user manual

MegaBACE

SNP Genotyping
Instrument Administrator's Guide

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Amersham
Biosciences

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.

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

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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 Administrator's Guide* describes how to configure the MegaBACE™ SNP genotyping system software for your laboratory. This guide is a supplement to the *MegaBACE Instrument Administrator's Guide.*

Administrators are responsible for configuring the MegaBACE system initialization files, for creating parameter templates, and for creating plate setup data files (.psd). Operators are responsible for performing runs on the instrument. If you have instrument operator responsibilities for the MegaBACE system in addition to being an administrator, see the *MegaBACE SNP Genotyping Instrument Operator's Guide* for additional guidelines.

Related publications

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

- Protocol booklets in the MegaBACE SNuPe™ genotyping kit and the SNuPe multiple-injection marker kit describe how to prepare the SNP samples before running them on the MegaBACE instrument.
- *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.
- *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 Administrat*or*'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.

Special notices

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

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.

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.

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
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USA
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Web site

http://www.amershambiosciences.com

Chapter 1 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 data collection throughput by repeated, time-spaced injections of short DNA samples into the same set of capillaries on the MegaBACE instrument. The system consists of the SNuPe (single nucleotide primer extension) genotyping reagents, the MegaBACE instrument, and software. This chapter provides an overview of the SNP genotyping system software. The topics are—

- SNP genotyping system software overview (section 1.1)
- Instrument Control Manager software features (section 1.2)
- Software workflow for performing a multi-injection run (section 1.3)
- Administrator tasks (section 1.4)

You should use this guide in conjunction with the *MegaBACE SNP Genotyping Instrument Operator's Guide* and the *MegaBACE Instrument Administrator's Guide.*

1.1 SNP genotyping system software overview

The MegaBACE SNP genotyping system software consists of the—

- **Instrument Control Manager software—**Contains the graphical user interface that allows an operator to control the MegaBACE instrument. See section 1.2 for an overview of the Instrument Control Manager features.

- **Host Scan Controller software—**Provides the communication between the MegaBACE instrument and the Instrument Control Manager.

- **MegaBACE Header Editor software—**Allows you to view and edit the data in the extended header of the raw sample data files (.rsd). The software allows you to view the standard header. For more information about MegaBACE Header Editor, see the Help available within the software.

- **SNP Profiler software—**Performs automated SNP genotyping of the data collected from the SNuPe samples during a multi-injection run on the MegaBACE instrument. SNP Profiler uses the SNuPe multi-injection marker to demarcate the samples in one plate from the other plates that are injected during the run. See the *MegaBACE SNP Profiler User's Guide* or the Help available within the software for details.
- **Sequencing software—**Supports sequencing analysis of the data collected during a sequencing run on the MegaBACE instrument. The sequencing software can also be used to verify the performance of the MegaBACE instrument for SNP genotyping. See the *MegaBACE SNP Genotyping Instrument Operator's Guide* for details on how to verify the instrument performance for the SNP genotyping application.
    - **Sequence Analyzer software—**Supports sequencing analysis of the data collected during a sequencing run on the MegaBACE instrument. See the *MegaBACE Sequence Analyzer User's Guide* or the Help available within the software for a description of how to use the software.
    - **MegaBACE Sequencing ScoreCard™ software—**Provides quality statistics for MegaBACE data that was base called using Sequence Analyzer. See the Help available within the software for details.

1.2 Instrument Control Manager software features

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 into the same set of capillaries for a single run.

The Instrument Control Manager allows you to—

- Specify the plate set definitions in plate setup data files (.psd) that the operator can import at the start of the Inject Samples and Run protocol. Alternatively, you or the operator can use the Plate Set Setup window to create plate set definitions. The operator can then select these precreated plate set definitions at the start of the Inject Samples and Run protocol.
- Specify the plate-specific attributes for each injection (plate ID, SNP marker names, and sample names) using a .psd file that the operator can import when the plate is injected. Alternatively, the operator can manually specify the plate ID and SNP marker name for each injection.
- Specify the default plate set setup parameters and instrument parameters using templates. The MegaBACE software includes templates (default) for the recommended SNP genotyping parameters.

- Provide full flexibility to the instrument operator to make changes or limit the operator's ability to modify the plate set setup parameters or instrument control parameters for a run.
- Change applications. The software supports SNP genotyping, sequencing, or microsatellite genotyping. **Note:** For each application, you need the applicable software license and the appropriate instrument filter set.
- Serially inject multiple sample plates into the same set of capillaries for a single run (*MegaBACE SNP Genotyping Instrument Operator's Guide*).
- Store the raw run folders in application-specific locations (SNP genotyping, sequencing, and microsatellite genotyping).

1.3 Software workflow for performing a multi-injection run

The SNP samples are prepared using the SNuPe genotyping kit and the SNuPe multi-injection marker kit. During a single run, the operator can serially inject multiple SNuPe sample plates into the same set of capillaries (multi-injection run). The Instrument Control Manager prompts the operator 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, the operator must provide—

- A plate set definition (section 1.3.1)
- The plate-specific attributes for each injection (section 1.3.2)

The workflow that the operator follows depends on how you configure 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, the operator can import the required information from .psd files during the Inject Samples and Run protocol (figure 1-1). As the administrator, you create the .psd files and provide the operator with the file names or bar codes to enter during the run:
  - A master .psd file containing the plate set definition and attributes for the first plate
  - Additional .psd files to import the plate-specific attributes for each subsequent sample injection

- **Alternative multi-injection workflow—**The alternative workflow can require the operator to perform more manual entries to provide the required information:
  - You or the operator can use the Plate Set Setup window to create the plate set definition before the run. The values for the plate set setup parameters can be provided from a template, imported from a .psd file, or manually entered in the window. See the *MegaBACE SNP Genotyping Instrument Operator's Guide* for a description of how to use the Plate Set Setup window.
  - At the start of the Inject Samples and Run protocol, the operator selects the plate set ID of the precreated plate set definition.
  - For each injection, the operator can manually enter a few plate-specific attributes when the plate is injected.

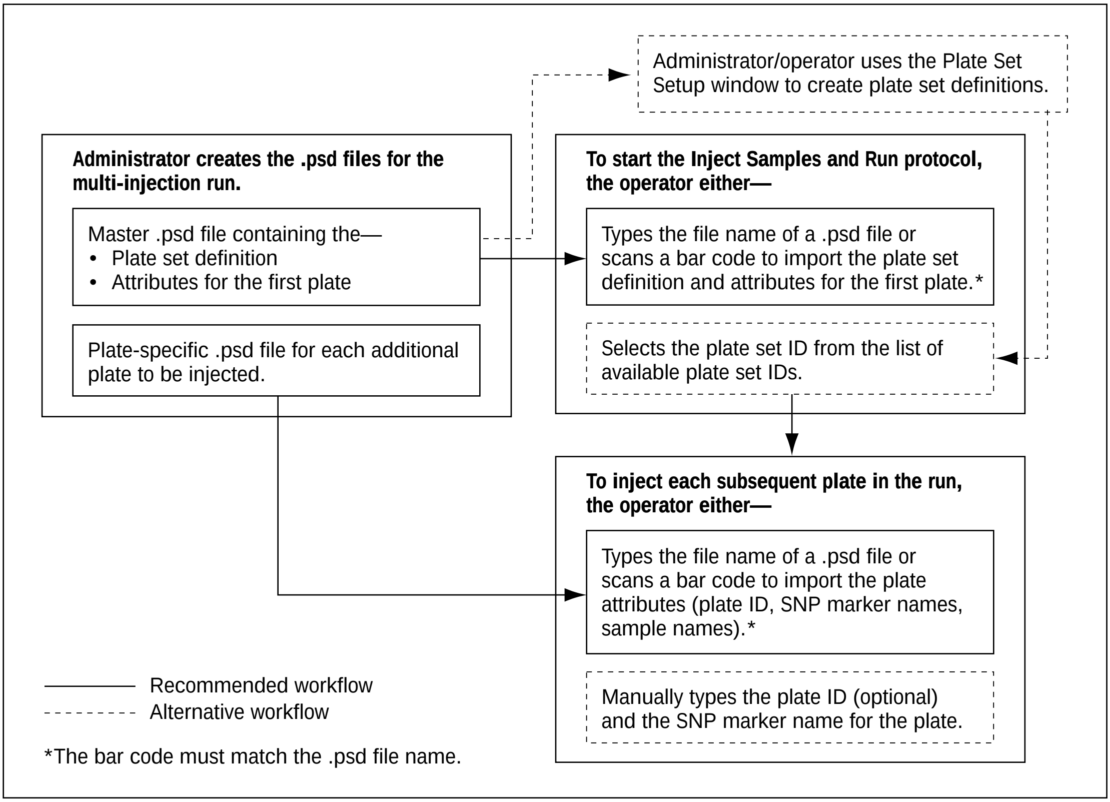


Figure 1-1. Typical workflows for providing the plate set definition and the plate-specific attributes.

1.3.1 About the plate set definition

A plate set definition consists of the—

- **Plate set ID—**The name for the set of plates injected 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. **Note:** If the plate set ID is missing, the software uses the ID of the plate that is injected first as the ID for the entire plate set. 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 injected in a single run. The values for these parameters can be imported from a .psd file, selected from a plate set setup template, or manually entered in the Plate Set Setup window.
- **(Optional) List of plates—**The list of bar codes representing 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.

1.3.2 The plate-specific attributes

The plate-specific attributes for the multi-injection run can include—

- **(Optional) Plate ID—**You can provide a plate ID in the .psd file that the operator imports for the injection or you can allow the operator to manually type the plate ID for the injection.
- **SNP marker names—**The Instrument Control Manager requires at least one SNP marker name for each injection. To specify more than one SNP marker name per injection (one name per well), the software requires a .psd file for the injection. Alternatively, you can allow the operator to manually type one SNP marker name for each plate they inject.
- **(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.
- **(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:** Comments about the plate set are included as part of the plate set setup parameters.

If the operator does not import the plate attributes from a .psd file, they can specify only the plate ID and one SNP marker name for each injection.

1.4 Administrator tasks

The administrator tasks vary depending on whether your laboratory uses .psd files. To automate the workflow during a multi-injection run, .psd files are recommended. Table 1-1 provides a task overview for the MegaBACE SNP genotyping system.

1.4.1 About configuring the system software for your laboratory

To tailor the SNP genotyping application for your laboratory, you can modify the system initialization files. The system software includes two initialization files that you can modify—

- **MegaBACE.ini file—**Determines the settings the Instrument Control Manager uses to control the operation of the MegaBACE instrument. For example, the MegaBACE.ini file can specify—
  - The default application (sequencing, genotyping, or SNP genotyping), if your laboratory uses more than one application on the same instrument.
  - Default plate setup and instrument control parameter templates for each application.
  - Whether an operator can manually edit the plate setup or instrument control parameters.
  - Whether the software enforces the order of the plates in a plate set.
  - Whether the software prompts the operator for a plate ID and SNP marker name, or whether the software provides the option of copying the sample names from the previous injection.
- **Chemistry.ini file—**Determines the chemistry parameter sets available in the Instrument Control Manager software. The Chemistry.ini file includes a chemistry parameter set for the SNuPe chemistry.

See chapter 2 for details on how to use these files.

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

Task	Frequency	Reference
(Optional) Although the Instrument Control Manager is already configured for SNP genotyping, the administrator can set additional preferences for the application.	• Once, when the laboratory first receives the system • Any time the system needs to be reconfigured	Chapter 2
(Optional) Administrator creates the plate setup data files (.psd) to automate how an operator provides the plate set definition and the attributes of plates within a plate set.	Every run	Section 3.2
(Optional) Although the software includes default templates for plate set setup parameters and instrument control parameters, the administrator can create additional templates.	–	Sections 3.5 and 3.6
Operator starts the MegaBACE system.	After any system shutdown	*MegaBACE Instrument Operator's Guide*
In the Instrument Control Manager, the operator selects the SNP genotyping application and checks that the correct filters are installed in the instrument for detecting the SNuPe dye set.	Any time the operator changes applications on the MegaBACE system	*MegaBACE SNP Genotyping Instrument Operator's Guide*
Operator performs a spectral calibration run, and then creates a spectral overlap matrix. SNP Profiler requires a spectral overlap matrix to perform automated SNP genotyping.	Periodically (monthly) on each instrument, depending on the throughput of your laboratory, and any time your laboratory— • Changes the run conditions, protocols, or chemistry within the selected application • Replaces the capillary arrays • Moves 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.	*MegaBACE SNP Genotyping Instrument Operator's Guide*

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

Task	Frequency	Reference
Operator performs a SNP genotyping run.	–	*MegaBACE SNP Genotyping Instrument Operator's Guide*
Analyst uses SNP Profiler to perform SNP genotyping on the collected data.	–	*MegaBACE SNP Profiler User's Guide*

1.4.2 About creating plate setup data files (.psd)

Important **The text or bar code that an operator enters (plate set ID or plate ID) must match the file name (case insensitive) of the .psd file for the software to import the file.**

For each multi-injection run, you can provide two types of .psd files (figure 1-1)—

- **Master .psd file—**Allows you to specify the plate set definition, including a list of the plates to be included in the plate set. You also use the master .psd file to specify the plate-specific attributes (section 1.3.2) for the first plate to be injected.
- **Additional plate .psd files—**Allows you to specify the plate-specific attributes (section 1.3.2) for each plate (injection).

For example, for a multi-injection run consisting of nine plates (injections), you would prepare—

- **One master .psd file—**To define the plate set and provide the attributes for the first plate to be injected.
- **Eight additional plate .psd files—**To provide the attributes for the second injection and all the subsequent injections in the run.

1.4.3 About the parameter templates

The Instrument Control Manager allows you to use templates to specify two different kinds of parameters—

- **Plate set setup template (.tpl)—**The plate set setup parameters are required for the plate set definition (section 1.3.1). The software includes a default plate set setup template (StdSNP.tpl) that includes the recommended parameters for SNP genotyping.

- **Instrument control parameters template (.icp)—**A combination of settings defining the instrument run conditions and matrix fill and flush cycles. The software includes a default instrument control parameters template (SNP_Typing.icp) that includes the recommended parameters for SNP genotyping.

The parameter templates can automate the operator tasks for the SNP genotyping application.

Chapter 2 Setting up the system software for SNP genotyping

To configure the system software for your laboratory's workflow, you might need to modify the system initialization files. This chapter describes the fields that have been added to the system initialization files to support SNP genotyping. The topics are—

- Overview of the system initialization files (section 2.1)
- The SNP genotyping fields in the MegaBACE.ini file (section 2.2)
- Setting SNP genotyping as the default application (section 2.3)
- Setting default parameter templates (section 2.4)
- Specifying limits for the injections (section 2.5)
- Enforcing the order in which an operator injects the plates (section 2.6)
- Prompting the operator for plate-specific information (section 2.7)
- The SNuPe chemistry parameters (section 2.8)

For additional details on the system initialization files, see the *MegaBACE Instrument Administrator's Guide*.

2.1 Overview of the system initialization files

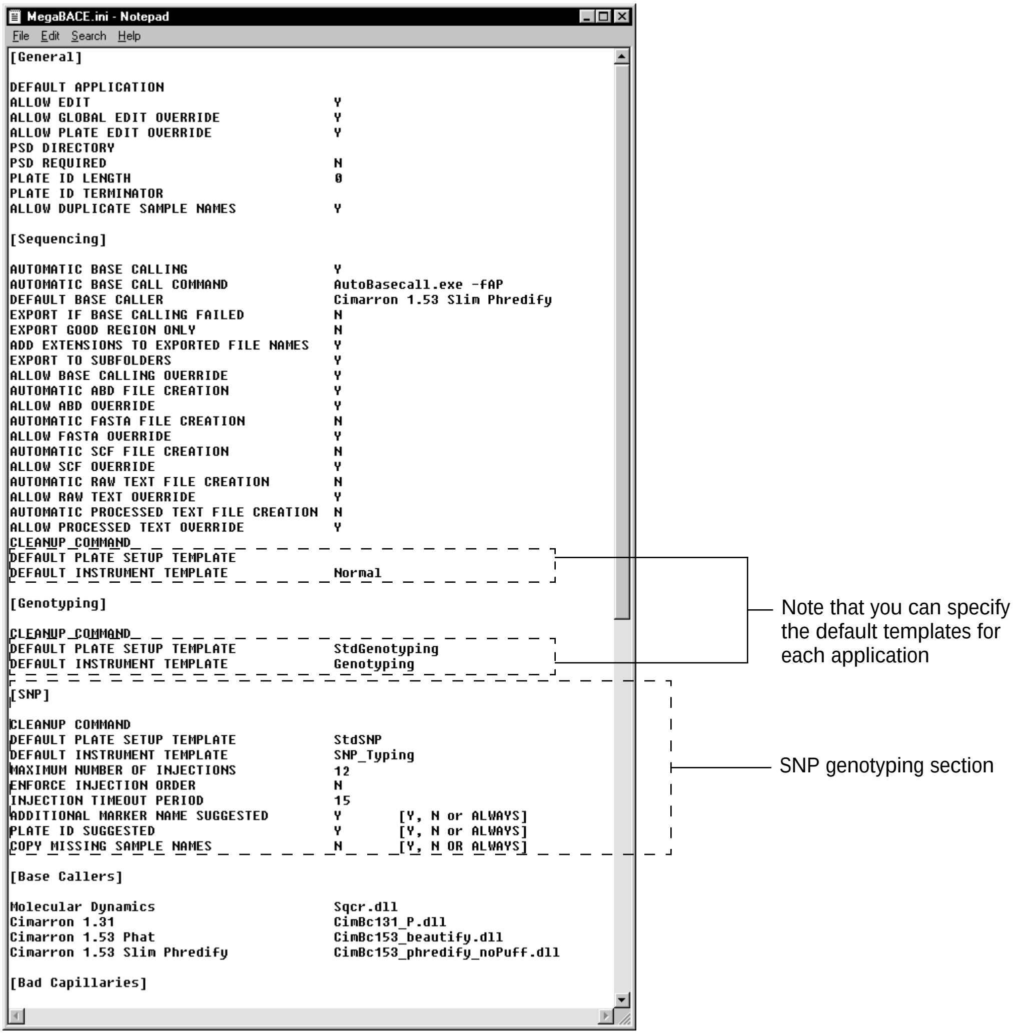
The MegaBACE system software includes the following files that you can modify to configure the Instrument Control Manager for your laboratory workflow and applications:

- **MegaBACE.ini file—**Determines the settings available in the Instrument Control Manager when you open the software.
- **Chemistry.ini—**Determines the choices for the chemistry parameter sets that are available in the Instrument Control Manager. The Instrument Control Manager uses only one Chemistry.ini file to define the available chemistry parameter sets for all applications (sequencing, genotyping, and SNP genotyping).

The system initialization files are stored in the …\MegaBACE\DataSystem folder. You can open the files using any software that supports tab-separated text, such as Microsoft Notepad or Excel.

2.2 The SNP genotyping fields in the MegaBACE.ini file

The MegaBACE.ini file determines the settings available in the Instrument Control Manager when you open the software. The file is divided into sections for sequencing, genotyping, and SNP genotyping (figure 2-1).



```
[General]

DEFAULT APPLICATION
ALLOW EDIT                              Y
ALLOW GLOBAL EDIT OVERRIDE              Y
ALLOW PLATE EDIT OVERRIDE               Y
PSD DIRECTORY
PSD REQUIRED                            N
PLATE ID LENGTH                         0
PLATE ID TERMINATOR
ALLOW DUPLICATE SAMPLE NAMES            Y

[Sequencing]

AUTOMATIC BASE CALLING                  Y
AUTOMATIC BASE CALL COMMAND             AutoBasecall.exe -fAP
DEFAULT BASE CALLER                     Cimarron 1.53 Slim Phredify
EXPORT IF BASE CALLING FAILED           N
EXPORT GOOD REGION ONLY                 N
ADD EXTENSIONS TO EXPORTED FILE NAMES   Y
EXPORT TO SUBFOLDERS                    Y
ALLOW BASE CALLING OVERRIDE             Y
AUTOMATIC ABD FILE CREATION             Y
ALLOW ABD OVERRIDE                      Y
AUTOMATIC FASTA FILE CREATION           N
ALLOW FASTA OVERRIDE                    Y
AUTOMATIC SCF FILE CREATION             N
ALLOW SCF OVERRIDE                      Y
AUTOMATIC RAW TEXT FILE CREATION        N
ALLOW RAW TEXT OVERRIDE                 Y
AUTOMATIC PROCESSED TEXT FILE CREATION  N
ALLOW PROCESSED TEXT OVERRIDE           Y
CLEANUP COMMAND
DEFAULT PLATE SETUP TEMPLATE
DEFAULT INSTRUMENT TEMPLATE             Normal

[Genotyping]

CLEANUP COMMAND
DEFAULT PLATE SETUP TEMPLATE            StdGenotyping
DEFAULT INSTRUMENT TEMPLATE             Genotyping

[SNP]

CLEANUP COMMAND
DEFAULT PLATE SETUP TEMPLATE            StdSNP
DEFAULT INSTRUMENT TEMPLATE             SNP_Typing
MAXIMUM NUMBER OF INJECTIONS            12
ENFORCE INJECTION ORDER                 N
INJECTION TIMEOUT PERIOD                15
ADDITIONAL MARKER NAME SUGGESTED        Y       [Y, N or ALWAYS]
PLATE ID SUGGESTED                      Y       [Y, N or ALWAYS]
COPY MISSING SAMPLE NAMES               N       [Y, N OR ALWAYS]

[Base Callers]

Molecular Dynamics                      Sqcr.dll
Cimarron 1.31                           CimBc131_P.dll
Cimarron 1.53 Phat                      CimBc153_beautify.dll
Cimarron 1.53 Slim Phredify             CimBc153_phredify_noPuff.dll

[Bad Capillaries]
```

Note that you can specify the default templates for each application

SNP genotyping section

Figure 2-1. The SNP genotyping fields in the MegaBACE.ini file. Note that the file contains a section for each application, which allows you to specify a default plate setup and instrument template.

Table 2-1 describes the fields in the SNP genotyping section of the MegaBACE.ini file. See the *MegaBACE Instrument Administrator's Guide* for a description of the other fields in the MegaBACE.ini file.

Table 2-1. The SNP genotyping fields in the MegaBACE.ini file

Field name	Description	Setting type	Default
Cleanup command	Allows an operator to enter a command, which the software performs at the end of the run, for transferring plate records and raw data files to another workstation for analysis or archiving.	String	none
Default plate setup template	Specifies a default plate set setup template.	String	StdSNP
Default instrument template	Specifies a default instrument control parameter template.	String	SNP_Typing
Maximum number of injections	Specifies an upper limit and default value for the NUMBER OF INJECTIONS field that is specified in the plate set setup (section 3.3.3) for a run. If a number is not specified, the software does not impose a limit.	Integer	12
Enforce injection order	Determines whether the operator must inject the plates in the order specified in the master .psd file. **Y =** If the plate set .psd file specifies a PLATE SET (section 3.3.2), the bar code the operator enters in every injection must match a specific item and the order specified in the PLATE SET field. **N =** If the plate set .psd file specifies a PLATE SET, the bar code the operator enters in every injection must match one of the items in the PLATE SET field. The order of injections does not matter.	Y/N	N
Injection timeout period	Determines the time (minutes) the software allows for completing another injection after the injection INTERVAL TIME, specified in the plate set setup, has expired. The software displays a timer in the Additional Injection window. If the time expires before the injection is complete, the software aborts the protocol. If the Sleep After This Run protocol was selected for the run, the software also puts the instrument in sleep mode.	Integer	15

Table 2-1. The SNP genotyping fields in the MegaBACE.ini file (continued)

Field name	Description	Setting type	Default
Additional marker name suggested	Determines if a window appears when an injection is missing a SNP marker name and prompts the operator for the information: **Y =** The window appears if an injection is missing a SNP marker name. The operator can provide a SNP marker name. The operator can also choose to suppress the reappearance of the window and use the latest provided SNP marker name for any injections missing this attribute in the run. **N =** The window does not appear. For any injections missing this attribute, the software automatically copies the latest SNP marker name provided in a previous injection. **ALWAYS =** The window appears for every injection for which there is a missing SNP marker name. The operator cannot suppress the reappearance of the window.	Y, N, or ALWAYS	Y
Plate ID suggested	Determines if a window appears when an injection is missing a plate ID and prompts the operator for the information: **Y =** The window appears if an injection is missing a plate ID, but the operator can suppress the reappearance of the window for the remaining injections in the run. **N =** The window does not appear. **ALWAYS =** The window appears for every injection for which there is a missing plate ID. The operator cannot suppress the reappearance of the window.	Y, N, or ALWAYS	Y

Table 2-1. The SNP genotyping fields in the MegaBACE.ini file (continued)

Field name	Description	Setting type	Default
Copy Missing Sample Names	For an injection missing all sample names, determines if the software proceeds without sample names, prompts the operator for input, or copies the sample names from a previous injection: **N =** The software does not prompt the operator for input and proceeds with the injection without sample names. **Y =** The software prompts the operator to choose whether to copy the sample names from the latest plate containing at least one sample name or proceed with the injection without sample names. **ALWAYS =** The software does not prompt the operator, but automatically copies the sample names from the latest plate containing at least one sample name.	Y, N, or ALWAYS	N

2.3 Setting SNP genotyping as the default application

The operator can change the selected application in the Instrument Control Manager by choosing the Applications command from the View menu. However, any time you restart the Instrument Control Manager, the software automatically resets the selected application to the default specified in the MegaBACE.ini file.

To set SNP genotyping as the default application, you use the following field and value in the MegaBACE.ini file:

Field in MegaBACE.ini file	Value
DEFAULT APPLICATION	SNP Genotyping

2.4 Setting default parameter templates

The Instrument Control Manager allows you to use templates to define the setup parameters for the plate set and the instrument control parameters for multiple runs. You can specify default templates in the MegaBACE.ini file.

If you enable the edit mode in the MegaBACE.ini file, the operator can modify the values for a given run or choose a different template in the Instrument Control Manager. However, any time the Instrument Control Manager is restarted, the software automatically resets the selected template to the default specified in the MegaBACE.ini file.

2.4.1 Setting the default plate set setup template

If you specify a default template in the MegaBACE.ini file, the values from the plate set setup template appear automatically in the Plate Set Setup window. As figure 2-1 and table 2-1 show, the software has been configured to use the StdSNP.tpl template as the default for SNP genotyping. If you need to specify a different default template for the SNP genotyping application, you can use the following field in the SNP section of the MegaBACE.ini file:

Field in MegaBACE.ini file	Value
DEFAULT PLATE SETUP TEMPLATE	Type the file name of the .tpl file, without the .tpl file extension.

If the edit mode is enabled, the operator can select a different default plate set setup template from the Templates menu in the Plate Set Setup window.

Note: If the operator uses a plate setup data file (.psd) to define the plate set for a run, the values in the .psd file override any duplicate parameters specified in a .tpl file.

2.4.2 Setting the default instrument control parameters template

If you specify a default template in the MegaBACE.ini file, the values from the instrument control template appear automatically in the Instrument Control window when the operator performs a run. As figure 2-1 and table 2-1 show, the software has been configured to use the SNP_Typing.icp template as the default for SNP genotyping. If you need to specify a different default template for the SNP genotyping application, you can use the following field in the SNP section of the MegaBACE.ini file:

Field in MegaBACE.ini file	Value
DEFAULT INSTRUMENT TEMPLATE	Type the file name of the .icp file, without the .icp file extension.

If the edit mode is enabled, the operator can select a different instrument template from the Templates menu in the Instrument Control window.

2.5 Specifying limits for the injections

2.5.1 Specifying a maximum number of injections

Your laboratory might want to specify a maximum number of injections to make sure an operator does not exceed the number of injections possible for the given run conditions. All the samples in a multi-injection run must be injected into the capillaries before the samples from the first injection reach the capillary detection windows. The injection interval time (plate set definition) affects how many injections an operator can complete before the first injection reaches the capillary detection window. The time required for an injection interval is related to the length of the SNuPe primer. See the *MegaBACE SNP Genotyping Instrument Operator's Guide* for guidelines on designing a multi-injection run.

To specify a maximum number of injections, you use the following field in the MegaBACE.ini file:

Field in MegaBACE.ini file	Value
MAXIMUM NUMBER OF INJECTIONS	12 or less **Note:** If a greater value is specified for the NUMBER OF INJECTIONS value, the software displays an error message when the operator provides the plate set definition (section 3.1).

If the MAXIMUM NUMBER OF INJECTIONS value is—

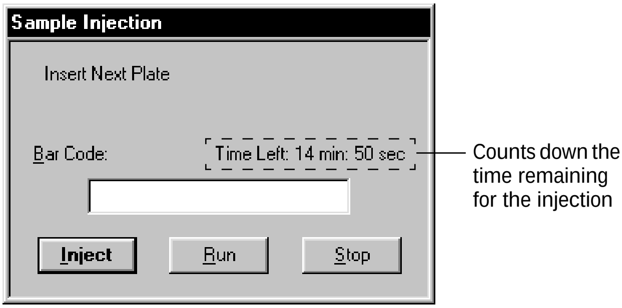
- Less than the NUMBER OF INJECTIONS value, the software displays an error message.
- Greater than the NUMBER OF INJECTIONS value, the software uses the NUMBER OF INJECTIONS value as the number of injections proposed to the operator during the run.

If no NUMBER OF INJECTIONS value is provided, the software uses the MAXIMUM NUMBER OF INJECTIONS value as the number of injections proposed to the operator during the run.

2.5.2 Specifying the injection timeout period

Typically, an operator should inject the plate within 1–2 minutes after the Sample Injection window appears (figure 2-2) during the Inject Samples and Run protocol. However, you should allow sufficient time in the injection period in case an operator needs to leave the instrument temporarily during one of the injections.

To specify a time limit for the injection, you use the INJECTION TIMEOUT PERIOD field in the MegaBACE.ini file. The value you specify for the INJECTION TIMEOUT PERIOD (15 minutes, default) is the maximum time the operator has to inject a plate for any single injection in the multi-injection run.



Sample Injection

Insert Next Plate

Bar Code:

Time Left: 14 min: 50 sec

Counts down the time remaining for the injection

Figure 2-2. The Sample Injection window showing the timer that counts down the time remaining for the injection.

If the operator does not inject the plate before the time in the Sample Injection window elapses, the software aborts the Inject Samples and Run protocol. In addition, if the Sleep After This Run option was selected in the Instrument Control window, the instrument stores the capillaries and lowers the temperature of the instrument.

2.6 Enforcing the order in which an operator injects the plates

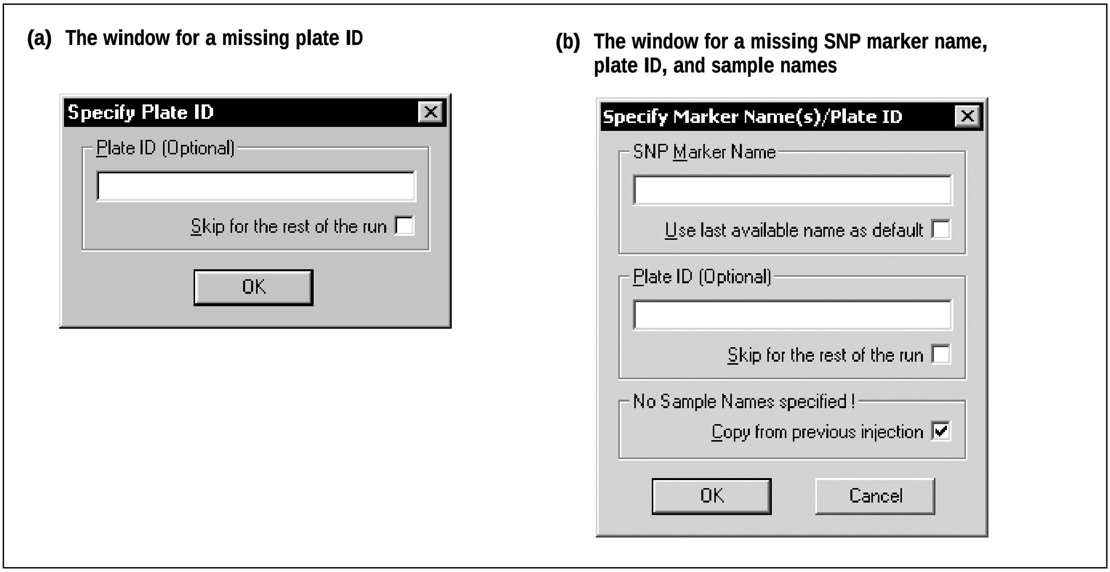
You can use the ENFORCE INJECTION ORDER field in the MegaBACE.ini file to specify the order in which the operator injects the plates in a plate set. The value you specify for the ENFORCE INJECTION ORDER field is only applicable if you use a master .psd file to specify the list of plates in the PLATE SET field (section 3.3.2). In this case, the following values apply for the ENFORCE INJECTION ORDER field—

- **Y—**The operator must provide the plate bar codes (.psd file names) for each injection in the order specified in the PLATE SET field of the .psd file.
- **N—**The bar code the operator enters for each injection must match one of the items in the PLATE SET field, but the order of injections does not matter.

In both cases, the software matches the bar code that the operator provides with a given item in the PLATE SET field only once. **Note:** If the NUMBER OF INJECTIONS value is greater than the number of items in the PLATE SET field (section 3.3), the operator can enter any bar codes for the remaining injections.

2.7 Prompting the operator for plate-specific information

If the plate ID, SNP marker name, or sample names are missing for an injection in a multi-injection run, you can configure the software to display a window that allows manual entry of the missing attributes (figure 2-3).


(a) The window for a missing plate ID

(b) The window for a missing SNP marker name, plate ID, and sample names

Figure 2-3. The Specify Marker Name/Plate ID window changes dynamically depending on which information is missing: (a) an example of the window requesting a missing plate ID, and (b) an example of the window requesting the SNP marker name, the plate ID, and sample names.

2.7.1 Providing missing SNP marker names

The software requires at least one SNP marker name per injection. To specify one plate-level SNP marker name per injection, you can allow the operator to import the information from a .psd file for each injection or type the name in the Specify Marker Name window (figure 2-3b). To specify a SNP marker name for a given well in an injection that is different than the plate-level (global) SNP marker name, the software requires a .psd file for the injection (section 3.2).

If the SNP marker name is missing for a given injection, the choices available to the operator vary depending on the value you specify for the ADDITIONAL MARKER NAME SUGGESTED field. The field is effective only if an injection is missing the plate-level marker name and does not have well-specific SNP marker names defined for all the wells in the plate. The value you specify for the ADDITIONAL MARKER NAME SUGGESTED field can depend on whether your laboratory uses—

- Different SNP markers in the same run
- The same SNP marker for multiple runs

Using different SNP marker names in the same run

If your laboratory uses multiple SNP markers in any given run, you might want the software to prompt the operator for any injections that are missing the SNP marker name. To do this, you can specify Y (default) or ALWAYS as the value for the ADDITIONAL MARKER NAME SUGGESTED field. The value that you specify depends on whether you want to allow the operator to automatically copy the last plate-level SNP marker name provided in the run for an injection that is missing the attribute.

- **Y—**Allows the operator to provide the SNP marker name and choose to automatically copy the latest plate-level SNP marker name for an injection that is missing the attribute. If an injection is missing the plate-level SNP marker name, a Specify Marker Name window appears (figure 2-3b) and allows the operator to type the SNP marker name.

  In addition, the operator can select the **Use last available name as default** check box. If the operator selects the check box, the Specify Marker Name window does not appear again during the run. Instead, the software automatically uses the SNP marker name the operator provided for the following consecutive injections that are missing the attribute. If the software encounters a plate in the subsequent injections that contains a plate-level SNP marker name, this latest SNP marker name is used for the subsequent consecutive injections that are missing the attribute until the software encounters an injection containing the attribute.

- **ALWAYS—**Allows the operator to provide the SNP marker name, but prevents the operator from choosing to copy the latest SNP marker name for any subsequent injections that are missing the SNP marker name.

  If a Specify Marker Name window appears, the **Use last available name as default** check box is unavailable. The operator must provide a SNP marker name and cannot suppress the reappearance of the window.

Using the same SNP marker name for multiple injections

If your laboratory uses the same SNP marker on many individuals, you might want to copy the SNP marker name for any injections that are missing this attribute. The default value (Y) for the ADDITIONAL MARKER NAME SUGGESTED field allows the operator to choose to copy the missing SNP marker names as described above.

Alternatively, you might want the software to copy the missing SNP marker name from the previous injection automatically instead of prompting the operator. To do this, specify N for the value of the ADDITIONAL MARKER NAME SUGGESTED field. If the first injection is missing the SNP marker name, a Specify Marker Name window appears, but the window does not reappear for subsequent injections in the same run. Instead, the software automatically uses the last plate-level SNP marker name provided during the run for the following consecutive injections that are missing the attribute. If the software encounters a plate in the subsequent injections that contains a plate-level SNP marker name, this latest SNP marker name is used for the subsequent consecutive injections that are missing the attribute until the software encounters an injection containing the attribute.

2.7.2 Providing missing plate IDs

If an injection is missing the plate ID, you can configure the software to prompt the operator for the missing plate ID or ignore the missing plate ID.

Prompting the operator for missing plate IDs

You use the PLATE ID SUGGESTED field to configure the software to display a Specify Plate ID window (figure 2-3a), which allows the operator to provide the missing plate ID. In addition, you can also specify whether the operator—

- Can suppress the reappearance of the Specify Plate ID window for the remaining injections. To enable this feature, specify Y as the value for the PLATE ID SUGGESTED field (default).

  If a Specify Plate ID window appears, the operator can select the **Skip for the rest of the run** check box. If the operator selects the check box, the software proceeds without a plate ID for any injections missing this attribute.

- Cannot suppress the reappearance of the Specify Plate ID window for the remaining injections. To do this, specify ALWAYS as the value for the PLATE ID SUGGESTED field.

  If a Specify Plate ID window appears, the **Skip for the rest of the run** check box is not available. A Specify Plate ID window appears for any injections in the run that are missing a plate ID.

Ignoring missing plate IDs

If you want the software to ignore any missing plate IDs and automatically proceed without prompting the operator, specify N as the value for the PLATE ID SUGGESTED field.

2.7.3 Copying sample names from a previous injection

The ability to copy all the sample names from a previous injection can be useful if your laboratory uses the same sample plate layout for multiple injections. To specify the sample names for a given injection, the software requires a .psd file (section 3.4.3). However, if all the sample names are missing for an injection, you can allow the operator to copy the sample names from a previous injection in the same run.

You can use the COPY MISSING SAMPLE NAMES field to specify how the software handles the injections for which no sample names are specified.

Ignoring the missing sample names

If you want the software to ignore the missing sample names and proceed without prompting the operator for information, specify N as the value for the COPY MISSING SAMPLE NAMES field (default). The software uses the well ID in place of the missing sample names for the given injection.

Allowing the operator to copy the missing sample names

If you want the software to prompt the operator to copy all the sample names from the latest injection that contained sample names, specify Y as the value for the COPY MISSING SAMPLE NAMES field.

If no sample names are provided for a given injection, a window appears (figure 2-3b). In the No Sample Names Specified area, the operator can select the **Copy from previous injection** check box. If the operator selects the check box, the software copies the sample names from the latest injection containing any sample names.

Automatically copying the missing sample names

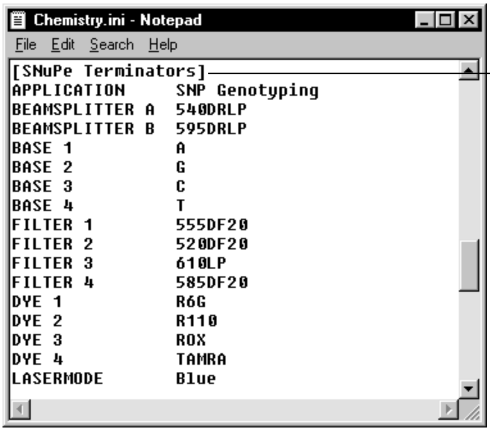
If you want the software to automatically copy all the sample names from the latest injection that contained sample names, specify ALWAYS as the value for the COPY MISSING SAMPLE NAMES field. In this case, the software does not prompt the operator to copy the missing sample names.

2.8 The SNuPe chemistry parameters

The Chemistry.ini file (figure 2-4a) contains all the chemistry parameter sets that are available in the Instrument Control Manager. The chemistry parameters are required for defining the plate set setup. The chemistry parameters appear in the Chemistry tab of the Plate Set Setup window (figure 2-4b). The Chemistry.ini file contains chemistry parameter sets for the sequencing, genotyping, and SNP genotyping applications that have been validated on the MegaBACE instrument. Figure 2-4 shows the SNuPe Terminators chemistry parameter set, which specifies the dye names, base order, and filters for the detecting SNuPe products.

If you use plate setup templates, you must specify the name of the chemistry parameter set in the template. You can also specify the chemistry name in a .psd file for a given plate. For instructions on how to add additional chemistry parameter sets to the Chemistry.ini file, see the *MegaBACE Instrument Administrator's Guide*.

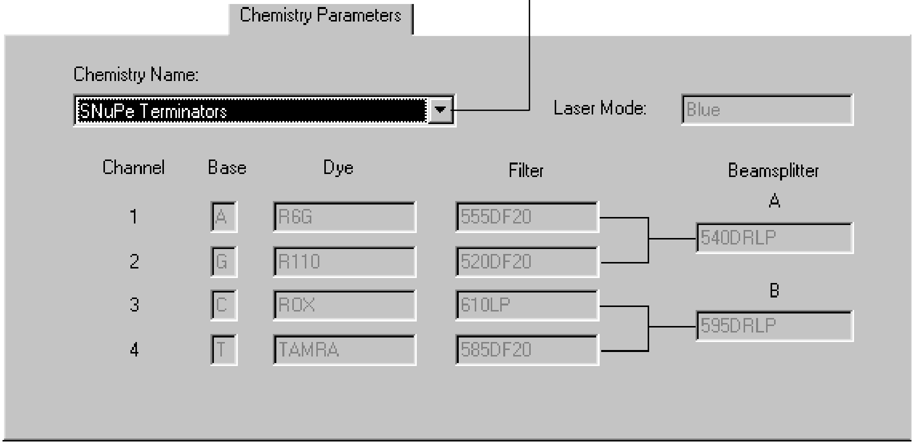
(a) The Chemistry.ini file



```
[SNuPe Terminators]
APPLICATION     SNP Genotyping
BEAMSPLITTER A  540DRLP
BEAMSPLITTER B  595DRLP
BASE 1          A
BASE 2          G
BASE 3          C
BASE 4          T
FILTER 1        555DF20
FILTER 2        520DF20
FILTER 3        610LP
FILTER 4        585DF20
DYE 1           R6G
DYE 2           R110
DYE 3           ROX
DYE 4           TAMRA
LASERMODE       Blue
```

The name used in the Chemistry.ini file appears as a name in the Chemistry Name list.

(b) The Chemistry Parameters tab



Chemistry Name: SNuPe Terminators

Laser Mode: Blue

Channel	Base	Dye	Filter	Beamsplitter
1	A	R6G	555DF20	A: 540DRLP
2	G	R110	520DF20	
3	C	ROX	610LP	B: 595DRLP
4	T	TAMRA	585DF20	

Figure 2-4. The chemistry parameters: (a) the Chemistry.ini file showing the chemistry parameter set for SNP genotyping, and (b) the Chemistry Parameters tab of the Plate Set Setup window in the Instrument Control Manager.

Chapter 3 Creating plate set definitions and .psd files

For each SNP genotyping run, the Instrument Control Manager requires a plate set definition, at least one SNP marker name for each injection, and the instrument control parameters. Although not required, plate setup data files (.psd) are recommended to automate how an operator provides the plate set definition and plate-specific attributes during a multi-injection run. This chapter describes how to create .psd files and provides examples of the plate set setup templates and instrument parameter templates for SNP genotyping. The topics are—

- About creating plate set definitions (section 3.1)
- Creating plate setup data files (.psd) for SNP genotyping (section 3.2)
- Specifying the plate set definition in a master .psd file (section 3.3)
- Specifying the attributes for a plate in a .psd file (section 3.4)
- Using a plate set setup template file (.tpl) for SNP genotyping (section 3.5)
- About the instrument control template (.icp) (section 3.6)

3.1 About creating plate set definitions

A plate set definition consists of the—

- **Plate set ID—**The software uses the plate set ID to name the run folder. If the plate set ID is missing, the software uses the plate ID of the first plate injected. If the plate ID is also missing, the software uses the file name of the master plate setup data file (.psd) to name the run folder (section 3.2).

- **Plate set setup parameters—**The run parameters that are common to all the plates in the plate set (section 3.1.1).

- **List of plates (optional)—**The list of bar codes representing the plates in the plate set (section 3.3.2).

3.1.1 The plate set setup parameters

For a SNP genotyping run, the plate setup parameters are common to the entire set of plates injected during the multi-injection run. Table 3-1 lists the plate set setup parameters. The software requires values for all the parameters, except the optional parameters and comments.

Table 3-1. The plate set setup parameters

Parameter	Description	Default value for SNP genotyping
Electrophoresis Parameters		
Sample injection voltage	The range is 1–20 kV.	12 kV
Sample injection time	The range is 1–600 s.	7 s
Run voltage	The range is 1–20 kV.	6 kV
Run time	The range is 1–720 min.	50 min
Interval voltage	The range is 1–20 kV.	9 kV
Interval time*	The range is 1–1800 s.	100 s
Chemistry Parameters	The combination of settings for the dyes, the laser excitation mode, the filters, and the beam-splitters. The Chemistry.ini file (section 2.8) must contain the chemistry parameter set before the chemistry parameter set is available for selection.	SNuPe Terminators
File Names	The name of the raw sample data file (.rsd) the software produces for each well in a plate. The software uses the well ID as the file name unless alternative file names are provided in the master .psd file, a plate set setup template, or the Plate Set Setup window. **Note:** Unlike sequencing or microsatellite genotyping, to specify the sample names for a SNP genotyping run, you must use .psd files.	Well IDs
Optional Parameters		
Run temperature	The temperature used for a special run. Typically defined as part of the instrument control parameters (section 3.6), the value provided here overwrites the value specified in the instrument control parameters.	No default specified
PMT1 voltage	The PMT1 voltage for a special run.Typically defined as part of the instrument control parameters (section 3.6), the value provided here overwrites the value specified in the instrument control parameters.	No default specified
PMT2 voltage	The PMT2 voltage for a special run. Typically defined as part of the instrument control parameters (section 3.6), the value provided here overwrites the value specified in the instrument control parameters.	No default specified

Table 3-1. The plate set setup parameters (continued)

Parameter	Description	Default value for SNP genotyping
Number of injections[*]	The number of injections the software prompts the operator for during a multi-injection run. The MegaBACE.ini file specifies an upper limit for this field (section 2.5.1).	No default specified
Comments	Any comments about the plate set.	No default specified

[*] See the *MegaBACE SNP Genotyping Instrument Operator's Guide* for guidelines on determining the interval time and the number of injections.

3.1.2 Methods for creating the plate set definitions

The Instrument Control Manager allows you to create the plate set definitions in various ways. You can use—

- A plate setup data file (.psd) that an operator can import at the start of the Inject Samples and Run protocol.
- The Plate Set Setup window (figure 3-1) to create the definitions before the run. The operator can select the precreated plate set definitions at the start of the Inject Samples and Run protocol.

Plate setup data file (.psd)

You can create a master .psd file (section 3.2) that an operator can import during the run. The .psd file can specify the plate set ID, the plate set setup parameters, and the set of plates included in the multi-injection run. In addition, the master .psd file can include the plate-specific attributes for the first plate to be injected.

Plate Set Setup window

You or an operator can create the plate set definition before the run using the Plate Set Setup window (figure 3-1). To do this, you or the operator can—

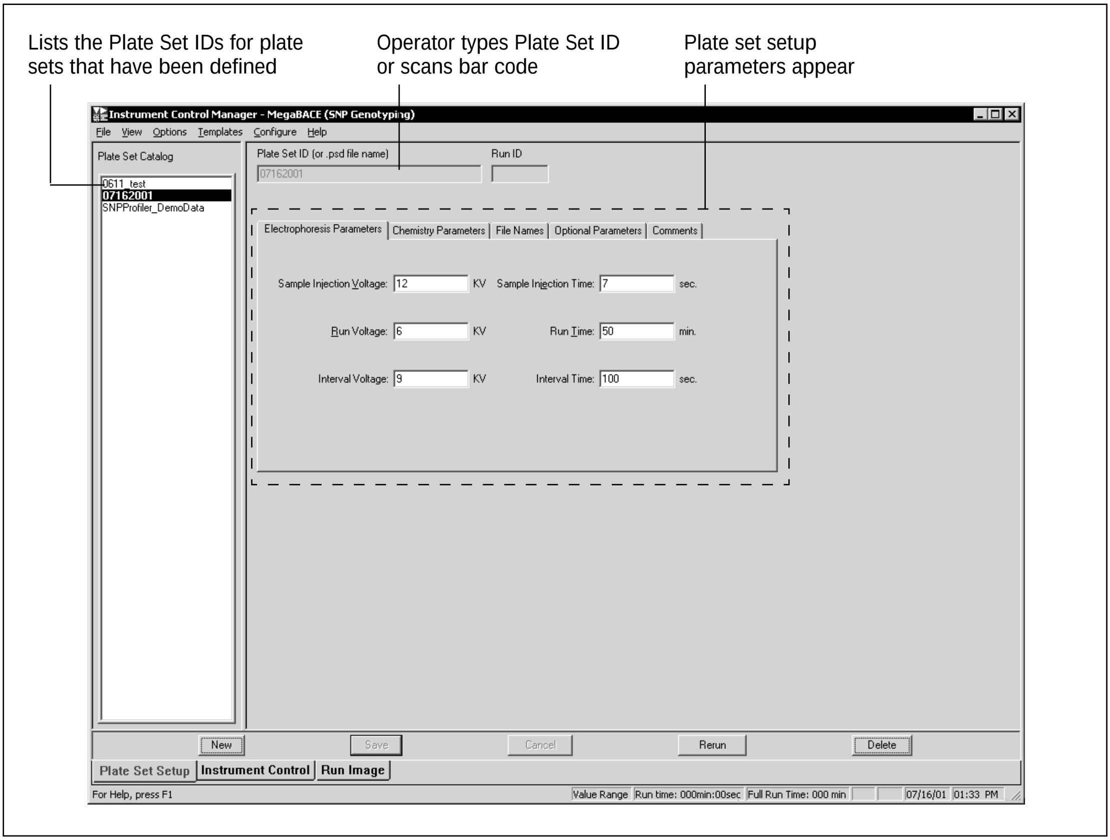
- **Import a master .psd file—**When the .psd file name is entered in the Plate Set ID box of the Plate Set Setup window, the software imports the information from the .psd file and automatically creates the plate set definition (section 3.2).
- **Select a plate set setup template (.tpl)—**Your laboratory can use a template to specify the plate set setup parameters that are visible in the Plate Set Setup window (section 3.5). In the MegaBACE.ini file, you can

specify a default template that appears automatically when an operator creates a new plate set definition in the Plate Set Setup window. The software includes a default template for the SNP genotyping application (StdSNP.tpl).

A plate set setup template cannot include fields for the plate set ID, the SNP marker name, or the list of plates in the run. However, you can use a master .psd file in addition to the template to specify these additional parameters.

- **Manually enter or modify the setup parameters—**To manually enter or change parameters in the Plate Set Setup window, you must enable the edit mode in the MegaBACE.ini file. See the *MegaBACE Instrument Administrator’s Guide* for a description of the edit mode.

See the *MegaBACE SNP Genotyping Instrument Operator’s Guide* for a description of how to create plate set definitions in the Plate Set Setup window.



0611_test
07162001
SNPProfiler_DemoData

Plate Set ID (or .psd file name): 07162001
Run ID:

Sample Injection Voltage: 12 KV
Sample Injection Time: 7 sec.
Run Voltage: 6 KV
Run Time: 50 min.
Interval Voltage: 9 KV
Interval Time: 100 sec.

For Help, press F1 | Value Range | Run time: 000min:00sec | Full Run Time: 000 min | 07/16/01 | 01:33 PM

Figure 3-1. The Plate Set Setup window displays the parameters shared by all the plates in the plate set.

3.2 Creating plate setup data files (.psd) for SNP genotyping

3.2.1 About the types of .psd files

For SNP genotyping you can use two types of .psd files, one as the master file for the plate set and the first plate you inject and one for each subsequent plate in the plate set—

- **Master .psd file—**The master .psd file for the plate set can list the set of plates included in a multiple-injection run and define the plate setup parameters shared by all the plates in the run (section 3.3). The master .psd file can also include the information for the first plate that you inject. For example, you can specify a plate ID and the SNP marker name for the plate and you can associate a sample name with each well in the plate.

  Note: If you want to associate a sample name with each well in a given plate, you must use a .psd file to define the sample names.

- **Additional plate .psd files—**For each plate in the plate set, you can use a plate-specific .psd file to specify the plate-level and well-level information for a given plate (section 3.4). If you want to associate a sample name with each well in a plate, you must use a .psd file to define the sample names.

3.2.2 Creating a plate setup data file (.psd)

The .psd files can be created using any software that supports tab-separated text. When the .psd file is imported, the Instrument Control Manager stores the information in the extended header of the raw sample data files (.rsd) for all the files in the run (chapter 5). The well-level information, such as sample name, becomes part of the extended header in the .rsd file for the well.

To create a .psd file—

1. Open a new document or an existing .psd file using software such as Notepad or Excel. **Note:** The MegaBACE software includes examples of .psd files that you can use as templates.
2. Type the fields and values for the plate set (section 3.3) or plate (section 3.4) using a two-column format. You must type the field names in the first column (the field column). You type the values for each field name in the second column (the value column). The field column can be separated from the values column by any number of tabs.

3. Save the file in an appropriate format—

   - **File name—**Make sure the file name is an exact match to the plate set ID or bar code that the operator will use to import the file. Make sure you use the .psd file extension.
   - **Storage location—**Make sure you store the .psd file in the …\MegaBACE\Psd folder (or the folder you specified as the Psd directory in the MegaBACE.ini file); otherwise, the Instrument Control Manager will not find the file.

   Note: If you use Excel, save the file as a tab-delimited file.

3.3 Specifying the plate set definition in a master .psd file

Figure 3-2 shows an example of a master .psd file that includes the plate set definition. Table 3-2 lists the fields for the master .psd file. In addition to these fields, the master .psd file can contain fields that define the attributes of the first plate injected in the run (table 3-3).

Note: All fields in a .psd file are optional. However, if the required plate set setup parameters are not provided in a .psd file, the Plate Set Setup window must be used to create the plate set definition.

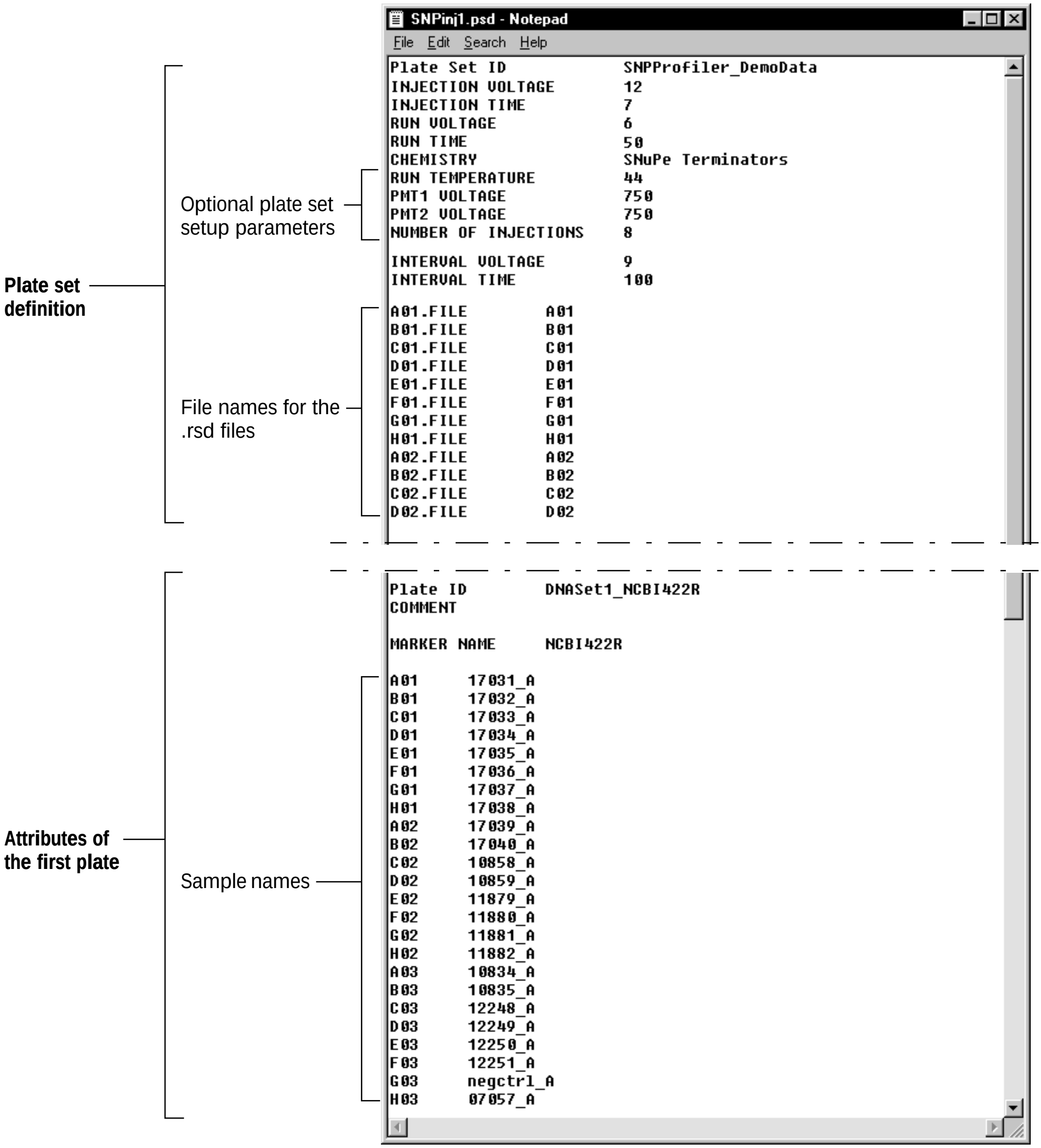
3.3.1 Specifying the plate set ID

To define the plate set ID, you can use the PLATE SET ID field in the .psd file. The software uses the plate set ID to name the run folder. If the PLATE SET ID field does not contain a value, the software uses the value provided for the PLATE ID field. If the PLATE ID is also missing, the software uses the .psd file name (bar code) as the plate set ID and to name the raw run folder.

3.3.2 Defining the list of plates in the plate set

To define the list of plates in the plate set, you use the PLATE SET field in the master .psd file. In the value column for this field, you list the bar code (.psd file name) for each .psd file in the plate set, including the first plate (master .psd file name). You list the bar codes in one line using a space to separate each bar code from the others in the list. Each bar code in the list must match the name of a .psd file (without the file extension) that you want the operator to import during the run.

The list of bar codes that you provide for this field determines the set of bar codes that the software can accept during the run. Each bar code can be used during the run only as many times as it is listed in the PLATE SET field.



```
Plate Set ID            SNPProfiler_DemoData
INJECTION VOLTAGE       12
INJECTION TIME          7
RUN VOLTAGE             6
RUN TIME                50
CHEMISTRY               SNuPe Terminators
RUN TEMPERATURE         44
PMT1 VOLTAGE            750
PMT2 VOLTAGE            750
NUMBER OF INJECTIONS    8

INTERVAL VOLTAGE        9
INTERVAL TIME           100

A01.FILE        A01
B01.FILE        B01
C01.FILE        C01
D01.FILE        D01
E01.FILE        E01
F01.FILE        F01
G01.FILE        G01
H01.FILE        H01
A02.FILE        A02
B02.FILE        B02
C02.FILE        C02
D02.FILE        D02
```

```
Plate ID        DNASet1_NCBI422R
COMMENT

MARKER NAME     NCBI422R

A01     17031_A
B01     17032_A
C01     17033_A
D01     17034_A
E01     17035_A
F01     17036_A
G01     17037_A
H01     17038_A
A02     17039_A
B02     17040_A
C02     10858_A
D02     10859_A
E02     11879_A
F02     11880_A
G02     11881_A
H02     11882_A
A03     10834_A
B03     10835_A
C03     12248_A
D03     12249_A
E03     12250_A
F03     12251_A
G03     negctrl_A
H03     07057_A
```

Figure 3-2. Example of master .psd file defining the plate set and the first plate to be injected.

Table 3-2. The fields in the master .psd file for a plate set

Field name	**Value**
Plate set ID	String consisting of any characters except spaces or other characters not allowed in file names.
Plate set	(Optional) String consisting of bar codes separated by spaces. The bar codes must match the file names of the .psd files in the plate set.
Injection voltage	Integer or fraction (kV)
Injection time	Integer (s)
Run voltage	Integer or fraction (kV)
Run time	Integer (min)
Interval voltage	Integer or fraction (kV)
Interval time	Integer (s)
Chemistry	String representing the name of a chemistry set that has been specified in the Chemistry.ini file.
Number of injections	Integer **Note:** The upper limit can be specified in the MegaBACE.ini file (section 2.2).
Run temperature	Integer (°C)
PMT1 voltage	Integer or fraction (kV)
PMT2 voltage	Integer or fraction (kV)
Well_ID.FILE (where well_ID is the location of the well, such as A01)	String representing the file name that you want to assign the designated well location. If you use spaces in the file names, the Instrument Control Manager converts the spaces to underscore characters in the file name. **Note:** An .rsd file for a given well location contains the sample data from all the plates injected during the run.
Plate Set.comment	String, including spaces, representing the comments for the plate set.
User-defined plate set fields	Fields that can be imported, but are not used by the software. The software stores the information from these fields in the extended header of the files.

Note: See table 3-1 for the value ranges and recommended values for the plate set setup parameters.

Table 3-3. The fields used in any .psd file for SNP genotyping

Field name	Value
Plate ID	String representing the plate ID or bar code for the plate.
Marker name	String representing the SNP marker name for the plate.
Comment	String, including spaces, representing comments about the plate or injection.
Well_ID or well_ID.NAME (where well_ID is the location of the well, such as A01)	String representing the sample name that you want to assign to the designated well location. If you use spaces in the sample names, the Instrument Control Manager converts the spaces to underscore characters in the file name. **Note:** The software does not use this label as a file name because the sample name is specific to a well for a single plate in the plate set.
Well_ID.MARKER NAME (where well_ID is the location of the well, such as A01)	String representing the SNP marker name that you want to assign to the designated well location.
User-defined fields	Fields that can be imported, but are not used by the software. The software stores the information from these fields in the extended header of the files.

The values you use for the PLATE SET field should agree with the values you specify for the following fields:

- NUMBER OF INJECTIONS field in the .psd file. If the NUMBER OF INJECTIONS value is—
  - Less than the number of bar codes in the PLATE SET, the NUMBER OF INJECTIONS value determines the number of injections. During each injection, the operator must provide one of the bar codes in the PLATE SET.
  - Greater than the number of bar codes in the PLATE SET, the operator can use any bar codes (.psd file names) for the injections remaining after using all the bar codes listed for the PLATE SET.
- ENFORCE INJECTION ORDER field in the MegaBACE.ini file (section 2.2), which allows you to enforce the order in which the operator injects the plates listed in the PLATE SET.

3.3.3 Defining the number of injections

To define the number of injections, you can use the NUMBER OF INJECTIONS field in the .psd file. The value you specify for this field should agree with the values specified for the following fields:

- PLATE SET field in the .psd file. See section 3.3.2 for details.
- MAXIMUM NUMBER OF INJECTIONS field in the MegaBACE.ini file. If the NUMBER OF INJECTIONS value exceeds the maximum number specified in the MegaBACE.ini file, the software displays an error message when the operator tries to import the .psd file.

3.4 Specifying the attributes for a plate in a .psd file

You can specify the attributes for a plate in a .psd file. For a multi-injection run, you can use one .psd file for each plate you inject after the first injection. The attributes for the first plate are specified in the master .psd file (section 3.3).

Figure 3-3 shows an example of a .psd file for one plate. Table 3-3 lists the fields you can use in a .psd file to define the attributes of a specific plate in the plate set. These fields can be defined only in a .psd file. **Note:** The software does not display these values in the Plate Set Setup window because the values are unique to a single plate in the plate set.

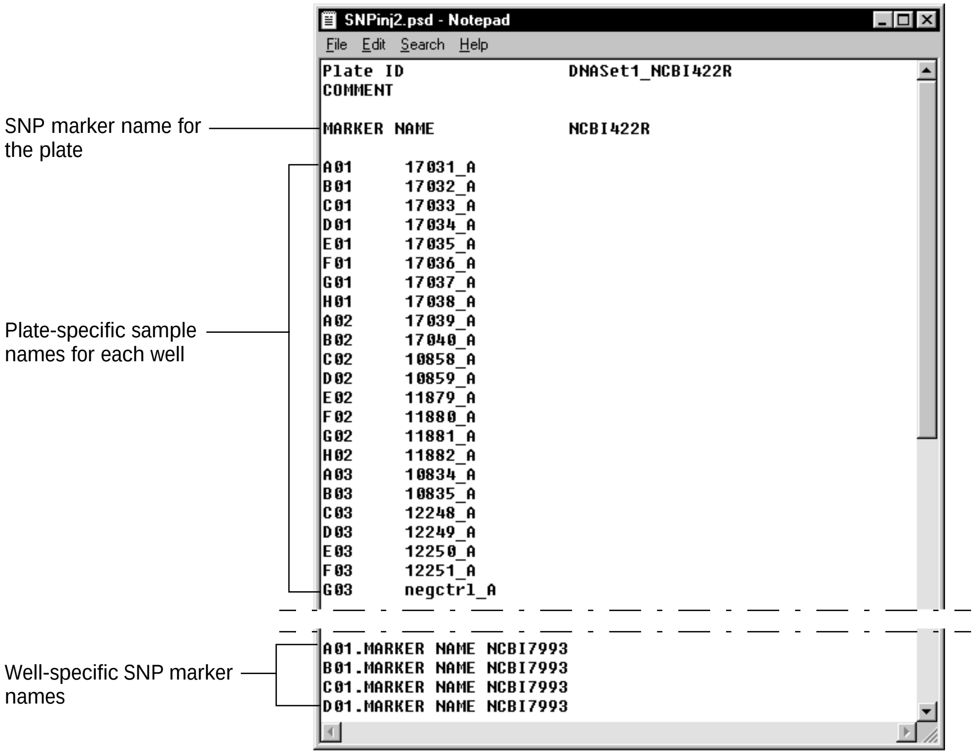
3.4.1 Specifying the plate ID

In the .psd file for a given plate, you can specify the plate ID. Alternatively, you can configure the MegaBACE.ini file so that the software always prompts the operator for the plate ID for each injection (section 2.7).

3.4.2 Specifying the SNP marker name

The Instrument Control Manager requires at least one SNP marker name to be specified for each injection in a SNP genotyping run. Multiple SNP marker names for each plate (injection) are allowed. To specify the names of the SNP markers for a plate, you can use the following fields in the .psd file:

- MARKER NAME—Specifies a SNP marker name for the entire plate.
- Well_ID.MARKER NAME—Specifies the SNP marker name for the selected well ID. The well-specific SNP marker name overrides the plate SNP marker name.



```
Plate ID                DNASet1_NCBI422R
COMMENT

MARKER NAME             NCBI422R

A01     17031_A
B01     17032_A
C01     17033_A
D01     17034_A
E01     17035_A
F01     17036_A
G01     17037_A
H01     17038_A
A02     17039_A
B02     17040_A
C02     10858_A
D02     10859_A
E02     11879_A
F02     11880_A
G02     11881_A
H02     11882_A
A03     10834_A
B03     10835_A
C03     12248_A
D03     12249_A
E03     12250_A
F03     12251_A
G03     negctrl_A
```

```
A01.MARKER NAME NCBI7993
B01.MARKER NAME NCBI7993
C01.MARKER NAME NCBI7993
D01.MARKER NAME NCBI7993
```

Figure 3-3. Example of a .psd file defining one plate (injection) in the plate set.

Alternatively, if you do not use .psd files, you can configure the MegaBACE.ini file (section 2.7) so that the software always prompts the operator for the SNP marker name for each injection during the run. This method allows the operator to enter only the global SNP marker name for each plate.

Note: SNP Profiler requires a profile for each SNP marker name found in the extended header of the .rsd files. See the *MegaBACE SNP Profiler User’s Guide* for a description of the SNP Marker Editor.

3.4.3 Specifying sample names for a given plate

To specify a sample name for a given well on a plate in a SNP genotyping run, you must use the well_ID.NAME field or well_ID field in a .psd file.

If your laboratory routinely uses the same sample plate layout for multiple injections, you might want to copy the sample names from one injection to the next. See section 2.7.3 for a description of how to configure this option.

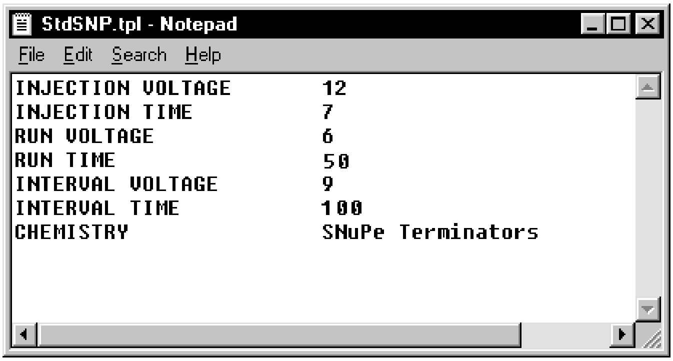
3.5 Using a plate set setup template file (.tpl) for SNP genotyping

To create a plate set definition (section 3.1.2), you can use a plate set setup template (.tpl) instead of a plate set .psd file. A plate set setup template allows you to specify the plate set setup parameters listed in table 3-1. Figure 3-4 shows an example of the StdSNP.tpl file for SNP genotyping, which is included with the software.

You can configure the MegaBACE.ini file (section 2.2) to load a default .tpl file automatically when an operator selects the SNP genotyping application.

The .tpl files can be created using any text editor that supports tab-separated text, but the .tpl files must be stored in the …\MegaBACE\Templates folder. Alternatively, you can specify the plate set setup parameters in the Plate Set Setup window and then save the parameters as a template *(MegaBACE SNP Genotyping Instrument Operator's Guide).*

Note: To specify the list of plates in a plate set before the run, the software requires the PLATE SET field in a plate set .psd file (section 3.3).



```
INJECTION VOLTAGE       12
INJECTION TIME          7
RUN VOLTAGE             6
RUN TIME                50
INTERVAL VOLTAGE        9
INTERVAL TIME           100
CHEMISTRY               SNuPe Terminators
```

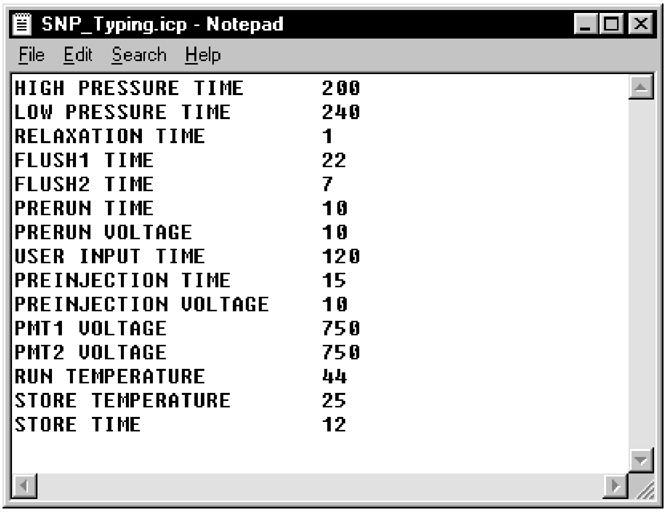
Figure 3-4. An example of a plate set setup template file (.tpl) for SNP genotyping.

3.6 About the instrument control template (.icp)

The instrument control parameters file (.icp) allows you to specify a set of instrument parameters as a template for use in multiple runs. Figure 3-5 shows an example of the SNP_Typing.icp file for SNP genotyping, which is included with the software.

You can configure the MegaBACE.ini file (section 2.2) to load a default .icp file automatically when an operator selects the SNP genotyping application.

The .icp files can be created using any text editor that supports tab-separated text. The .icp files must be stored in the …\MegaBACE\Templates folder. Alternatively, you can save the parameters that you specify in the Instrument Control window as a template *(MegaBACE Instrument Administrator's Guide)*. Note that the same fields are used for all the applications (sequencing, genotyping, or SNP genotyping).



```
HIGH PRESSURE TIME      200
LOW PRESSURE TIME       240
RELAXATION TIME         1
FLUSH1 TIME             22
FLUSH2 TIME             7
PRERUN TIME             10
PRERUN VOLTAGE          10
USER INPUT TIME         120
PREINJECTION TIME       15
PREINJECTION VOLTAGE    10
PMT1 VOLTAGE            750
PMT2 VOLTAGE            750
RUN TEMPERATURE         44
STORE TEMPERATURE       25
STORE TIME              12
```

Figure 3-5. An example of an instrument control parameter file (.icp) for SNP genotyping.

Chapter 4 File storage

This chapter describes the file storage conventions for SNP genotyping. The topics are—

- Raw sample data file content (section 4.1)
- Naming and storage conventions for raw sample data (section 4.2)
- System initialization file storage (section 4.3)
- Parameter configuration file storage (section 4.4)

Refer to the *MegaBACE SNP Profiler User's Guide* for a description of the file storage conventions for the SNP Profiler data.

4.1 Raw sample data file content

The Instrument Control Manager creates a raw sample data file (.rsd) for the data collected from each capillary during the SNP genotyping run. Each .rsd file represents a specific well location and contains the data for all the plates injected during the run. Each file contains—

- Plate set ID
- Well location
- Plate set setup parameters and instrument control parameters
- Run data: run ID, instrument ID, and date
- Raw electropherogram

You can use the MegaBACE Header Editor software to view the file header of any .rsd file. See chapter 5 for a description of the SNP genotyping fields in the extended header.

4.2 Naming and storage conventions for raw sample data

By default, the software uses the well locations as the file names for the .rsd files from a SNP genotyping run. Alternative .rsd file names can be specified in a master plate setup data file (.psd), a plate set setup template file (.tpl), or the File Names tab of the Plate Set Setup window (chapter 3).

Each time you run a plate set on the MegaBACE instrument, the Instrument Control Manager creates a raw run folder that includes the .rsd files for the run. The MegaBACE system uses the plate set ID and the run ID to name the raw run folders (plate_setID_runID):

- If the plate set ID is missing, the software uses the plate ID of the first plate injected. If the plate ID is also missing, the software uses the file name of the master .psd file (bar code) to name the run folder.
- The run ID starts with Run01. The number indicates how many times the plate set ID has been run.

Table 4-1 summarizes the conventions for naming and storing the raw sample data files and folders.

Table 4-1. Naming and storage conventions for the raw sample data

File or folder type	Name	Location
Raw sample data file (.rsd)	Well_ID.rsd (default)	PlateID_runID (raw run folder)
Raw run folder	Plate_setID_runID	…\MegaBACE\Data (unless you select a different location)

The File Storage command in the Options menu allows the operator to specify an application-specific file storage location for the raw data and to change the storage location of the raw data at any time. This feature only affects the data storage of subsequent runs. It does not change the location of data already stored on the computer. See the *MegaBACE SNP Genotyping Instrument Operator's Guide* for a description of how to specify an application-specific file storage location.

4.3 System initialization file storage

Table 4-2 lists the system initialization files that you can modify to configure the Instrument Control Manager.

Table 4-2. The system initialization files

File name	Description	Storage location
MegaBACE.ini	Defines the options available in the Instrument Control Manager.	…\MegaBACE\DataSystem folder
Chemistry.ini	Defines the available chemistry parameter sets, including the dyes, bases, filters, and laser modes.	…\MegaBACE\DataSystem folder

4.4 Parameter configuration file storage

Table 4-3 lists the parameter configuration files that you can use to automate the operator's tasks during the run.

Table 4-3. The parameter configuration files

File extension	Description	Storage location
.psd	For SNP genotyping, you can use two types of plate setup data files (.psd): • A master .psd file, which specifies the plate set definition shared by all plates in the plate set, and plate-specific attributes for the first plate to be injected in a set. • Additional .psd files, which specify the plate-specific attributes only. (See section 3.2.)	…\MegaBACE\Psd folder
.tpl	A plate set setup template file (.tpl) can be used to define the plate set setup parameters shared by a group of plates (section 3.5).	…\MegaBACE\Templates folder
.icp	For use in multiple runs, an instrument control parameter file (.icp) is used as a template to specify a set of parameters that control the instrument (section 3.6).	…\MegaBACE\Templates folder

Chapter 5 The extended header for SNP genotyping

This chapter describes the labels that have been added to the extended header of the raw sample data files (.rsd) to support SNP genotyping. The topics in this chapter are—

- Additional extended header labels for SNP genotyping (section 5.1)
- Viewing and editing the data in the extended header (section 5.2)

See the *MegaBACE Instrument Administrator's Guide* for a description of the basic fields that appear in the extended header and for a description of the standard file header. See the Help available within the MegaBACE Header Editor for a description of how to use the software.

5.1 Additional extended header labels for SNP genotyping

The additional labels that appear in the raw sample data files (.rsd) from a SNP genotyping run are—

- **Plate set attributes—**The attributes that apply to the entire run appear at the top level of the extended file header. Table 5-1 lists the additional top-level labels for SNP genotyping.
- **Injection-specific (plate) attributes—**The attributes for an injection appear under the subsection for the given injection. Injection-specific subsections are labeled as INJECTION 1, INJECTION 2, and so forth. Table 5-2 lists the labels for the injection-specific attributes.

Table 5-1. Plate set fields in the extended header

Field	Description
INTERVAL VOLTAGE	Plate set definition setting (section 3.1).
INTERVAL TIME	Plate set definition setting (section 3.1).
NUMBER OF INJECTIONS	Actual number of sample injections performed during the run.
PLATE SET ID	Always present. If a .psd file is used for the plate set and the plate set ID is missing, the software uses the plate ID, if available. Otherwise, the software uses the bar code or the .psd file name.
PLATE SET	(Optional) Values may be present if a .psd file is used (section 3.3.2).
All user-defined plate set labels	(Optional) Values may be present if a .psd file is used.

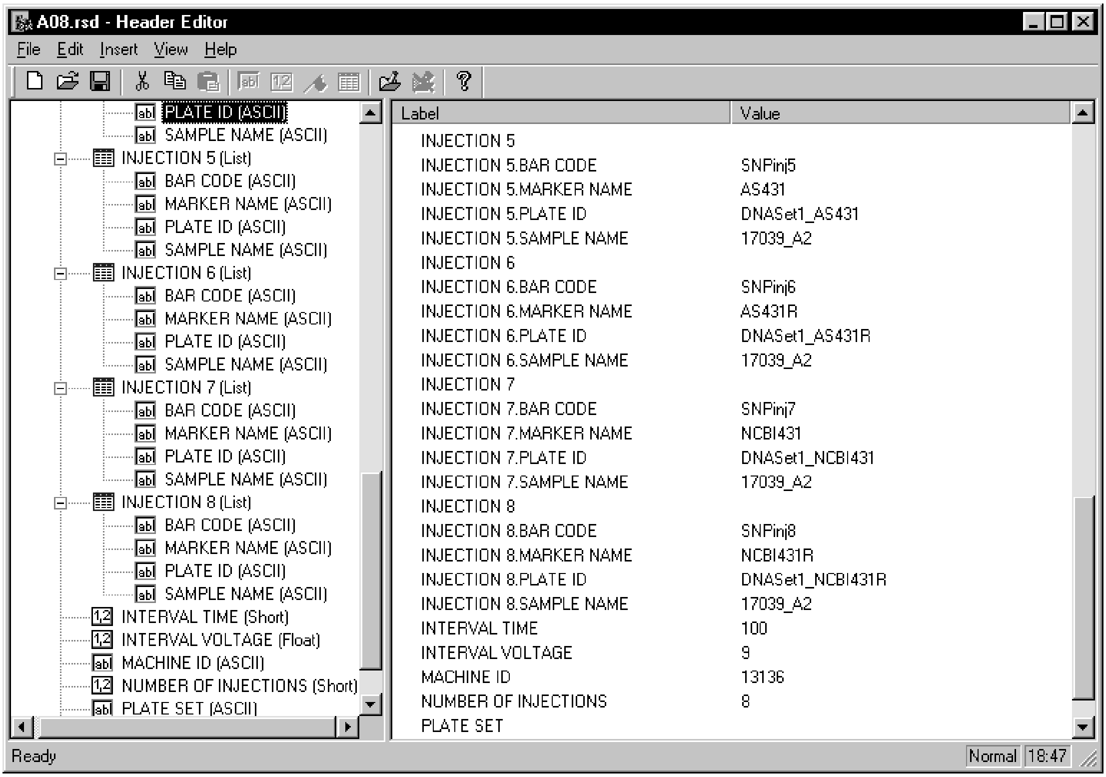
Table 5-2. Injection-specific fields in the extended header

Field	Description
BAR CODE	Injection-specific attribute for SNP genotyping, but a top-level, run-wide attribute in other applications, such as sequencing and genotyping. The value may be missing for injections other than the first injection if a .psd file is not used.
PLATE ID	(Optional) Injection-specific attribute for SNP genotyping, but a top-level, run-wide attribute in other applications, such as sequencing and genotyping. The value may be missing.
MARKER NAME	Always present for each injection of a SNP genotyping run. If only one SNP marker name is provided, the software copies that name for each injection in the run.
SAMPLE NAME	(Optional) Injection-specific attribute for SNP genotyping, but a top-level, run-wide attribute in other applications, such as sequencing and genotyping. For SNP genotyping, a .psd file must be used to specify sample names.
All user-defined labels	(Optional) Values may be present if a .psd file is used.

5.2 Viewing and editing the data in the extended header

You use the MegaBACE Header Editor software to open the file and display the contents of the extended header. If necessary, you can edit information, such as SNP marker names.

To start MegaBACE Header Editor, double-click the **Header Editor icon** in the MegaBACE shortcut folder on the Windows desktop. The Header Editor window appears (figure 5-1).



- PLATE ID (ASCII)
- SAMPLE NAME (ASCII)
- INJECTION 5 (List)
  - BAR CODE (ASCII)
  - MARKER NAME (ASCII)
  - PLATE ID (ASCII)
  - SAMPLE NAME (ASCII)
- INJECTION 6 (List)
  - BAR CODE (ASCII)
  - MARKER NAME (ASCII)
  - PLATE ID (ASCII)
  - SAMPLE NAME (ASCII)
- INJECTION 7 (List)
  - BAR CODE (ASCII)
  - MARKER NAME (ASCII)
  - PLATE ID (ASCII)
  - SAMPLE NAME (ASCII)
- INJECTION 8 (List)
  - BAR CODE (ASCII)
  - MARKER NAME (ASCII)
  - PLATE ID (ASCII)
  - SAMPLE NAME (ASCII)
- INTERVAL TIME (Short)
- INTERVAL VOLTAGE (Float)
- MACHINE ID (ASCII)
- NUMBER OF INJECTIONS (Short)
- PLATE SET (ASCII)

Label	Value
INJECTION 5	
INJECTION 5.BAR CODE	SNPinj5
INJECTION 5.MARKER NAME	AS431
INJECTION 5.PLATE ID	DNASet1_AS431
INJECTION 5.SAMPLE NAME	17039_A2
INJECTION 6	
INJECTION 6.BAR CODE	SNPinj6
INJECTION 6.MARKER NAME	AS431R
INJECTION 6.PLATE ID	DNASet1_AS431R
INJECTION 6.SAMPLE NAME	17039_A2
INJECTION 7	
INJECTION 7.BAR CODE	SNPinj7
INJECTION 7.MARKER NAME	NCBI431
INJECTION 7.PLATE ID	DNASet1_NCBI431
INJECTION 7.SAMPLE NAME	17039_A2
INJECTION 8	
INJECTION 8.BAR CODE	SNPinj8
INJECTION 8.MARKER NAME	NCBI431R
INJECTION 8.PLATE ID	DNASet1_NCBI431R
INJECTION 8.SAMPLE NAME	17039_A2
INTERVAL TIME	100
INTERVAL VOLTAGE	9
MACHINE ID	13136
NUMBER OF INJECTIONS	8
PLATE SET	

Figure 5-1. The Header Editor window displaying the information for a selected well from the run.

The MegaBACE Header Editor software includes a Help file that describes how to use the software. To access the Help file, in the MegaBACE Header Editor software, choose **Help Topics** from the Help menu.

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.

channels—see spectral channel.

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 do not provide a plate set ID, the software uses the ID of the plate that is injected first as the ID for the entire plate set. If the plate ID is also missing, the software uses the master .psd file name (bar code).

plate set setup parameters—the plate 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 IDs or 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 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 sample 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 names—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.

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 genotyping 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 cross-talk is caused by overlaps in the emission spectra of the different dyes. Each dye is detected through a specific spectral channel, but cross-talk signals are 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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