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user manual

# MegaBACE 4000

Instrument Administrator's Guide
Version 3.2

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Amersham Biosciences

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### Glossary



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# Preface

## About this guide

The *MegaBACE 4000 Instrument Administrator's Guide* describes how to configure the MegaBACE™ system software for your laboratory. This guide describes how to use the system initialization files, how to create plate setup and instrument control templates, and how to create the plate setup data files (.psd) to automate the workflow for the instrument operator.

If you have instrument operator responsibilities for the MegaBACE 4000 system in addition to being an administrator, see the *MegaBACE 4000 Instrument User's Guide* for additional guidelines.

## Related publications

In addition to the *MegaBACE 4000 Instrument Administrator's Guide*, the following publications are available for the MegaBACE 4000 system:

- *MegaBACE 4000 Instrument User's Guide* describes how to use the MegaBACE system to perform runs and how to use the instrument protocols. This guide also provides instructions on maintaining the instrument and guidelines on troubleshooting problems.
- *MegaBACE 4000 Site Preparation Guide* provides instructions for setting up the installation site for the MegaBACE 4000 instrument. Reading the site preparation guide is a prerequisite for the installation of the MegaBACE 4000 system.
- The MegaBACE analysis software user's guides and the Help available within the software provide instructions on how to use the software to analyze the data collected from the MegaBACE 4000 instrument.

## Assumptions

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



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## Assistance

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

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# Part one

# General system configuration

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# Chapter 1 Introduction to the MegaBACE 4000 system software

The MegaBACE 4000 DNA Analysis System is a high-throughput automated gene analysis system with 384-capillary capacity. The system consists of reagents for sample preparation, the MegaBACE instrument, and the software.

This chapter provides an overview of the system software. The topics are—

- The MegaBACE 4000 system software components (section 1.1)
- Overview of the Instrument Control Manager features (section 1.2)
- Overview of the sequencing setup (section 1.3)
- Overview of the STR genotyping setup (section 1.4)
- Overview of the SNP genotyping setup (section 1.5)
- File format overview (section 1.6)
- File storage overview (section 1.7)

## 1.1 The MegaBACE 4000 system software components

The MegaBACE 4000 instrument control software consists of the—

- **Instrument Control Manager—**Contains the graphical interface that allows you to control the instrument.
- **Host Scan Controller—**Provides the communication between the MegaBACE 4000 instrument and the Instrument Control Manager.

Analysis software is available for use with the MegaBACE 4000 instrument to support various types of applications, including:

- Sequencing analysis
- Microsatellite genotyping, also known as short-tandem-repeat (STR) genotyping, and fragment analysis
- Single-nucleotide-polymorphism (SNP) genotyping

The MegaBACE Header Editor allows you to view and edit the data in the extended header of the raw sample data files (.rsd) (section A.2).



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## 1.2 Overview of the Instrument Control Manager features

The Instrument Control Manager allows you to—

- Specify the plate 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 Setup window to create plate definitions. The operator can then select these precreated plate definitions at the start of the Inject Samples and Run protocol.
- Specify the default plate setup parameters and instrument parameters using templates. The MegaBACE 4000 software includes templates (default) for the recommended parameters.
- Enable or disable the edit mode. You can allow the instrument operator to edit or prevent the ability to edit the plate setup and instrument parameters for running a plate.
- Change applications. The software supports sequencing, STR genotyping (or fragment analysis), and SNP genotyping.
- (SNP genotyping only) Serially inject multiple sample plates into the same set of capillaries for a single run.
- Collect data.
- (Sequencing only) Automatically call bases on the collected data and export the data to other file formats after base calling.
- Change the data storage location, and store the raw run folders in application-specific locations (sequencing, STR genotyping, and SNP genotyping).

## 1.3 Overview of the sequencing setup

This section provides an overview of the administrator's tasks and the system setup for the sequencing application.



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### 1.3.1 Administrator tasks for sequencing

Table 1-1 provides an administrator's task overview for the sequencing application on the MegaBACE 4000 system. The administrator tasks vary depending on whether your laboratory uses plate setup data files (.psd). The .psd files automate the operator's workflow for providing the plate definition for a run.

**Table 1-1.** Administrator's task overview for sequencing

| Task | Frequency | Reference |
|---|---|---|
| To tailor the Instrument Control Manager to the laboratory's workflow, you can modify the—<br>• MegaBACE.ini file<br>• Basecall.ini file<br>• Chemistry.ini file | Once, when the laboratory first receives the system, or whenever the system needs to be reconfigured | Chapters 2, 3, and 5 |
| The software includes default template files for the—<br>• Plate setup parameters (.tpl files)<br>• Instrument control parameters (.icp files)<br>You can create additional templates based on the needs of your laboratory, and you can specify defaults. | Once, when the laboratory first receives the system, or whenever you change the run conditions for multiple plates | Chapters 4 and 6 |
| (Optional) You can create plate setup data files (.psd) to automate how an operator provides the plate definition for a run. For each .psd file you create, you provide the operator with a .psd file name or bar code to import the file. | For each plate you run | Chapter 7 |

### 1.3.2 The system initialization files for sequencing

To meet the needs of your laboratory, you might need to modify the system initialization files. The system software includes three initialization files that you can modify—

- **MegaBACE.ini file—**Determines the settings the Instrument Control Manager software uses to control the operations of the MegaBACE 4000 instrument (chapters 2 and 5). For example, the MegaBACE.ini file can specify—
  - The default application (sequencing, STR genotyping, or SNP genotyping) and the default plate setup and instrument control templates for each application
  - Whether an operator can manually edit the plate setup or instrument control parameters



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- Whether the software performs base calling automatically after a run is completed

- **Chemistry.ini file—**Determines the chemistry parameter sets available in the Instrument Control Manager software. See chapter 3 for details on how to use the Chemistry.ini file.
- **Basecall.ini file—**Determines the base callers available in the Instrument Control Manager software, and specifies a default base caller. See chapter 5 for details on how to use the Basecall.ini file.

### 1.3.3 About data collection with automatic base calling

The Instrument Control Manager allows you to perform continuous base calling and file export after data collection without operator intervention. To do this, you specify automatic base calling, select a default base caller, and specify the file formats for the base-called data (SCF, FASTA, ASCII text, or ABD). For a description of how to set up the base calling feature, see chapter 5.

### 1.3.4 The parameter template files for sequencing

The parameter templates are optional files that can be used to define the plate setup parameters and instrument control parameters that an operator can import at run time. The software includes templates that are ready for you to use or modify. You can also create new templates. The Instrument Control Manager uses two kinds of parameter templates—

- **Plate setup template file (.tpl)—**You can use a .tpl file for multiple plates to specify only the plate setup parameters that appear in the Plate Setup window of the Instrument Control Manager. The software includes a selection of templates for sequencing. See chapter 6 for a description of how to create a plate setup template.
- **Instrument control template file (.icp)—**You can use an .icp file to specify the instrument control parameters for multiple runs. The instrument control parameters define the instrument run conditions and the matrix fill and flush cycles. The software includes a default template for sequencing (Normal.icp). See chapter 4 for a description of how to create an instrument control template.



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### 1.3.5 The plate setup data files (.psd) for sequencing

**Important**

**For the software to import the .psd file, the text or bar code that an operator enters must match the file name (case insensitive) of the .psd file.**

An operator can automatically import the plate definition from a .psd file. A .psd file can specify the plate ID, plate setup parameters, and other plate- and well-level attributes, such as sample names and file names.

The .psd files can be used, either alone or in combination with a .tpl file, to automate the operator's task of defining a plate setup. If you use a .psd file to specify all the plate setup parameters, you do not need to use a .tpl file. Similarly, if you use a .tpl file to specify all the plate setup parameters for multiple plates, you do not need to use a .psd file unless you want to specify additional parameters for a particular plate.

The parameters entered last, whether entered manually in the Plate Setup window or automatically using a .psd or .tpl file, overwrite any previously entered parameters. The parameters that you specify in the .psd file become part of the extended header of each raw sample data (.rsd) file created during the run. Section 7.1 provides examples of .psd files for sequencing.

### 1.3.6 Workflow alternatives for the sequencing plate setup

During a run on the MegaBACE 4000 instrument, the Instrument Control Manager prompts the operator for the appropriate steps. The software includes protocols for rinsing the capillaries, filling the capillaries with sieving matrix, performing a prerun, and performing the sample injection for the electrophoresis run.

For each run, the operator must provide a plate definition. A plate definition consists of the—

- **Plate ID—**The name of the plate. The software uses the plate ID to name the raw run folder that stores the data from the run.
- **Plate setup parameters—**The electrophoresis parameters, the chemistry parameters, the sample names, plate comments, and other parameters.



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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). Figure 1-1 shows the basic workflows, which are:

- **Automated workflow—**For the most automated workflow, the operator can import the plate definition from a .psd file at the start of the Inject Samples and Run protocol. As the administrator, you create the .psd file (chapter 7) and provide the operator with the file name or the bar code to enter for the run.
- **Alternative workflow—**
  - You or the operator can use the Plate Setup window to create the plate definition before the run. The values for the plate setup parameters can be provided from a template, imported from a .psd file, or manually entered in the Plate Setup window. See chapter 6 of this guide for a description of how to create a template. See the *MegaBACE 4000 Instrument User's Guide* for a description of how to use the Plate Setup window to create plate definitions.
  - At the start of the Inject Samples and Run protocol, the operator selects the plate ID for the precreated plate definition.

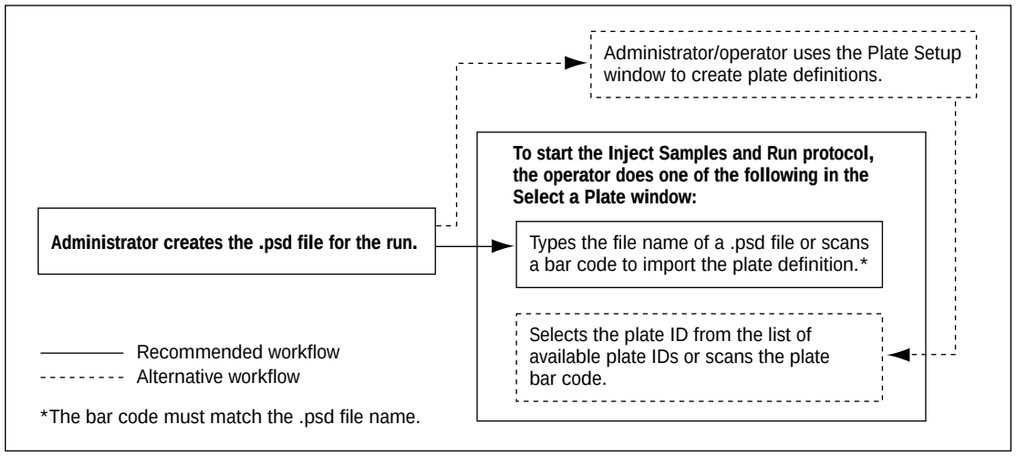



**Figure 1-1.** Workflow alternatives for providing the sequencing plate definition for a run.



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## 1.3.7 How the software uses the sequencing parameters

See figure 1-2 for an overview of how the Instrument Control Manager uses the parameters specified in the .psd files, in the parameter templates (.tpl and .icp), and by keyboard entry.

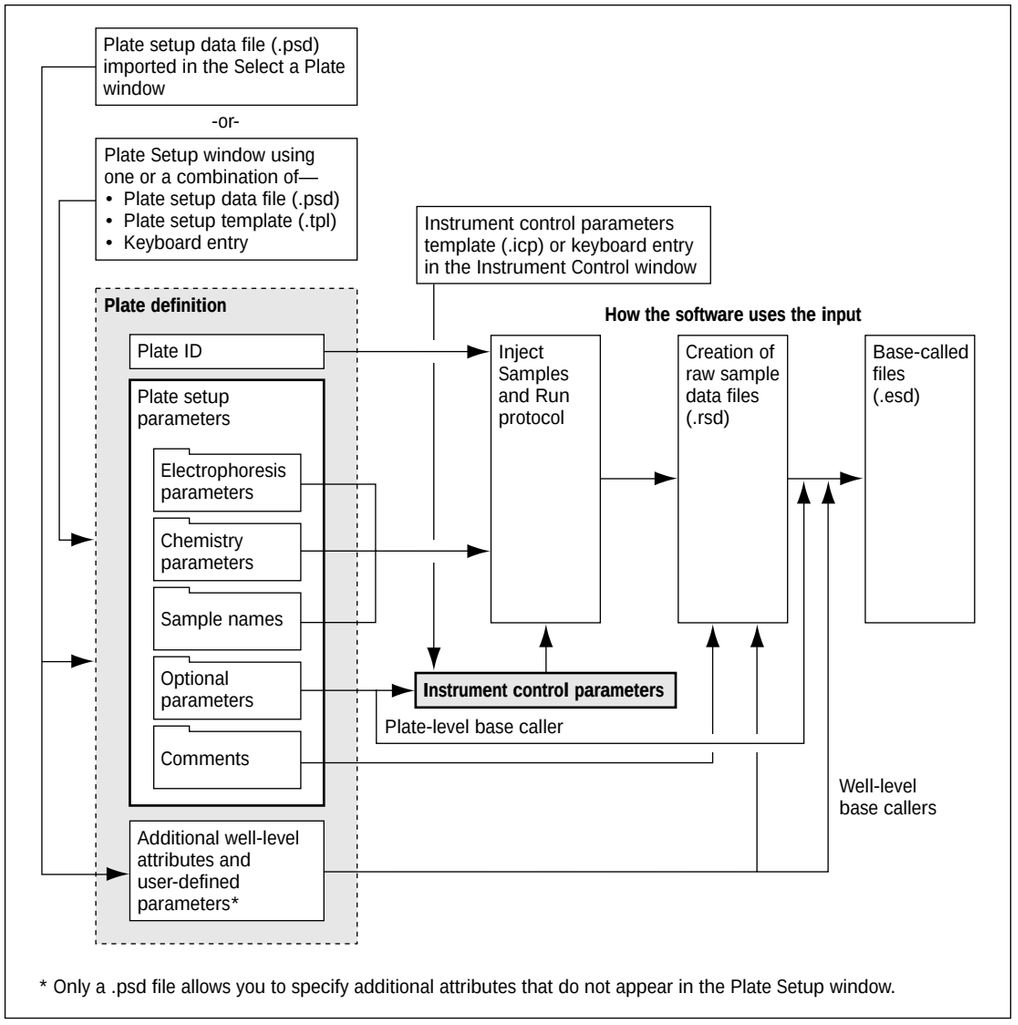



**Figure 1-2.** Overview of how the software uses the parameters specified for a sequencing run.



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## 1.4 Overview of the STR genotyping setup

This section provides an overview of the workflow and the system setup for the STR genotyping application.

### 1.4.1 Administrator tasks for STR genotyping

Table 1-2 provides an administrator's task overview for the STR genotyping application on the MegaBACE 4000 system. The administrator tasks vary depending on whether your laboratory uses plate setup data files (.psd). The .psd files automate the operator's workflow for providing the plate definition for a run.

**Table 1-2.** Administrator's task overview for STR genotyping

| Task | Frequency | Reference |
|---|---|---|
| To tailor the Instrument Control Manager to the laboratory's workflow, you can modify the—<br>• MegaBACE.ini file<br>• Chemistry.ini file | Once, when the laboratory first receives the system, or whenever the system needs to be reconfigured | Chapters 2, 3, and 8 |
| The software includes default template files for the—<br>• Plate setup parameters (.tpl files)<br>• Instrument control parameters (.icp files)<br>You can create additional templates based on the needs of your laboratory, and you can specify defaults. | Once, when the laboratory first receives the system, or whenever you change the run conditions for a set of plates | Chapters 4 and 9 |
| (Optional) You can create plate setup data files (.psd) to automate how an operator provides the plate definition. For each .psd file you create, you provide the operator with a .psd file name or bar code to import the file. | For each plate you run | Chapter 10 |



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### 1.4.2 The system initialization files for STR genotyping

To meet the needs of your laboratory, you might need to 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 operations of the MegaBACE 4000 instrument (chapters 2 and 8). For example, the MegaBACE.ini file can specify—
  - The default application (sequencing, STR genotyping, or SNP genotyping) and the default plate setup and instrument control templates for each application
  - Whether an operator can manually edit the plate setup or instrument control parameters
- **Chemistry.ini file—**Determines the chemistry parameter sets available in the Instrument Control Manager software. See chapter 3 for details on how to use the Chemistry.ini file.

### 1.4.3 The parameter template files for STR genotyping

The parameter templates are optional files that can be used to define the plate setup parameters and instrument control parameters that an operator can import at run time. The software includes templates that are ready for you to use or modify, or you can create new ones. The Instrument Control Manager uses two kinds of parameter templates—

- **Plate setup template file (.tpl)—**You can use a .tpl file for multiple plates to specify only the plate setup parameters that appear in the Plate Setup window of the Instrument Control Manager. The software includes a default template for genotyping (StdGenotyping.tpl). See chapter 9 for a description of how to create a plate setup template for STR genotyping.
- **Instrument control parameter template file (.icp)—**You can use an .icp file to specify the instrument control parameters for multiple runs. The instrument control parameters define the instrument run conditions and the matrix fill and flush cycles. The software includes a default template for genotyping (Genotyping.icp). See chapter 4 for a description of how to create an instrument control template.



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### 1.4.4 The plate setup data files (.psd) for STR genotyping

**Important** **For the software to import the .psd file, the text or bar code that an operator enters must match the file name (case insensitive) of the .psd file.**

An operator can automatically import the plate definition from a .psd file. A .psd file can specify the plate ID, plate setup parameters, and other plate- and well-level attributes, such as sample names and file names.

The .psd files can be used, either alone or in combination with a .tpl file, to automate the operator's task of defining a plate. If you use a .psd file to specify all the plate setup parameters, you do not need to use a .tpl file. Similarly, if you use a .tpl file to specify all the plate setup parameters for multiple plates, you do not need to use a .psd file unless you want to specify additional parameters for a particular plate.

The parameters entered last, whether entered manually in the Plate Setup window or automatically using a .psd or .tpl file, overwrite any previously entered parameters. The parameters that you specify in the .psd file become part of the extended header of each raw sample data (.rsd) file created during the run. Section 10.1 provides examples of .psd files for STR genotyping.

### 1.4.5 Workflow alternatives for the STR genotyping plate setup

During a run on the MegaBACE 4000 instrument, the Instrument Control Manager prompts the operator for the appropriate steps. The software includes protocols for rinsing the capillaries, filling the capillaries with sieving matrix, performing a prerun, and performing the sample injection for the electrophoresis run.

For each run, the operator must provide a plate definition. A plate definition consists of the—

- **Plate ID—**The name of the plate. The software uses the plate ID to name the raw run folder that stores the data from the run.
- **Plate setup parameters—**The electrophoresis parameters, the chemistry parameters, the sample names, plate comments, and other parameters.



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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). Figure 1-3 shows the workflows, which are—

- **Automated workflow—**For the most automated workflow, the operator can import the plate definition from a .psd file at the start of the Inject Samples and Run protocol. As the administrator, you create the .psd file (chapter 10) and provide the operator with the file name or the bar code to enter for the run.
- **Alternative workflow—**
  - You or the operator can use the Plate Setup window to create the plate definition before the run. The values for the plate setup parameters can be provided from a template, imported from a .psd file, or manually entered in the Plate Setup window. See chapter 9 of this guide for a description of how to create a template. See the *MegaBACE 4000 Instrument User's Guide* for a description of how to use the Plate Setup window to create plate definitions.
  - At the start of the Inject Samples and Run protocol, the operator selects the plate ID of the precreated plate definition.

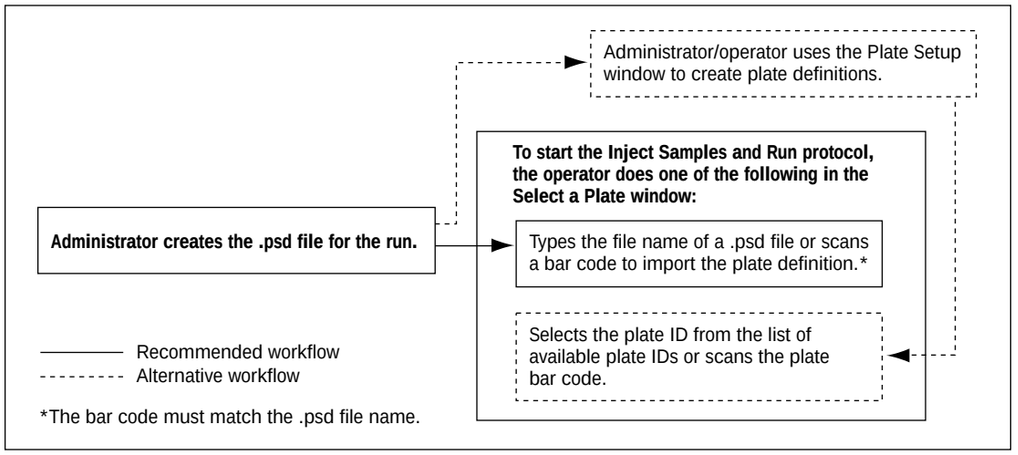



**Figure 1-3.** Typical STR genotyping workflows for providing the plate definition for a run.



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## 1.4.6 How the software uses the STR genotyping parameters

See figure 1-4 for an overview of how the Instrument Control Manager uses the parameters specified in the .psd files, in the parameter templates (.tpl and .icp), and by keyboard entry.

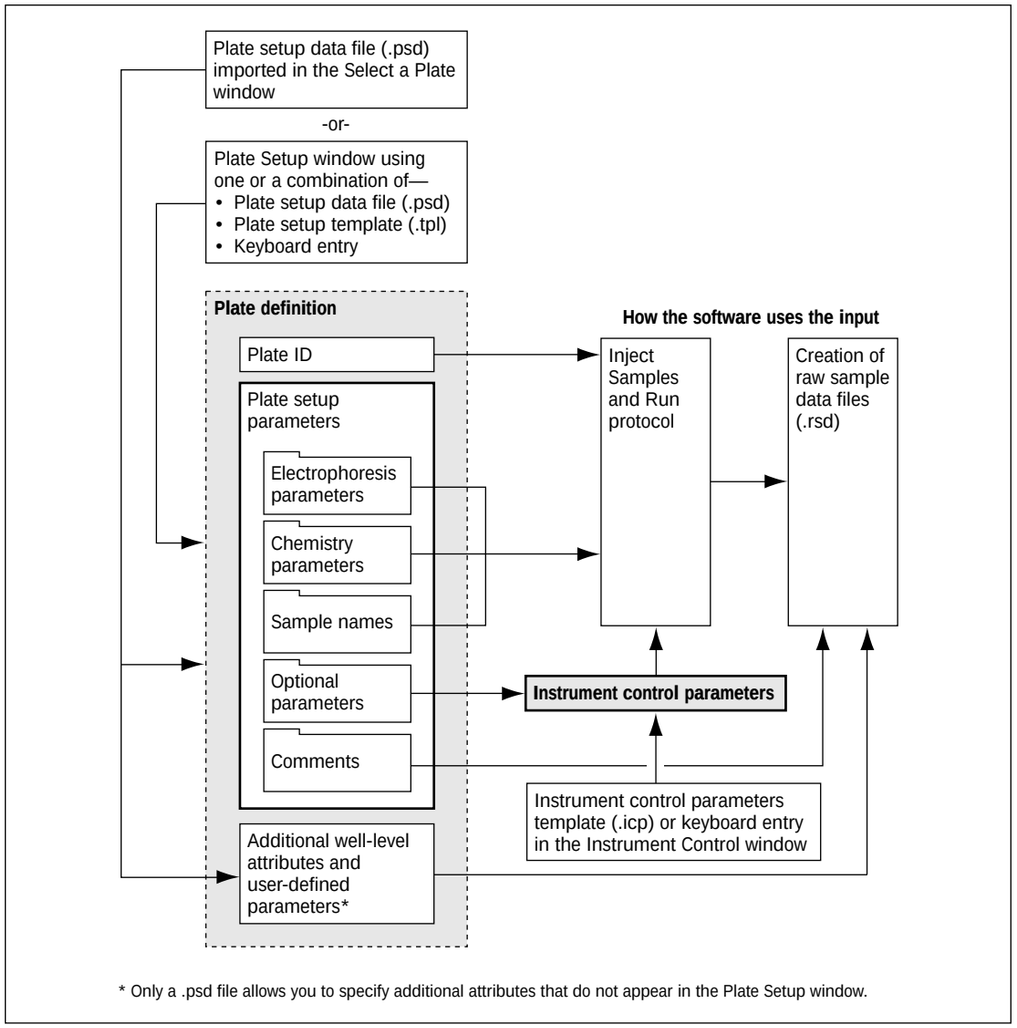



**Figure 1-4.** Overview of how the software uses the parameters specified for an STR genotyping run.



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## 1.5 Overview of the SNP genotyping setup

This section provides an overview of the administrator's tasks and the system setup for the SNP genotyping application.

### 1.5.1 Administrator tasks for SNP genotyping

Table 1-3 provides an administrator's task overview for the SNP genotyping application on the MegaBACE 4000 system. The administrator tasks vary depending on whether your laboratory uses plate setup data files (.psd). To automate the workflow during a multi-injection run, .psd files are recommended.

**Table 1-3.** Administrator's task overview for SNP genotyping

| Task | Frequency | Reference |
|---|---|---|
| To tailor the Instrument Control Manager to the laboratory's workflow, you can modify the—<br>• MegaBACE.ini file<br>• Chemistry.ini file | Once, when the laboratory first receives the system, or whenever the system needs to be reconfigured | Chapters 2, 3, and 11 |
| The software includes default template files for the—<br>• Plate set setup parameters (.tpl files)<br>• Instrument control parameters (.icp files)<br>You can create additional templates based on the needs of your laboratory, and you can set defaults. | Once, when the laboratory first receives the system, or whenever you change the run conditions for multiple plates | Chapters 4 and 12 |
| (Optional) You can create plate setup data files (.psd) to automate how an operator provides the plate set* definition and the attributes for each plate injected during a run. For each .psd file you create, you provide the operator with a .psd file name or bar code to import the file. | For each plate set and the associated plates in the set | Chapter 13 |

* A plate set includes all the plates injected during a multi-injection run for the SNP genotyping application. See section 1.5.6 for an overview of the plate set definition and the plate set setup parameters.



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## 1.5.2 The system initialization files for SNP genotyping

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) and the default plate setup and instrument control templates for each application
  - Whether an operator can manually edit the plate set setup or instrument control parameters
  - Whether the software enforces the injection 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.

## 1.5.3 The parameter templates for SNP genotyping

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

- **Plate set setup template (.tpl)—**You can use a .tpl file for multiple plate sets to specify only the parameters that appear in the Plate Set Setup window of the Instrument Control Manager. The plate set setup parameters are required for the plate set definition (section 1.5.6). The software includes a default plate set setup template for SNP genotyping (StdSNP.tpl). See chapter 12 for a description of how to create a plate set setup template for SNP genotyping.
- **Instrument control template (.icp)—**You can use an .icp file to specify the instrument control parameters for multiple runs. The instrument control parameters define the instrument run conditions and the matrix fill and flush cycles. The software includes a default instrument control template for SNP genotyping (SNP_Typing.icp). See chapter 4 for a description of how to create a default instrument control template.



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

### 1.5.4 The plate setup data files (.psd) for SNP genotyping

**Important** **For the software to import the .psd file, the text or bar code that an operator enters must match the file name (case insensitive) of the .psd file.**

For each multi-injection run, you can provide two types of .psd files (chapter 13)—

- **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 for the first plate to be injected.
- **Plate-specific .psd files—**Allow you to specify the plate-specific attributes for each 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.

### 1.5.5 Workflow alternatives for the SNP genotyping plate setup

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, which includes the plate set ID and the setup parameters for the plate set
- The plate-specific attributes for each injection



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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). Figure 1-5 shows the workflow alternatives.

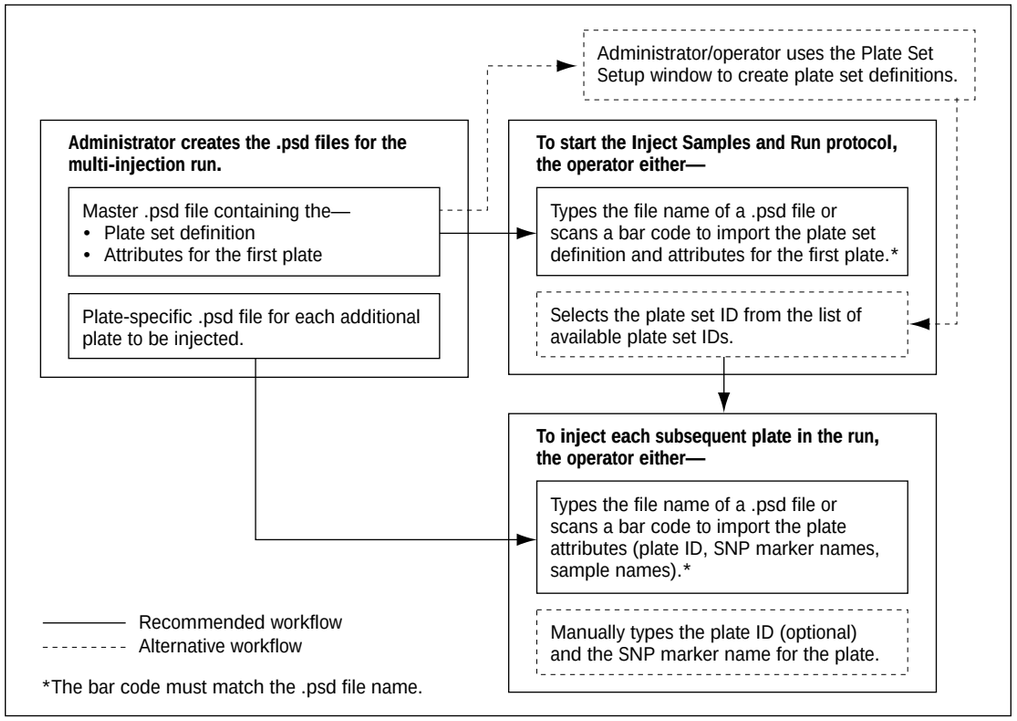



**Figure 1-5.** Workflow alternatives for providing the plate set definition and the plate-specific attributes for SNP genotyping runs.



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As figure 1-5 shows, the workflow alternatives include—

- **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. 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—**
  - 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 4000 Instrument User'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 for the precreated plate set definition.
  - For each injection, the operator can manually enter a few plate-specific attributes when the plate is injected.

## 1.5.6 SNP genotyping plate sets and plate attributes

### The plate set definition for SNP genotyping

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 master .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 master .psd file, selected from a plate set setup template, or manually entered in the Plate Set Setup window.



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- **(Optional) List of plates—**The list of bar codes representing the plates contained in the plate set. To specify the list of plates and the injection order for a plate set, you must use a master .psd file.

### The plate-specific attributes for SNP genotyping

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 different SNP marker names for different wells on a plate, the software requires a .psd file for the injection. Alternatively, you can allow the operator to manually type one SNP marker name for each injected plate.
- **(Optional) Sample names—**To specify the sample names for a plate, the Instrument Control Manager requires a .psd file for the sample plate injection.
- **(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 a .psd file is not used for a given injection, the operator can specify only the plate ID and one SNP marker name for the injection.

## 1.5.7 How the software uses the SNP genotyping parameters

See figure 1-6 for an overview of how the Instrument Control Manager uses the parameters specified in the .psd files, in the parameter templates (.tpl and .icp), and by keyboard entry.



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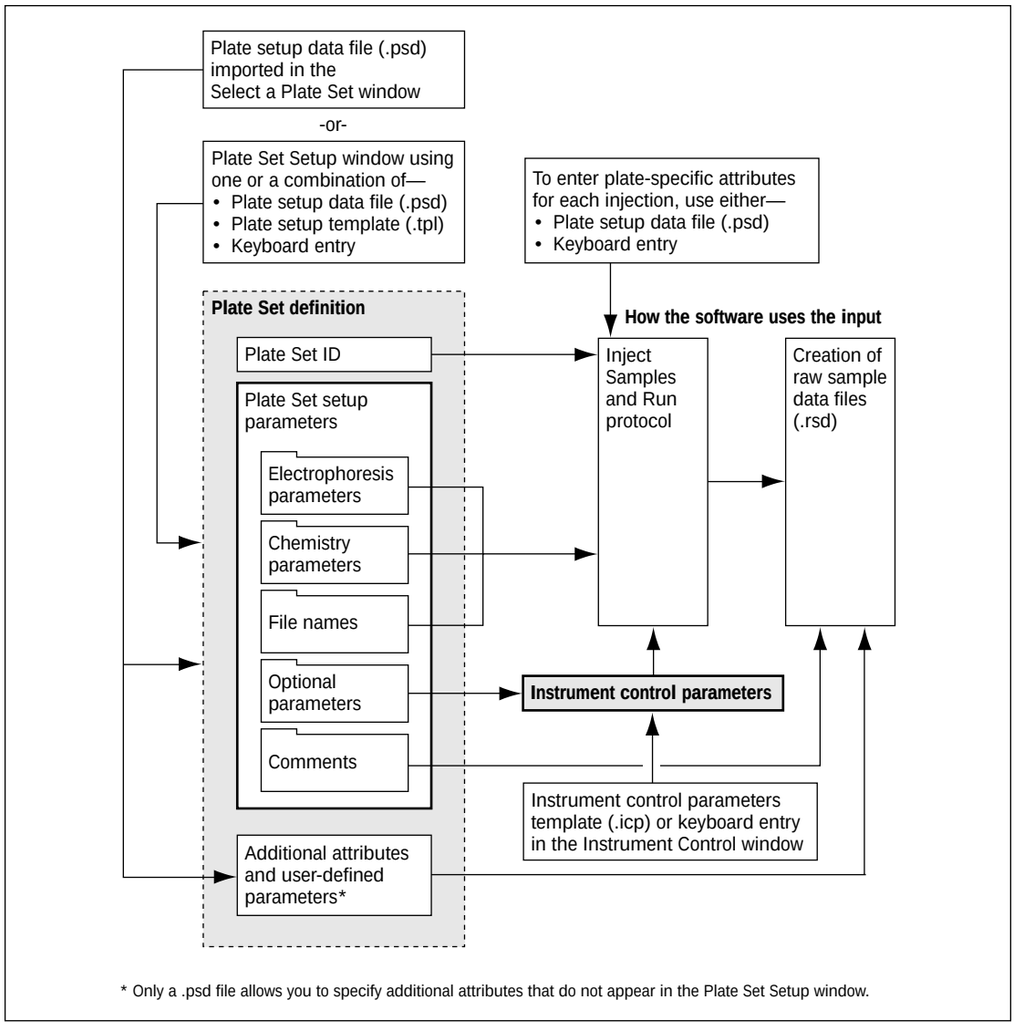



**Figure 1-6.** Overview of how the software uses the parameters specified for a SNP genotyping run.



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## 1.6 File format overview

The initialization files (MegaBACE.ini, Chemistry.ini, and Basecall.ini), the parameter template files (.tpl and .icp), and the .psd files all share the same format. The file format consists of two columns (field column and value column) separated by one or more tabs. The software ignores additional character spaces. The order of the fields is arbitrary, and the field names are not case-sensitive. The Instrument Control Manager ignores any unrecognized fields or any blank rows between fields.

If you type a value that is invalid (for example an alpha character when the software requires a number, or a value that is out of range), the Instrument Control Manager displays an error message when the software loads the file. If this occurs, you must correct the value in the file before it can be loaded successfully.

## 1.7 File storage overview

The Instrument Control Manager looks for each type of file in a specific location—

- The MegaBACE.ini and Chemistry.ini files must be in the MegaBACE\Instrument Control\DataSystem folder.
- (Sequencing only) The Basecall.ini file must be in the MegaBACE\Base Calling folder.
- The template files (.tpl and .icp) must be stored in the MegaBACE\Instrument Control\Templates folder.
- The .psd files must be stored in the MegaBACE\Instrument Control\Psd folder or a location that you specify in the MegaBACE.ini file.

See chapter 15 for details on file storage.

**Important** **The system initialization files must be stored in the appropriate MegaBACE folder. The software will not function properly unless these files are stored properly. See chapter 15 for details.**



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# Chapter 2 Specifying basic system preferences

This chapter describes the system initialization file that you can modify to configure the software for your laboratory. The topics are—

- Using the MegaBACE.ini file (section 2.1)
- Specifying a default application (section 2.2)
- Enabling or disabling the edit mode (section 2.3)
- Specifying how plate setup data (.psd) files are used (section 2.4)
- Using automated bar coding (section 2.5)
- Suppressing file creation for empty or bad capillaries (section 2.6)

## 2.1 Using the MegaBACE.ini file

The MegaBACE.ini file determines the initial settings the Instrument Control Manager uses to control the instrument. The Instrument Control Manager uses only one MegaBACE.ini file that loads automatically when you start the software. Table 2-1 describes the sections in the MegaBACE.ini file, and figure 2-1 shows the MegaBACE.ini file.

**Table 2-1.** Sections in the MegaBACE.ini file

| Section name | Description | Reference |
|---|---|---|
| [General] | Lists the basic settings the Instrument Control Manager uses to run the instrument. The fields are active for all applications (sequencing, STR genotyping, and SNP genotyping). | Sections 2.1 through 2.5 |
| [Sequencing] | Lists the default plate setup and instrument control templates for sequencing and all the base calling and file export modes. | Chapter 5 |
| [Genotyping] | Lists the default plate setup and instrument control templates for STR genotyping. | Chapter 8 |
| [SNP] | Lists the default plate set setup and instrument control templates for SNP genotyping and preferences for the injections during a multi-injection run. | Chapter 11 |
| [Bad Capillaries] | Lists the well IDs of any bad capillaries (broken or clogged). The Instrument Control Manager does not create data files for capillaries that are empty, broken, or clogged.<br>**Note:** You can specify empty capillaries in a plate setup template (.tpl) or a plate setup data file (.psd). | Section 2.6 |



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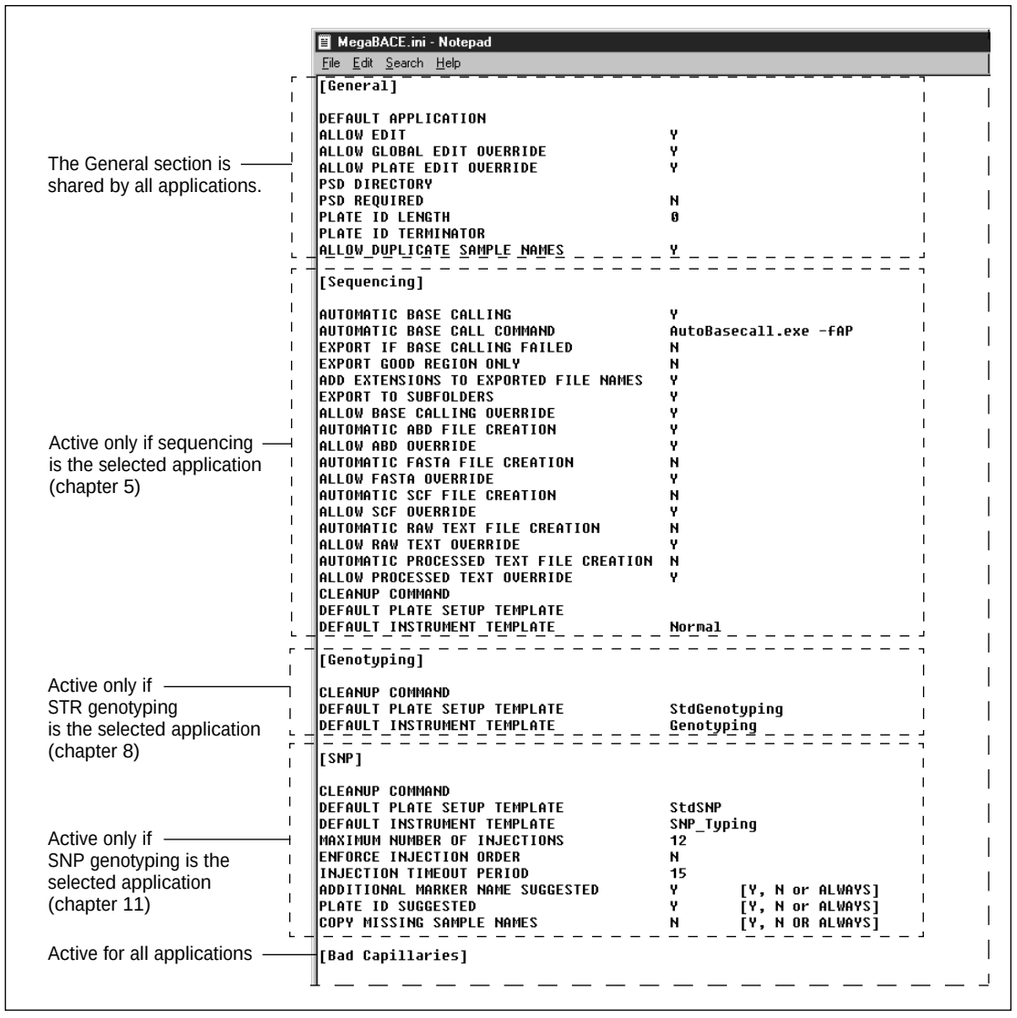



**Figure 2-1.** The MegaBACE.ini file opened in Notepad.



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## 2.1.1 Modifying the MegaBACE.ini file

**Important** **The Instrument Control Manager can use the MegaBACE.ini file only if the file name is MegaBACE.ini and the file is stored in the …\MegaBACE\Instrument Control\DataSystem folder.**

The MegaBACE.ini file is in a two-column, tab-separated format (figure 2-1). The first column (field column) contains the various modes and conditions that you can customize for the laboratory. The second column (value column) defines the setting. See section 1.6 for additional details on file formats.

To modify the MegaBACE.ini file—

1. Open the **MegaBACE.ini file** in the …\MegaBACE\Instrument Control\DataSystem folder using any text editor. See figure 2-1 for an example of the MegaBACE.ini file opened in Notepad.
2. Change the values in the value column as needed. Each field in the file has a specified data type. The data types for the value column include—
   - **Y/N—**You type **Y** for yes or **N** for no to set the conditions.
   - **String—**You type text.
   - **Integer—**You type a number.

   Table 2-2 provides a description of the field names and the associated data type required for each field in the General section of the MegaBACE.ini file. For details on the application-specific sections of the MegaBACE.ini file, see the corresponding chapter for each application (chapter 5 for sequencing, chapter 8 for STR genotyping, or chapter 11 for SNP genotyping).
3. Save the file in the …\MegaBACE\Instrument Control\DataSystem folder.

**Important** **You must restart the Instrument Control Manager in order for the changes you make in the MegaBACE.ini file to take effect.**



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## 2.1.2 General field names

**Table 2-2.** The general field names and default settings of the MegaBACE.ini file

| Field name | Description | Data type | Default |
|---|---|---|---|
| DEFAULT APPLICATION | Specifies the application to use as the default when an operator opens the Instrument Control Manager. | String* | Sequencing is the default. |
| ALLOW EDIT | Determines whether an operator can edit the parameters in the Plate Setup window and Instrument Control window.<br>Y = operator can change parameters (edit mode is turned on)<br>N = operator cannot change parameters (edit mode is turned off) | Y or N | Y |
| ALLOW GLOBAL EDIT OVERRIDE | Determines whether an operator can change the Edit Mode command on the Configure menu at any time.<br>Y = operator can change mode<br>N = operator cannot change mode | Y or N | Y |
| ALLOW PLATE EDIT OVERRIDE | Determines whether an operator can change the Edit Mode command on the Configure menu only for the plate definition that is currently being created (if Allow Global Edit Override is N).<br>Y = operator can change mode<br>N = operator cannot change mode | Y or N | Y |
| PSD DIRECTORY | Specifies the path for the psd folder. | Path† | …\MegaBACE\ Instrument Control\Psd |
| PSD REQUIRED | Determines whether an operator can proceed without a .psd file (see section 2.4.2).<br>Y = operator cannot proceed without a .psd file<br>N = to create a plate definition, the operator can choose whether to import a .psd file | Y or N | Y |
| PLATE ID LENGTH | Determines the number of characters allowed in the bar code and eliminates the need to press ENTER. | Integer‡ | N/A |



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**Table 2-2.** The general field names and default settings of the MegaBACE.ini file (continued)

| Field name | Description | Data type | Default |
|---|---|---|---|
| PLATE ID TERMINATOR | Determines what terminates the entry of the plate ID, for example a character or group of characters. Eliminates the need to press ENTER (section 2.5.2). | String | None |
| ALLOW DUPLICATE SAMPLE NAMES | Allows an operator to use duplicate sample names.<br>Y = allow duplicate names (Well IDs are appended to the sample names to create unique file names.)<br>N = do not allow duplicate names | Y or N | Y |

* String = A text string
† Path = The complete path to a file folder
‡ Integer = A number

## 2.2 Specifying a default application

Whenever you restart the Instrument Control Manager, the software automatically resets the selected application (sequencing, STR genotyping, or SNP genotyping) to the default specified in the MegaBACE.ini file. After the software starts, the operator can change the application selected in the Instrument Control Manager by choosing the Applications command from the Configure menu. The name of the selected application appears in the title bar of each of the Instrument Control Manager windows.

The Instrument Control Manager windows display only the parameters that are applicable for the selected application. For example, if sequencing is selected, the Plate Setup window displays only the sequencing chemistry names, and the Plate Catalog displays only the sequencing plate IDs.

To set the default application, you use the following field in the MegaBACE.ini file:

| Field in MegaBACE.ini file | Value | Results |
|---|---|---|
| DEFAULT APPLICATION | (blank) | The sequencing application is the selected default. |
| | Genotyping | The STR genotyping application is the selected default. |
| | SNP Genotyping | The SNP genotyping application is the selected default. |



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For each application, you can specify the default plate setup template and the default instrument control template. For details on the application-specific sections of the MegaBACE.ini file, see the chapter for each application (chapter 5 for sequencing, chapter 8 for STR genotyping, or chapter 11 for SNP genotyping).

## 2.3 Enabling or disabling the edit mode

The edit mode determines whether an operator can manually edit the parameters displayed in the Plate Setup window or Plate Set Setup window and the Instrument Control window. The edit mode also determines whether an operator can change templates or select templates. If the edit mode is turned on, a check mark appears next to the Edit Mode command on the Configure menu. If the edit mode is turned off, no check mark appears.

The MegaBACE.ini file contains the default settings for the edit mode conditions (table 2-3). You can use the MegaBACE.ini file to enable or disable the edit mode menu command, which allows an operator to turn on or off the edit mode using the Configure menu. You can also specify whether the edit mode remains on or applies to one plate only.

Table 2-3 provides an overview of how to specify the edit mode conditions in the MegaBACE.ini file and how the Edit Mode command appears in the Configure menu.

**Table 2-3.** The edit mode fields in the MegaBACE.ini file

| Allow Edit | Allow Global Edit Override | Allow Plate Edit Override | Edit Mode command in the Configure menu |
|---|---|---|---|
| Y | N/A | N/A | Turned on (checked). Can be turned off. |
| N | Y | N/A | Turned off (unchecked). Can be turned on at will. |
| N | N | Y | Turned off (unchecked). Can be turned on for one newly created plate only, and turns off automatically for all other plates. |
| N | N | N | Turned off (unchecked). No editing is allowed. An operator can create a new plate definition only by importing a .psd file or by using the default plate setup template. |



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The MegaBACE.ini file contains the following fields that allow you to specify the edit mode conditions:

- **Allow Edit—**Specifying **Y** turns the edit mode on and allows an operator to edit any of the plate setup or instrument parameters. The Edit Mode command on the Configure menu has a check mark to indicate that it is turned on. Specifying **N** turns off the edit mode, but an operator can turn on the edit mode if a **Y** is specified for the ALLOW GLOBAL EDIT OVERRIDE field.
- **Allow Global Edit Override—**Specifying **Y** allows an operator to turn on or off the edit mode in the Configure menu at will. While in the edit mode, an operator can edit the plate setup parameters or instrument parameters. Specifying **N** turns off the edit mode, which prevents the operator from turning on the edit mode, unless a **Y** is specified for the ALLOW PLATE EDIT OVERRIDE field.
- **Allow Plate Edit Override—**Specifying **Y** allows an operator to choose the edit mode for the current plate only (newly created plate). The edit mode becomes unavailable when the operator selects another plate. Specifying **N** disables the edit mode feature.

If all the above conditions are set to **N** in the MegaBACE.ini file, an operator cannot edit any of the plate setup parameters (plate set setup for SNP genotyping) or instrument parameters in the Instrument Control Manager windows. In this case, an operator can set up plate definitions (plate set definitions for SNP genotyping) only by importing .psd files or by using the default plate setup template.

When you turn on the edit mode, an operator can create a plate setup template, or select or set a default template from the Plate Setup Template menu. An operator can also create an instrument parameter template, or select a template from the Instrument Templates menu.

## 2.4 Specifying how plate setup data (.psd) files are used

A plate setup data file (.psd) allows you to define—

- **Sequencing and STR genotyping—**A plate ID, the plate setup parameters, and other plate- and well-level attributes so that an operator can automatically import the plate definition. The operator can import the .psd file either before the run in the Plate Setup window or at the start of the run in the Select a Plate window *(MegaBACE 4000 Instrument User's Guide).*



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- **SNP genotyping—**A plate set ID, the plate set setup parameters, and other plate- and well-level attributes so that an operator can automatically import the plate set definition and plate-attributes for each injection. The operator can import the master .psd file containing the plate set definition and the attributes for the first plate either before the run in the Plate Set Setup window or at the start of the run in the Select a Plate Set window *(MegaBACE 4000 Instrument User's Guide)*. The operator can import a plate-only .psd file for each subsequent injection.

With automatic import, the plate setup parameters or plate set setup parameters and plate attributes are specified with less operator intervention. The parameters that you specify in the .psd file become part of the extended header of each raw sample data file (.rsd) created during the run.

### 2.4.1 Specifying a new .psd file folder location

By default, the .psd files are saved in the …\MegaBACE\Instrument Control\Psd folder. To specify a new storage location for the .psd files, you use the following field in the MegaBACE.ini file:

| Field in MegaBACE.ini file | Value |
|---|---|
| PSD DIRECTORY | …\MegaBACE\Instrument Control\Psd |

You can specify a file path on a network so that the .psd files can be shared by multiple workstations. To do this, use the full network path location. For example—

| Field in MegaBACE.ini file | Value |
|---|---|
| PSD DIRECTORY | \\computer_name\folder_name\Psd |

### 2.4.2 Specifying that .psd files are required

The operator can always import a .psd file. However, the PSD REQUIRED field determines whether the operator can proceed without a .psd file. By default, the value for this field is N. If the PSD REQUIRED field is set to Y, the operators must use a .psd file if they use the Plate Setup window to create plate definitions. If the software cannot find or load the .psd file, an error message appears and the operation stops.



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If the PSD REQUIRED field is set to N, the operator is not required to use a .psd file when creating the plate definitions in the Plate Setup window. The software still accepts .psd files.

| Field in MegaBACE.ini file | Value |
|---|---|
| PSD REQUIRED | Y or N |

## 2.5 Using automated bar coding

**Important** **Regardless of the method used to enter the plate ID, the final text entry or bar code (less a possible plate ID terminator) must match the name (case insensitive) of the .psd file in order for a .psd file to be imported.**

To eliminate the need to press ENTER when importing a .psd file, you can use the following fields in the MegaBACE.ini file:

- PLATE ID LENGTH
- PLATE ID TERMINATOR

(Sequencing and STR genotyping) When an operator types a .psd file name or scans a bar code in the Plate ID box of the Plate Setup window or the Select a Plate window, the software automatically imports the .psd file when the PLATE ID LENGTH or PLATE ID TERMINATOR conditions are met.

(SNP genotyping) When an operator types a .psd file name or scans a bar code in the Plate Set Setup window, the Select a Plate Set window, or the Bar Code box of the Sample Injection window, the software automatically imports the .psd file when the PLATE ID LENGTH or PLATE ID TERMINATOR conditions are met.

### 2.5.1 Specifying the plate ID length

You can predefine the number of characters in the bar code or .psd file name using the PLATE ID LENGTH field.

| Field in MegaBACE.ini file | Value |
|---|---|
| PLATE ID LENGTH | Integer |

When the number of characters entered reaches the predefined value, the software automatically imports the .psd file.



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### 2.5.2 Specifying the plate ID terminator

You can determine which character or group of characters terminates the entry of the bar code or .psd file name. You can use any printable character as the plate ID terminator. After detecting the terminator in the string entered, the Instrument Control Manager stops accepting characters, removes the terminator from the end of the string, and automatically imports the .psd file.

| Field in MegaBACE.ini file | Value |
|---|---|
| PLATE ID TERMINATOR | String |

## 2.6 Suppressing file creation for empty or bad capillaries

The Instrument Control Manager allows you to suppress the creation of raw sample data files (.rsd) for capillaries that are empty, broken, or clogged. You can list the well ID for each defective capillary in the [Bad Capillaries] section of the MegaBACE.ini file. This is a global setting. For every capillary listed, a red arrow appears above the capillary button in the Run Image window (figure 2-2).

You can specify empty capillaries in a .psd file, a .tpl file, or manually in the Plate Setup window (sequencing and STR genotyping) or the Plate Set Setup window (SNP genotyping). By specifying NOT USED as the file name, an empty capillary appears as black, and a yellow arrow appears above the well button in the Run Image window.



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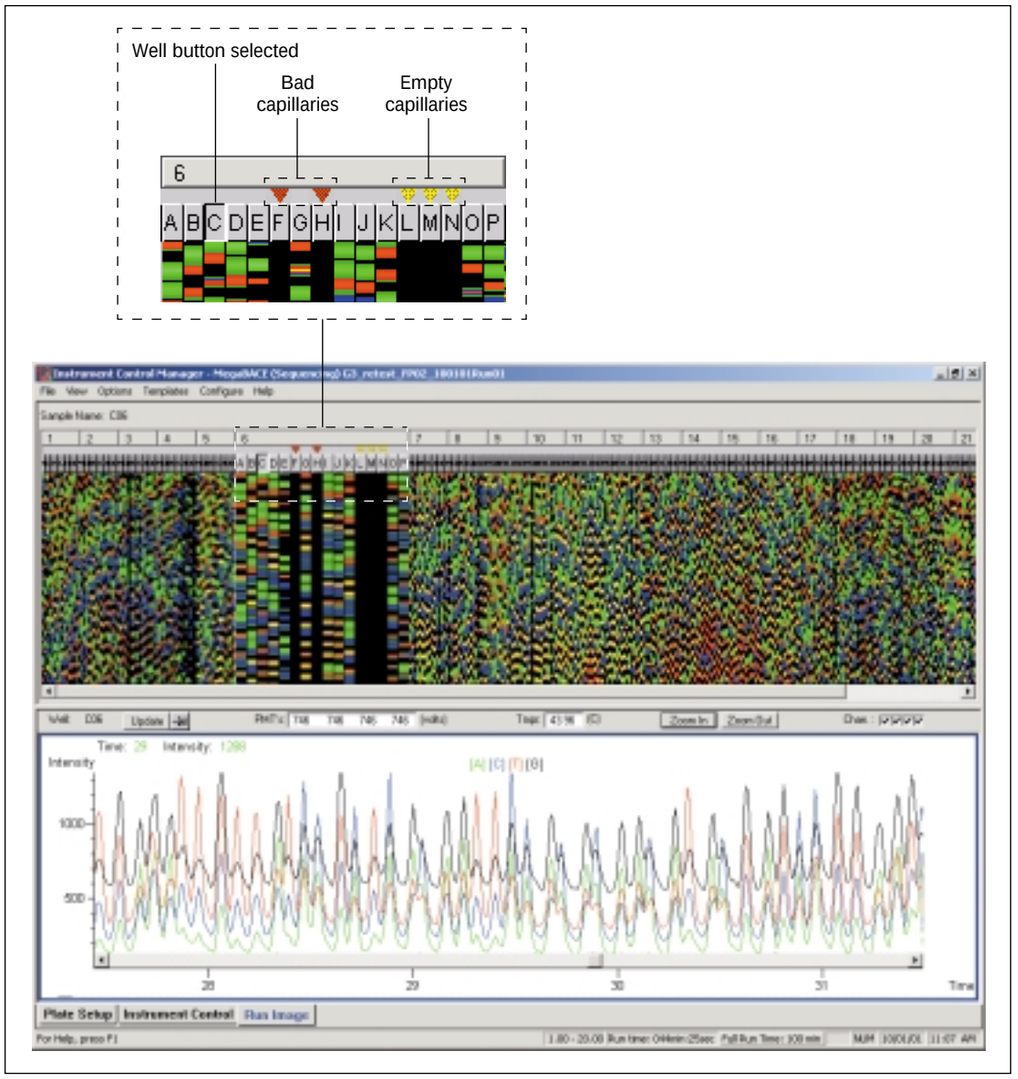



**Figure 2-2.** The Run Image window for an instrument with bad capillaries and empty capillaries indicated.



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# Chapter 3 Specifying the chemistry parameters

This chapter describes the system chemistry initialization file that you can modify to configure the system for your laboratory workflow. The topics are—

- How the software uses the Chemistry.ini file (section 3.1)
- About specifying the chemistry (section 3.2)
- The fields in the Chemistry.ini file (section 3.3)
- Adding a new set of chemistry parameters (section 3.4)

## 3.1 How the software uses the Chemistry.ini file

The Chemistry.ini file contains all the chemistry parameter sets that are available in the Instrument Control Manager. The Instrument Control Manager uses only one Chemistry.ini file to define all the available chemistry parameter sets. See section 1.6 for a description of the file format.

## 3.2 About specifying the chemistry

To use one of the available chemistry parameter sets, you must specify a chemistry name that is recognized by the Instrument Control Manager. You can specify the chemistry name in a—

- Plate setup data file (.psd)

  **Note:** For SNP genotyping, if you are using a .psd file to specify the chemistry name, it must be a master .psd file.

- Plate setup template file (.tpl)

If you turn on the edit mode, an operator can select the chemistry name in the Plate Setup window (sequencing and STR genotyping) or the Plate Set Setup window (SNP genotyping). Only the names of the chemistry parameter sets for the selected application appear in the Chemistry Name list in the Chemistry Parameters tab (figure 3-1).



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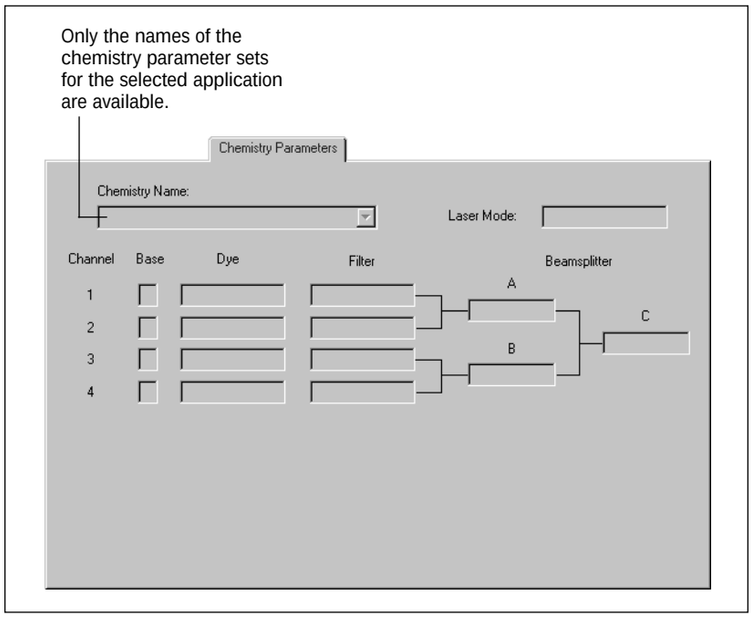



**Figure 3-1.** The Chemistry Parameters tab of the Plate Setup window (sequencing and STR genotyping) or the Plate Set Setup window (SNP genotyping).

## 3.3 The fields in the Chemistry.ini file

The fields in the Chemistry.ini file include the following:

- **Application**—Required field if you are defining a parameter set for an application other than sequencing. If you do not specify the application, the Instrument Control Manager assumes that the chemistry parameter set is for the sequencing application. The Instrument Control Manager recognizes the following three application types:
  - Sequencing
  - Genotyping (STR genotyping)
  - SNP genotyping
- **Beamsplitters—**BEAMSPLITTER A indicates the beamsplitter used in spectral channels 1 and 2. BEAMSPLITTER B indicates the beamsplitter used in spectral channels 3 and 4. BEAMSPLITTER C splits the emission light between BEAMSPLITTER A and BEAMSPLITTER B.



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- **Filters—**The filter number indicates the spectral channel number. For example, FILTER 1 indicates the filter used to detect the emissions in spectral channel 1.
- **Bases—**Required field for the sequencing and SNP genotyping applications. The base number indicates the channel number used to detect the base. For example, BASE 1 indicates the base detected through spectral channel 1. Valid values are A, G, C, or T.
- **Dyes—**Required field for the STR genotyping application. The dye number indicates the channel number used to detect the dye. For example, DYE 1 indicates the dye detected through spectral channel 1.
- **Laser mode—**Required field. The only valid value for the MegaBACE 4000 instrument is blue.

## 3.4 Adding a new set of chemistry parameters

**Important** **If the application is not specified in the chemistry parameter set, the Instrument Control Manager assumes the chemistry is for the sequencing application, and you must specify the bases.**

To add an additional chemistry parameter set to the Chemistry.ini file—

1. Open the Chemistry.ini file using a text editor or Excel. The Chemistry.ini file is located in the …\MegaBACE\Instrument Control\DataSystem folder. Figure 3-2 shows a section of the Chemistry.ini file.

   The name of each chemistry parameter set appears in brackets, for example [TSII-Version 2 Terminators]. The field names appear in the left column, and the values appear in the right column. The columns are separated by one or more tabs.

2. Scroll to the end of the file and type a name for the new chemistry parameter set. You must provide a unique name for each chemistry parameter set. Make sure you put the name in brackets (figure 3-2).
3. Copy and paste the field names and values from a chemistry parameter set that is similar to the one you are adding. Make any required changes to the field values for the new parameter set.
4. Save the file. The Instrument Control Manager can use the file only if the file name is Chemistry.ini and the file is stored in the …\MegaBACE\Instrument Control\DataSystem folder.

   **Note:** The changes appear immediately in the Instrument Control Manager without restarting the software.



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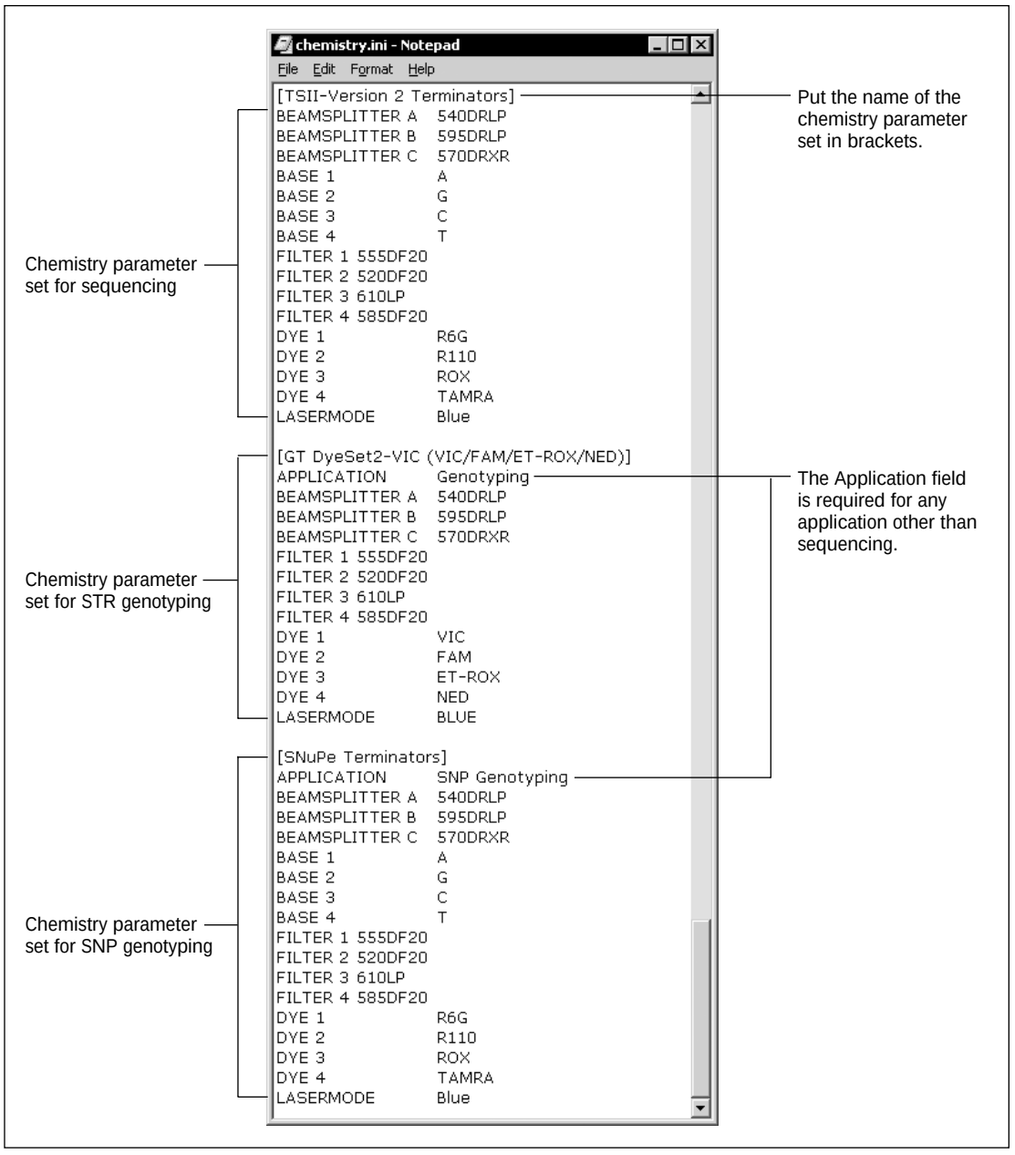



**Figure 3-2.** A section of the Chemistry.ini file opened in Notepad.



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5. Verify that the changes appear in the Chemistry Parameters tab (figure 3-1). If the name for a new or revised chemistry parameter set does not appear in the Chemistry Name list for the selected application, check the parameters in the Chemistry.ini file for syntax errors.

   Syntax errors can include—

   - The required field is missing. The required fields for sequencing are laser mode and base names.
   - The required field has an out-of-range value. For example, a base name has a letter other than A, G, C, or T for the value.
   - Duplicate values exist for different fields, for example, two bases are named A.
   - The parameter set includes a misspelled field name or value for a required field.



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# Chapter 4 Creating templates for the instrument control parameters

The Instrument Control Manager allows you to save the frequently used instrument control parameters as templates so that you do not have to reenter the parameters. This chapter describes how to create the instrument control templates. The topics are—

- About instrument control templates (section 4.1)
- Using the Instrument Control window to create a template (section 4.2)
- Using a text file to create an instrument control template (.icp) (section 4.3)

## 4.1 About instrument control templates

The instrument control parameters are displayed in the Run Parameters tab and Sleep Parameters tab of the Instrument Control window (figure 4-1). The software includes the following instrument control template files (.icp):

- **Sequencing—**The Normal.icp template is provided.
- **STR genotyping—**The Genotyping.icp template is provided.
- **SNP genotyping—**The SNP_Typing.icp template is provided.

You can use the templates provided, or modify the existing templates to create new templates. You can also create a template from the values entered in the Instrument Control window, or you can create a template using a text editor or Excel. Tables 4-1 and 4-2 describe the instrument control parameters.

You can specify a default .icp file in the MegaBACE.ini file for each application (chapter 5 for sequencing, chapter 8 for STR genotyping, or chapter 11 for SNP genotyping). The default .icp file is loaded into the Instrument Control window at startup. If you enable the edit mode (section 2.3), an operator can also select a template from the Instrument Templates menu.



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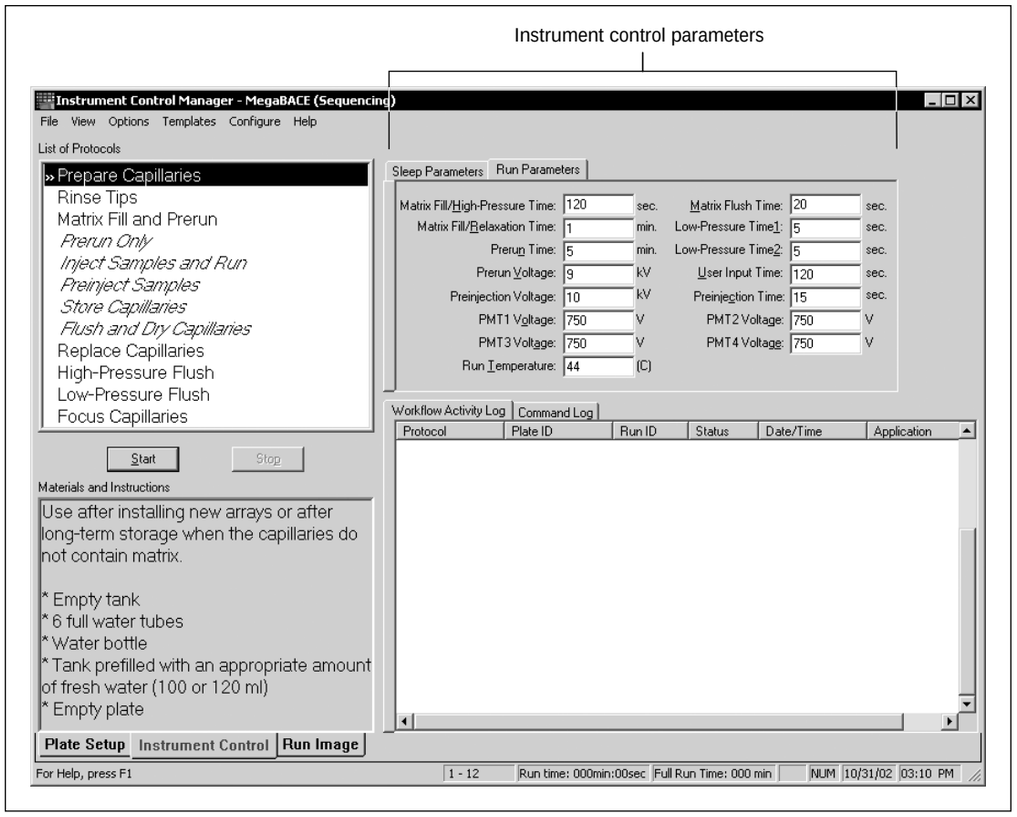



**Figure 4-1.** The Instrument Control window with the sequencing instrument parameters displayed.



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**Table 4-1.** Instrument control run parameters

| Field name | Description (value range) |
|---|---|
| Matrix Fill/High-Pressure Time | The time (seconds) for applying high pressure to fill the capillaries with matrix (range: 1–600 s). |
| Matrix Fill/Relaxation Time | The time (minutes) to allow the matrix to equilibrate (range: 1–120 min). |
| Prerun Time | The time (minutes) for the electrophoresis prerun (range: 1–120 min). |
| Prerun Voltage | The voltage for the prerun (range: 1–15 kV). |
| Preinjection Voltage | The voltage for preinjecting samples (range: 1–15 kV). |
| PMT1 Voltage | The voltage for PMT1 (range: 500–900 V). |
| PMT2 Voltage | The voltage for PMT2 (range: 500–900 V). |
| PMT3 Voltage | The voltage for PMT3 (range: 500–900 V). |
| PMT4 Voltage | The voltage for PMT4 (range: 500–900 V). |
| Run Temperature | The electrophoresis compartment temperature for the run (range 27–44 ºC). |
| Matrix Flush Time | The time (seconds) for the first matrix flush (range: 1–60 s). |
| Low-Pressure Time1 | The time (seconds) for the nitrogen pressure to flush water through the capillaries (range: 1–60 s). |
| Low-Pressure Time2 | The time (seconds) for the nitrogen pressure to dry the capillaries (range: 1–300 s). |
| User Input Time | The time (seconds) you need to open and close the anode and cathode drawers after the display on the instrument gives the instruction to load the plate, tank, or tubes in either the anode or the cathode side of the instrument (range: 10–240 s). |
| Preinjection Time | The time (seconds) for preinjecting samples (range: 1–600 s). |



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**Table 4-2.** Instrument control sleep parameters

| Field name | Description and value range |
|---|---|
| Sleep After This Run | This setting is not saved in the template. The check box that you check if you want the instrument set in sleep mode after the current or next run. |
| Sleep Temperature | Although the Sleep Temperature field requires a value, the software turns off the electrophoresis compartment heater during the Sleep After This Run and the Store Capillaries protocols. The value range is 25–30. |
| Sleep Time | The length of time (hours) the instrument will be left idle, during which the software turns off the electrophoresis compartment heater and the laser. The range is 1–168 h (Store Capillaries protocol) or 1–16 h (Sleep After This Run option). |

## 4.2 Using the Instrument Control window to create a template

The MegaBACE software includes instrument control templates (.icp files) that you can use. You can modify the existing templates to create new templates. You can create a template from the values entered in the Instrument Control window (figure 4-1), or you can create a template using a text editor or Excel (section 4.3). See tables 4-1 and 4-2 for a list of the parameters.

**Important** **The edit mode must be turned on to allow creating, editing, saving, or importing instrument control templates in the Instrument Control Manager. See section 2.3 for information about enabling the edit mode in the MegaBACE.ini file.**

To create an instrument control template—

1. Start the Instrument Control Manager. In the **MegaBACE** folder on the Windows desktop, double-click the **Instrument Control Manager** icon.
2. Click the **Instrument Control** tab to display the Instrument Control window (figure 4-1).
3. To modify an existing template—
   - On the **Templates** menu, point to **Instrument Template,** and click **Select Template.** The Select Template window appears and displays the list of available templates (.icp).



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- Select the template (.icp) you want to modify, and click **Open.** The values of the selected template appear in the Instrument Parameters area (figure 4-1).

4. Modify any of the parameters.
5. To save the current instrument control parameters as a template—
   - On the **Templates** menu, point to **Instrument Template,** and click **Save Template**. The Save window appears.
   - Type a new name, and click **Save**. The template is saved in the …\MegaBACE\Instrument Control\Templates folder. The software adds the .icp file extension.

## 4.3 Using a text file to create an instrument control template (.icp)

You can also create an .icp file in a two-column format in Excel or in any text editor using tabs as column separators. You must use the field names that the Instrument Control Manager recognizes and type them in the first column (field column) as shown in figure 4-2. You type the values for each field in the second column (value column). The Instrument Control Manager uses the values only for the fields it recognizes. See table 4-1 for a list of the run parameter (field) names and a description of the expected values. See table 4-2 for a list of sleep parameters.



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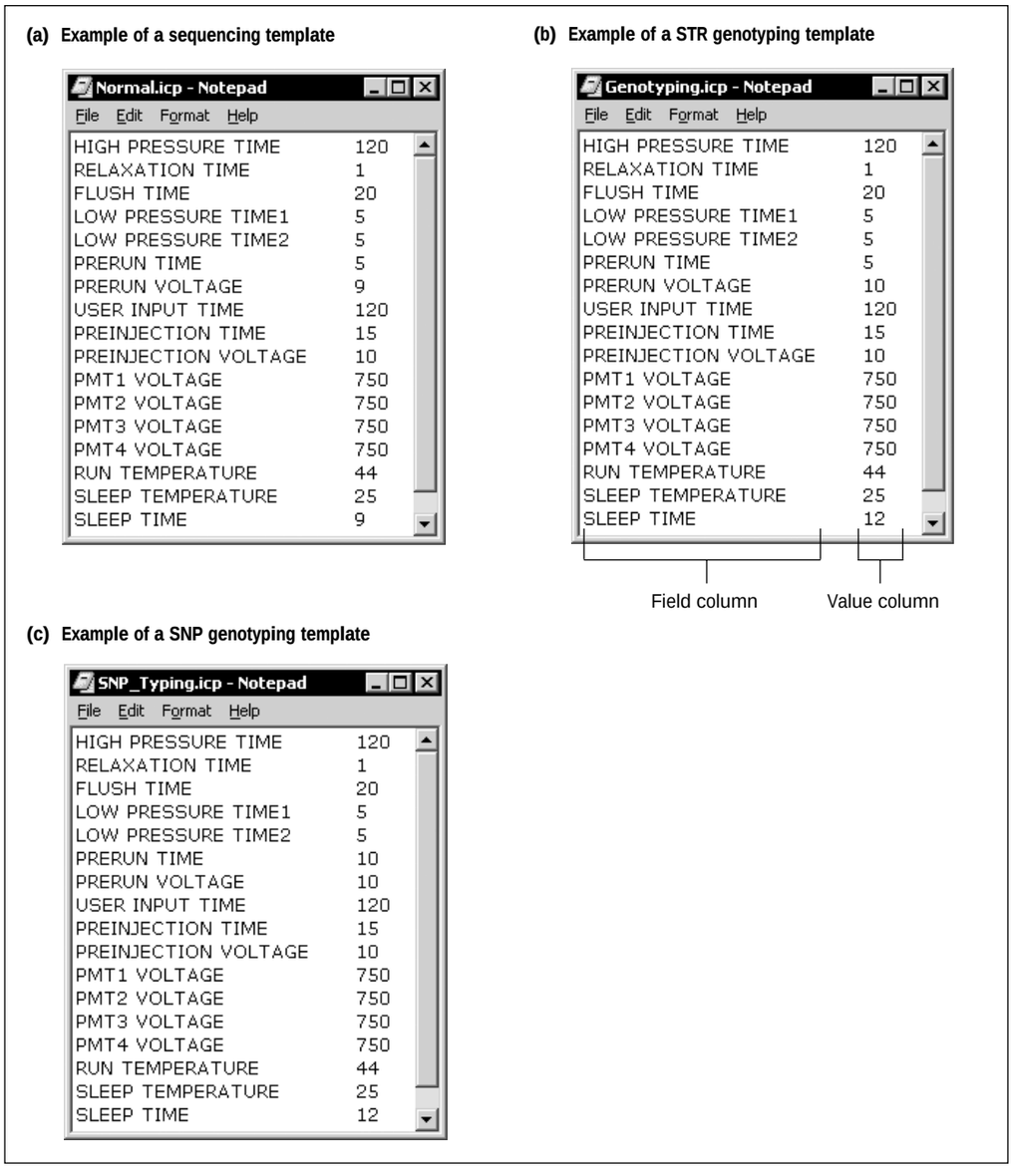



**Figure 4-2.** Examples of instrument control template files (.icp) created using Notepad.



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# Part two

# Setting up the system for sequencing

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# Chapter 5 Specifying preferences for sequencing runs

This chapter describes how to set up the automatic base calling feature and how to automatically export files to other file formats. The topics are—

- The sequencing fields in the MegaBACE.ini file (section 5.1)
- Setting default parameter templates (section 5.2)
- Configuring the automatic base calling feature (section 5.3)
- Turning on or off the automatic base calling feature (section 5.4)
- Setting up the automatic file export feature (section 5.5)
- Settings in the Basecall.ini file (section 5.6)
- Specifying which base caller to use (section 5.7)

Make sure you configure the General section of the MegaBACE.ini file (chapter 2) for your laboratory. To create additional chemistry parameters, see chapter 3.

## 5.1 The sequencing 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: General, Sequencing, Genotyping (STR genotyping), and SNP (SNP genotyping). Figure 5-1 shows the MegaBACE.ini file with the Sequencing fields highlighted.

Table 5-1 lists the field names and default settings in the Sequencing section of the MegaBACE.ini file. See chapter 2 for a description of the other fields in the MegaBACE.ini file.



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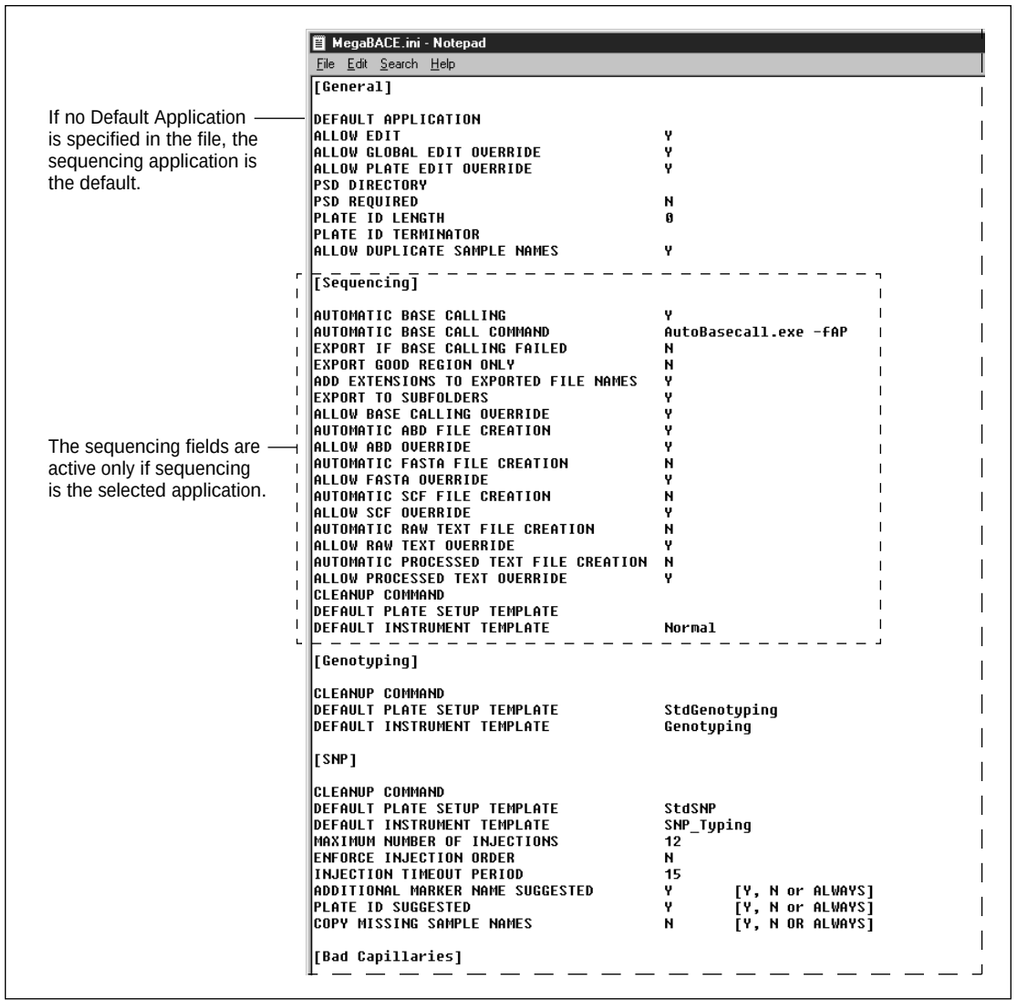



**Figure 5-1.** The MegaBACE.ini file with the sequencing fields highlighted.



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**Table 5-1.** The field names and default settings for the sequencing section of the MegaBACE.ini file

| Field name | Description | Setting type | Default |
|---|---|---|---|
| AUTOMATIC BASE CALLING | Determines whether the Instrument Control Manager launches automatic base calling.<br>Y = launch base calling<br>N = do not launch base calling | Y or N | Y |
| AUTOMATIC BASE CALL COMMAND | Allows automatic base calling to start right after data collection.<br>**Caution: If this field is blank, automatic base calling will fail.**<br>The field also allows you to specify a FASTA option: -fa, -fap, and -fag (section 5.5.2). | String | AutoBasecall.exe -fap |
| EXPORT IF BASE CALLING FAILED | Allows an operator to specify whether to export files for which base calling failed.<br>Y = export files<br>N = do not export files | Y or N | N |
| EXPORT GOOD REGION ONLY | Allows an operator to specify whether to export only the good region of sequence.<br>Y = export good region<br>N = export all | Y or N | N |
| ADD EXTENSIONS TO EXPORTED FILE NAMES | Adds standard extensions to exported file names.<br>Y = add extensions<br>N = do not add extensions | Y or N | Y |
| EXPORT TO SUBFOLDERS | Exports specified files to subfolders named with the file extensions.<br>Y = use subfolders<br>N = do not use subfolders | Y or N | Y |
| ALLOW BASE CALLING OVERRIDE | Determines whether an operator can override (turn on or turn off) automatic base calling from the Automatic Base Calling window, which opens from the Configure menu.<br>Y = operator can override<br>N = operator cannot override | Y or N | Y |



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**Table 5-1.** The field names and default settings for the sequencing section of the MegaBACE.ini file (continued)

| Field name | Description | Setting type | Default |
|---|---|---|---|
| AUTOMATIC ABD FILE CREATION | Determines whether the Instrument Control Manager automatically creates ABD files after base calling.<br>Y = create ABD files<br>N = do not create ABD files | Y or N | Y |
| ALLOW ABD OVERRIDE | Determines whether an operator can override automatic ABD file creation from the Automatic Base Calling window, which opens from the Configure menu.<br>Y = operator can override<br>N = operator cannot override | Y or N | Y |
| AUTOMATIC FASTA FILE CREATION | Determines whether the Instrument Control Manager automatically creates FASTA files (.seq) after base calling.<br>Y = create FASTA files<br>N = do not create FASTA files | Y or N | N |
| ALLOW FASTA OVERRIDE | Determines whether an operator can override automatic FASTA file creation from the Automatic Base Calling window, which opens from the Configure menu.<br>Y = operator can override<br>N = operator cannot override | Y or N | Y |
| AUTOMATIC SCF FILE CREATION | Determines whether the Instrument Control Manager automatically creates SCF files after base calling.<br>Y = create SCF files<br>N = do not create SCF files | Y or N | N |
| ALLOW SCF OVERRIDE | Determines whether an operator can override automatic SCF file creation from the Automatic Base Calling window, which opens from the Configure menu.<br>Y = operator can override<br>N = operator cannot override | Y or N | Y |



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**Table 5-1.** The field names and default settings for the sequencing section of the MegaBACE.ini file (continued)

| Field name | Description | Setting type | Default |
|---|---|---|---|
| AUTOMATIC RAW TEXT FILE CREATION | Determines whether the Instrument Control Manager automatically creates raw text files after base calling.<br>Y = create raw text files<br>N = do not create raw text files | Y or N | N |
| ALLOW RAW TEXT OVERRIDE | Determines whether an operator can override automatic raw text file creation from the Automatic Base Calling window, which opens from the Configure menu.<br>Y = operator can override<br>N = operator cannot override | Y or N | Y |
| AUTOMATIC PROCESSED TEXT FILE CREATION | Determines whether the Instrument Control Manager automatically creates processed text files after base calling.<br>Y = create processed text files<br>N = do not create processed text files | Y or N | N |
| ALLOW PROCESSED TEXT OVERRIDE | Determines whether an operator can override automatic processed text file creation from the Automatic Base Calling window, which opens from the Configure menu.<br>Y = operator can override<br>N = operator cannot override | Y or N | Y |
| CLEANUP COMMAND | Allows the administrator to specify a program or batch file to be executed at the end of the run after base calling. This can be useful for transferring plate records and raw data files to another workstation for analysis or archiving. See appendix C for a more detailed description. | String consisting of the path to the program or batch file (full or relative path to the instrument control folder) | None |
| DEFAULT PLATE SETUP TEMPLATE | Specifies a default template for the plate setup parameters. | String | None |
| DEFAULT INSTRUMENT TEMPLATE | Specifies a default template for the instrument control parameters. | String | Normal |



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## 5.2 Setting default parameter templates

In the MegaBACE.ini file, you can specify the—

- Default plate setup template
- Default instrument template

Whenever the Instrument Control Manager is restarted, the software automatically resets the templates selected for a given application to the default templates specified in the MegaBACE.ini file. However, if the edit mode is on, the operator can choose a different template in the Instrument Control Manager.

### 5.2.1 Setting the default plate setup template

If you specify a default plate setup template in the MegaBACE.ini file, the values from the plate setup template appear automatically in the Plate Setup window when the operator clicks **New.** To specify a default template for sequencing, use the following field in the sequencing 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. |

**Note:** If a plate setup data file (.psd) is used to define the plate for a run, the values for any duplicate parameters overwrite the values specified on the screen which typically come from the default plate setup template. The original default values are automatically restored at the completion of the run.

### 5.2.2 Setting the default instrument control template

If you specify a default instrument control template in the MegaBACE.ini file, the values appear automatically in the Instrument Control window when the Instrument Control Manager starts. To specify a default template for sequencing, use the following field in the sequencing 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. |



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## 5.3 Configuring the automatic base calling feature

The software can perform base calling automatically after the data collection is completed for a run. To configure the automatic base calling feature, you use a combination of the following files:

- **MegaBACE.ini file—**The Sequencing section of this file allows you to specify if the automatic base calling feature is on and whether an operator can turn on or off the feature in the Instrument Control Manager (section 5.4). This file also allows you to configure the file export feature (section 5.5).
- **Basecall.ini file—**The Base Callers section of the file lists the available base callers, and the Default section of the file can specify a default base caller (sections 5.6 and 5.7.1).
- **Plate setup template (.tpl)—**If used, a template can specify the base caller to be used for multiple plates (section 5.7.2 and chapter 6).
- **Plate setup data file (.psd)—**If used, a .psd file can specify both plate-level and well-level base callers for a given plate (section 5.7.2 and chapter 7).

## 5.4 Turning on or off the automatic base calling feature

The Sequencing section of the MegaBACE.ini file allows you to turn on the automatic base calling mode or to allow an operator to turn on or off the automatic base calling from the Configure menu in the Instrument Control Manager. The settings in the MegaBACE.ini file are tied directly to the settings in the Automatic Base Calling window in the Instrument Control Manager. Table 5-2 provides an overview of how you specify the automatic base calling conditions in the MegaBACE.ini file and how the Automatic Base Calling command appears in the Configure menu and in the Automatic Base Calling window (figure 5-2). See table 5-1 for a full list of the fields in the Sequencing section of the MegaBACE.ini file.



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**Table 5-2.** The automatic base calling mode conditions in the MegaBACE.ini file

| AUTOMATIC BASE CALLING field | ALLOW BASE CALLING OVERRIDE field | Automatic Base Call command in the Configure menu and the Automatic Base Calling window |
|---|---|---|
| Y | Y | Turned on (checked). Can be turned off in the Automatic Base Calling window. |
| Y | N | Turned on (checked). Cannot be turned off. |
| N | Y | Turned off (unchecked). Can be turned on at will in the Automatic Base Calling window. |
| N | N | Turned off and unchecked. Cannot be turned on. |

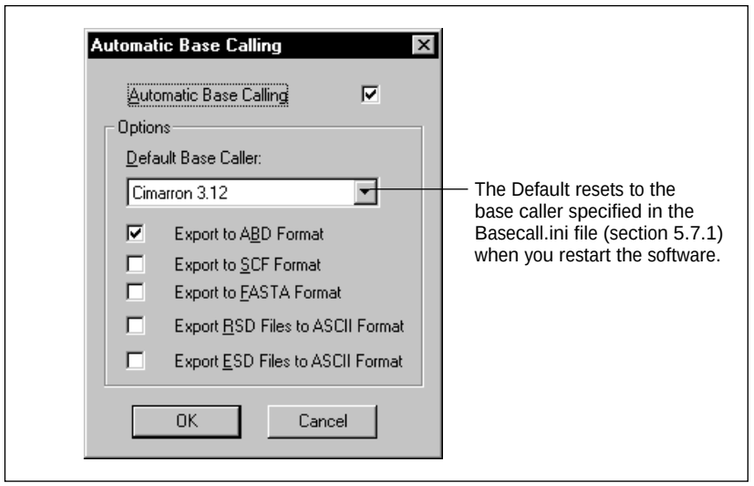



**Figure 5-2.** The Automatic Base Calling window, which is accessed from the Configure menu in the Instrument Control Manager.

**Note:** For a description of how to use the Automatic Base Calling window, see the *MegaBACE 4000 Instrument User's Guide.*



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## 5.5 Setting up the automatic file export feature

After automatic base calling, the software can automatically export the resulting files to other file formats in addition to the base-called sample data files (.esd). You can also specify file export options, such as whether to export if base calling fails or whether to add extensions to the file names.

### 5.5.1 Specifying the file formats for export

The additional format options for automatic file export are—

- **ABD—**Contains all the raw data, including the electropherogram and spectral separation table. If base-called, the file also contains all the raw and base-called data, including the called sequence.
- **FASTA—**Contains the file name, file location, and the called sequence for each well in the base-called plate (default). See section 5.5.2.
- **SCF—**Contains the raw data and the electropherograms. If base-called, includes the raw and base-called data, and the called sequence. See section 5.5.3.
- **ASCII text—**Contains the plate name, sample name, instrument ID, base caller name, electrical current information, scan rate, instrument parameters. If base-called, includes the sequence starting and ending points and the number of bases called.

You can use the fields in the Sequencing section of the MegaBACE.ini file (table 5-1) to select the file formats you want the Instrument Control Manager to create. You also use the fields in the MegaBACE.ini file to specify whether the operator can override the MegaBACE.ini file export selections that appear in the Automatic Base Calling window (figure 5-2). In the MegaBACE.ini file, if you specify—

- **Y** for any of the export file formats, the corresponding check boxes for the specified file formats are selected in the Automatic Base Calling window.
- **N** for any of the export file formats, the corresponding check boxes for the specified file formats are not selected in the Automatic Base Calling window.
- **Y** to ALLOW [FILE_FORMAT] OVERRIDE, an operator can select or deselect the corresponding export options in the Automatic Base Calling window.

**Note:** Automatic base calling must be turned on to export the selected file format(s).



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### 5.5.2 The FASTA file format

The FASTA file creation has three different options, and you can specify one, two, or all three options. If you want to use the FASTA export feature, you must select at least one of the following options:

- **-fa** for creation of one .seq file for each analyzed .esd file. These files are stored in the …\MegaBACE\AnalyzedData\plateID_runID folder.
- **-fap** for creation of one .seq file for each base-called plate. This file is stored in the …\MegaBACE\AnalyzedData\plateID_runID folder. This is the default switch used for FASTA in the Automatic Base Call Command in the MegaBACE.ini file.
- **-fag** for global creation of one .seq file for all the analyzed plates. This file is stored in the …\MegaBACE\AnalyzedData\plateID_runID folder.

To specify the FASTA file format, you use the AUTOMATIC BASE CALL COMMAND field in the MegaBACE.ini file. For example, to specify the -fap format, you would use the following:

| Field in MegaBACE.ini file | Value |
|---|---|
| AUTOMATIC BASE CALL COMMAND | AutoBasecall.exe -fap |

### 5.5.3 Changing the SCF version

For the SCF format, you can choose whether to export in SCF 2.00 or SCF 3.00 (default) file format. To change the SCF version, you use the Basecall.ini file (section 5.6).

### 5.5.4 Using subfolders for the exported files

You can use the EXPORT TO SUBFOLDERS field in the MegaBACE.ini file (table 5-1) to specify whether to export the selected file formats to subfolders. If you specify—

- **Y**—The software creates subfolders, within the raw or analyzed run folder, respectively, for the specified export files. Each subfolder has the name of the export file format (ABD, SCF, FASTA, ASCII text). See table 15-3 for a detailed description of each file format.
- **N**—The software exports the files in the selected formats to the top-level raw or analyzed run folder, respectively.



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### 5.5.5 Using the file export options

If you turn on the automatic base calling feature, the Instrument Control Manager provides the following export options:

- **EXPORT IF BASE CALLING FAILED—**Default is **N**.
- **EXPORT GOOD REGION ONLY—**Default is **N**.
- **ADD EXTENSIONS TO EXPORTED FILE NAMES—**Default is **Y**.

You turn on or off the options in the MegaBACE.ini file by typing **Y** or **N**.

## 5.6 Settings in the Basecall.ini file

Figure 5-3 shows the Basecall.ini file. The Basecall.ini file is in a two-column, tab-separated format. Table 5-3 provides a description of each section and field in the Basecall.ini file.

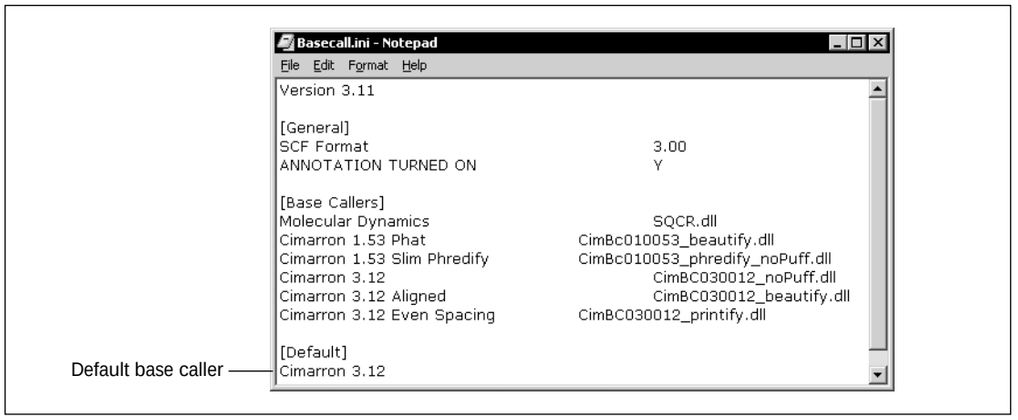



**Figure 5-3.** The Basecall.ini file.



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**Table 5-3.** The sections and fields in the Basecall.ini file

| Section and field name | Description | Setting type | Default |
|---|---|---|---|
| [General] | | | |
| SCF FORMAT | Determines the SCF file format version. | 2.00 or 3.00 | 3.00 |
| ANNOTATION TURNED ON | Determines whether the base caller produces annotation information, which is needed by the Sequencing Diagnostics software.<br>**Note:** The base calling is slower if the setting is Y instead of N. | Y or N | Y |
| [Base Callers] | Lists the available base callers. The first column lists the base caller names, and the second column lists the corresponding file names of the base callers. | – | – |
| [Default] | Specifies the default base caller for the laboratory. | String* | Name of the latest base caller |

* The text must match the name of one of the available base callers in the Base Callers section of the file.

## 5.7 Specifying which base caller to use

There are a variety of ways in which you can specify the base caller(s) to use. You can specify a—

- Default base caller for all plates
- Base caller(s) as part of the plate definition

### 5.7.1 Specifying a default base caller

If the plate definition for a given run does not specify a base caller, the software uses the default base caller. The software allows you to specify a default base caller in the Basecall.ini file (figure 5-3) and in the Automatic Base Calling window.

#### The Automatic Base Calling window

To open the Automatic Base Calling window (figure 5-2), on the **Configure** menu, click **Automatic Base Calling.** See the *MegaBACE 4000 Instrument User's Guide* for details on the Automatic Base Calling window.

The default base caller specified in the Automatic Base Calling window overwrites the default base caller specified in the Basecall.ini file. When you restart the software, the default resets to the base caller specified in the Basecall.ini file.



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### Specifying the default base caller in the Basecall.ini file

The Base Callers section of the Basecall.ini file lists the names of the available base callers. The base callers are provided during installation. The Default section lists the name of the default base caller. To specify a default base caller, you type the base caller name in column one of the Default section (figure 5-3). The software recognizes only the base caller names listed in column one of the Base Callers section.

## 5.7.2 About specifying a base caller(s) in the plate definition

The Plate Setup window, a plate setup template (.tpl), and/or a plate setup data file (.psd) can be used in creating a plate definition. Each of these methods allows you to specify a base caller or base callers. If you want to specify—

- **Base caller(s) for multiple plates—**Use a plate setup template (.tpl) (chapter 6) and the Plate Setup window to create plate definitions.
- **Plate-specific and well-specific base callers—**Use a .psd file to create the plate definition. A .psd file allows you to define both a plate-level base caller(s) and well-level base callers for given wells on the plate (section 7.3).

If you use a combination of methods when creating plate definitions (for example, .tpl and .psd files) and the plate-level base caller is defined in more than one source, the software uses the plate-level base caller specified last.



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# Chapter 6 Creating templates for sequencing plate setup parameters

For each sequencing run, the Instrument Control Manager requires a plate definition and the instrument control parameters. This chapter describes plate definitions and how to create the plate setup templates for sequencing. The topics are—

- About creating the plate definitions (section 6.1)
- About plate setup templates for sequencing (section 6.2)
- Using the Plate Setup window to create a plate setup template (section 6.3)
- Creating a plate setup template in a text file (section 6.4)
- Using a template to suppress .rsd file creation for empty capillaries (section 6.5)
- Setting and clearing the default plate setup template (section 6.6)

For a description of the instrument control templates, see chapter 4.

## 6.1 About creating the plate definitions

A plate definition consists of the—

- **Plate ID—**The software uses the plate ID to name the run folder.
- **Plate setup parameters—**The electrophoresis parameters, chemistry parameters, sample names, comments and optional parameters for the plate.

### 6.1.1 Methods for creating the plate definitions

The Instrument Control Manager allows you to create the plate 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 .psd file can specify the plate ID and the plate setup parameters. In addition, the .psd file can include the well-specific attributes. For a description of the .psd files for sequencing, see chapter 7.
- The Plate Setup window (figure 6-1) to create the definitions before the run. The operator can select the precreated plate definitions at the start of the Inject Samples and Run protocol.



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### 6.1.2 The methods for creating plate definitions in the Plate Setup window

You or an operator can create the plate definition before the run using the Plate Setup window (figure 6-1). To do this, you or the operator can—

- **Import a .psd file—**You can use a .psd file to enter the plate definition. When the .psd file name is entered in the Plate ID box of the Plate Setup window, the software imports the information from the .psd file and automatically creates the plate definition.
- **Select a plate setup template (.tpl)—**You can use a template to enter the plate setup parameters that are visible in the Plate Setup window for multiple plates (section 6.2). In the MegaBACE.ini file, you can specify a default template that appears automatically when an operator creates a new plate definition in the Plate Setup window. The software includes templates for various sequencing chemistries.
- **Manually enter or modify the setup parameters—**To manually enter or change parameters in the Plate Setup window, you must enable the edit mode in the MegaBACE.ini file. See section 2.3 for a description of the edit mode.

See the *MegaBACE 4000 Instrument User's Guide* for a description of how to create plate definitions in the Plate Setup window.



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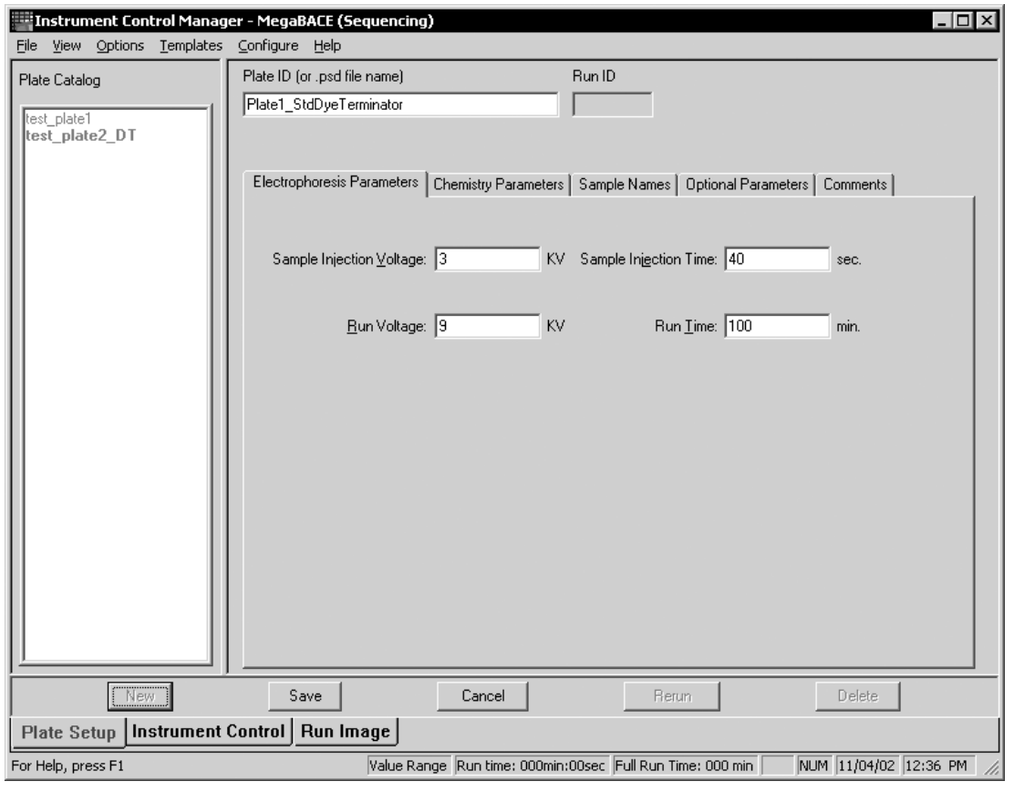



**Figure 6-1.** The Plate Setup window for sequencing.

## 6.2 About plate setup templates for sequencing

**Important**

**The edit mode must be turned on to allow you to create, edit, select, or save plate setup templates (.tpl) in the Instrument Control Manager. See section 2.3 for information about enabling the edit mode.**

You can simplify the process of creating plate definitions by using plate setup templates (.tpl files). In a plate setup template, you specify parameters that are common to multiple plates. You can create templates in the Plate Setup window (section 6.3), or you can create a .tpl file using any text editor or Excel.

See table 6-1 for a description of the plate setup parameters for sequencing.



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**Table 6-1.** The recognized fields for sequencing plate setup templates

| Field name | Value |
|---|---|
| **The values for the following fields can be imported into the Plate Setup window or typed manually. When importing the values, the fields can be defined in either a .psd file or a plate setup template (.tpl).** | |
| **Note:** If both a .psd file and a plate setup template are used to define the same parameter, the values provided in the file imported last overwrite the values for any identical fields. | |
| **Electrophoresis Parameters tab** | |
| Injection voltage | Integer or fraction (kV). The range is 1–15 kV. |
| Injection time | Integer (s). The range is 1–600 s. |
| Run voltage | Integer or fraction (kV). The range is 1–15 kV. |
| Run time | Integer (min). The range is 1–720 min. |
| **Chemistry Parameters tab** | |
| Chemistry | String. The name of a chemistry parameter set.<br>**Note:** The name must match a name in the Chemistry.ini file (chapter 3) for the sequencing application. Otherwise, the software will not recognize the name. |
| **Sample Names tab** | |
| Well_ID<br>(for example, A01)<br>or Well_ID.NAME<br>(.psd file only) | String. The sample name that you want to assign to each well location (ID) and use as the .rsd file name (default), for example, Sample1.<br>**Note:** If you want to use names other than sample names as the file names, you must also use the Well_ID.FILE field in the .psd file. |
| **Optional Parameters tab** | |
| **Note:** The Run temperature and PMT voltages are typically defined as part of the instrument control parameters (chapter 4). The values can be defined here for a special run. If defined here, the values temporarily overwrite the same values defined in an .icp file or the Instrument Control window. | |
| Run temperature | Integer (°C). The range is 27–44 °C. |
| PMT1 voltage | Integer or fraction (kV). The range is 500–900 V. |
| PMT2 voltage | Integer or fraction (kV). The range is 500–900 V. |
| PMT3 voltage | Integer or fraction (kV). The range is 500–900 V. |
| PMT4 voltage | Integer or fraction (kV). The range is 500–900 V. |



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**Table 6-1.** The recognized fields for sequencing plate setup templates (continued)

| Field name | Value |
|---|---|
| Base caller | String. The optional name of a plate-specific base caller that overwrites the default base caller specified in the Basecall.ini file or the Automatic Base Calling window.<br>**Note:** You can designate multiple base callers in the .psd file or .tpl file for specified plates. You must separate each base caller name with a comma in the same value column for the BASE CALLER field. |
| **Comments tab** | |
| Comments | String, including spaces, representing the comments about the plate that you want to include. |

## 6.3 Using the Plate Setup window to create a plate setup template

You can use the Plate Setup window (figure 6-1) to create a plate setup template. To do this—

1. In the Plate Setup window, click **New.**
2. In the following tabs, enter the parameters that you want to use for the template. See table 6-1 for a description of the parameters.
   - **Electrophoresis Parameters tab—**Type the values for the parameters in each of the boxes.
   - **Chemistry Parameters tab—**Select the name of the chemistry parameters from the **Chemistry Name** list.
   - **Sample Names tab—**By default, the software uses the well IDs to name the files (for example, A01.rsd). If you want to use sample names other than the well IDs, you can type the names in the Sample Names tab. You can also specify any empty wells for which you want to suppress the raw sample data file (.rsd) creation, see section 6.5.
   - **Optional Parameters tab—**(Optional) If you want to overwrite the Run Temperature and PMT Voltage values, which are provided in the instrument control parameters, you can type the values in this tab. You can also specify a base caller for the plate in this tab.
   - **Comments tab—**You can type any comments about the plate.

   See the *MegaBACE 4000 Instrument User's Guide* for details on using the Plate Setup window.



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3. (Optional) If you want to suppress the raw sample data file (.rsd) creation for empty capillaries, see section 6.5.
4. On the **Templates** menu, point to **Plate Setup Templates,** and click **Save Template**. The Save Template window appears.
5. Type the template name, and click **Save**. The template is saved in the …\MegaBACE\Instrument Control\Templates folder. The software adds the .tpl file extension.

## 6.4 Creating a plate setup template in a text file

**Important** **When you create a plate setup template, you must type the .tpl file extension and save it in the …\MegaBACE\Instrument Control\Templates folder. Otherwise, the Instrument Control Manager will not find the file.**

To create a plate setup template—

1. Open a new document or an existing .tpl file using a text editor (such as Notepad) or Excel.
2. Type the field names in the first column (field column), and type the values for each field in the second column (value column). Make sure the field column and value column are separated by a tab. See table 6-1 for a list of the recognized fields for the plate setup parameters. Figure 6-2 shows an example of a plate setup template file (.tpl).
3. Save the file in an appropriate format. You must type the .tpl file extension.

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

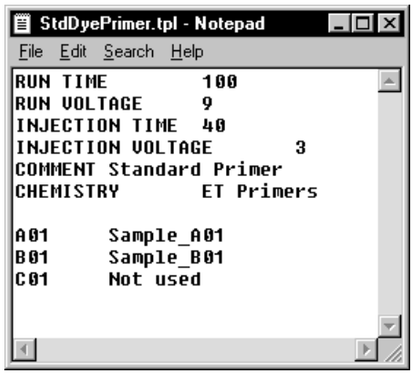



**Figure 6-2.** An example of a plate setup template file (.tpl) for sequencing.



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## 6.5 Using a template to suppress .rsd file creation for empty capillaries

To specify an empty capillary, you designate the corresponding well as unused. The software does not create a raw sample data file (.rsd) for any wells that are specified as not used. In the Run Image window (figure 2-2), an unused well is indicated by a yellow arrow that appears above the capillary button.
**Note:** To specify bad capillaries, see section 2.6.

You can specify unused wells in either a—

- **Plate setup data file (.psd)—**You can use a .psd file to specify the unused wells if the unused wells occur for a single plate (section 7.6).
- **Plate setup template—**You can use a plate setup template if the unused wells occur for multiple plates.

You can specify unused wells in a plate setup template by typing **NOT USED** (case insensitive) as the sample name specified in either the Sample Names tab of the Plate Setup window or the .tpl file (section 6.4). To specify an unused well in the Sample Names tab of the Plate Setup window—

1. In the **Sample Names** tab of the Plate Setup window (figure 6-3), adjust the width of the column that includes the unused well. To increase the column width, click the **RIGHT ARROW** key in the Sample Names tab. To decrease the column width, click the **LEFT ARROW** key in the Sample Names tab.
2. Type **NOT USED** (case insensitive) as the sample name for the unused well.



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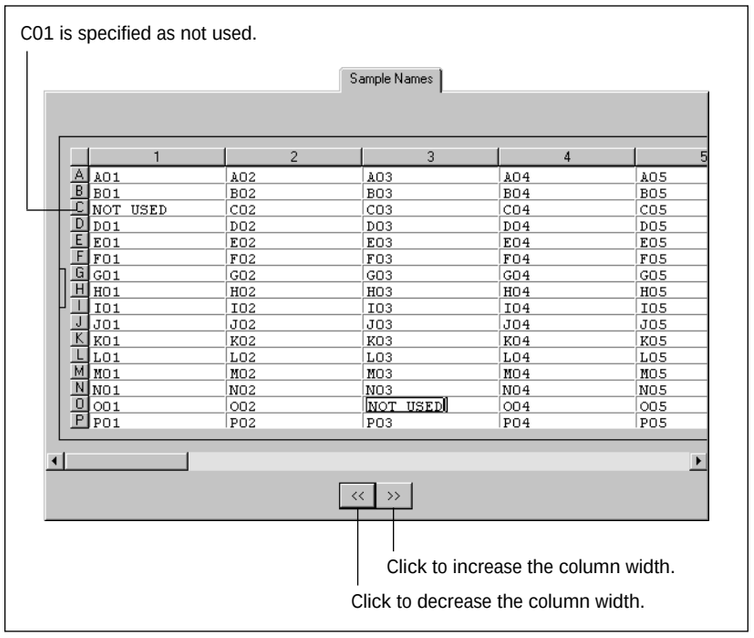



**Figure 6-3.** The Sample Names tab of the Plate Setup window.

## 6.6 Setting and clearing the default plate setup template

You can set a default plate setup template in the—

- **Sequencing section of the MegaBACE.ini file—**To set a default plate setup template for sequencing in the MegaBACE.ini file, see section 5.2.1.
- **Templates menu in the Plate Setup window—**The default template is active only until you close the Instrument Control Manager. When the software is restarted, the default plate setup template specified in the Sequencing section of the MegaBACE.ini file is active until you change it again.



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### 6.6.1 Using the Templates menu to set a default template

To set the default plate setup template—

1. In the Instrument Control Manager, click the **Plate Setup** tab to display the Plate Setup window (figure 6-1).
2. On the **Templates** menu, point to **Plate Setup Template,** and click **Set Default**. The Set Default window appears.
3. Select the name of the template you want to use as the default, and click **Open.** The Instrument Control Manager designates the template you selected as the default template.

The next time you click New in the Plate Setup window, the values from the default template appear in the parameter boxes.

### 6.6.2 Clearing the default plate setup template

To clear the default plate setup template—

1. In the Instrument Control Manager, click the **Plate Setup** tab to display the Plate Setup window (figure 6-1).
2. On the **Templates** menu, point to **Plate Setup Templates,** and click **Clear Default.** The template you cleared is no longer the default template.

When you click New in the Plate Setup window, no values appear in the parameter boxes. You can select another default template (section 6.6.1), or use the Plate Setup window to create another template, save it, and then select it as the default template.

**Important** **When you restart the software, the default templates specified in the MegaBACE.ini file are active.**



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# Chapter 7 Creating plate setup data files (.psd) for sequencing

This chapter describes the plate setup data files (.psd) for sequencing. The topics are—

- Using plate setup data files (.psd) for sequencing (section 7.1)
- Creating a plate setup data file (.psd) (section 7.2)
- Specifying a base caller in a .psd file (section 7.3)
- Annotating well-specific information in a .psd file (section 7.4)
- Designating the sample names and file names in the .psd file (section 7.5)
- Using a .psd file to suppress .rsd file creation for empty capillaries (section 7.6)

## 7.1 Using plate setup data files (.psd) for sequencing

You can define a plate ID, the plate setup parameters, and other plate- and well-level attributes in a .psd file so that an operator can automatically import the plate definition. With automatic import, the plate setup parameters are specified with less operator intervention.

The operator can import the .psd file either before the run in the Plate Setup window or during the run in the Select a Plate window (*MegaBACE 4000 Instrument User's Guide*). The parameters that you specify in the .psd file become part of the extended header of each raw sample data (.rsd) file created during the run.

### 7.1.1 About the plate ID

When an operator enters the text or bar code in the plate ID area of the Plate Setup window or the Select a Plate window (described in the *MegaBACE 4000 Instrument User's Guide*), the Instrument Control Manager looks for a .psd file with a file name that matches the text entered and automatically imports the plate setup parameters that are contained in the .psd file.



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An operator can enter a plate ID in the following ways:

- **Type the text** in the Plate ID box in the Plate Setup window or the Select a Plate window.
- **Scan a bar code** into the Plate ID box in the Plate Setup window or the Select a Plate window.

**Important** **Regardless of the method used to enter the plate ID, the text entry or bar code (less a possible plate ID terminator) must match the name of the .psd file in order for a .psd file to be imported.**

Figure 7-1 shows an example of a .psd file and the Plate Setup window. The .psd file name matches the plate ID typed in the Plate Setup window.

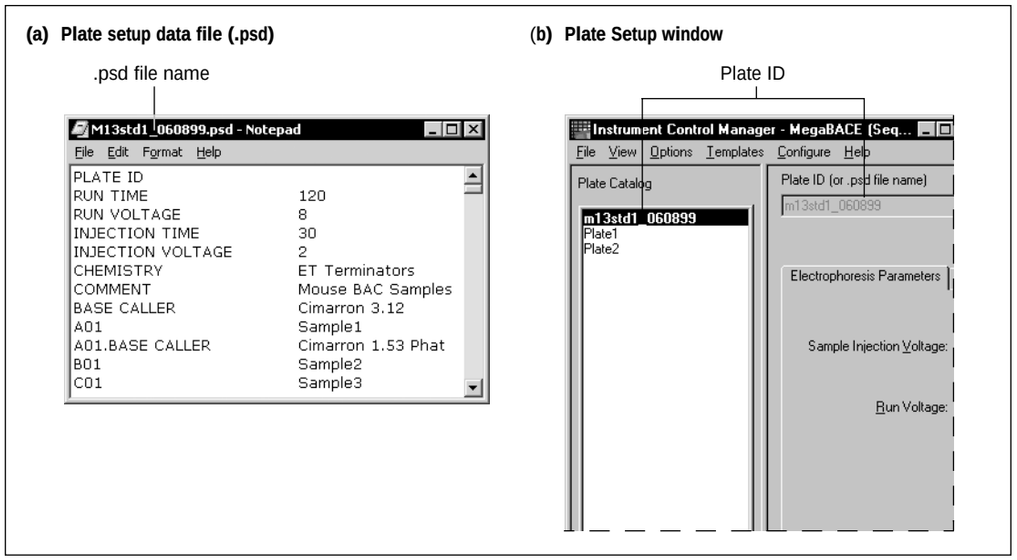



**Figure 7-1.** Using a .psd file: (a) Example of a .psd file. (b) The Plate Setup window with a plate ID that matches the .psd file name.



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## 7.1.2 Using a plate ID that is different from the scanned ID

If the bar code is not what you want to use as the plate ID, you can use the .psd file to replace a bar code scanned into the Plate ID box in the Plate Setup window. To do this, you type a plate ID in the .psd file opposite the PLATE ID field (figure 7-2a).

The plate ID you type opposite the PLATE ID field in the .psd file replaces the bar code and becomes the real plate ID in the Plate Setup window (figure 7-2b).

For example, in figure 7-2, the—

- Plate ID scanned into the Plate Setup window is **10303385858**
- Plate setup data file name is **10303385858.psd**
- Plate ID opposite the PLATE ID field name inside the .psd file is **M13std40_092499** (figure 7-2a)
- Actual plate ID that appears in the Plate Setup window is **M13std40_092499** (figure 7-2b)

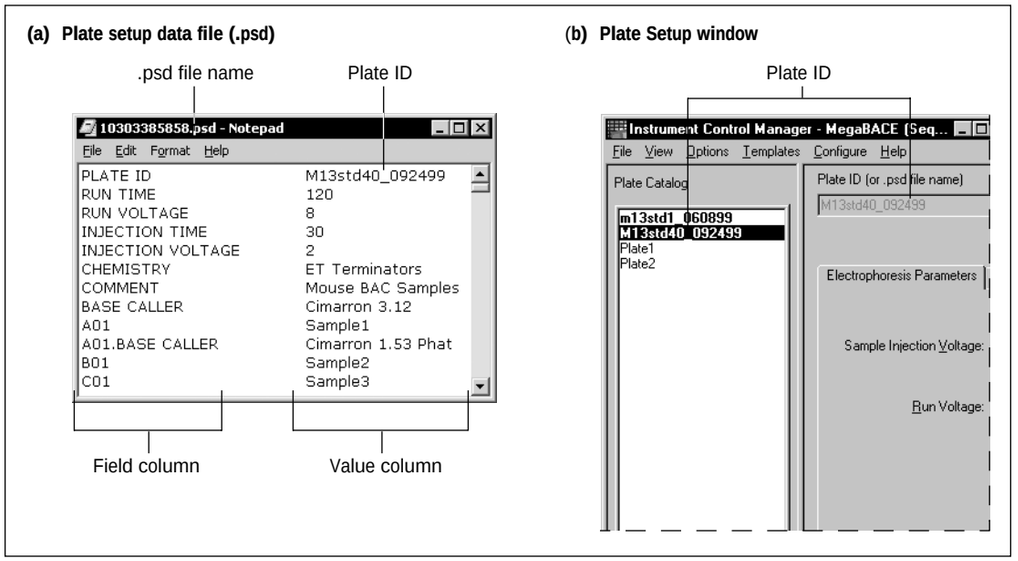



**Figure 7-2.** Replacing the scanned plate ID: (a) Example of a .psd file with a plate ID listed in the file. (b) The Plate Setup window with a plate ID that replaced the original bar code that was scanned.



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## 7.2 Creating a plate setup data file (.psd)

**Important**

**When you create a .psd file, you must type the .psd file extension and save it in the …\MegaBACE\Instrument Control\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.**

**Regardless of the method the operator uses to enter the plate ID in the Plate Setup window or Select a Plate window, the text entry or bar code (less a possible plate ID terminator) must match the name of the .psd file in order for a .psd file to be imported.**

To create a .psd file—

1. Open a new document or an existing .psd file using a text editor (such as Notepad) or Excel.
2. Type the field names in the first column (field column) and the values for each field name in the second column (value column). The order of the fields is arbitrary, and the field names are case-insensitive. Figure 7-3 shows an example of a .psd file created in Excel. Section 7.2.1 describes the fields that you can use in a .psd file.

   **Note:** If you use spaces in the sample or file names, the Instrument Control Manager converts the spaces to underscore characters in the file name.
3. Save the file in an appropriate format. Make sure you use the .psd file extension.

   **Note:** If you use Excel, save the file as a tab-delimited file (figure 7-3).



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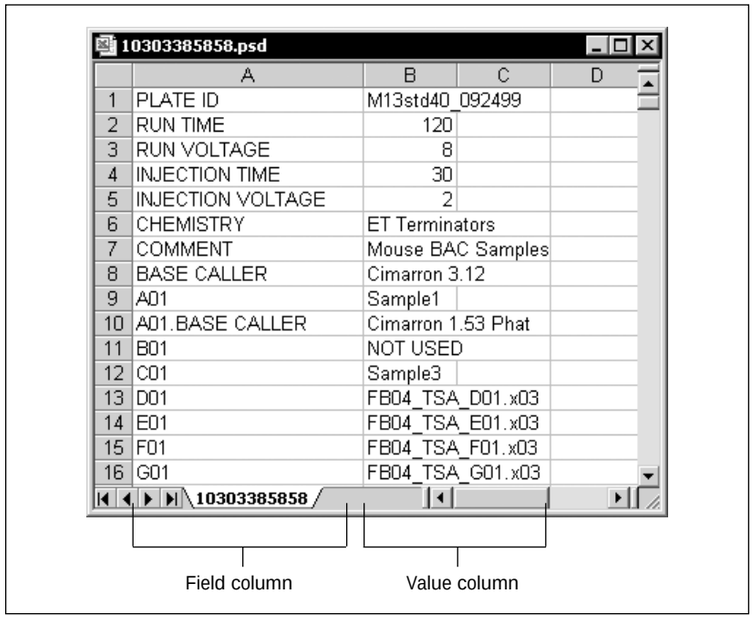



**Figure 7-3.** An example of a .psd file created in Excel.

## 7.2.1 The .psd field names for sequencing

The .psd file can include the following fields:

- All the fields that appear in the tabs of the Plate Setup window or in a plate setup template (.tpl). Table 6-1 lists the available fields.

  **Note:** If both a .psd file and a plate setup template (.tpl) are used to define the same plate setup parameters, the values provided in the file imported last overwrite the values for any identical fields.

- Additional plate-specific fields and well-specific fields listed in table 7-1.



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**Table 7-1.** Additional fields available for the sequencing .psd files

| Field name | Value |
|---|---|
| Plate ID | String. If the plate ID entered here is different from the bar code of the .psd file name, the plate ID entered here becomes the real plate ID that appears in the Plate Setup window. |
| Well_ID.FILE (for example, A01.FILE) | String. The .rsd file name that you want to assign to a given well location. For example, A01 if you want to use the well location as the file name. This parameter overwrites the sample name as the file name. |
| Well_ID.BASE CALLER (for example, A01.BASE CALLER) | String. The name(s) of the base caller(s) that you want to use for a well that differ from the plate-level base caller(s). To specify multiple base callers, you separate each name with a comma. |
| User-defined fields | Fields that can be imported, but are not used by the software. These data are stored in the extended header of the files. |

Tables 6-1 and 7-1 list the field names that the Instrument Control Manager recognizes, except for the user-defined fields. For additional plate information you want included in the output file (that will not appear in the Plate Setup window), you can use field names the Instrument Control Manager does not recognize (for example, Operator). You must also provide a name in the value column (for example, Mary Smith).

### 7.2.2 The .psd file value constraints

If you type a value that is invalid (for example, an alpha character when the software requires a number, or a value that is out of range), the Instrument Control Manager displays an error message when you import the .psd file. If this occurs, you must correct the value in the .psd file before it can be imported successfully. Alternatively, if the edit mode is on, you can enter the correct value in the Plate Setup window.



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## 7.3 Specifying a base caller in a .psd file

You can specify a base caller or multiple base callers for a plate and for wells on a plate in the .psd file. The base caller(s) specified in the .psd file overwrite the base caller(s) specified in the default plate setup template (.tpl) (section 6.4) and the default base caller specified in the Basecall.ini file (section 5.6).

### 7.3.1 Designating a base caller for the plate

To designate a plate-specific base caller in a .psd file, you use the following field:

| Field name | Value |
|---|---|
| BASE CALLER | The base caller name(s) |

To designate a plate-level base caller, you can use the BASE CALLER field in either the .psd file or the .tpl file. To designate multiple plate-level base callers, separate each base caller name with a comma in the value column of the BASE CALLER field.

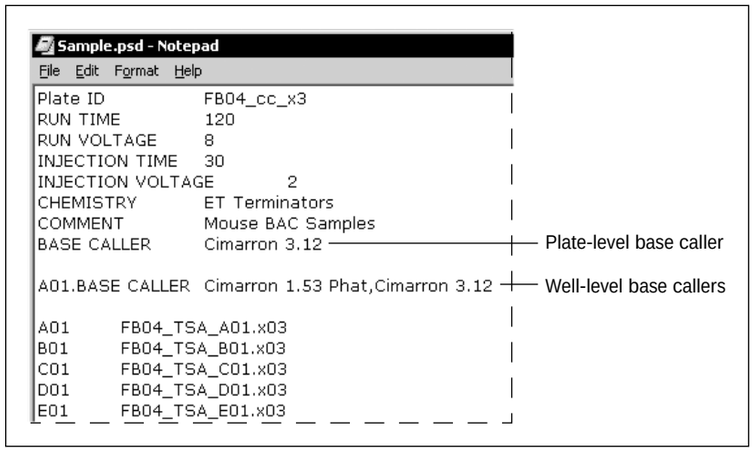



**Figure 7-4.** Example of a .psd file with entries to designate plate- and well-specific base callers.



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### 7.3.2 Specifying a base caller for a selected well on a plate

To specify one or more well-level base callers that differ from the plate-level base caller, you use the following field in the .psd file. If you specify multiple base callers, separate each base caller name with a comma (figure 7-4).

| Field name | Value |
|---|---|
| Well_ID.BASE CALLER | The base caller name(s) |

## 7.4 Annotating well-specific information in a .psd file

The values for the field names that begin with a well ID (for example, A01) are well specific. For example, you can specify the following types of information for a given well:

- Sample name, which becomes the .rsd file name by default
- Base callers (section 7.3)
- Explicit file name, if you need the .rsd file name to be different from the sample name (section 7.5)
- User-defined information

If you want additional well-specific information for your records, the Instrument Control Manager allows you to include a dot (.) character after the well ID followed by an additional name to annotate the well ID. The following and figure 7-2 show examples of what you might enter in the .psd file for annotation of well-specific information:

| Field name | Value |
|---|---|
| A01.BASE CALLER | Cimarron 1.53 Phat,Cimarron 3.12 |
| A01.SAMPLE TYPE | Tissue |

**Note:** To designate multiple well-specific base callers, separate each base caller name with a comma in the .psd file.

The Instrument Control Manager writes the additional well-specific information only into the .rsd file header for the corresponding well. The well-specific annotation does not appear in the Plate Setup window.



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## 7.5 Designating the sample names and file names in the .psd file

If no file names are specified, but the sample names are provided for a plate, the software uses the sample names as the file names, for example, sample1.rsd. If no sample or file names are provided for a plate, the Instrument Control Manager uses the well location (well IDs) as the sample file names, for example, A01.rsd.

You can provide sample names and designate explicit file names that are different from the sample names. To do this, you use two fields—Well_ID.FILE and Well_ID or Well_ID.NAME. The following and figure 7-5 show examples of what you might enter in the .psd file to designate a file name that is different from the sample name.

| Field name | Value | Comment |
|---|---|---|
| A01.NAME | 14660 | Sample name |
| A01.FILE | A01 | File name |

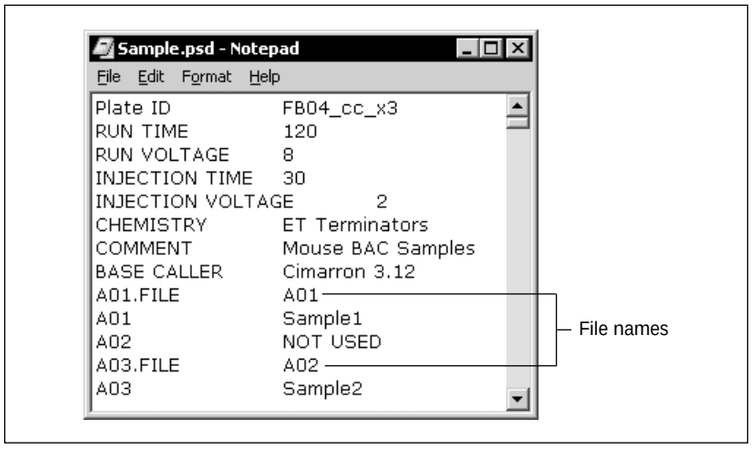



**Figure 7-5.** Example of a .psd file with entries to designate the well ID as the file names.



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## 7.6 Using a .psd file to suppress .rsd file creation for empty capillaries

You can indicate unused wells by typing **NOT USED** (case-insensitive) as the sample name specified in the .psd file (figure 7-5). For every unused well, a yellow arrow appears above the capillary button in the Run Image window (figure 2-2). The software does not create an .rsd file for an unused well. For details on suppressing .rsd files for defective capillaries, see section 2.6.



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# Part three

# Setting up the system for STR genotyping

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# Chapter 8 Specifying preferences for STR genotyping

This chapter describes the fields that have been added to the system initialization files to support STR genotyping. The topics are—

- The STR genotyping fields in the MegaBACE.ini file (section 8.1)
- Setting STR genotyping as the default application (section 8.2)
- Setting default parameter templates for STR genotyping (section 8.3)

Make sure you configure the General section of the MegaBACE.ini file (chapter 2) for your laboratory. To create additional chemistry parameters, see chapter 3.

## 8.1 The STR 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: General, Sequencing, Genotyping (STR genotyping), and SNP (SNP genotyping). Figure 8-1 shows the MegaBACE.ini file with the Genotyping fields highlighted.

Table 8-1 describes the fields in the Genotyping section of the MegaBACE.ini file. See chapter 2 for a description of the other fields in the MegaBACE.ini file.



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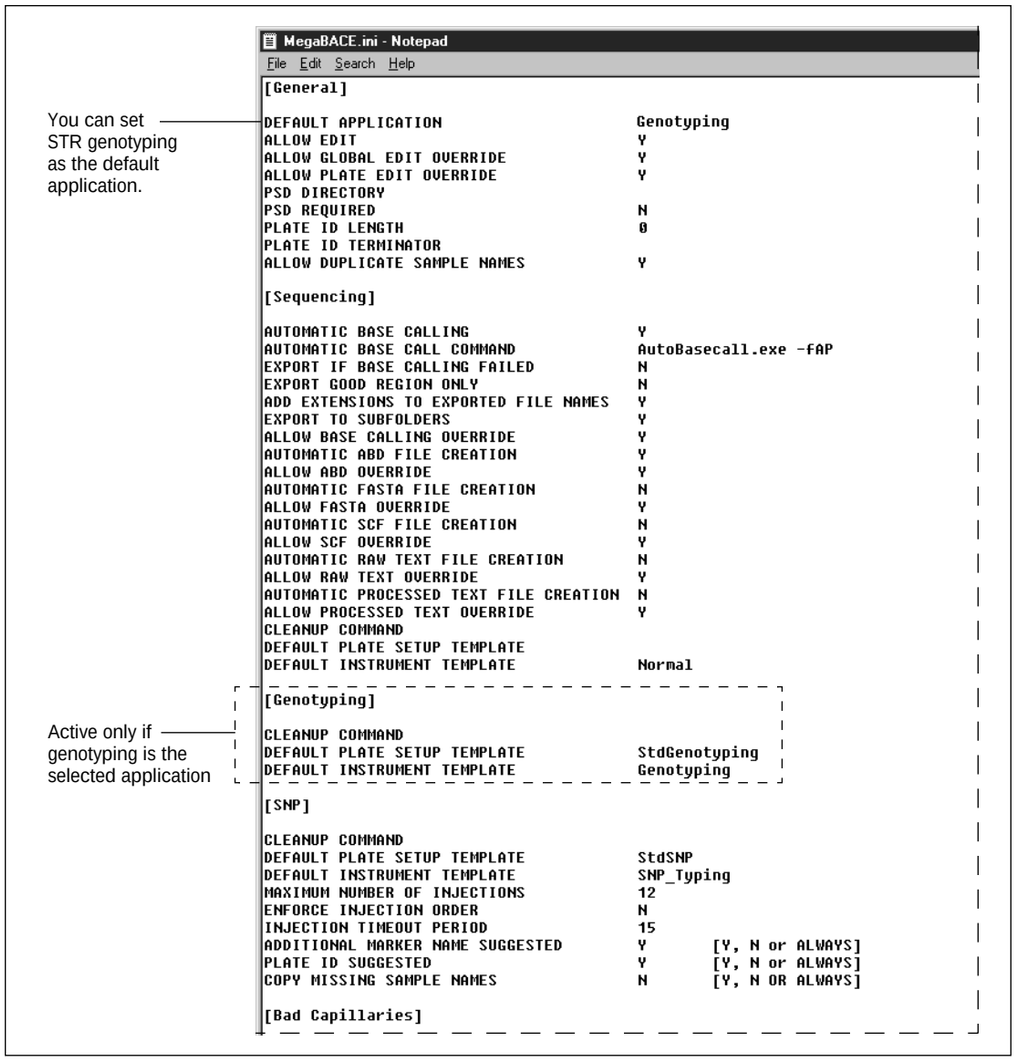



**Figure 8-1.** The STR genotyping fields in the MegaBACE.ini file. Note that the file contains a section for each application, which allows you to specify a default plate setup and instrument template.



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**Table 8-1.** The STR genotyping fields in the MegaBACE.ini file

| Field name | Description | Setting type | Default |
|---|---|---|---|
| Cleanup command | Allows the administrator to specify a program or batch file to be executed at the end of the run. This can be useful for transferring the raw data files to another workstation for analysis or archiving. See appendix C for a more detailed description. | String consisting of the path to the program or batch file (full or relative path to the instrument control folder) | none |
| Default plate setup template | Specifies a default plate setup template. | String | StdGenotyping |
| Default instrument template | Specifies a default instrument control template. | String | Genotyping |

## 8.2 Setting STR genotyping as the default application

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

To set STR 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 | Genotyping |

## 8.3 Setting default parameter templates for STR genotyping

The Instrument Control Manager allows you to use templates to define the plate setup parameters and the instrument control parameters for multiple runs. You can specify default templates in the Genotyping section of the MegaBACE.ini file. The software includes the following templates for the STR genotyping application, which are specified as the default templates:

- **Plate setup template—**StdGenotyping.tpl
- **Instrument control template—**Genotyping.icp



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If you enable the edit mode in the MegaBACE.ini file (section 2.3), the operator can modify the values for a given run or choose a different template in the Instrument Control Manager. However, when the Instrument Control Manager is restarted, the software automatically resets the selected templates to the defaults specified in the MegaBACE.ini file.

You can use the templates provided, or modify the existing templates to create new templates. To create a new plate setup template for STR genotyping, see chapter 9. To create a new instrument control template, see chapter 4. To set new STR genotyping defaults in the MegaBACE.ini file, see the following sections.

### 8.3.1 Setting the default plate setup template for STR genotyping

If you specify a default template in the MegaBACE.ini file, the values from the plate setup template appear automatically in the Plate Setup window. As figure 8-1 and table 8-1 show, the software has been configured to use the StdGenotyping.tpl as the default template for STR genotyping. If you need to specify a different default template for the STR genotyping application, you can use the following field in the Genotyping 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 setup template from the Templates menu in the Plate Setup window.

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

### 8.3.2 Setting the default instrument control template for STR genotyping

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 8-1 and table 8-1 show, the software has been configured to use the Genotyping.icp template as the default for STR genotyping. If you need to specify a different default template for the STR genotyping application, you can use the following field in the Genotyping section of the MegaBACE.ini file:



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| 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.



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# Chapter 9 Creating templates for STR genotyping plate setup parameters

For each STR genotyping run, the Instrument Control Manager requires a plate definition and the instrument control parameters. This chapter describes plate definitions and how to create the plate setup templates for STR genotyping. The topics are—

- About creating the plate definitions (section 9.1)
- About plate setup templates for STR genotyping (section 9.2)
- Using the Plate Setup window to create a template (section 9.3)
- Creating a plate setup template in a text file (section 9.4)
- Using a template to suppress .rsd file creation for empty capillaries (section 9.5)
- Setting and clearing the default plate setup template (section 9.6)

For a description of the instrument control templates, see chapter 4.

## 9.1 About creating the plate definitions

A plate definition consists of the—

- **Plate set ID—**The software uses the plate ID to name the run folder.
- **Plate setup parameters—**The electrophoresis parameters, chemistry parameters, sample names, comments and optional parameters for the plate.

### 9.1.1 Methods for creating the plate definitions

The Instrument Control Manager allows you to create the plate 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 .psd file can specify the plate ID and the plate setup parameters. In addition, the .psd file can include the well-specific attributes. For a description of the .psd files for STR genotyping, see chapter 10.
- The Plate Setup window (figure 9-1) to create the definitions before the run. The operator can select the precreated plate definitions at the start of the Inject Samples and Run protocol.



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### 9.1.2 The methods for creating plate definitions in the Plate Setup window

You or an operator can create the plate definition before the run using the Plate Setup window (figure 9-1). To do this, you or the operator can—

- **Import a .psd file—**You can use a .psd file to enter the plate definition specific to a single plate. The .psd file can specify plate attributes in addition to the parameters that are visible in the Plate Setup window. When the .psd file name is entered in the Plate ID box of the Plate Setup window, the software imports the information from the .psd file and automatically creates the plate definition.
- **Select a plate setup template (.tpl)—**You can use a template to enter the plate setup parameters for multiple plates (section 9.2). The template can specify only the parameters that are visible in the Plate Setup window. In the MegaBACE.ini file, you can specify a default template that appears automatically when an operator creates a new plate definition in the Plate Setup window. The software includes a default template for the STR genotyping application (StdGenotyping.tpl).
- **Manually enter or modify the setup parameters—**To manually enter or change parameters in the Plate Setup window, you must enable the edit mode in the MegaBACE.ini file. See section 2.3 for a description of the edit mode.

See the *MegaBACE 4000 Instrument User's Guide* for a description of how to create plate definitions in the Plate Setup window.



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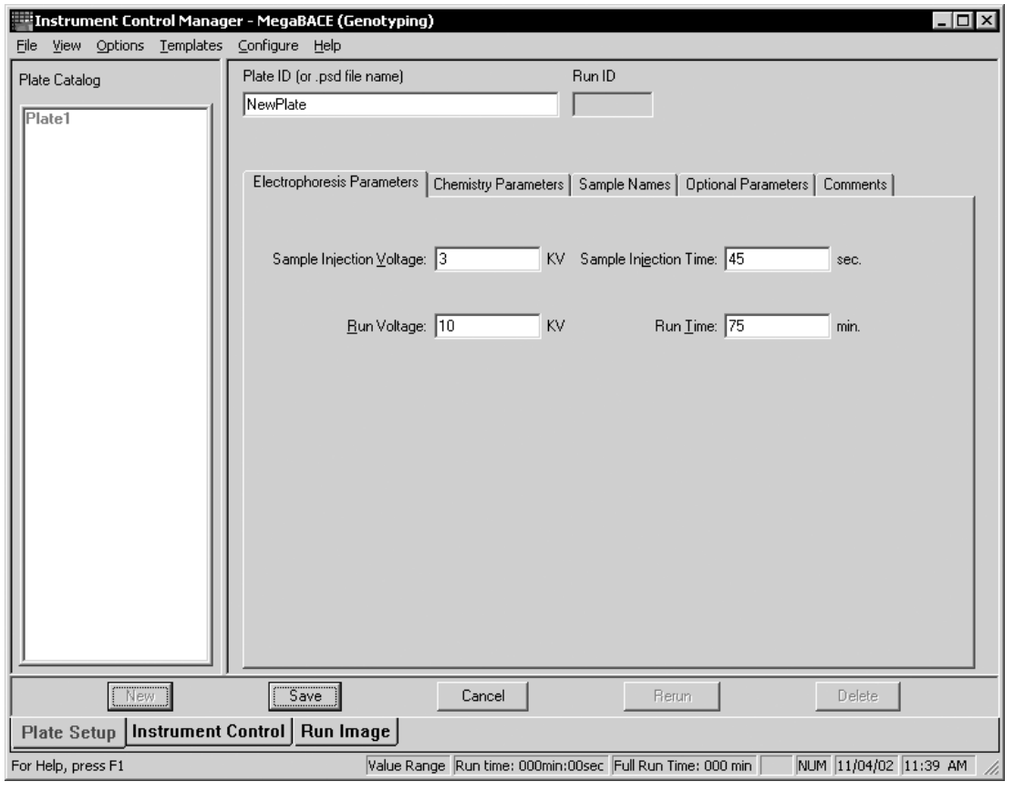



**Figure 9-1.** The Plate Setup window for the STR genotyping application.

## 9.2 About plate setup templates for STR genotyping

**Important**

**The edit mode must be turned on to allow you to create, edit, select, or save plate setup templates (.tpl) in the Instrument Control Manager. See section 2.3 for information about enabling the edit mode.**

You can simplify the process of creating plate definitions by using plate setup templates (.tpl). The templates allow you to save the frequently used plate setup parameters so that an operator does not have to re-enter the required parameters for every plate. In a plate setup template, you specify parameters that are common to multiple plates. You can create templates in the Plate Setup window (section 9.3), or you can create a .tpl file using any text editor or Excel (section 9.4).



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See table 9-1 for a list of the recognized fields for the plate setup parameters.

**Table 9-1.** The recognized fields for the STR genotyping plate setup templates

| Field name | Value |
|---|---|
| **The values for the following fields can be imported into the Plate Setup window or typed manually. When importing the values, the fields can be defined in either a .psd file or a plate setup template (.tpl).** | |
| **Note:** If both a .psd file and template are used to define the same parameter, the values in the file imported last overwrite the values for any identical fields. | |
| **Electrophoresis Parameters tab** | |
| Injection voltage | Integer or fraction (kV). The range is 1–15 kV. |
| Injection time | Integer (s). The range is 1–600 s. |
| Run voltage | Integer or fraction (kV). The range is 1–15 kV. |
| Run time | Integer (min). The range is 1–720 min. |
| **Chemistry Parameters tab** | |
| Chemistry | String. The name of a chemistry parameter set.<br>**Note:** The name must match a name in the Chemistry.ini file (chapter 3) for the STR genotyping application. Otherwise, the software will not recognize the name. |
| **Sample Names tab** | |
| Well_ID<br>(for example, A01)<br>or<br>Well_ID.NAME<br>(.psd file only) | String. The sample name that you want to assign to each well location (ID) and use as the .rsd file name (default), for example, Sample1.<br>**Note:** If you want to use names other than sample names as the file names, you must also use the Well_ID.FILE field in the .psd file. |
| **Optional Parameters tab** | |
| **Note:** The Run temperature and PMT voltages are typically defined as part of the instrument control parameters (chapter 4). The values can be defined here for a special run. If defined here, the values temporarily overwrite the same values defined in an .icp file or the Instrument Control window. | |
| Run temperature | Integer (°C). The range is 27–44 °C. |
| PMT1 voltage | Integer or fraction (kV). The range is 500–900 V. |
| PMT2 voltage | Integer or fraction (kV). The range is 500–900 V. |
| PMT3 voltage | Integer or fraction (kV). The range is 500–900 V. |
| PMT4 voltage | Integer or fraction (kV). The range is 500–900 V. |
| **Comments tab** | |
| Comments | String, including spaces, representing the comments about the plate that you want to include. |



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## 9.3 Using the Plate Setup window to create a template

You can use the Plate Setup window (figure 9-1) to create a plate setup template. To do this—

1. In the Plate Setup window, click **New.**
2. In the following tabs, enter the parameters that you want to use for the template. See table 9-1 for a description of the parameters.
   - **Electrophoresis Parameters tab—**Type the values for the parameters in each of the boxes.
   - **Chemistry Parameters tab—**Select the name of the chemistry parameters from the **Chemistry Name** list.
   - **Sample Names tab—**By default, the software uses the well IDs to name the files (for example, A01.rsd). If you want to use sample names other than the well IDs, you can type the names in the Sample Names tab.

     If you want to use the Sample Names tab to specify empty wells for which you want to suppress the raw sample data file (.rsd) creation, see section 9.5.
   - **Optional Parameters tab—**(Optional) If you want to overwrite the Run Temperature and PMT Voltage values, which are provided in the instrument control parameters, you can type the values in this tab.
   - **Comments tab—**You can type any comments about the plate.

   See the *MegaBACE 4000 Instrument User's Guide* for details on using the Plate Setup window.
3. On the **Templates** menu, point to **Plate Setup Templates,** and click **Save Template**. The Save Template window appears.
4. Type the template name, and click **Save**. The template is saved in the …\MegaBACE\Instrument Control\Templates folder. The software adds the .tpl file extension.



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## 9.4 Creating a plate setup template in a text file

**Important**

**When you create a plate setup template, you must type the .tpl file extension and save it in the …\MegaBACE\Instrument Control\Templates folder. Otherwise, the Instrument Control Manager will not find the file.**

To create a plate setup template—

1. Open a new document or an existing .tpl file using a text editor (such as Notepad) or Excel.
2. Type the field names in the first column (field column), and type the values for each field in the second column (value column). Make sure the field column and value column are separated by a tab. See table 9-1 for a list of the recognized fields for the plate setup parameters. Figure 9-2 shows an example of a plate setup template file (.tpl).
3. Save the file in an appropriate format. You must type the .tpl file extension.

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

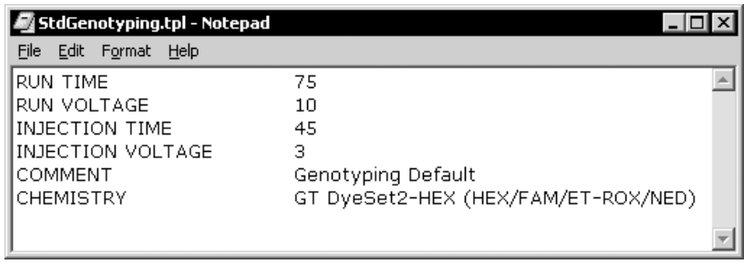



**Figure 9-2.** An example of a plate setup template file (.tpl) for STR genotyping.

## 9.5 Using a template to suppress .rsd file creation for empty capillaries

To specify an empty capillary, you designate the corresponding well as unused. The software does not create a raw sample data file (.rsd) for any wells that are specified as not used. In the Run Image window (figure 2-2), an unused well is indicated by a yellow arrow that appears above the capillary button.

**Note:** To specify bad capillaries, see section 2.6.

You can specify unused wells in either a—

- **Plate setup data file (.psd)—**You can use a .psd file to specify the unused wells if the unused wells occur for a single plate (section 7.6).



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- **Plate setup template—**You can use a plate setup template if the unused wells occur for multiple plates.

You can specify unused wells in a plate setup template by typing **NOT USED** (case-insensitive) as the sample name specified in either the Sample Names tab of the Plate Setup window or the .tpl file (section 9.4).

To specify an unused well in the Sample Names tab of the Plate Setup window—

1. In the **Sample Names** tab of the Plate Setup window (figure 9-3), adjust the width of the column that includes the unused well. To increase the column width, click the **RIGHT ARROW** key in the Sample Names tab. To decrease the column width, click the **LEFT ARROW** key in the Sample Names tab.
2. Type **NOT USED** (case-insensitive) as the sample name for the unused well.

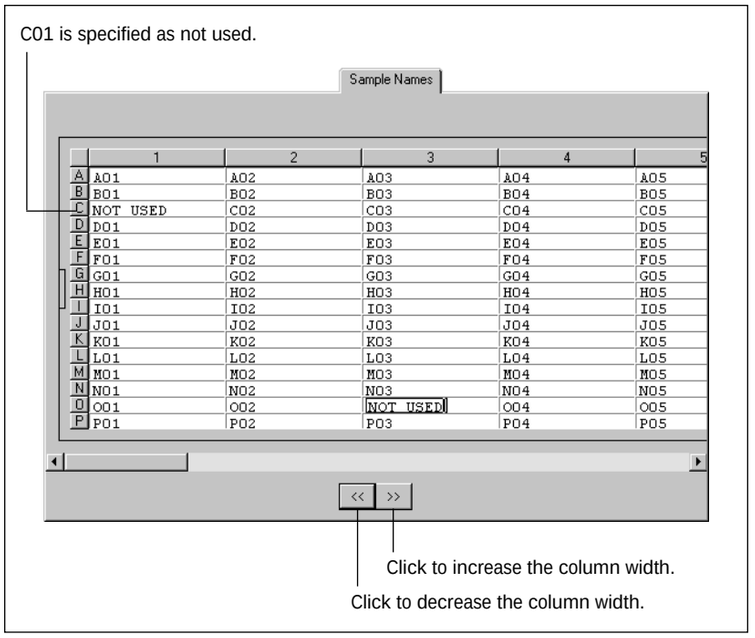



**Figure 9-3.** The Sample Names tab of the Plate Setup window.



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## 9.6 Setting and clearing the default plate setup template

You can set a default plate setup template in the—

- **Genotyping section of the MegaBACE.ini file—**To set a default plate setup template for STR genotyping in the MegaBACE.ini file, see section 8.3.1.
- **Templates menu in the Plate Setup window—**The default template is active only until you close the Instrument Control Manager. When the software is restarted, the default plate setup template specified in the Genotyping section of the MegaBACE.ini file is active until you change it again.

### 9.6.1 Using the Templates menu to set a default template

To set the default plate setup template—

1. In the Instrument Control Manager, click the **Plate Setup** tab to display the Plate Setup window (figure 9-1).
2. On the **Templates** menu, point to **Plate Setup Template,** and click **Set Default**. The Set Default window appears.
3. Select the name of the template you want to use as the default, and click **Open.** The Instrument Control Manager designates the template you selected as the default template.

The next time you click New in the Plate Setup window, the values from the default template appear in the parameter boxes.

### 9.6.2 Clearing the default plate setup template

To clear the default plate setup template—

1. In the Instrument Control Manager, click the **Plate Setup** tab to display the Plate Setup window (figure 9-1).
2. On the **Templates** menu, point to **Plate Setup Templates,** and click **Clear Default.** The template you cleared is no longer the default template.

When you click New in the Plate Setup window, no values appear in the parameter boxes. You can select another default template (section 9.6.1), or use the Plate Setup window to create another template, save it, and then select it as the default template.

**Important** **When you restart the software, the default templates specified in the MegaBACE.ini file are active.**



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# Chapter 10 Creating plate setup data files (.psd) for STR genotyping

This chapter describes the plate setup data files (.psd) for STR genotyping. The topics are—

- Using plate setup data files (.psd) for STR genotyping (section 10.1)
- Creating a plate setup data file (.psd) (section 10.2)
- Annotating well-specific information in a .psd file (section 10.3)
- Designating the sample names and file names in the .psd file (section 10.4)
- Using a .psd file to suppress .rsd file creation for empty capillaries (section 10.5)

## 10.1 Using plate setup data files (.psd) for STR genotyping

You can define a plate ID, the plate setup parameters, and other plate- and well-level attributes in a .psd file so that an operator can automatically import the plate definition. With automatic import, the plate setup parameters are specified with less operator intervention.

The operator can import the .psd file either before the run in the Plate Setup window or during the run in the Select a Plate window (*MegaBACE 4000 Instrument User's Guide*). The parameters that you specify in the .psd file become part of the extended header of each raw sample data (.rsd) file created during the run.

### 10.1.1 About the plate ID

When an operator enters the text or bar code in the plate ID area of the Plate Setup window or the Select a Plate window (described in the *MegaBACE 4000 Instrument User's Guide*), the Instrument Control Manager looks for a .psd file with a file name that matches the text entered and automatically imports the plate setup parameters that are contained in the .psd file.



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An operator can enter a plate ID in the following ways:

- **Type the text** in the Plate ID box in the Plate Setup window or the Select a Plate window.
- **Scan a bar code** into the Plate ID box in the Plate Setup window or the Select a Plate window.

**Important** **Regardless of the method used to enter the plate ID, the text entry or bar code (less a possible plate ID terminator) must match the name of the .psd file in order for a .psd file to be imported.**

Figure 10-1 shows an example of a .psd file and the Plate Setup window. The .psd file name matches the plate ID typed in the Plate Setup window.

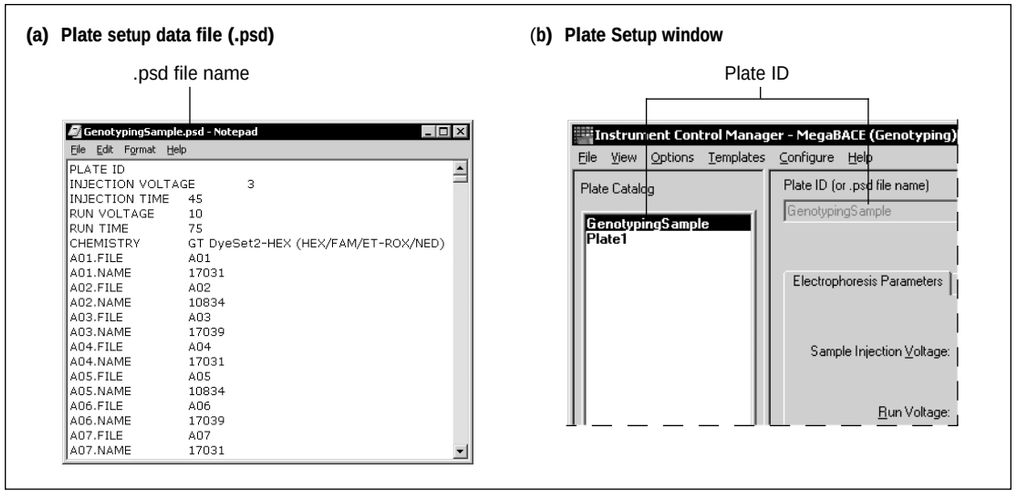



**Figure 10-1.** Using a .psd file: (a) Example of a .psd file. (b) The Plate Setup window with a plate ID that matches the .psd file name.

## 10.1.2 Using a plate ID that is different from the scanned ID

If the bar code is not what you want to use as the plate ID, you can use the .psd file to replace a bar code scanned into the Plate ID box in the Plate Setup window. To do this, you type a plate ID in the .psd file opposite the PLATE ID field (figure 10-2a).

The plate ID you type opposite the PLATE ID field in the .psd file replaces the bar code and becomes the real plate ID in the Plate Setup window (figure 10-2b).



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For example, in figure 10-2, the—

- Plate ID scanned into the Plate Setup window is **GenotypingSample**
- Plate setup data file name is **GenotypingSample.psd**
- Plate ID opposite the PLATE ID field name inside the .psd file is **Genotyping_Demo_Panel6** (figure 10-2a)
- Actual plate ID that appears in the Plate Setup window is **Genotyping_Demo_Panel6** (figure 10-2b)

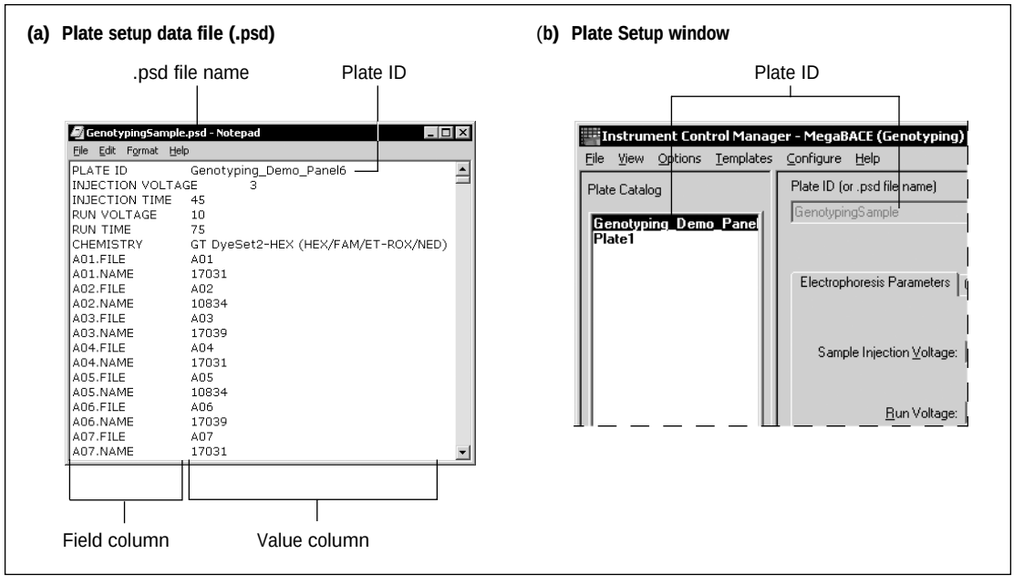



**Figure 10-2.** Replacing the scanned plate ID: (a) Example of a .psd file with a plate ID listed in the file. (b) The Plate Setup window with a plate ID that replaced the original bar code that was scanned.



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## 10.2 Creating a plate setup data file (.psd)

**Important**

**When you create a .psd file, you must type the .psd file extension and save it in the …\MegaBACE\Instrument Control\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.**

**Regardless of the method the operator uses to enter the plate ID in the Plate Setup window or Select a Plate window, the text entry or bar code (less a possible plate ID terminator) must match the name of the .psd file in order for a .psd file to be imported.**

To create a .psd file—

1. Open a new document or an existing .psd file using a text editor (such as Notepad) or Excel.
2. Type the field names in the first column (field column) and the values for each field name in the second column (value column). The order of the fields is arbitrary, and the field names are case-insensitive. Figure 10-3 shows an example of a .psd file created in Excel. Section 10.2.1 describes the fields that you can use in a .psd file.

   **Note:** If you use spaces in the sample or file names, the Instrument Control Manager converts the spaces to underscore characters in the file name.
3. Save the file in an appropriate format. You must type the .psd file extension.

   **Note:** If you use Excel, save the file as a tab-delimited file (figure 10-3).



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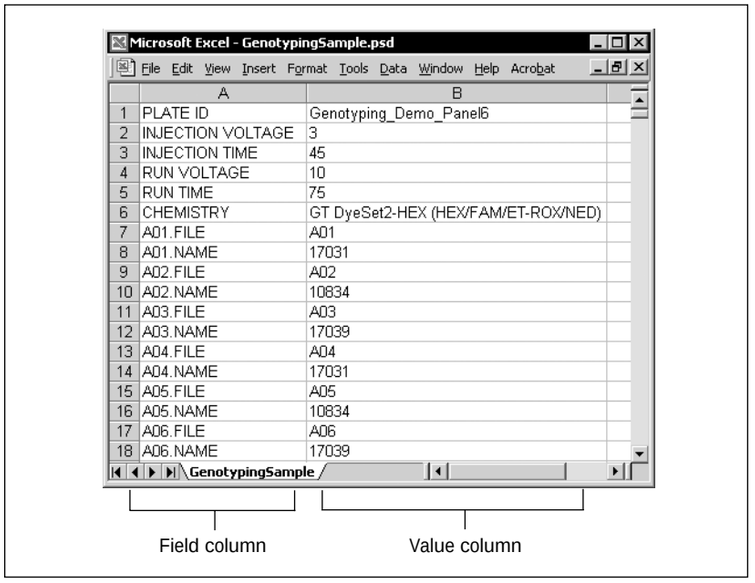



**Figure 10-3.** An example of a .psd file created in Excel.

## 10.2.1 The .psd field names

The .psd file can include the following fields:

- All the fields that appear in the tabs or Plate Setup window or in a plate setup template (.tpl). Table 9-1 lists the available fields.

  **Note:** If both a .psd file and a plate setup template (.tpl) are used to define the same plate setup parameters, the values provided in the file imported last overwrite the values for any identical fields.

- The plate- and well-specific fields listed in table 10-1.



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**Table 10-1.** Additional fields available for the STR genotyping .psd files

| Field name | Value |
|---|---|
| Plate ID | The string for the plate ID. If the plate ID entered here is different from the bar code of the .psd file name, the plate ID entered here becomes the real plate ID that appears in the Plate Setup window. |
| Well_ID.FILE (for example, A01.FILE) | The .rsd file name that you want to assign for a given well location. For example, A01 if you want to use the well location as the file name. This parameter overwrites the sample name as the file name. |
| User-defined fields | Fields that can be imported, but are not used by the software. These data are stored in the extended header of the files. |

Tables 9-1 and 10-1 list the field names that the Instrument Control Manager recognizes, except for the user-defined fields. For additional plate information you want included in the output file (that will not appear in the Plate Setup window), you can use field names the Instrument Control Manager does not recognize (for example, Operator). You must also provide a name in the value column (for example, Mary Smith).

### 10.2.2 The .psd file value constraints

If you type a value that is invalid (for example, an alpha character when the software requires a number, or a value that is out of range), the Instrument Control Manager displays an error message when you import the .psd file. If this occurs, you must correct the value in the .psd file before it can be imported successfully. Alternatively, if the edit mode is on, you can enter the correct value in the Plate Setup window.

## 10.3 Annotating well-specific information in a .psd file

The values for the field names that begin with a well ID (for example, A01) are well specific. If you want additional well-specific information for your records, the Instrument Control Manager allows you to include a dot (.) character after the well ID followed by an additional name to annotate the well ID. Figure 10-3 shows examples of what you might enter in the .psd file for annotation of well-specific information.

The Instrument Control Manager writes the additional well-specific information only into the .rsd file header for the corresponding well. The well-specific annotation does not appear in the Plate Setup window.



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## 10.4 Designating the sample names and file names in the .psd file

If no file names are specified, but the sample names are provided for a plate, the software uses the sample names as the file names, for example, sample1.rsd. If no sample or file names are provided for a plate, the Instrument Control Manager uses the well location (well IDs) as the sample file names, for example, A01.rsd.

You can provide sample names and designate explicit file names that are different from the sample names. To do this, you use two fields—Well_ID.FILE and Well_ID.NAME. The following and figure 10-4 show examples of what you might enter in the .psd file to designate a file name that is different from the sample name.

| Field name | Value | Comment |
|---|---|---|
| A01.NAME | 14660 | Sample name |
| A01.FILE | A01 | File name |

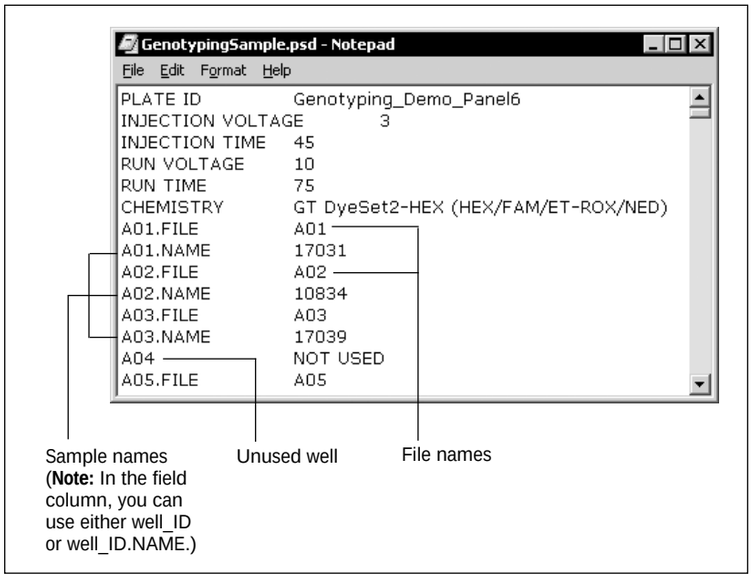



**Figure 10-4.** Example of a .psd file with entries to designate the well ID as the file names.



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## 10.5 Using a .psd file to suppress .rsd file creation for empty capillaries

You can indicate unused wells by typing **NOT USED** (case-insensitive) as the sample name specified in the .psd file (figure 10-4). For every unused well, a yellow arrow appears above the capillary button in the Run Image window (figure 2-2). The software does not create an .rsd file for an unused well. For details on suppressing .rsd files for defective capillaries, see section 2.6.



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# Part four

# Setting up the system for SNP genotyping

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# Chapter 11 Specifying preferences for SNP genotyping

This chapter describes the fields in the MegaBACE.ini file that you can use to configure the software for SNP genotyping. The topics are—

- The SNP genotyping fields in the MegaBACE.ini file (section 11.1)
- Setting SNP genotyping as the default application (section 11.2)
- Setting default parameter templates for SNP genotyping (section 11.3)
- Specifying limits for the injections (section 11.4)
- Enforcing the order in which an operator injects the plates (section 11.5)
- Prompting the operator for plate-specific information (section 11.6)

Make sure you configure the General section of the MegaBACE.ini file for your laboratory (chapter 2). To create additional chemistry parameters, see chapter 3.

## 11.1 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: General, Sequencing, Genotyping (STR genotyping), and SNP (SNP genotyping). Figure 11-1 shows the MegaBACE.ini file with the SNP fields highlighted.

Table 11-1 describes the fields in the SNP section of the MegaBACE.ini file. See chapter 2 for a description of the other fields in the MegaBACE.ini file.



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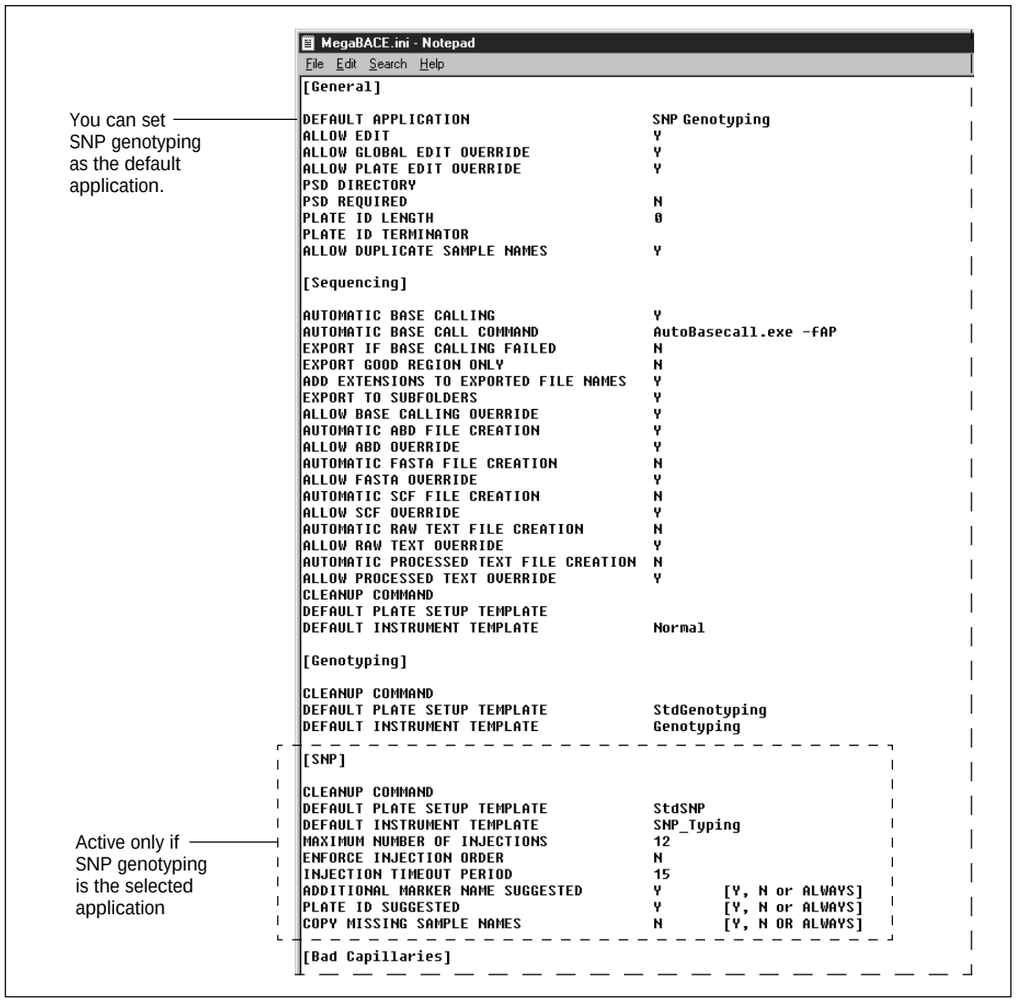



**Figure 11-1.** The SNP fields in the MegaBACE.ini file. Note that the file contains a section for each application, which allows you to specify a default plate setup and instrument template.



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**Table 11-1.** The SNP genotyping fields in the MegaBACE.ini file

| Field name | Description | Setting type | Default |
|---|---|---|---|
| Cleanup command | Allows the administrator to specify a program or batch file to be executed at the end of the run. This can be useful for transferring the raw data files to another workstation for analysis or archiving. See appendix C for a more detailed description. | String consisting of the path to the program or batch file (full or relative path to the instrument control folder) | none |
| Default plate setup template | Specifies a default plate set setup template. | String | StdSNP |
| Default instrument template | Specifies a default instrument control 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 13.2.3) 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.<br>Y = If the plate set .psd file specifies a PLATE SET (section 13.4.2), the bar code the operator enters in every injection must match a specific item and the order specified in the PLATE SET field.<br>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.<br>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 |



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**Table 11-1.** 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:<br>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.<br>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.<br>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:<br>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.<br>N = The window does not appear.<br>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 |



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**Table 11-1.** 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:<br>N = The software does not prompt the operator for input and proceeds with the injection without sample names.<br>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.<br>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 |

## 11.2 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, when 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 |



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## 11.3 Setting default parameter templates for SNP genotyping

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, when the Instrument Control Manager is restarted, the software automatically resets the selected template to the default specified in the MegaBACE.ini file.

### 11.3.1 Setting the default plate set setup template for SNP genotyping

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 11-1 and table 11-1 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 overwrite any duplicate parameters specified in a .tpl file.



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### 11.3.2 Setting the default instrument control template for SNP genotyping

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 11-1 and table 11-1 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.

## 11.4 Specifying limits for the injections

### 11.4.1 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 4000 Instrument User'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 | Integer (12 or less)<br>**Note:** If the NUMBER OF INJECTIONS value is greater than the maximum specified by this field, the software displays an error message when the operator provides the plate set definition (section 12.2). |



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The software checks to see if a NUMBER OF INJECTIONS value is provided with the plate set setup parameters (tables 12-1 and 13-2). 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.

## 11.4.2 Specifying the injection timeout period

Typically, an operator should inject the plate within 1–2 minutes after the Sample Injection window appears (figure 11-2) 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.

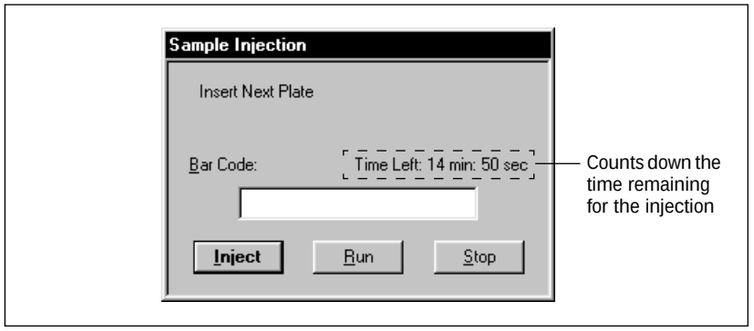



**Figure 11-2.** The Sample Injection window showing the timer that counts down the time remaining for the injection.



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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 software turns off the laser and the heater in the electrophoresis compartment.

## 11.5 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 13.2.2). 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 the 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 13.2), the operator can enter any bar codes for the remaining injections.

## 11.6 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 11-3).



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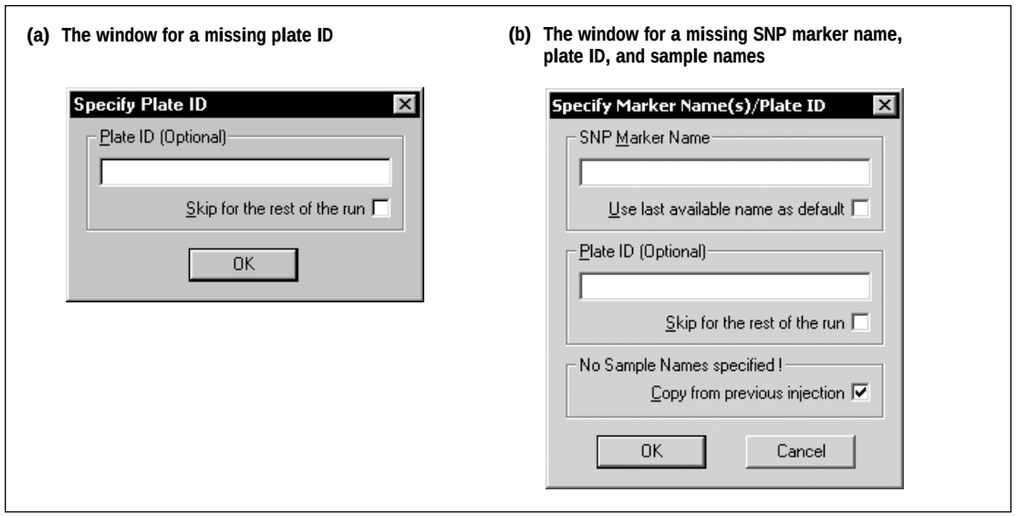



**Figure 11-3.** The Specify Marker Name(s)/Plate ID window changes dynamically depending on which information is missing: (a) an example of the window requesting a missing plate ID, and (b) an example of the window requesting the SNP marker name, the plate ID, and sample names.

### 11.6.1 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(s) window (figure 11-3b). 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 13.2).

If the SNP marker name is missing for a given injection, the choices available to the operator vary depending on the value you specify in 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 in 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



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### 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(s) window appears (figure 11-3b) 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(s) 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(s) 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(s) window appears, but the window does not



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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.

## 11.6.2 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 11-3a), 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.

## 11.6.3 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 13.2.3). However, if all the sample names are missing for an injection,



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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 11-3b). 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.



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# Chapter 12 Creating templates for SNP genotyping plate set setup parameters

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. This chapter describes plate set definitions and how to create the plate set setup templates for SNP genotyping. The topics are—

- About creating the plate set definitions (section 12.1)
- About the plate set setup parameters for SNP genotyping (section 12.2)
- Using the Plate Set Setup window to create a template (section 12.3)
- Creating a plate set setup template in a text file (.tpl) (section 12.4)
- Using a template to suppress .rsd file creation for empty capillaries (section 12.5)
- Setting and clearing the default plate set setup template (section 12.6)

For a description of the instrument control templates, see chapter 4.

## 12.1 About creating the 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 .psd to name the run folder.
- **Plate set setup parameters—**The run parameters that are common to all the plates in the plate set.
- **List of plates (optional)—**The list of bar codes representing the plates in the plate set. The list of plates can only be specified in a master .psd file (section 13.2.2).



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### 12.1.1 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 master plate setup data file (.psd) that an operator can import at the start of the Inject Samples and Run protocol. 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. For a description of the .psd files for SNP genotyping, see chapter 13.
- The Plate Set Setup window (figure 12-1) 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.

### 12.1.2 The methods for creating plate set definitions in the Plate Set Setup window

You or an operator can create the plate set definition before the run using the Plate Set Setup window (figure 12-1). To do this, you or the operator can—

- **Import a master .psd file—**You can use a master .psd file to enter the plate set definition for a single plate set. 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. See chapter 13 for a description of how to create .psd files.
- **Select a plate set setup template (.tpl)—**You can use a template to enter the plate set setup parameters that are visible in the Plate Set Setup window for multiple plate sets (section 12.2). 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 section 2.3 for a description of the edit mode.

See the *MegaBACE 4000 Instrument User's Guide* for a description of how to create plate set definitions in the Plate Set Setup window.



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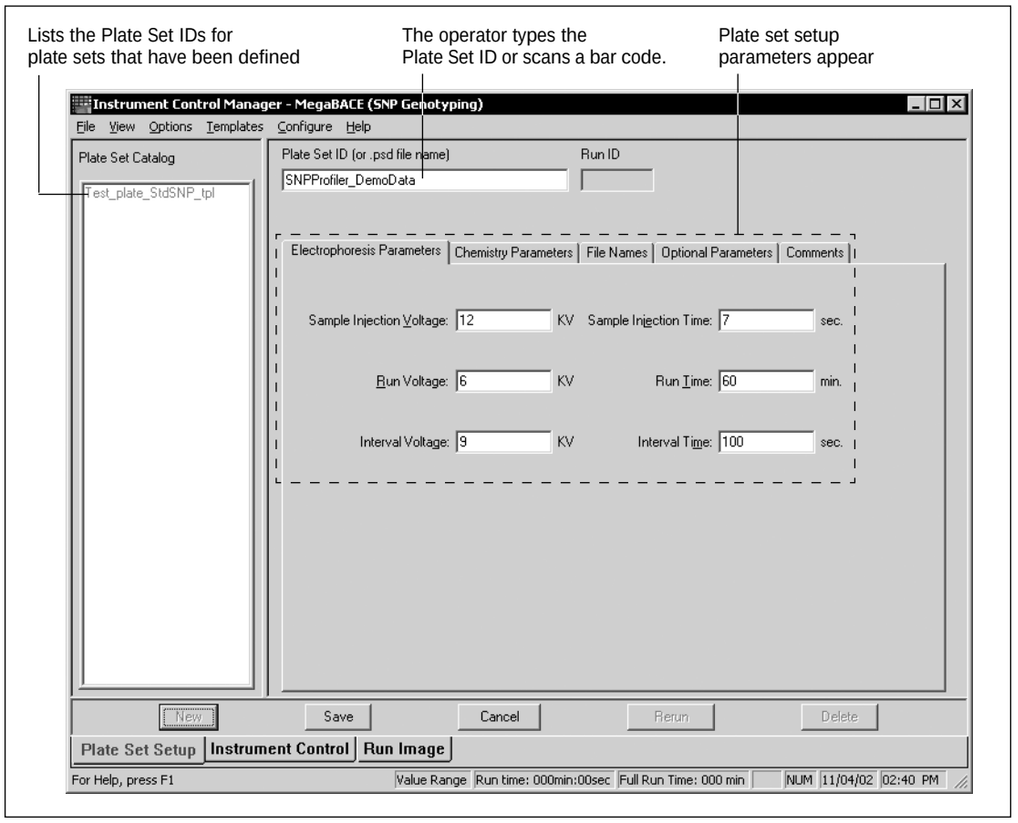



**Figure 12-1.** The Plate Set Setup window displays the parameters shared by all the plates in the plate set.



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## 12.2 About the plate set setup parameters for SNP genotyping

For a SNP genotyping run, the plate set setup parameters are common to the entire set of plates injected during the multi-injection run. The plate set setup parameters consist of the electrophoresis parameters, the chemistry parameters, the file names, plate set comments, and other parameters shared by all the plates injected in the 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.

Table 12-1 lists the plate set setup parameters. The software requires values for all the parameters, except the optional parameters and comments.

**Table 12-1.** The plate set setup parameters

| Field name | Value | Default value for SNP genotyping |
|---|---|---|
| **Electrophoresis Parameters tab** | | |
| Sample injection voltage | Integer or fraction (kV). The range is 1–15 kV. | 12 kV |
| Sample injection time | Integer (s). The range is 1–600 s. | 7 s |
| Run voltage | Integer or fraction (kV). The range is 1–15 kV. | 6 kV |
| Run time | Integer (min). The range is 1–720 min. | 60 min |
| Interval voltage | Integer or fraction (kV). The range is 1–15 kV. | 9 kV |
| Interval time* | Integer (s). The range is 1–1800 s. | 100 s |
| **Chemistry Parameters tab** | | |
| Chemistry | String. The name of a chemistry parameter set.<br>**Note:** The name must match a name in the Chemistry.ini file (chapter 3) for the SNP genotyping application. Otherwise, the software will not recognize the name. | SNuPe Terminators |
| **File Names tab** | | |
| Well_ID | String. 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 parameters template, or the Plate Set Setup window.<br>**Note:** Unlike sequencing and STR genotyping, to specify the sample names for a SNP genotyping run, you must use .psd files. | Well IDs |



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**Table 12-1.** The plate set setup parameters (continued)

| Field name | Value | Default value for SNP genotyping |
|---|---|---|
| **Optional Parameters tab** | | |
| **Note:** The Run temperature and PMT voltages are typically defined as part of the instrument control parameters (chapter 4). The values can be defined here for a special run. If defined here, the values temporarily overwrite the same values defined in an .icp file or the Instrument Control window. | | |
| Run temperature | Integer (°C). The range is 27–44 °C. | No default specified |
| PMT1 voltage | Integer or fraction (kV). The range is 500–900 V. | No default specified |
| PMT2 voltage | Integer or fraction (kV). The range is 500–900 V. | No default specified |
| PMT3 voltage | Integer or fraction (kV). The range is 500–900 V. | No default specified |
| PMT4 voltage | Integer or fraction (kV). The range is 500–900 V. | No default specified |
| Number of injections* | Integer. The range is 1–12. The MegaBACE.ini file specifies an upper limit for this field (section 11.4.1).<br>**Note:** This value specified the number of injections the software prompts the operator for during a multi-injection run. | No default specified |
| **Comments tab** | | |
| Comments | String, including spaces, representing the comments that you want to include about the plate set. | No default specified |

* See the *MegaBACE 4000 Instrument User's Guide* for guidelines on determining the interval time and the number of injections.

## 12.3 Using the Plate Set Setup window to create a template

You can use the Plate Set Setup window (figure 12-1) to create a plate set setup template. To do this—

1. In the Plate Set Setup window, click **New.**
2. In the following tabs, enter the parameters that you want to use for the template. See table 12-1 for a description of the parameters.
   - **Electrophoresis Parameters tab—**Type the values for the parameters in each of the boxes.
   - **Chemistry Parameters tab—**Select the name of the chemistry parameters from the **Chemistry Name** list.



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- **File Names tab—**By default, the software uses the well IDs to name the files (for example, A01.rsd). If you want to use file names other than the well IDs, you can type the names in the File Names tab.

  If you want to use the File Names tab to specify empty wells for which you want to suppress the raw sample data file (.rsd) creation, see section 12.5.

- **Optional Parameters tab—**(Optional) If you want to overwrite the Run Temperature and PMT Voltage values, which are provided in the instrument control parameters, you can type the values in this tab. You can also type a value for the Number of Injections.

- **Comments tab—**You can type any comments about the plate set.

See the *MegaBACE 4000 Instrument User's Guide* for details on using the Plate Set Setup window.

3. On the **Templates** menu, point to **Plate Setup Templates,** and click **Save Template**. The Save Template window appears.

4. Type the template name, and click **Save**. The template is saved in the …\MegaBACE\Instrument Control\Templates folder. The software adds the .tpl file extension.

## 12.4 Creating a plate set setup template in a text file (.tpl)

**Important** **When you create a plate setup template, you must type the .tpl file extension and save it in the …\MegaBACE\Instrument Control\Templates folder. Otherwise, the Instrument Control Manager will not find the file.**

To create a plate set setup template—

1. Open a new document or an existing .tpl file using a text editor (such as Notepad) or Excel.

2. Type the field names in the first column (field column), and type the values for each field in the second column (value column). Make sure the field column and value column are separated by a tab. See table 12-1 for a list of the recognized fields for the plate set setup parameters. Figure 12-2 shows an example of the StdSNP.tpl file for SNP genotyping, which is included with the software.

3. Save the file in an appropriate format. If you use Excel, save the file as a tab-delimited file. You must type the .tpl file extension.



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**Note:** If you enter the optional parameters listed in table 12-1, the values will overwrite the values for the same parameters defined in the Instrument Control window.

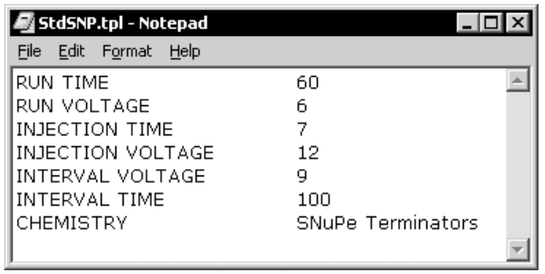



**Figure 12-2.** An example of a plate set setup template file (.tpl) for SNP genotyping.

**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 13.2.2).

## 12.5 Using a template to suppress .rsd file creation for empty capillaries

To specify an empty capillary, you designate the corresponding well as unused. The software does not create a raw sample data file (.rsd) for any wells that are specified as not used. In the Run Image window (figure 2-2), an unused well is indicated by a yellow arrow that appears above the capillary button.

**Note:** To specify bad capillaries, see section 2.6.

You can specify unused wells in either a—

- **Plate setup data file (.psd)—**You can use a .psd file to specify the unused wells if the unused wells occur for a single plate (chapter 13).
- **Plate setup template—**You can use a plate setup template if the unused wells occur for multiple plates.

You can specify unused wells in a plate setup template by typing **NOT USED** (case insensitive) as the sample name specified in either the File Names tab of the Plate Set Setup window or the .tpl file (section 12.4).



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To specify an unused well in the File Names tab of the Plate Set Setup window—

1. In the **File Names** tab of the Plate Set Setup window (figure 12-3), adjust the width of the column that includes the unused well. To increase the column width, click the **RIGHT ARROW** key in the File Names tab. To decrease the column width, click the **LEFT ARROW** key in the File Names tab.
2. Type **NOT USED** (case-insensitive) as the sample name for the unused well.

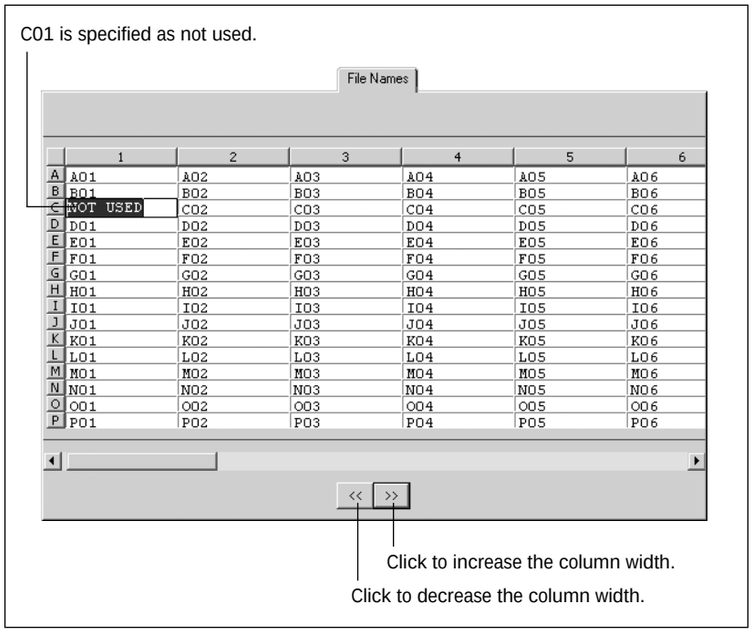



**Figure 12-3.** The File Names tab of the Plate Set Setup window.



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## 12.6 Setting and clearing the default plate set setup template

You can set a default plate set setup template in the—

- **SNP section of the MegaBACE.ini file—**To set a default plate set setup template for SNP genotyping in the MegaBACE.ini file, see section 11.3.1.
- **Templates menu in the Plate Set Setup window—**The default template is active only until you close the Instrument Control Manager. When the software is restarted, the default plate set setup template specified in the SNP section of the MegaBACE.ini file is active until you change it again.

### 12.6.1 Using the Templates menu to set a default template

To set the default plate set setup template—

1. In the Instrument Control Manager, click the **Plate Set Setup** tab to display the Plate Set Setup window (figure 12-1).
2. On the **Templates** menu, point to **Plate Setup Template** and click **Set Default**. The Set Default window appears.
3. Select the name of the template you want to use as the default, and click **Open.** The Instrument Control Manager designates the template you selected as the default template.

The next time you click New in the Plate Set Setup window, the values from the default template appear in the parameter boxes.

### 12.6.2 Clearing the default plate set setup template

To clear the default plate set setup template—

1. In the Instrument Control Manager, click the **Plate Set Setup** tab to display the Plate Set Setup window (figure 12-1).
2. On the **Templates** menu, point to **Plate Setup Templates,** and click **Clear Default.** The template you cleared is no longer the default template.

When you click New in the Plate Set Setup window, no values appear in the parameter boxes. You can select another default template (section 12.6.1), or use the Plate Set Setup window to create another template, save it, and then select it as the default template.

**Important** **When you restart the software, the default templates specified in the MegaBACE.ini file are active.**



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# Chapter 13 Creating plate setup data files (.psd) for SNP genotyping

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—

- Creating plate setup data files (.psd) for SNP genotyping (section 13.1)
- Specifying the plate set definition in a master .psd file (section 13.2)
- Using a .psd file to suppress .rsd file creation for empty capillaries (section 13.3)
- Specifying the attributes for a plate in a .psd file (section 13.4)

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

### 13.1.1 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—**Figure 13-1 shows a 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. The master .psd file can also include the plate-attribute fields for the first plate that you inject. For example, you can specify a plate ID and the SNP marker name for the first plate, and you can associate a sample name with each well in the first plate.

  **Note:** If you want to define the sample names for a plate in a multi-injection run, a .psd file is required.



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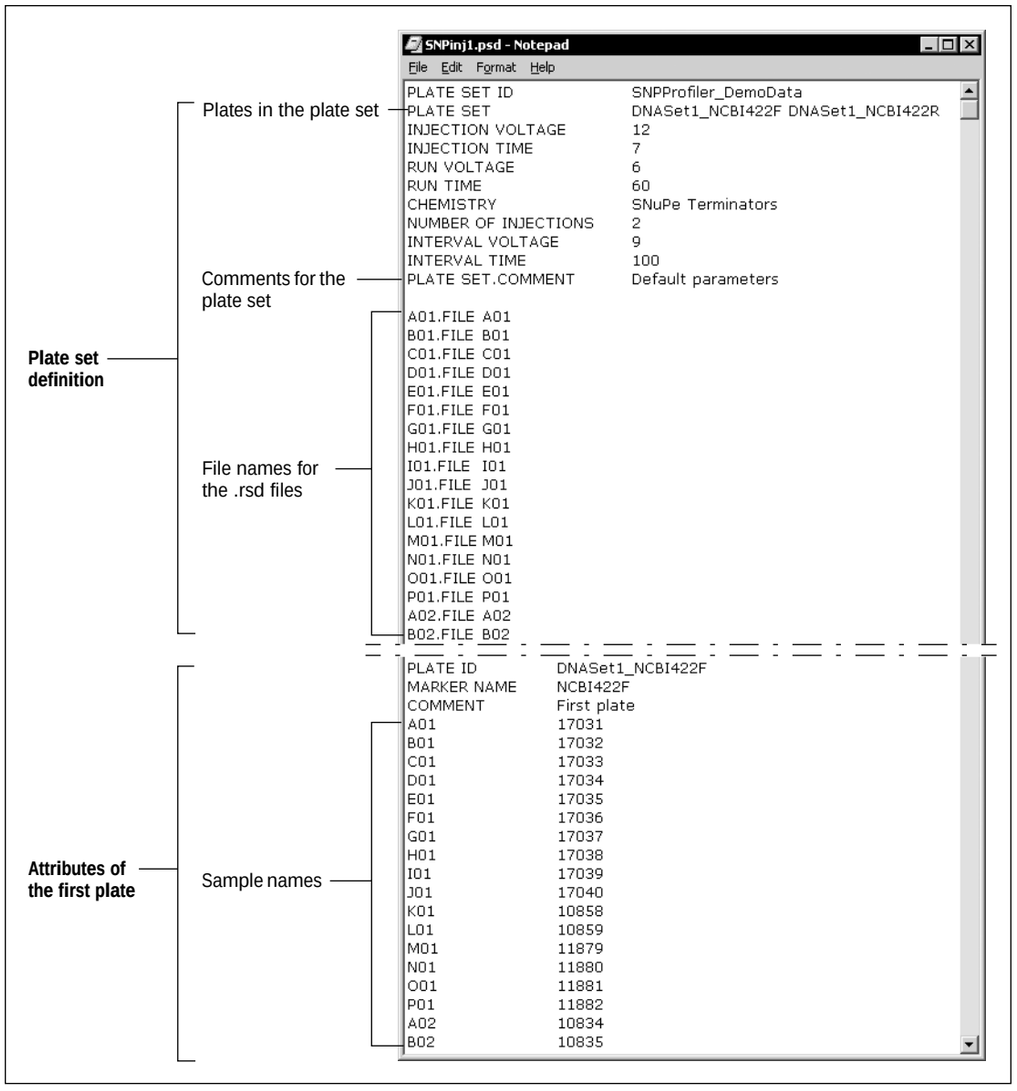
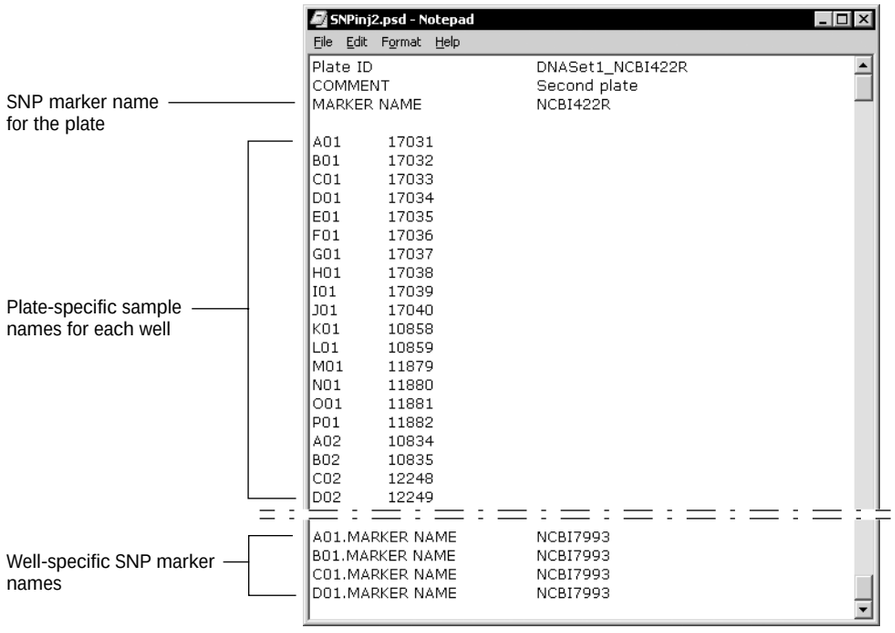



**Figure 13-1.** Example of master .psd file that defines both the plate set and the first plate to be injected.



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- **Plate-only .psd files—**For each plate in the plate set, you can use a plate-only .psd file to specify the plate-level and well-level information for a given plate (figure 13-2). 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.




**Note:** You can use a plate-only .psd file to provide the plate attributes for a single injection. If you are using .psd files for a multi-injection run, a master .psd file is also required to define the plate set setup parameters and the attributes for the first plate injected.

**Figure 13-2.** Example of a plate-only .psd file defining one plate (injection) in the plate set.



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### 13.1.2 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. The well-level information, such as sample name, becomes part of the extended header in the .rsd file for the well.

**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 (section 12.1).

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. Using a two-column format, type the appropriate fields and values for the .psd file. The fields and values that you can use depend on whether you are creating a—
   - **Master .psd file—**Figure 13-1 shows an example of a master .psd file that includes the plate set definition. Table 13-1 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 13-2).

     **Note:** If both a .psd file and template are used to define the same plate set setup parameter, the values provided in the file imported last overwrite the values for any identical fields.
   - **Plate-only .psd file—**Figure 13-2 shows an example of a .psd file that defines a single plate (injection). Table 13-2 lists the plate-attribute fields you can use in a .psd file for a single plate.

   Make sure you type the field names in the first column (the field column), and the values for each field name in the second column (the value column). Separate the field column from the value column using a tab.
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.



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- **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 cannot find the file.

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

**Table 13-1.** 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. |
| 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. |
| **Fields that can be specified in either a master .psd file or a plate set setup template (chapter 12):** | |
| Injection voltage | Integer or fraction (kV). The range is 1–15 kV. |
| Injection time | Integer (s). The range is 1–600 s. |
| Run voltage | Integer or fraction (kV). The range is 1–15 kV. |
| Run time | Integer (min). The range is 1–720 min. |
| Interval voltage | Integer or fraction (kV). The range is 1–15 kV. |
| Interval time | Integer (s). The range is 1–1800 s. |
| Chemistry | String. The name of a chemistry parameter set.<br>**Note:** The name must match a name in the Chemistry.ini file (chapter 3) for the SNP genotyping application. Otherwise, the software will not recognize the name. |
| **Optional parameters:** The run temperature and PMT voltages are typically defined as part of the instrument control parameters (chapter 4). If the values are defined here for a special run, the values temporarily overwrite the same values defined in an .icp file or the Instrument Control window. | |
| Run temperature | Integer (°C). The range is 27–44 °C. |
| PMT1 voltage | Integer (kV). The range is 500–900 V. |
| PMT2 voltage | Integer (kV). The range is 500–900 V. |
| PMT3 voltage | Integer (kV). The range is 500–900 V. |
| PMT4 voltage | Integer (kV). The range is 500–900 V. |
| Number of injections | Integer<br>**Note:** The upper limit can be specified in the SNP section of the MegaBACE.ini file (section 11.4). |



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**Table 13-1.** The fields in the master .psd file for a plate set (continued)

| Field name | Value |
|---|---|
| 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.<br>**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. |

**Table 13-2.** The plate-attribute 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.<br>**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. |

* To define plate attributes such as sample names, you must use a .psd file for the injection. If you use a .psd file for the first injection, the .psd file typically includes the plate set setup parameters as well as the attributes for the first plate.



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## 13.2 Specifying the plate set definition in a master .psd file

This section describes the fields you can use in a master .psd file for a SNP genotyping run. See section 13.1 for instructions on how to create the .psd file.

### 13.2.1 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.

When an operator enters the text or bar code in the Plate Set ID area of the Plate Set Setup window or the Select a Plate Set window, the Instrument Control Manager looks for a .psd file with a file name that matches the text entered and automatically imports the plate set setup parameters that are contained in the .psd file.

**Important** **Regardless of the method used to enter the plate ID, the text entry or bar code must match the name of the .psd file in order for a .psd file to be imported.**

Figure 13-3 shows an example of a master .psd file and the Plate Set Setup window.



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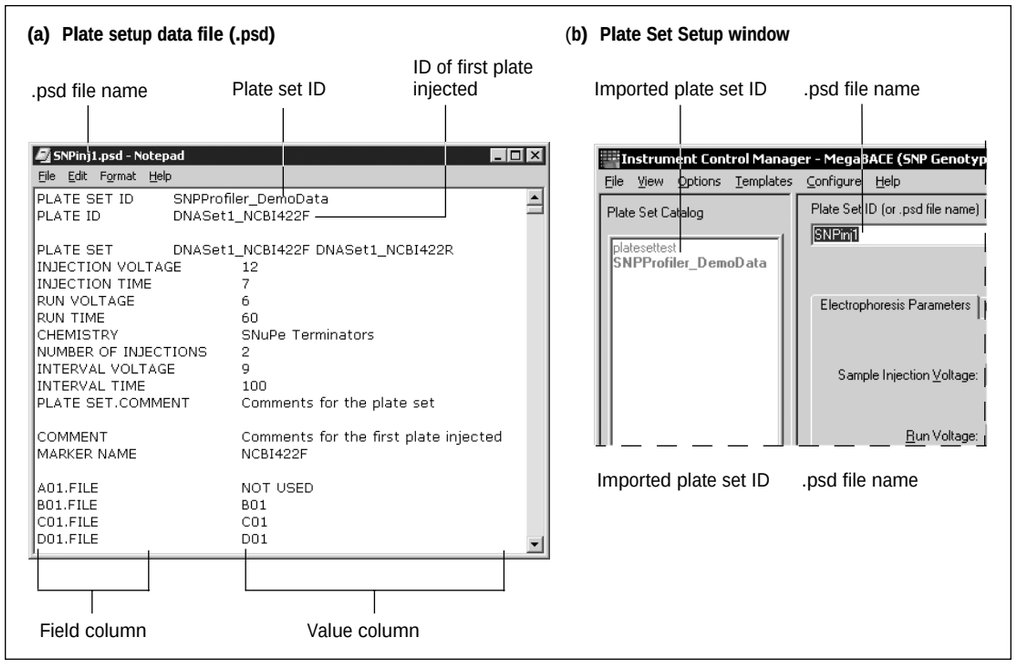



**Figure 13-3.** Replacing the scanned plate set ID: (a) Example of a master .psd file with a plate set ID listed in the file. (b) The Plate Set Setup window with a plate set ID that replaced the original bar code that was scanned.

## 13.2.2 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 (figures 13-1 and 13-3a). 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.



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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 SNP section of the MegaBACE.ini file (section 11.5), which allows you to enforce the order in which the operator injects the plates listed in the PLATE SET.

### 13.2.3 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 13.2.2 for details.
- MAXIMUM NUMBER OF INJECTIONS field in the MegaBACE.ini file (section 11.4.1). 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.

## 13.3 Using a .psd file to suppress .rsd file creation for empty capillaries

You can indicate unused wells by typing **NOT USED** (case-insensitive) as the file name specified in the .psd file (figure 13-3a). For every unused well, a yellow arrow appears above the capillary button in the Run Image window (figure 2-2). The software does not create an .rsd file for a well specified as not used. For details on suppressing .rsd files for defective capillaries, see section 2.6.



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## 13.4 Specifying the attributes for a plate in a .psd file

This section describes the fields you can use in a .psd file to describe the attributes of a single plate (injection) in a SNP genotyping run. The software does not display the plate-attribute fields in the Plate Set Setup window, because the fields apply to a single plate in the plate set. You can use the plate-attribute fields in both the master .psd file and the plate-only .psd files.

**Note:** If you do not use a master .pdf file to define the setup parameters for the plate set and the attributes for the first plate, you cannot use the plate-only .psd files to define the plate attributes for each injection.

Figure 13-2 shows an example of a plate-only .psd file. Table 13-2 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. See section 13.1 for instructions on how to create the .psd file.

### 13.4.1 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 11.6).

### 13.4.2 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 (figure 13-2). The well-specific SNP marker name overwrites the plate SNP marker name for the given well.

Alternatively, if you do not use .psd files, you can configure the MegaBACE.ini file (section 11.6) 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.



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### 13.4.3 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 11.6.3 for a description of how to configure this option.



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# Part five

# Protocol modification and file storage

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# Chapter 14 Bypassing or overriding a protocol

This chapter describes how to bypass or override a protocol. The topics are—

- Bypassing the selected protocol (section 14.1)
- Overriding the contents of a protocol (section 14.2)

In a typical operation, the software selects the next protocol in the workflow. You might need to use a protocol that is different from the one selected in the List of Protocols in the Instrument Control window (figure 14-1).

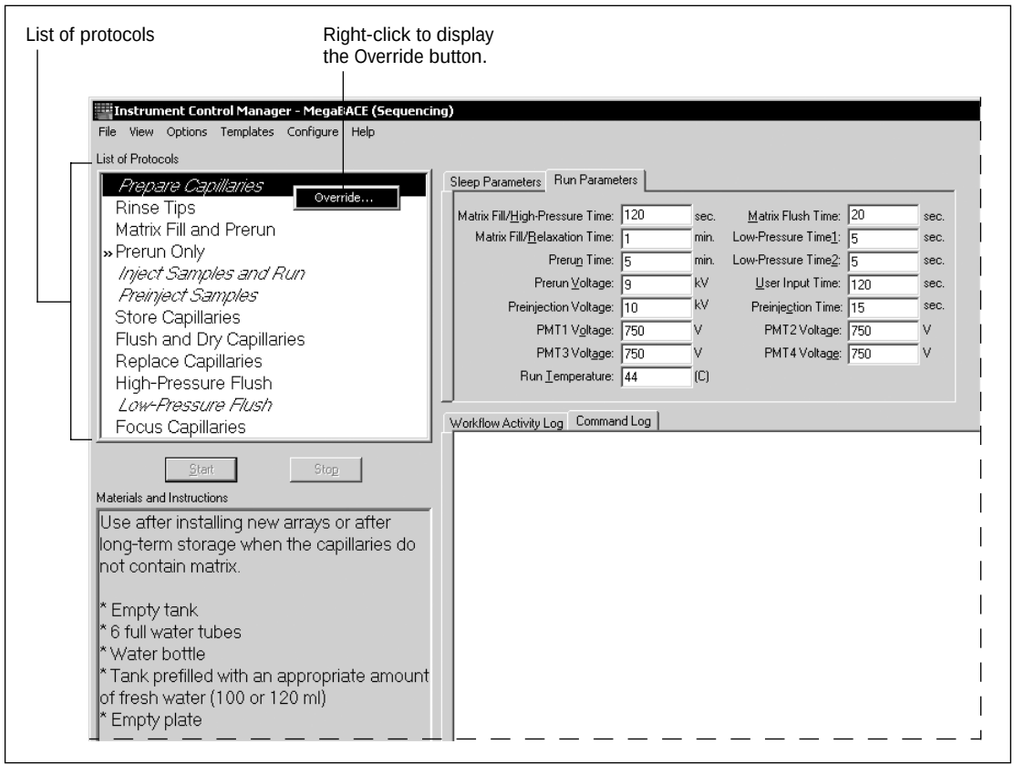



**Figure 14-1.** The List of Protocols in the Instrument Control window.



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The Instrument Control Manager allows you to—

- Bypass the protocol the software selects. You can select any protocol, even if it is disabled (section 14.1).
- Override all or part of the protocol (section 14.2).

## 14.1 Bypassing the selected protocol

To bypass the selected protocol—

1. In the Instrument Control window, select the protocol you want to run, and click the **right mouse button.** The Override button appears (figure 14-1).
2. Click the **Override** button. A window appears and asks for a password (figure 14-2).

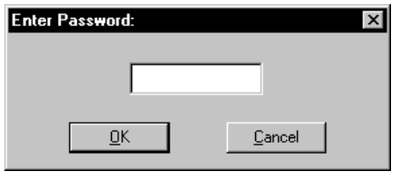



**Figure 14-2.** The Enter Password window.

3. Type the appropriate password and click **OK.** The Override Commands window appears (figure 14-3).

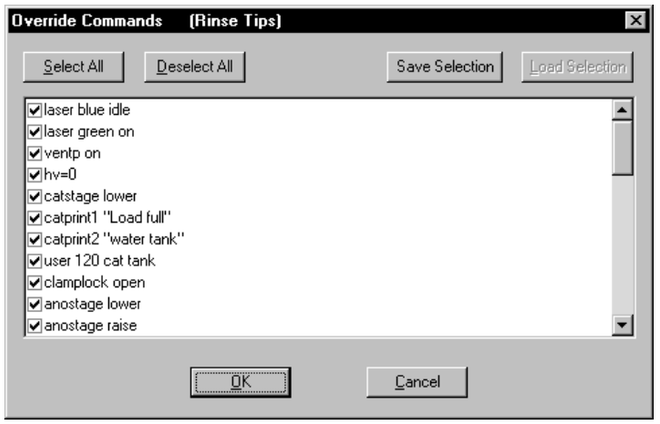



**Figure 14-3.** The Override Commands window.



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4. Click **OK** to run all the commands for the selected protocol. The window closes. In the Instrument Control window (figure 14-1), the selected protocol name appears bold to show that it is running.

## 14.2 Overriding the contents of a protocol

You can override all or part of the protocol the software selects as the next logical protocol. To do this—

1. With the protocol selected, click the **right mouse button**. The Override button appears (figure 14-1).
2. Click the **Override** button. A window appears and asks for a password (figure 14-2).
3. Type the appropriate password and click **OK**. The Override Commands window appears (figure 14-3).
4. In the Override Commands window—
   - Select the commands you want to run. You can click **Deselect All** and select the desired commands. Alternatively, you can deselect only the commands you do not want to run.
   - (Optional) Click **Save Selection** to save a specific set of commands you want to re-select later. You can save one set of selected commands for each protocol. To re-select a previously saved set of selected commands, click **Load Selection.**
   - Click **OK** to close the window.

In the Instrument Control window (figure 14-1), the selected protocol name appears bold to show that it is running.



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# Chapter 15 File storage

This chapter describes—

- Raw sample data storage (section 15.1)
- Base-called sample data storage (sequencing only) (section 15.2)
- System initialization file storage (section 15.3)
- Parameter configuration file storage (section 15.4)

## 15.1 Raw sample data storage

This section describes how the sample data files are stored and what the files contain.

### 15.1.1 Raw sample data file content

For each run, the Instrument Control Manager creates a raw sample data file (.rsd) for the data collected from each well. Each .rsd file contains—

- Plate ID (sequencing and STR genotyping) or plate set ID (SNP genotyping)
- Well location
- If provided, sample name (sequencing and STR genotyping) or sample names (SNP genotyping)
- Run data: run ID, instrument ID, instrument control parameters, plate setup parameters, and date
- Information about the electrical current
- Raw electropherogram (signal intensities)

### 15.1.2 Naming and storage conventions (sequencing and STR genotyping only)

By default, the software uses the sample names that you provide as the file names for the .rsd files. If the sample names that you assign for a given plate are not unique, the Instrument Control Manager appends the well ID to the file name (sample_name_well_ID) only if the ALLOW DUPLICATE SAMPLE NAMES field in the MegaBACE.ini file is set to Y. If no sample names are provided, the software uses the well ID as the file name for the .rsd files.



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Alternatively, you can use a plate setup data file (.psd) to specify the explicit file names for the .rsd files. For sequencing, see section 7.5. For STR genotyping, see section 10.4.

Each time you run a plate 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 ID and the run ID to name the raw run folders (plateID_runID). You assign the plate ID when you set up the plate in the Instrument Control Manager software. The run ID defaults to Run01. Table 15-1 summarizes the conventions for naming and storing the raw sample data files and folders.

**Table 15-1.** Naming and storage conventions for the raw sample data (sequencing and STR genotyping only)

| File or folder type | Name | Location |
|---|---|---|
| Raw sample data file (.rsd) | Sample_name.rsd, well_ID.rsd, or a combination of both | PlateID_runID (raw run folder) |
| Raw run folder | PlateID_runID | …\MegaBACE\Data (unless you select a different location) |

The File Storage command on the Options menu allows you to change the storage location of the data at any time. This feature only affects the data storage of subsequent runs. It does not change the location of the data already stored on the computer. See the *MegaBACE 4000 Instrument User's Guide* for a description of how to change the file storage location.

### 15.1.3 Naming and storage conventions (SNP genotyping only)

By default, the software uses the well ID as the .rsd file name, for example, A01.rsd. Alternatively, you can use a master .psd file, a plate set setup template, or the Plate Set Setup window to specify the file names for the .rsd files (chapter 13).

**Note:** Unlike sequencing and STR genotyping, to specify the sample names for a SNP genotyping run, you must use .psd files.

Each time you perform a multi-injection run, the Instrument Control Manager creates a raw run folder that includes the .rsd files for the run. The software uses the plate set ID and the run ID to name the raw run folders (plate set ID_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



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uses the file name of the master plate setup data file (.psd) to name the run folder. The run ID defaults to Run01. Table 15-2 summarizes the conventions for naming and storing the raw sample data files and folders.

**Table 15-2.** Naming and storage conventions for the raw sample data (SNP genotyping only)

| File or folder type | Name | Location |
|---|---|---|
| Raw sample data file (.rsd) | Well_ID.rsd | Plate set ID_runID (raw run folder) |
| Raw run folder | Plate set ID_runID | …\MegaBACE\Data (unless you select a different location) |

The File Storage command on the Options menu allows you to change the storage location of the data at any time. This feature only affects the data storage of subsequent runs. It does not change the location of the data already stored on the computer. See the *MegaBACE 4000 Instrument User's Guide* for a description of how to change the file storage location.

## 15.2 Base-called sample data storage (sequencing only)

You can specify that the software create a subfolder for each export file format that you use after automatic base calling (chapter 5). Sections 15.2.1 and 15.2.2 describe the naming and storage conventions. See the *MegaBACE 4000 Instrument User's Guide* for a description of how to change the storage location for the base-called data.

### 15.2.1 Naming and storage conventions for the base-called and exported files

Table 15-3 provides an overview of the naming and storage conventions for the base-called and exported files. Note that the base-called sample data files obtain the file names from the .rsd files.



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**Table 15-3.** Naming and storage conventions for the base-called and exported files

| File type | File contents | File name | Folder storage |
|---|---|---|---|
| Base-called | Plate ID, well location, sample name, instrument ID, base caller name, called sequence, sequence starting and ending points, read length for the high-quality data, quality values, electrical current information, base-called electropherogram for the sample. | Sample_name.esd, well_ID.esd | PlateID_basecallerID (analyzed run folder) |
| Exported—ABD | All the raw data, including electropherogram and spectral separation table. If base-called, contains all the raw and base-called data, including the called sequence. | Sample_name.abd, well_ID.abd | ABD subfolder (default) **Note:** In the MegaBACE.ini file, you can specify that the files be stored at the top level of the run folder (table 5-1 and chapter 5). |
| Exported—SCF | Raw data and the electropherograms. If base-called, includes the raw and base-called data, and the called sequence. | Sample_name.scf, well_ID.scf **Note:** The .scf extension might not display in Windows Explorer. | SCF subfolder (default) **Note:** In the MegaBACE.ini file, you can specify that the files be stored at the top level of the run folder (table 5-1 and chapter 5). |
| Exported—FASTA | File name, file location, and the called sequence for each well in the base-called plate (default). You can use the MegaBACE.ini file to specify other options (section 5.5.2):<br>• One .seq file for each .esd file<br>• One .seq file for each base-called plate<br>• One .seq file for all base-called plates | .seq | PlateID_basecallerID folder (default) |
| Exported—ASCII text | Plate name, sample name, instrument ID, base caller name, sequence starting and ending points, electrical current information, scan rate, instrument parameters, the number of bases called. | Sample_name.txt, well_ID.txt | Text subfolder (default) **Note:** In the MegaBACE.ini file, you can specify that the files be stored at the top level of the run folder (table 5-1 and chapter 5). |



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### 15.2.2 Naming and storage conventions for the base-called run folders

The different types of base-called data (section 15.1.2) are stored in different folders. Table 15-4 lists the run folders, exported data folders, and the naming conventions for these folders.

The software uses the plate ID, the base caller ID, the base caller version number (n_nnn), and the base caller method to name the analyzed run folders. The base caller IDs are—

- M = Molecular Dynamics™
- Cp = All Cimarron™ base callers

**Table 15-4.** Naming conventions for the analyzed run folders

| Folder type | Folder name | Storage location |
|---|---|---|
| Analyzed (Molecular Dynamics base caller) | PlateID_Mn_nnn_MD1 | …\MegaBACE\AnalyzedData folder (default) |
| Analyzed (Cimarron 1.53 Phat base caller) | PlateID_Cp1_53000_MD1 | …\MegaBACE\AnalyzedData folder (default) |
| Analyzed (Cimarron 1.53 Slim Phredify base caller) | PlateID_Cp1_53000_sl_ph_MD1 | …\MegaBACE\AnalyzedData folder (default) |
| Analyzed (Cimarron 3.12) | PlateID_Cp312_MD1 | …\MegaBACE\AnalyzedData folder (default) |
| Analyzed (Cimarron 3.12 Even Spacing) | PlateID_Cp312_es_MD1 | …\MegaBACE\AnalyzedData folder (default) |
| Analyzed (Cimarron 3.12 Aligned base caller) | PlateID_Cp312_a_MD1 | …\MegaBACE\AnalyzedData folder (default) |
| Exported ABD | Abd | PlateID_basecallerID folder (default) |
| Exported SCF | Scf | PlateID_basecallerID folder (default) |
| Exported text | Text | PlateID_basecallerID folder (default) |



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## 15.3 System initialization file storage

Figure 15-1 shows the system initialization file and parameter configuration file locations. Table 15-5 lists the system initialization files that you can modify to configure the Instrument Control Manager.

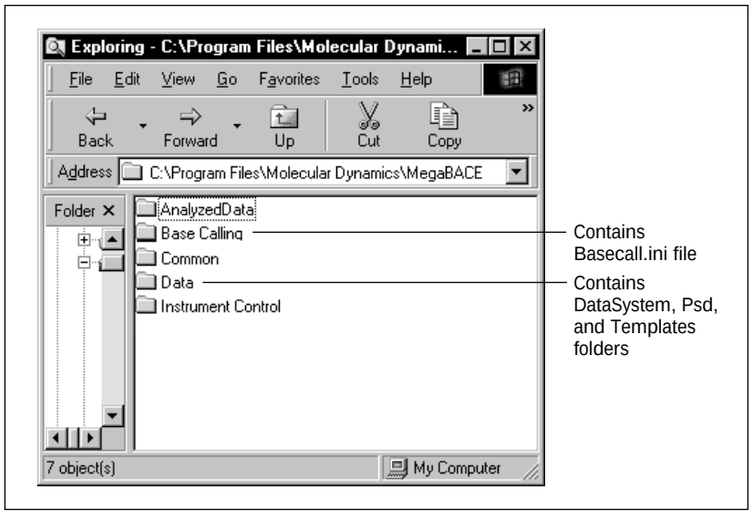



**Figure 15-1.** Storage locations of the system initialization files and the parameter configuration files.

**Table 15-5.** The system initialization files

| File name | Description | Location |
|---|---|---|
| MegaBACE.ini | Defines the options available in the Instrument Control Manager. | …\MegaBACE\Instrument Control\DataSystem folder |
| Chemistry.ini | Defines the available chemistry parameter sets, including the bases, filters, and laser modes. | …\MegaBACE\Instrument Control\DataSystem folder |
| Basecall.ini | Defines the default base caller and lists all the base callers available in the Instrument Control Manager. | …\MegaBACE\Base Calling folder |



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## 15.4 Parameter configuration file storage

Figure 15-1 shows the system initialization file and parameter configuration file locations. Table 15-6 lists the parameter configuration files that you can use.

**Table 15-6.** The parameter configuration files

| File extension | Description | Storage location |
|---|---|---|
| .psd | (Sequencing and STR genotyping only) Used for a given plate, a plate setup data file (.psd) allows you to specify the plate ID, plate setup parameters, and plate- and well-level attributes.<br>(SNP genotyping only) Used for a given plate set, a master.psd file allows you to specify the plate set ID, plate set setup parameters, and the attributes for the first plate injected. Additional, plate-specific .psd files can specify the attributes for each subsequent plate injected during the run. | …\MegaBACE\Instrument Control\Psd folder |
| .tpl | (Sequencing and STR genotyping only) Used for multiple plates, a plate setup template file (.tpl) allows you to specify the plate setup parameters. The .tpl file uses the same field names as the .psd file for the plate setup parameters.<br>(SNP genotyping only) Used for multiple plate sets, a plate setup template file (.tpl) allows you to specify the plate set setup parameters. The .tpl file uses the same field names as the .psd file for the plate set setup parameters. | …\MegaBACE\Instrument Control\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. | …\MegaBACE\Instrument Control\Templates folder |



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# Part six

# Appendixes

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# Appendix A Header formats

This appendix provides the standard header format and describes the extended header format used in the MegaBACE raw sample data files (.rsd) and base-called sample data files (.esd). The topics are—

- The standard header format (section A.1)
- Viewing and editing the data in the extended header (section A.2)
- The basic extended header format (section A.3)
- The extended header labels in sequencing files (section A.4)
- The extended header labels in STR genotyping files (section A.5)
- The extended header labels in SNP genotyping files (section A.6)

## A.1 The standard header format

Figure A-1 provides the format of the standard header format for the MegaBACE raw sample files (.rsd).



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| Offset | Size | Type | Variable | |
|---|---|---|---|---|
| 0 | 24 | char | m_tcSampleName[24] ; | // deprecated |
| 24 | 30 | char | m_tcOperator [30] ; | // unused |
| 54 | 4 | float | m_fRunVoltage ; | |
| 58 | 4 | float | m_fRunTime ; | // minutes |
| 62 | 4 | float | m_fInjectionTime ; | |
| 66 | 4 | float | m_fInjectionVoltage ; | |
| 70 | 4 | float | m_fTemperature ; | |
| 74 | 4 | int | m_nLaserIndex ; | // deprecated |
| 78 | 4 | float | m_fPMTVoltage1 ; | |
| 82 | 4 | float | m_fPMTVoltage2 ; | |
| 86 | 30 | char | m_csBeamSplitterA[30] ; | // deprecated |
| 116 | 30 | char | m_csBeamSplitterB[30] ; | // deprecated |
| 146 | 30 | char | m_csFilter1[30] ; | // deprecated |
| 176 | 30 | char | m_csFilter2[30] ; | // deprecated |
| 206 | 30 | char | m_csFilter3[30] ; | // deprecated |
| 236 | 30 | char | m_csFilter4[30] ; | // deprecated |
| 266 | 4 | float | m_fFillTime ; | |
| 270 | 4 | float | m_fFlushTime1 ; | |
| 274 | 4 | float | m_fFlushTime2 ; | |
| 278 | 4 | float | m_fFlushTime3 ; | |
| 282 | 4 | float | m_fPrerunVoltage ; | |
| 286 | 4 | float | m_fRelaxationTime ; | |
| 290 | 4 | float | m_fPrerunTime ; | |
| 294 | 64 | float | m_fSpecSepMx[4][4] ; | |
| 358 | 40 | char | m_csBaseOrder[4][10] ; | // deprecated |
| 398 | 22 | char | m_InstID[22] ; | |
| 420 | 30 | char | m_csParamSetName[30] ; | // deprecated |
| 450 | 4 | DWORD | m_dMagicNumber ; | // 826561349 |
| 454 | 4 | DWORD | m_dExtHeaderOffset ; | // new |
| 458 | 4 | DWORD | m_dExtHeaderLen ; | // new |
| 462 | 4 | float | m_fPreinjectionTime ; | // new |
| 466 | 4 | float | m_fPreinjectionVoltage ; | // new |
| 470 | 4 | float | m_fLowPressureTime ; | // deprecated |
| 474 | 4 | float | m_fUserInputTime ; | // new |
| 478 | 4 | float | m_fSleepTime ; | // new |
| 482 | 4 | float | m_fSleepTemperature ; | // new |
| 486 | 10 | char | m_csBuildNumber[10] ; | //format: 19990927A, includes \0 |
| 496 | 4 | int | m_nNumColumns ; | // maximum number of letter labels on the plate (depends on instrument type) |
| 500 | 4 | int | m_nNumRows ; | // maximum number of number labels on the plate (depends on instrument type) |
| 504 | 4 | float | m_fIntervalVoltage | |
| 508 | 2 | WORD | m_nIntervalTime | |
| 510 | 4 | float | m_fPMTVoltage3 | |
| 514 | 4 | float | m_fPMTVoltage4 | |
| 518 | 2 | WORD | m_wNumberOfCapillaries | |
| 520 | 64 | BYTE | m_byReserved[48] ; | |
| 568 | 6 | char | m_csRunID[6] ; | |
| 574 | 18 | char | m_csPlateID[18] ; | // deprecated |
| 592 | 4 | DWORD | m_dwPrevRunID ; | // unused |
| 596 | 4 | BOOL | m_bDWORDFile ; | // 1=raw,0=processed |
| 600 | 4 | BOOL | m_bSaturated ; | |
| 604 | 4 | float | m_fVersion ; | // 2.000 |
| 608 | 4 | time_t | m_tRunStartTime ; | |
| 612 | 4 | time_t | m_tRunStopTime ; | |
| 616 | 4 | DWORD | m_dwNumberOfLines ; | |
| 620 | 4 | int | m_nScanRate ; | // 0=1.75, 1=3.0 |
| 624 | 1 | BYTE | m_byFileType ; | // reserved |
| 625 | 1 | BYTE | m_byNumberOfCapillaries ; | // deprecated |
| 626 | 1 | BYTE | m_byCapUsage ; | |
| 627 | 1 | BYTE | m_byDummy ; | |
| 628 | 4 | UINT | m_uiSecurityCode ; | |

**Figure A-1.** Standard header format.



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## A.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 the chemistry names.

To start MegaBACE Header Editor, in the **MegaBACE** shortcut folder on the Windows desktop, double-click the **Header Editor** icon. The Header Editor window appears (figure A-2).

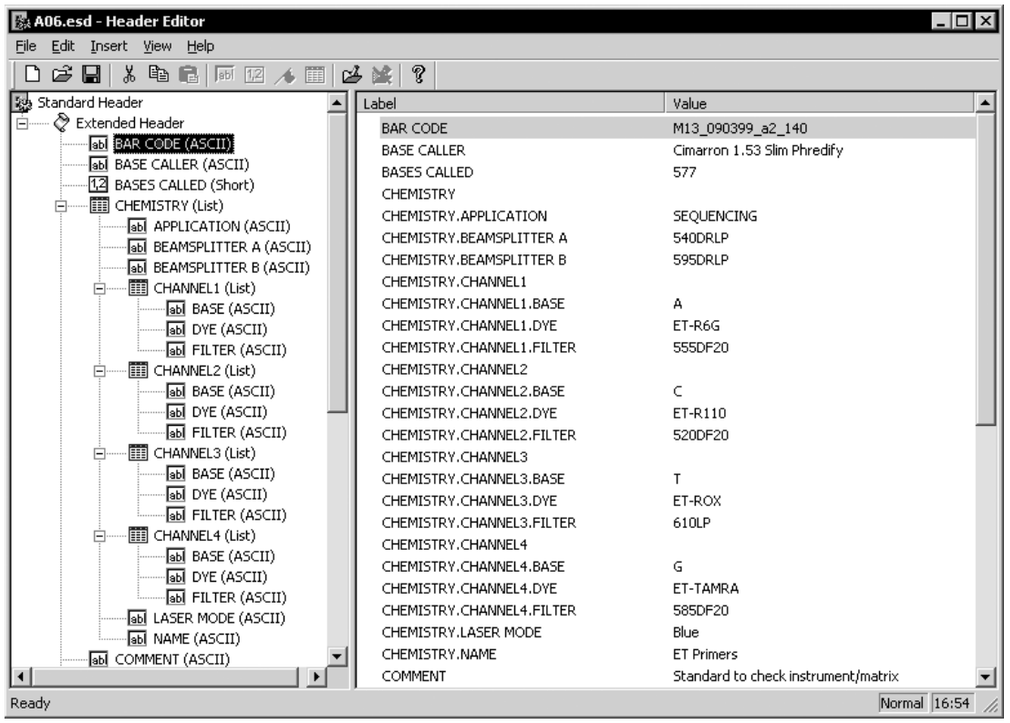



**Figure A-2.** The Header Editor window displaying the information for a selected well from a sequencing run.

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



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## A.3 The basic extended header format

The extended header is a variable length, variable content data block that is encoded using the Amersham Biosciences self-describing data protocol (appendix B).

All entries in the extended header are label-value pairs. The label is always an ASCII string. The values may be in any supported format.

The value associated with a label may be an array or a list. The entries in a list are also packed as label-value pairs. Therefore, the length of any list is always double the number of entries in the list.

The entries in the extended header are alphabetized by label. The entries within a list are also alphabetized.

The application programming interface (API) for retrieving values from the extended header accesses items in a list using a dot-delimited hierarchical name.

For example, CHEMISTRY.CHANNEL1.BASE would return the value associated with the label BASE from the CHANNEL1 list inside the CHEMISTRY value. (Labels are treated as case-independent.)

Any user-defined data imported from a plate setup data file (.psd) will be included in the extended header.

## A.4 The extended header labels in sequencing files

For sequencing runs, you can view the extended header labels in the raw sample data files (.rsd) and the base-called data files.

### A.4.1 The extended header in the sequencing .rsd files

The following labels will always appear in the raw sample data files (.rsd) for sequencing runs.



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**Note:** The indentations within CHEMISTRY denote nested lists.

BAR CODE
BASE CALLER
CHEMISTRY
    APPLICATION
    BEAMSPLITTER A
    BEAMSPLITTER B
    BEAMSPLITTER C
    CHANNEL1
        BASE
        DYE
        FILTER
    CHANNEL2
        BASE
        DYE
        FILTER
    CHANNEL3
        BASE
        DYE
        FILTER
    CHANNEL4
        BASE
        DYE
        FILTER
    LASER MODE
    NAME
COMMENT
MACHINE ID
PLATE ID
SAMPLE NAME
WELL ID



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### A.4.2 The bar code

BAR CODE is the string entered into the Plate ID field on the Plate Setup window. The bar code can be entered either by keyboard entry or with a bar code scanner. If there is no .psd file associated with the name, or if there is no PLATE ID entry in the .psd file, the plate ID will match the bar code.

### A.4.3 The extended header in base-called sample data files

All entries in a raw sample data file (.rsd) are preserved in the corresponding base-called sample data file (.esd). The following additional entries appear in base-called sample data files:

BASES CALLED
END GOOD SEQUENCE
END SEQUENCE
END SIGNAL
END TRACE PROCESSING
PEAK POSITIONS
QUALITY INDEX
QUALITY SCORES
SEQUENCE
START GOOD SEQUENCE
START SEQUENCE
START SIGNAL
START TRACE PROCESSING



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## A.5 The extended header labels in STR genotyping files

The following labels will always appear in the raw sample data files (.rsd) for STR genotyping runs.

**Note:** The indentations within CHEMISTRY denote nested lists.

BAR CODE
CHEMISTRY
    APPLICATION
    BEAMSPLITTER A
    BEAMSPLITTER B
    BEAMSPLITTER C
    CHANNEL1
        DYE
        FILTER
    LASER MODE
    NAME
COMMENT
MACHINE ID
PLATE ID
SAMPLE NAME
WELL ID

Note that BAR CODE is the string entered into the Plate ID field on the Plate Setup screen, either by keyboard entry or with a bar code scanner. If there is no .psd file associated with the name, or if there is no PLATE ID entry in the .psd file, the plate ID will match the bar code.

## A.6 The extended header labels in SNP genotyping files

The following labels will always appear in the raw sample data files (.rsd) for sequencing runs.

### A.6.1 Additional extended header labels for SNP genotyping

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

- **Plate set attributes—**The attributes that apply to the entire run appear at the top level of the extended file header. Table A-1 lists the additional top-level labels for SNP genotyping.



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- **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 A-2 lists the labels for the injection-specific attributes.

**Table A-1.** Plate set fields in the extended header

| Field | Description |
|---|---|
| CHEMISTRY<br>APPLICATION<br>BEAMSPLITTER A<br>BEAMSPLITTER B<br>BEAMSPLITTER C<br>CHANNEL<br>BASE<br>DYE<br>FILTER<br>LASER MODE<br>NAME | Always present. The chemistry parameters are a plate set definition setting (section 12.2).<br>**Note:** The indentations within CHEMISTRY denote nested lists. |
| COMMENT | (Optional) Plate set definition setting (section 12.2). |
| MACHINE ID | Always present, the software adds the ID of the MegaBACE instrument. |
| WELL ID | Always present, the well ID associated with the corresponding .rsd file. |
| INTERVAL VOLTAGE | Always present, a plate set definition setting (section 12.2). |
| INTERVAL TIME | Always present, a plate set definition setting (section 12.2). |
| NUMBER OF INJECTIONS | Always present, the 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 13.2.2). |
| All user-defined plate set labels | (Optional) Values may be present if a .psd file is used. |



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**Table A-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 STR 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 STR 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 STR 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. |



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# Appendix B Protocol for self-describing data

This appendix describes a protocol for packing self-describing data into a file. The topics are—

- Introduction (section B.1)
- The 1-byte header (section B.2)
- The length value (section B.3)
- The data buffer (section B.4)
- Notes about the protocol (section B.5)

## B.1 Introduction

The extended header consists of a list of keyword-value pairs. However, the protocol is completely general and can be used to pack data of almost any form.

In the protocol, a data block consists of a series of elements, where each element contains:

- A 1-byte header that contains two fields.
- A length value that consists of 1 to 3 bytes (except for Terminator, which is always zero) as determined by the number of length bytes field in the header.
- A data buffer that contains the number of bytes specified in the length value.

## B.2 The 1-byte header

The 1-byte header contains two fields:

- A 6-bit format field that defines the type of data contained in the element. The format field consists of the most significant 6 bits of the header byte.
- A 2-bit field that states the number of length bytes used to specify the size of the data storage. The number of length bytes field consists of the least significant 2 bits of the header byte. This field is zero for Terminator. For all other formats it must be nonzero.



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Figure B-1 shows the protocol.

| Format (octal) | Symbol | Description |
|---|---|---|
| 00 | Ln | List (where n is a number) |
| 01 | An | ASCII |
| 02 | Cn | Unicode |
| 10 | B | Boolean ( 1 byte: 1=true, 0=false ) |
| 31 | U1 | 1-byte unsigned integer (unsigned char) |
| [32 | U2 | 2-byte unsigned integer (unsigned short) ] |
| [34 | U4 | 4-byte unsigned integer (unsigned long) ] |
| 40 | F8 | 8-byte floating (double) |
| 44 | F4 | 4-byte floating (float) |
| [51 | S1 | 1-byte signed integer (char) ] |
| 52 | S2 | 2-byte signed integer (short) |
| 54 | S4 | 4-byte signed integer (long) |
| 77 | T | Terminator |

**Figure B-1.** The protocol for self-describing data.

**Notes to figure B-1:** The symbol that represents a format is used only in readable dumps of the data block. The symbol is not embedded in the data structure itself.

The lines delimited with square brackets indicate format codes that are not allowed in the extended header because those data types are not supported by the (COM) interface that enables the header.

## B.3 The length value

The length value consists of 1 to 3 bytes (except for Terminator) as determined by the number of length bytes field in the header.

If the format is List, this field contains the number of elements in the list. Otherwise, it contains the number of bytes of data storage used in the element.

**Note:** The number of individual data values in the element (that is, the array size) is the length divided by the size of each value.

## B.4 The data buffer

For all formats except List, the data buffer contains the number of bytes specified in length. For List, the number of elements is specified in length.



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## B.5 Notes about the protocol

The protocol packs the elements as a byte stream. In all cases, multibyte data are packed with the least significant byte first. This means that the packing/unpacking software will differ depending on whether the platform implements a big endian or little endian architecture (x86 is little endian).

The size of the data block may not be known a priori. If the size is indeterminate, the Terminator format code can be used to tell the unpacking algorithm where to stop. If the size of the data block can be unambiguously determined, the use of the Terminator is optional.

Character strings (A and C formats) are null terminated. (See figure B-1 for notations.)

The List format is used for creating complex data structures, which are treated as single elements. If the value requires multiple parameters of different data types (for example, character, string, or int, with int to represent the base, filter center, and filter width for each channel in the Chemistry specifier), the parameters can be specified inside a List, thereby making them a single value associated with the corresponding Keyword.

Human-readable representation (dump format) is as follows:

- By convention, the format and data for each element are listed between angle brackets. If the element is represented on a single line, the brackets appear on the same line. Otherwise, the brackets are vertically aligned. Nesting is indicated by indentation.
- For List elements, the number of elements in the List is appended to the L format identified. For example, L3 denotes a list containing three elements (figure B-2).

  **Note:** An embedded List counts as a single element for its enclosing List. Lists may be nested to any depth.

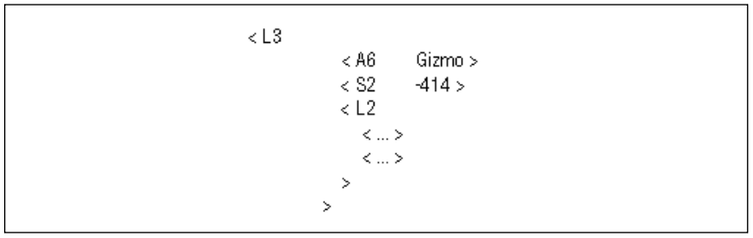



**Figure B-2.** Example of the List format. (See figure B-1 for notations.)



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For character elements (A and C formats), the number of characters is appended to the format identifier. For example, A6 denotes a five-character ASCII string with its terminating null. Strings are not surrounded by quotes unless the quote characters are supposed to be encoded as part of the string.

All other data types are treated as arrays if the element contains more than one item, where the array size is shown in square brackets. For example, U2[4] indicates an array of four 2-byte unsigned integers (figure B-3).

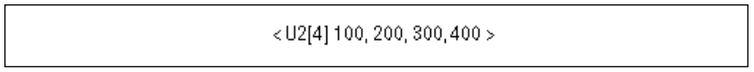



**Figure B-3.** Example of an array. (See figure B-1 for notations.)



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# Appendix C Launching a cleanup script after a run

**Important** **To write scripts that are launched using the CLEANUP COMMAND field, you should be familiar with Windows Shell programming.**

The CLEANUP COMMAND field in the MegaBACE.ini file allows you to specify a program or batch file to be executed at the end of the run for the selected application (sequencing, STR genotyping, or SNP genotyping). (For the sequencing application, the program or batch file can also be executed after automated base calling.) For instance, you can use the CLEANUP COMMAND feature to transfer plate records and raw data files to another workstation for analysis or archiving. This appendix describes how to use the CLEANUP COMMAND field in the MegaBACE.ini file. The topics are—

- Using the CLEANUP COMMAND (section C.1)
- Batch file examples (section C.2)

## C.1 Using the CLEANUP COMMAND

To use the CLEANUP COMMAND feature:

1. Create the program executable file (.exe) or batch file (.bat) that you want the Instrument Control Manager to launch. See section C.2 for examples of batch files (.bat).
   - Make use of the program arguments that the Instrument Control Manager supplies at run time. See tables C-1 and C-2 for a list of arguments.
   - Make a note of the location where you store the .exe or .bat file.
2. Open the MegaBACE.ini file, and locate the section for the application you are running (Sequencing, Genotyping, or SNP). In the value column for the CLEANUP COMMAND field, type the file path of the .exe or .bat file. For example:

| Field in MegaBACE.ini file | Value |
|---|---|
| CLEANUP COMMAND | ..\test.bat |

Make sure you use a tab (or tabs) between the CLEANUP COMMAND field and the text you type.



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The file path that you type in the Value column must be the full path of the .exe or .bat file or a path relative to the MegaBACE\Instrument Control folder. For example, if the file is stored in:

- **"...\MegaBACE\Instrument Control" folder—**Type only the file name, for example, test.bat.
- **Any folder other than the Instrument Control folder—**Type either the:

  Relative path, for example, ..\test.bat for a file stored in the MegaBACE folder

  Full path, for example, D:\test.bat

**Note:** You do not necessarily need to use quotes around the path even if it contains spaces.

3. Restart the Instrument Control Manager, and perform the run. See the *MegaBACE 4000 Instrument User's Guide* for details.

At the end of the run (after base calling, if any, for sequencing), the Instrument Control Manager runs the command with the program arguments (tables C-1 and C-2), which the Instrument Control Manager provides dynamically. The command runs in the background (without displaying a window).

**Table C-1.** Instrument Control Manager arguments for the sequencing and STR genotyping applications

| Argument number | Description* |
|---|---|
| 1 | The plate ID. |
| 2 | The full path of the run folder (in quotes). |
| 3 | The application name, Sequencing or Genotyping.<br>**Note:** The application name appears in the title bar of the Instrument Control Manager windows. |
| 4 | The bar code of the plate. This is the text that was entered in the Plate ID field during plate setup. |

* Items allowed to contain spaces are passed in quotes.



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**Table C-2.** Instrument Control Manager arguments for the SNP genotyping application

| Argument number | Description* |
|---|---|
| 1 | The plate set ID. |
| 2 | The full path of the run folder (in quotes). |
| 3 | The application name, SNP Genotyping.<br>**Note:** The application name appears in the title bar of the Instrument Control Manager windows. |
| 4 | The bar code used for the plate set definition. This is the text that was entered in the Plate Set ID field during plate set setup. |

* Items allowed to contain spaces are passed in quotes.

## C.2 Batch file examples

This section describes some simple examples of batch files (.bat) that could be specified for the CLEANUP COMMAND field.

### C.2.1 Displaying the full set of arguments after the run

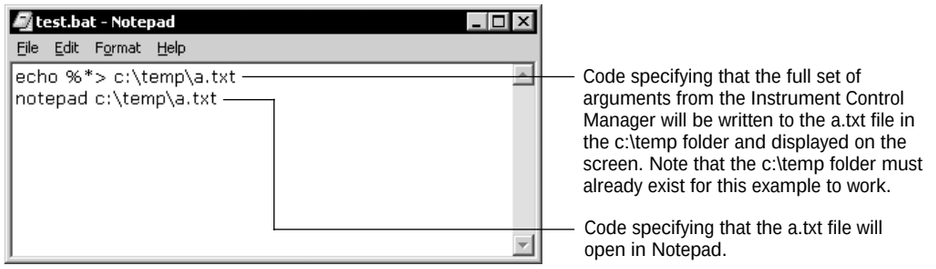
In the test.bat file example (figure C-1a), the code specifies that the full set of arguments, supplied by the Instrument Control Manager, will appear on screen in a Notepad file entitled, a.txt. Figure C-1b shows the resulting file that appears after the run.

In order for the Instrument Control Manager to launch the test.bat file (figure C-1a) after the run, the .bat file name and file path must be specified in the MegaBACE.ini file (section C.1), and the .bat file must reside at the specified location.



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**(a) An example of the code to display the full set of arguments in a Notepad file**




**(b) The resulting display that appears on the computer screen at the end of the run**

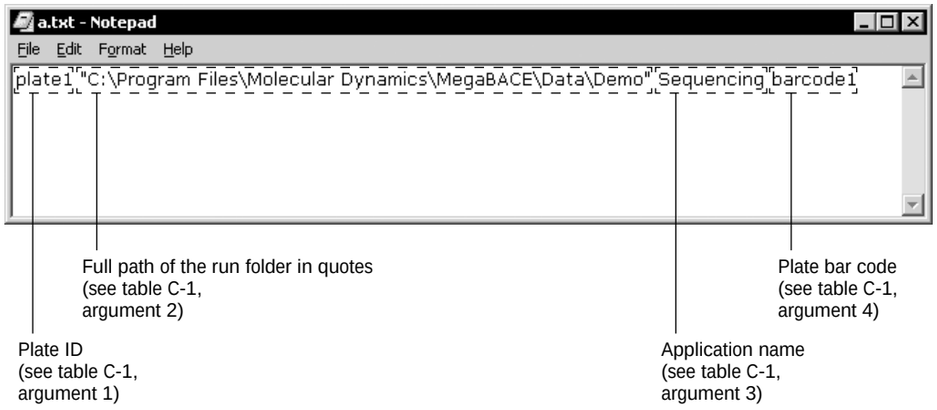



**Figure C-1.** An example of a .bat file displayed in Notepad (a), and the on-screen display that appears as a result of the code (b).



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## C.2.2 Coping the raw sample data files to another folder

Figure C-2 shows an example of the code to copy the raw sample data files (.rsd) from the run folder to another folder after the run. Figure C-2 shows the .bat file name and file path specified in the MegaBACE.ini file.

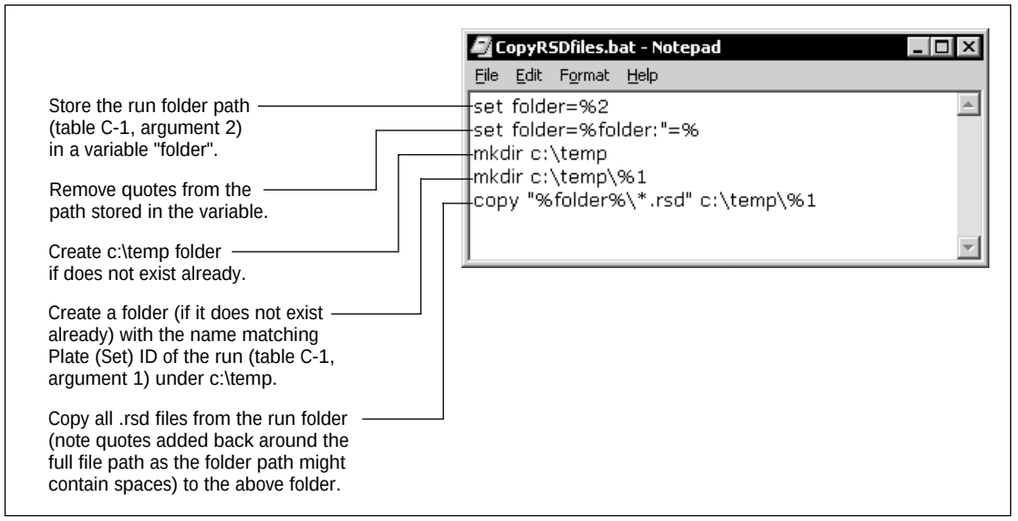



**Figure C-2.** An example of a .bat file displayed in Notepad and the functional explanation for each line of code.



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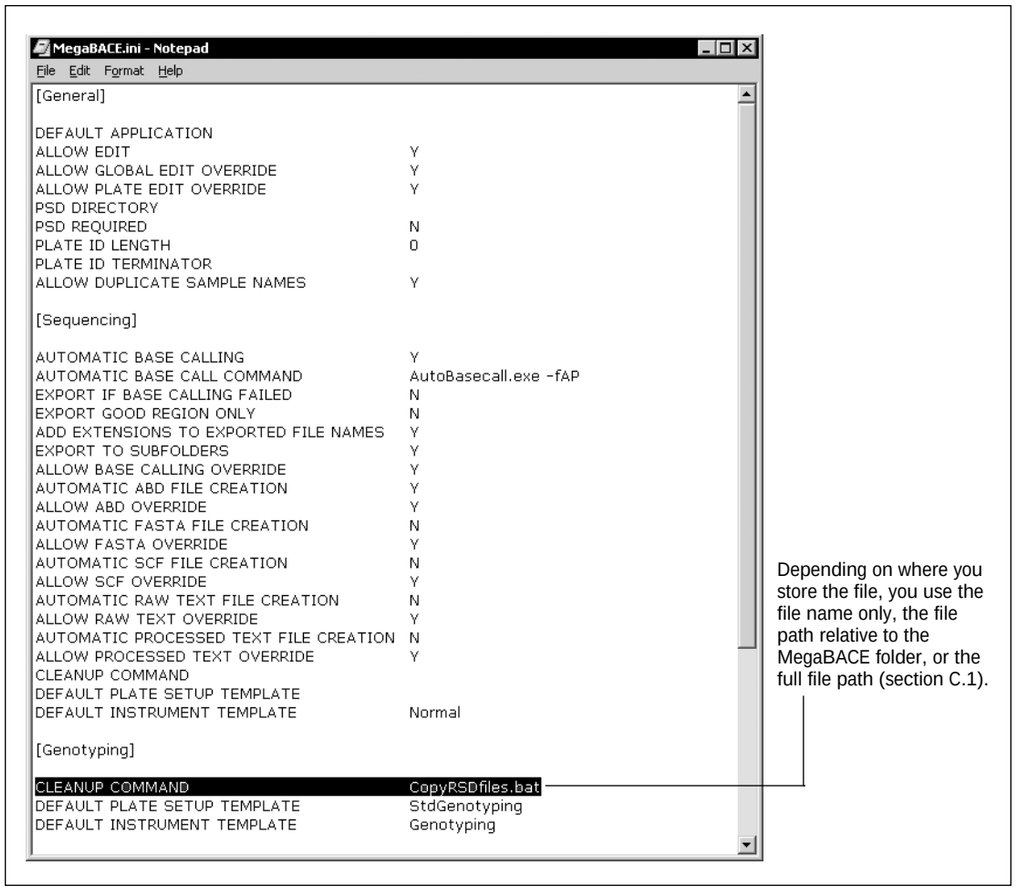



**Figure C-3.** The MegaBACE.ini file showing the CLEANUP COMMAND field for STR genotyping. Note that only the file name is required if the .bat file is stored in the MegaBACE\Instrument Control folder.



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# Glossary

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

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

**.icp files—**the instrument control 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 automatically import attributes for a plate and for individual wells on the plate, such as sample names. The Instrument Control Manager includes the information from the .psd file in the extended header of each raw sample data file (.rsd).

(SNP genotyping only) The plate setup data files allow you to specify attributes for a plate set or a plate.

**.rsd files—**the raw sample data files, each of which contains the raw data for a given well on a plate (for example, A01). The storage location is in a corresponding raw run folder in the …\MegaBACE\Data folder (default) or the location you specify.

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

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



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**.tpl files—**the plate setup template files. For sequencing and STR genotyping, the .tpl file can specify the plate setup parameters, including the electrophoresis conditions, the chemistry parameters, the file names, and optional parameters, such as the base caller.

For SNP genotyping, the .tpl file can specify the plate set setup parameters, which apply to the entire set of plates injected during a single run.

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

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

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

**channels—**see spectral channel.

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

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

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

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



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

**marker**—see SNP marker of STR marker.

**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.

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

**plate definition—**includes the plate ID and plate or plate set setup parameters.

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

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

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



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

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

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

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

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

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

**sample name—**(sequencing and STR genotyping only) the designation you provide for a given well in the Sample Names tab or in a plate setup template or .psd file.

(SNP genotyping only) the sample name is the designation you provide for a given well for a given injection (plate) in the multi-injection run. A plate setup data (.psd) file is required for each injection for which you want to assign sample names.

**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).

**SNP (single nucleotide polymorphism)**—(SNP genotyping only) 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, you perform a multi-injection run, and then you use SNP Profiler to perform the SNP genotyping analysis.

**SNP marker**—(SNP genotyping only) any genetic locus containing a single-base variation or polymorphism.

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



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**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.

**STR marker—**short-tandem-repeat (STR) marker, also known as a microsatellite marker, refers to any genetic locus containing a repeating variation on a chromosome. An STR marker consists of two primers that are used in the PCR reaction.

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



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