Multi-injection run workflow verification	Section 7.3
SNuPe reaction chemistry	SNuPe genotyping kit protocol
Results of SNP genotyping	*MegaBACE SNP Profiler User's Guide*

7.2 Verifying the SNP genotyping instrument

Table 7-2 provides the typical workflow for verifying that the MegaBACE instrument is functioning properly for the SNP genotyping application. After verifying that the instrument is functioning properly, you should verify that there are no problems with the workflow. See the *MegaBACE SNP Profiler User's Guide* for instructions on troubleshooting the workflow.

Table 7-2. Verifying the instrument for SNP genotyping

Task	Description	Reference
Verify that the run was performed following the SNP genotyping protocols for a multi-injection run.	Check the following— • The sequencing filter set was installed in the instrument during the run, and each filter was installed in the correct location.	Section 4.2.1
	• In the Instrument Control Manager, the SNP Genotyping application was selected and the appropriate plate set setup and instrument parameters were selected.	Section 4.2.2
	• The SNP genotyping versions of the Matrix Fill and Prerun protocol and the Inject Matrix and Run protocol were followed.	Chapter 6
Verify optimal performance of the MegaBACE instrument by performing a sequencing run of the M13 standards.	To verify the instrument performance— • Prepare a plate of the MegaBACE 4-Color Sequencing Standard Set (M13 standards). Make sure you follow the guidelines in the kit protocol booklet. (See the *MegaBACE Planning Guide* for ordering information.)	MegaBACE 4-Color Sequencing Standard Set kit protocol booklet
	• Make sure you are set up for the sequencing application.	Chapter 4
	• Perform a sequencing run	*MegaBACE Instrument Operator's Guide*
	• The plate of M13 sequencing standards should give an average read-length >500 bases with a 98.5% accuracy as determined using the ReadCheck utility.	MegaBACE Technical Support
	• If the run does not meet this specification, you can use Sequence Analyzer to view the electropherograms and determine if the problem is related to a specific spectral channel or limited to specific wells on the plate.	*MegaBACE Sequence Analyzer User's Guide*
	• If required, perform routine maintenance on the instrument.	*MegaBACE Instrument Maintenance and Troubleshooting Guide*

7.3 Verifying the multi-injection run workflow

To verify the multi-injection run workflow, you perform a multi-injection run using a plate containing the SNuPe multi-injection marker without any SNuPe products. To do this—

1. Prepare a SNuPe multi-injection marker plate. Make sure you substitute 0.1× TE buffer for the sample to achieve the appropriate volume. (See the SNuPe Multiple Injection Marker Kit Protocol for details.)
2. Make sure the sequencing filter set is installed in the instrument.
3. In the Instrument Control Manager, make sure the **SNP Genotyping** application is selected (section 4.2.2). To specify the plate set setup parameters, use the **StdSNP.tpl** template. Make sure the plate set setup parameters specify—
   - Injection interval at 9 kV for 100 s
   - 9 sample injections
4. Use the Matrix Fill and Prerun protocol for SNP genotyping (section 6.3.2).
5. Use the alternative workflow for the Inject Samples and Run protocol (section 6.5.2).
   - When the software prompts you for a SNP marker name, use the term *injection* as a placeholder.
   - Make sure you inject the same multi-injection marker plate 9 times. Then reinject the plate for the final injection.
6. Analyze the run data using the SNP Profiler software. **Note:** Make sure the SNP Marker Editor contains an entry for a SNP marker named *injection*. Verify that SNP Profiler can identify all the injections for all the capillaries. For details see the *MegaBACE SNP Profiler User's Guide* or the Help available within SNP Profiler.

Glossary

.icp files—the instrument control parameter template files, which can be used as templates to specify the instrument run conditions and the matrix fill and flush cycles for multiple runs.

.psd files—the plate setup data files that you can use to specify attributes for a plate set or a plate. The Instrument Control Manager attaches the information from the .psd file to each sample file during the run.

.rsd files—the raw sample data files that contain the data for given wells on a plate (for example, A01 through H12). The storage location is in a corresponding raw run folder (plate set ID_run ID) in the …\MegaBACE\Data folder (default) or the location you specify. For SNP genotyping, the file name is based on the well location (default), for example, A01.rsd. Alternatively, the file names can be specified in a plate set setup template, a .psd file for the plate set, or in the Plate Set Setup window.

.tpl files—the plate setup parameters template files. For SNP genotyping, the plate setup applies to the entire set of plates injected during a single run. A .tpl file can specify the electrophoresis conditions, the chemistry parameters, the file names, and optional parameters, such as number of injections. See the *MegaBACE SNP Genotyping Instrument Administrator's Guide* for details.

channels—see spectral channels.

dye set—the fluorescent dyes used to label samples in your experiment. The MegaBACE system is capable of detecting and distinguishing the emissions of dyes of different colors in a single capillary. The SNuPe dye set consists of the R6G, R110, ROX, and TAMRA dyes.

electropherograms—the digitized graphs that represent the signal intensities (rfu) recorded from the capillaries for the duration of 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.

LPA (linear polyacrylamide)—see sieving matrix.

marker—see multi-injection marker or SNP marker.

matrix—see sieving matrix or spectral overlap matrix.

multi-injection marker—fluorescently labeled pair of DNA fragments used to distinguish one injection from the next during a multi-injection run on a MegaBACE instrument. By default, the multi-injection marker is detected in spectral channel 2 and appears in the black trace of the electropherogram. The signal from the multi-injection marker appears as a characteristic doublet peak. SNP Profiler uses the multi-injection marker during analysis to demarcate the injection intervals.

multiple injection—a proprietary method of increasing SNP detection throughput by a more efficient use of capillary volume. Multiple plates are injected into the same set of capillaries, at time-spaced intervals, during the waiting period before the first injected samples appear at the detector.

plate ID—the name you give to the sample plate when you create a plate definition in the Instrument Control Manager. For SNP genotyping, the plate ID is optional.

plate set—a group of plates that share plate setup parameters and are serially injected into the same set of capillaries during a single run on the instrument.

plate set definition—the combination of the plate set ID and plate set setup parameters for a group of plates that you inject during a multi-injection run. The plate set definition can also include the list of bar codes for plates in the plate set and the injection order.

plate set ID—the name you give to the set of plates you inject in a multi-injection run. The software uses the plate set ID to name the raw run folder that stores the data from the run. If you import the plate set definition from a master .psd file that does not include the plate set ID, the software uses the plate ID of the first plate (if included in the master .psd file) as the plate set ID. If the plate ID is also missing, the software uses the .psd file name.

plate set setup parameters—the plate set setup parameters for a multiple-injection run, where all the setup parameters are common to all injections (plates). The plate set can include electrophoresis conditions, the chemistry parameters, the file names, and optional parameters. In addition, the plate set can include the list of all the plates (plate bar codes) to be injected during the run.

raw data—the original unprocessed data collected by the instrument. The Instrument Control Manager software creates a raw run folder for the raw sample data files (.rsd) for each run.

raw run folder—the directory on the computer that contains the raw sample data files (.rsd) for a run. The Instrument Control Manager creates a raw run folder for the data from each run and stores the folders in the …\MegaBACE\Data folder (default) or the location you specify.

run—the process of injecting samples in the instrument, performing capillary electrophoresis separation, and detecting the resulting signal from each capillary. Each run has a unique date and user ID.

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

sample name—the ID or designation you provide for the contents of a given well in the sample plate. If you want the sample names to appear in the SNP Profiler windows, you must use a plate setup data (.psd) file for each injection to assign the sample names before the run. See the *MegaBACE SNP Genotyping Instrument Administrator's Guide* for details.

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

SNP (single nucleotide polymorphism)—a base position in the genome that is variable in a population. To detect SNPs on the MegaBACE instrument, you use the SNuPe reagent kit to assay your samples, perform a multi-injection run, and then use SNP Profiler to perform automated SNP genotyping.

SNP marker—any genetic locus containing single-base variations or polymorphisms.

SNuPe genotyping kit—a set of components used to assay SNP samples based on single-nucleotide primer extension (SNuPe) technology.

spectral calibration run—a 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 SNP 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 matrix standards—the reagents you use to perform a calibration run and calculate the spectral overlap for the instrument and the run conditions. You use a spectral standard for each dye in the dye set.

spectral overlap matrix—the dye-to-channel mathematical matrix that lists which dye is detected through each spectral channel and measures the amount of unwanted signal (spectral overlap) present in each spectral channel from the other dyes in the dye set.

spectral separation—the software process that for each spectral channel removes the unwanted cross-talk signals. The unwanted cross-talk is caused by overlaps in the emission spectra of the different dyes. 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.

traces—the four digitized graphs 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 the associated base: green indicates A, black indicates G, blue indicates C, and red indicates T. Typically, the black trace also displays the peaks for the multi-injection marker.

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