ABI PRISM® GeneMapper™ Software Version 3.0

User's Manual

Applied Biosystems

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About GeneMapper Software

1

Chapter Overview

Introduction

This chapter describes the ABI PRISM® GeneMapper™ Software Version 3.0, the relationship of GeneMapper software to the current ABI PRISM® genotyping software, and the requirements for installing and starting GeneMapper software.

In This Chapter

This chapter contains the following topics:

Overview of GeneMapper Software

About GeneMapper Software

The GeneMapper software v3.0 is a new release of GeneMapper software. This release of GeneMapper software has additional features and enhancements similar to the functionality of ABI PRISM® GeneScan® and Genotyper® analysis software. GeneMapper software provides automated genotyping for linkage analysis, SNP validation, population genetics, and human identification users.

Supported Instruments

The GeneMapper software v3.0 supports data generated from the following instruments:

- ABI PRISM® 310 Genetic Analyzer
- ABI PRISM® 377 DNA Sequencer
- ABI PRISM® 3100-*Avant* Genetic Analyzer
- ABI PRISM® 3100 Genetic Analyzer
- ABI PRISM® 3700 DNA Analyzer
- Applied Biosystems 3730 DNA Analyzer
- Applied Biosystems 3730xl DNA Analyzer (for 48 capillary instruments only)

Supported Chemistry Kits

The GeneMapper software v3.0 is specifically designed to work with the following applications and reagents:

- ABI PRISM® Linkage Mapping Set v.2.5
- ABI PRISM® Mouse Mapping Primers v.1.0
- ABI PRISM® SNaPshot® Multiplex Kit
- ABI PRISM® SNaPshot® Primer Focus™ Kit
- AmpFℓSTR® Kits
- StockMarks® Animal Parentage Typing Kits
- ABI PRISM® GeneScan™ Size Standards
- Custom Microsatellites

Unique Features of GeneMapper Software v3.0

Several new features and enhancements of GeneMapper software v3.0 include:

- Display of polyploid genotypes
- Remote database connection
- Microsoft® Windows® 2000 operating system support
- A clean installation along with upgrades for the 3100 and 3100-*Avant* Data Collection computers
- Full integration with the Applied Biosystems 3730 DNA Analyzer including automation capabilities
- Expanded technical portfolio (*i.e.*, mutation screening)

Descriptions of Benefits

GeneMapper software using ABI instruments, Linkage Mapping Set (LMS), AmpFℓSTR® HID markers, or SNaPshot® Multiplex, provides capabilities that go beyond the ABI PRISM® GeneScan® and Genotyper® software products. It provides a genotyping capability that sets new standards for high-throughput and automated genotyping, with the following benefits.

Genotyping benefits of the GeneMapper software

Benefit	Description
High accuracy for size- and allele-calling	Sophisticated algorithms such as Multiple Allele Peak Determination (MAPDA), Automatic Bin Builder (ABB), and Automatic Bin Assignment Algorithms (ABAA) assure highly accurate allele calls .
High-throughput	In a recommended computing platform, GeneMapper software processes over 50,000 genotypes in 1 hour. This is sufficient capacity to analyze all the sample files accumulated by an Applied Biosystems® 3730 DNA Analyzer run overnight at peak capacity in 1 to 2 hours.

Genotyping benefits of the GeneMapper software *(continued)*

Benefit	Description
Fully automated operation	Process Component-Based Quality Values (PQV) monitor major components of the size- and allele-calling process, informing a user of the source of problems anywhere along the data analysis process.
Ease of use	One-button operation is provided with fully integrated, multi-step size-calling, allele-calling, and intelligent data management (using the GeneMapper database).

Unique features of the GeneMapper software v3.0

Unique Feature	Description
Multiple Allele Peak Determination Algorithms (MAPDA)	For non-forensic data, the MAPDA are used. For forensic data, the Human Identity Caller (HIC) algorithm is specifically optimized to handle data containing tetranucleotide repeats and allelic ladders.
Automatic Bin Builder (ABB) Automatic Bin Assignment Algorithms (ABAA)	After allele peaks are called using MAPDA, the ABB is used to create the bins, optimizing the distances between bins and the precise locations of bin centers. The ABAA then completes the allele-calling process by assigning allele peaks to their corresponding bins.
Process Component-Based Quality Values (PQV) (See Appendix A for more information.)	After bin building and allele assignment, bin quality values are assigned to these bins based on the success rate of the bins to locate allele peaks correctly from the sample file collection. The PQV system facilitates fully automated operation with only questionable allele calls, those with minor component related problems, requiring any intervention by the user. Bad samples, defined as being below the threshold of acceptability, can be discarded without any examination.

Unique features of the GeneMapper software v3.0 *(continued)*

Unique Feature	Description
GeneMapper Database	The GeneMapper database stores the following data: ♦ Predefined size standard definitions included with installation and custom size standard definitions ♦ Panel, marker, and allele bin definitions ♦ Analysis methods ♦ Table profiles (for generating tabular reports) ♦ Projects containing analyzed data (allele calls, confidence values, analysis methods, and size standard.)
Automated operation for all but problematic data	Automated allele calling is made possible by instrument and software calibration and setup steps, done once for many samples (including user-defined criteria). It is also made possible by the PQV system described above. Bad or "out-of-bounds" data can be safely discarded, and only data with minor component problems is deferred for user intervention.

Important Features

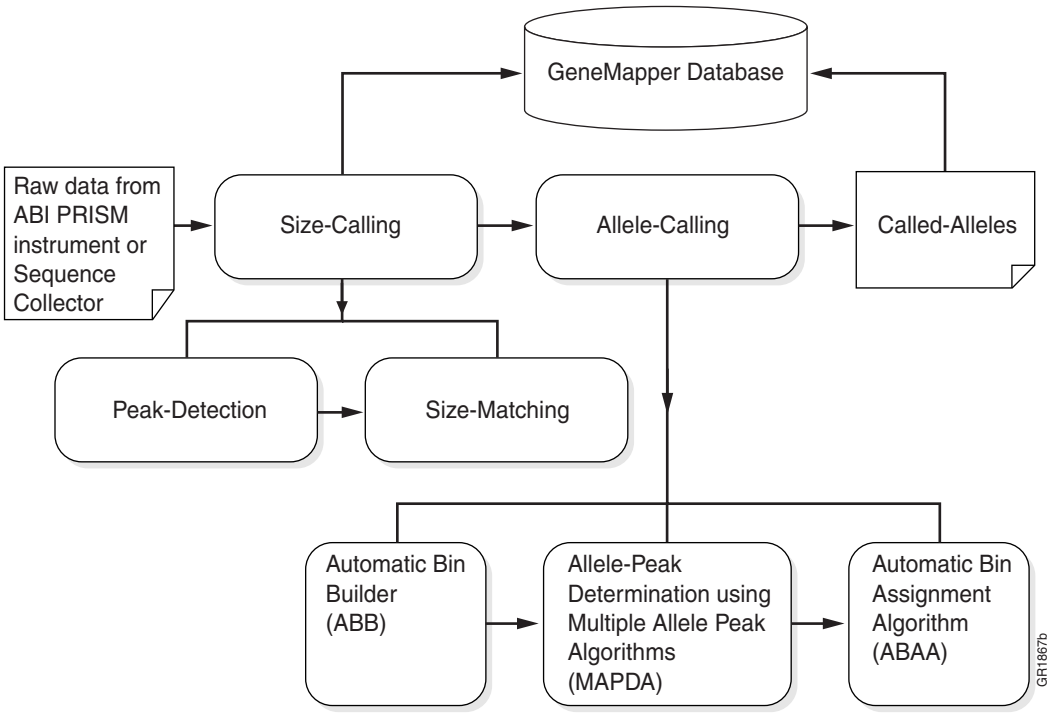
GeneMapper software v3.0 has a number of features that are built on the automated genotyping capabilities of the ABI PRISM GeneScan® and Genotyper applications. The table below lists important features of the GeneMapper software.

Feature	Description
Ability to read genetic analysis data produced by earlier ABI PRISM genotyping applications	You can import sample files directly into the GeneMapper database from either the ABI PRISM® Data Collection software or Sequence Collector database. It is not necessary to use separate analysis tools such as GeneScan software and Genotyper software for peak quantitation and allele calling. **Note** GeneMapper software has read-only access to the Sequence Collector Version 3.0 database. Therefore, GeneMapper database results cannot be written back to the Sequence Collector Version 3.0 database. Instead, they are stored in individual GeneMapper projects within the GeneMapper database.
Ability to read and process sample (.fsa) files containing a fifth dye. Provides fourth and fifth dye support.	Data containing a fifth dye can be analyzed just like the other four dyes.
Ability to export results in a user defined format	The final results from the GeneMapper software table can be exported as user-defined text files.
Ability to import panel, marker, and Bin definitions	Marker and bin definitions can be imported into the GeneMapper database using tab-delimited text files.
Ability to export panel, marker, and bin definitions	Marker and bin definitions can be exported from the GeneMapper database as tab-delimited files.

Feature	Description
Ability to create bin sets for different instruments	Bin definitions, which may vary between instrument types, can be stored as separate bin sets allowing simultaneous analysis of data from different instrument types in the same project.
Ability to correct failed in-lane size standards	Individual samples that fail size standard matching can be corrected as soon as they are incurred without redefining the size standard definition.

How GeneMapper Software Works

The following figure shows how data flows through the GeneMapper software system.



Hardware and Software Requirements

Hardware and Software Requirements

The following table describes the components your computer system requires to run GeneMapper software.

IMPORTANT The Oracle database has an embedded license which only permits five named users.

Note In order to perform all functions in GeneMapper software (with its embedded Oracle database), please ensure there are at least 500 MB of free space on the partition where the GeneMapper database is installed. It is possible to export your projects to a different drive or partition, and then delete those projects from the database to make room for new projects. (See Chapter 3, "Using the Project Window.")

System Component	Minimum Requirement	Recommended
Computer	♦ Intel® Pentium® III processor minimum of 540 MHz ♦ 256 MB of RAM[a] ♦ 6 GB hard disk (free space)	♦ Intel Pentium III processor faster than 700 MHz ♦ 512 MB RAM ♦ 10 GB EIDE hard drive ♦ 20/48X IDE CD-ROM ♦ 10/100 NIC with RWV (internal)
Monitor	800 x 600 pixels size 17-inch monitor	Larger monitor
Operating System	Microsoft® Windows NT® version 4.0 (Service Pack 5)	Microsoft Windows NT 4.0 Service Pack 5 or Windows 2000 Professional Service Pack 2
Ethernet Capability	Network card for Oracle® installation. TCP/IP must be installed prior to Oracle installation.	—

a. There are functions within the GeneMapper software that run faster with more than 256 MB RAM. Applied Biosystems recommends using at least 512 MB RAM for the best results.

Special Considerations

The following is a list of special considerations for running the GeneMapper software v3.0:

- The ABI PRISM® 3100 Genetic Analyzers require the 3100 Data Collection Software version 1.0 and a minimum of 512 MB RAM.
- The Applied Biosystems 3730/3730xl Genetic Analyzers require the 3730 Data Collection Software version 1.0.
- When using the GeneMapper software v3.0 with the ABI PRISM® 3700 DNA Analyzer, the software must be installed on a separate computer.
- GeneMapper software v3.0 requires a single-processor computer.
- GeneMapper software v3.0 runs on Windows NT® and Windows® 2000 platforms only. Conversion utilities are included for analyzing data from MacIntosh®-based sample files.
- GeneMapper software v3.0 is not compatible with Xeon chips on upgrades from GeneMapper software v.1.0.2.
- The version of Oracle® database in the GeneMapper software v3.0 is an embedded license for use by five (named) users only.

IMPORTANT To accommodate more than five users, additional GeneMapper software or Oracle database licenses must be purchased.

- When installing GeneMapper software v3.0, the user must log into the local machine and have administrator privileges.
- GeneMapper software v3.0 can exist with other Oracle clients but not other Oracle servers. The only exception to this rule is when the ABI PRISM® 3100 Data Collection Software version 1.0 and/or the Applied Biosystems 3730 Data Collection Software version 1.0 is installed on the system.

Registering GeneMapper Software

How to Register

To register, fill out the registration card included in this package and return it to Applied Biosystems, and read the information in Chapter 2, “Installing and Registering the GeneMapper Software” on page 2-3.

For Applied Biosystems technical support telephone and address information, see “Obtaining Technical Support” on page 1-12.

Note Installation privileges are available only if you have returned your registration card.

Obtaining Technical Support

Applied Biosystems Website

A services and support page is available on the Applied Biosystems Web site. To access this, go to:

http://www.appliedbiosystems.com

and click the link for services and support.

At the services and support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the services and support page provides worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Using GeneMapper Software 2

Chapter Overview

Introduction

This chapter describes techniques for collecting and preparing sample data that will help you improve the overall quality of the fragment analysis sample files you import into the ABI PRISM® GeneMapper™ Software Version 3.0. It also describes how to plan for use of the GeneMapper software features in your genotyping application.

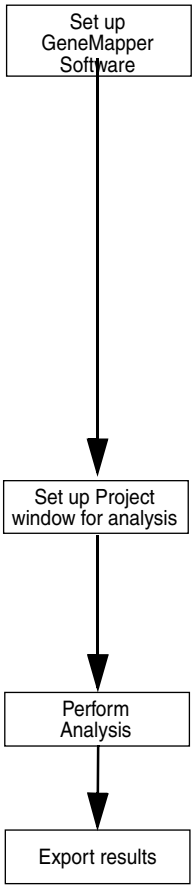
In This Chapter

This chapter contains the following topics:

Steps Required to Use GeneMapper Software

Using GeneMapper

The following steps are required to use the GeneMapper software. You will be guided through this process in more detail by reading the *ABI PRISM® GeneMapper™ ID Software v3.0 Human Identification Analysis Tutorial* (P/N 4335523), *Microsatellite Analysis with ABI PRISM® GeneMapper™ Software v3.0 Tutorial* (P/N 4335525), and *SNP Genotyping with ABI PRISM® GeneMapper™ Software v3.0 Tutorial* (P/N 4335524).



- Set up the GeneMapper software the first time for each analysis application:
  - Import or create panels and bins in the Panel Manager.
  - Create an analysis method suitable for your application with appropriate bin sets. No bin set is needed if you are performing a sizing-only application.
  - Create the appropriate size standard(s) if your data uses size standards other than those provided with GeneMapper software.
  - Set up options so that the same setup will apply the next time you use GeneMapper software, if desired.
  - Convert any Macintosh®-generated sample files to the .fsa format. (See Appendix E for details.)
- Set up the Project window for analysis of a given set of data:
  - Import the fragment analysis sample files.
  - Select the appropriate analysis method.
  - Choose the appropriate panels.
  - Select the size standard.
- Perform analysis and examine the results:
  - Initiate analysis.
  - Examine analysis results using the Plot window(s).
- Export results:
  - Set up table format for export (using Table Settings Editor).
  - Export (using Export Table command).

Installing and Registering the GeneMapper Software

No Prior Oracle Installation

An important requirement for the GeneMapper software installation is that no previous Oracle® installation be in place at the time of installation except for 3100 Data Collection Software (DCS), the 3100-Avant DCS, and the 3730 DCS.

CD-ROM Contents

IMPORTANT Do not work off of the CD-ROM. Install the software to the selected drive.

GeneMapper software comes on a CD-ROM and includes:

- Microsatellite, Human Identification (HID) marker, bin definitions and example data, and SNP genotyping tutorial data
- AppleScript® sample file conversion utilities
- Electronic (PDF)® version of the user's manual
- Size standard folder which contains various size standards
- Panel folder which contains Linkage Mapping and AmpFℓSTR panels and bins

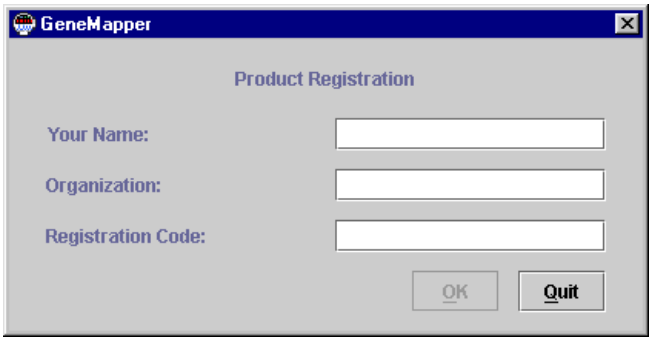
Installing GeneMapper Software

To install GeneMapper Software version 3.0, please use the instructions in the ABI PRISM® GeneMapper™ 3.0 Software Installation Instructions, P/N 4338561, found in the kit contents or use the *GeneMapper 3.0 Installation.txt* file on the CD-ROM.

Registering GeneMapper Software

When you are ready to use the GeneMapper software, follow the instructions in the table below for registration.

To register GeneMapper software:

Step	Action
1	Click **Start** > **Programs** > **Applied Biosystems** > **GeneMapper**. The first time you start the GeneMapper software, the Product Registration dialog box opens. 
2	Enter your name, organization, and registration code, and click **OK**. **Note** The first time you use the application, you are asked to enter the registration code found on your registration card. GeneMapper software then verifies the code. Keep your registration code in a place where you can easily retrieve it. If you need to reinstall the software at any time, you will be prompted for the registration code again.

Logging on to and Logging out of the GeneMapper Software

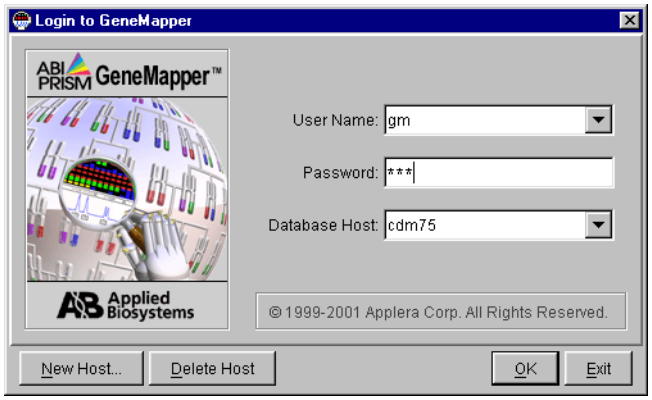
Introduction

This section provides information on logging on to and logging out of the GeneMapper software.

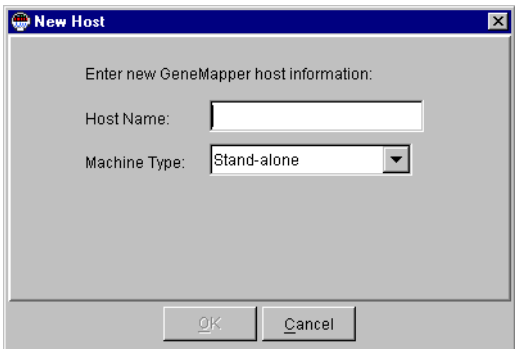
Logging on to the GeneMapper Software

You must log on to the GeneMapper software program with a user name, so that the system can log information to the database and preserve the options for each user.

To log on to the GeneMapper software:

Step	Action
1	When you start the GeneMapper software application, the following dialog box opens. Select your user name, if it exists, enter your password, and click **OK**.  **IMPORTANT** If you do not have a User Name, select the default **gm**, enter the password ***ifa,*** and click **OK** to launch the GeneMapper software. You can go to **Options** from the **Tools** menu and create new users and passwords.

To log on to the GeneMapper software: *(continued)*

Step	Action
2	Click the **New Host** button to open the New Host dialog box.  This option allows you to connect to GeneMapper software v3.0 databases on other computers.
3	In the **Host Name** field, enter the machine name or IP address of the database host. The window displays the new database host and its user names list. **Note** If the GeneMapper software cannot connect to the database host you entered, the following error message opens: "You have entered an invalid host." Click **OK** to exit and reenter the database host information.
4	Select the appropriate machine type from the drop-down list.
5	Click **OK**.

GeneMapper Software Access Rules

The following rules apply to access the GeneMapper software.

- The first time the GeneMapper software application is launched after installation, user registration is required.
- Once registered, all users can read, write, and edit everything in the database (except predefined content) because there are no access privileges.
- Sequence Collector access information is specified in the Options dialog box in the Tools menu.
- Only one user can access a database at a time.
- Database connections can only be made across computers using GeneMapper software v3.0 and not earlier versions of the software.

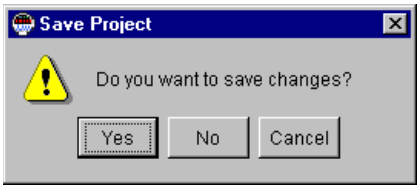
Logging Out

Close the GeneMapper software in one of three ways.

- Click **File > Logout**,
- Click **File > Exit**, or
- Click the **Close** button  .

If you close GeneMapper software with a new or blank Project window (or no changes in your Project), the Save Project (logout) dialog box does not open.

When you log out of the GeneMapper software, you have the option of saving or discarding the changes you made since the last time you updated the project..



If you close the GeneMapper software using the Logout command, the login window reopens after you select either “Yes” or “No”. GeneMapper software closes completely if you use either the Exit command or the Close button.

Importing Data from the Analysis Sample Sheet

Introduction

The sample sheet output from the ABI PRISM instrument used to collect data provides important input to GeneMapper software to identify the lane number and contents of each sample to undergo analysis. This section identifies the sample sheet information used by GeneMapper software so that you can correctly set up the sample sheet.

For detailed information on how to fill out a sample sheet correctly, see the user's manual for the appropriate ABI PRISM instrument.

Transfer of Information

Data is transferred from a sample sheet into GeneMapper software as described in the following table.

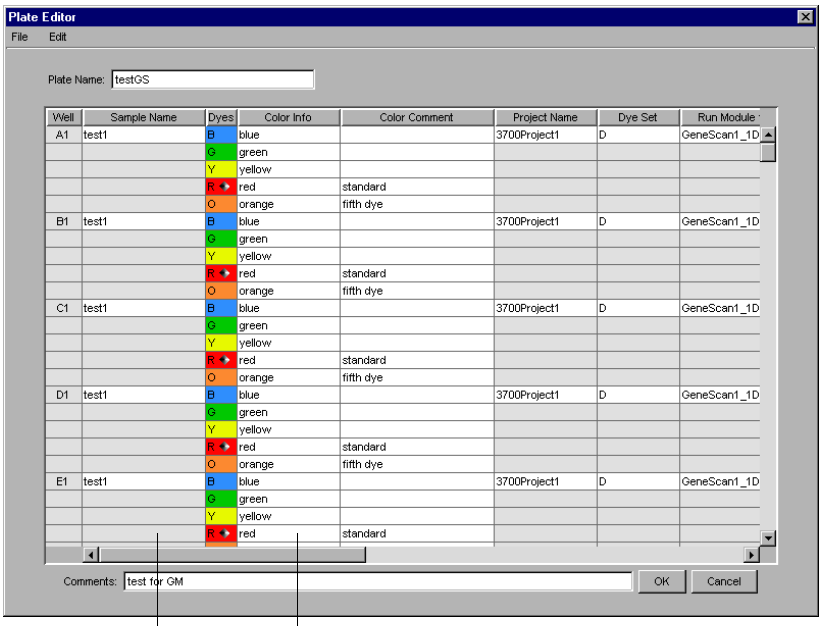
Sample Sheet	GeneMapper Software
Sample file name and Sample name **Note** These names are the same between a sample sheet and GeneMapper software.	Sample file name and Sample name
Sample Info or Color Info	Sample type (Control, Allelic Ladder, Sample, Negative Control)
Comment	Panel name
Comment after Panel name. Use the pipebar (|) to separate text into user defined columns. **Note** The 3730 has user defined columns 1-3 in the sample sheet.	User Defined Columns 1-3

Using Sample Sheet Information

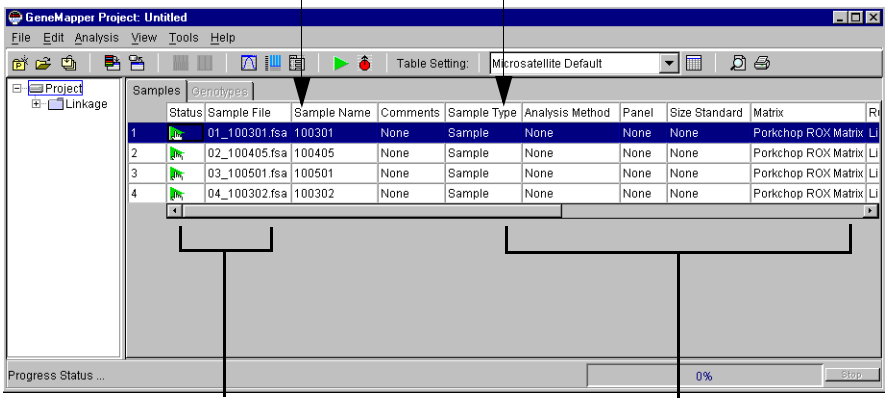
The sample sheet information is essential for associating the nature of sample fragments with individual dye/lanes and tables in the GeneMapper software.

For example, the following figures show how the GeneMapper software incorporates information entered in the Sample Name, Color Info, and the Color Comment fields of a sample sheet into GeneMapper software Project tables. It is your responsibility to ensure that the entries on the sample sheet for your particular ABI PRISM instrument are correctly set up to provide GeneMapper software with the necessary information.

Sample Sheet



GeneMapper Software Project Table



User defined

Read from Sample File

Find Command You can increase the utility of the Find (Ctrl+F) command in GeneMapper software by carefully planning the format of the

information you put into the Sample Info or Color Info field of the sample sheet.

Example of How To Use the Find Command

One Method	
If...	**Then...**
you have 12 samples, numbered 1, 2, 3,..., 12, and you enter these numbers into the Sample Info field	when you search for all dye/lanes containing a **1** in the Sample Info field, not only will you select sample 1, you will also get samples 10, 11, and 12.
A Better Plan	
If...	**Then...**
you number the samples 01, 02, 03, and so on	a search for the text **01** would select only the desired dye/lanes.
In Addition	
You can place key words in the Sample Comment or Color Comment field that distinguish samples from each other.	

GeneMapper Manager

Introduction

The GeneMapper Manager is a centralized interface within the GeneMapper software v3.0 for managing and organizing projects. The GeneMapper Manager window contains six tabs that point to the following views:

- Projects - see Chapter 3, “Using the Project Window”
- Analysis Methods - Chapter 5, “Using Analysis Methods”
- Table Settings - Chapter 6, “Using Table Settings”
- Plot Settings - Chapter 7, “Using Plot Windows - Samples and Genotypes”
- Matrices - Chapter 9, “Creating and Evaluating a Matrix”
- Size Standards - Chapter 10, “Using the Size Standard Settings”

Overview of the User's Manual

Organization of the User Manual

This manual provides technical information to support the GeneMapper software user.

- The following chapters explain how to use the major application components:
  - Chapter 3, "Using the Project Window," describes how to use the Project window, including the Project Manager to delete and rename Projects.
  - Chapter 4, "Using Panel Manager," describes how to use the Panel Manager to manage all of the chemistry kits, panels, markers, and bin definitions required for automated allele calling.
  - Chapter 5, "Using Analysis Methods," describes how to use the Analysis Methods tab to create custom analysis methods for use in analyzing sample files.
  - Chapter 6, "Using Table Settings," describes how to use the Table Settings tab to create new profiles, hide and show table columns, and filter the table entries (rows) in Project windows.
  - Chapter 7, "Using Plot Windows - Samples and Genotypes," describes how to view data graphically and edit allele calls.
  - Chapter 8, "Using the Plot Settings Editor," describes how to create a custom plot profile for viewing data.
  - Chapter 9, "Creating and Evaluating a Matrix," describes how to create a matrix file.
  - Chapter 10, "Using the Size Standard Settings," describes how to create a size standard and how to use the Size Match Editor.
- The following appendices contain reference information:
  - Appendix A, "Process Quality Values," explains how to use the Process Component-Based Quality Values.
  - Appendix B, "Software Genotyping Algorithms," provides a description of the genotyping algorithms used in GeneMapper software.
  - Appendix C, "Project Window Software Interface," describes the dialog boxes and menu commands associated with the Project Window.

- Appendix D, “GeneScan Size Standards,” provides information on the Size Standards provided with the GeneMapper software.
- Appendix E, “Sample File Conversion,” describes how to use the Macintosh® AppleScript® program to convert Macintosh-generated fragment analysis sample files to the Windows format.
- Appendix F, “Software Warranty Information,” describes the Applied Biosystems warranty that comes with the software package.

Using the Project Window 3

Chapter Overview

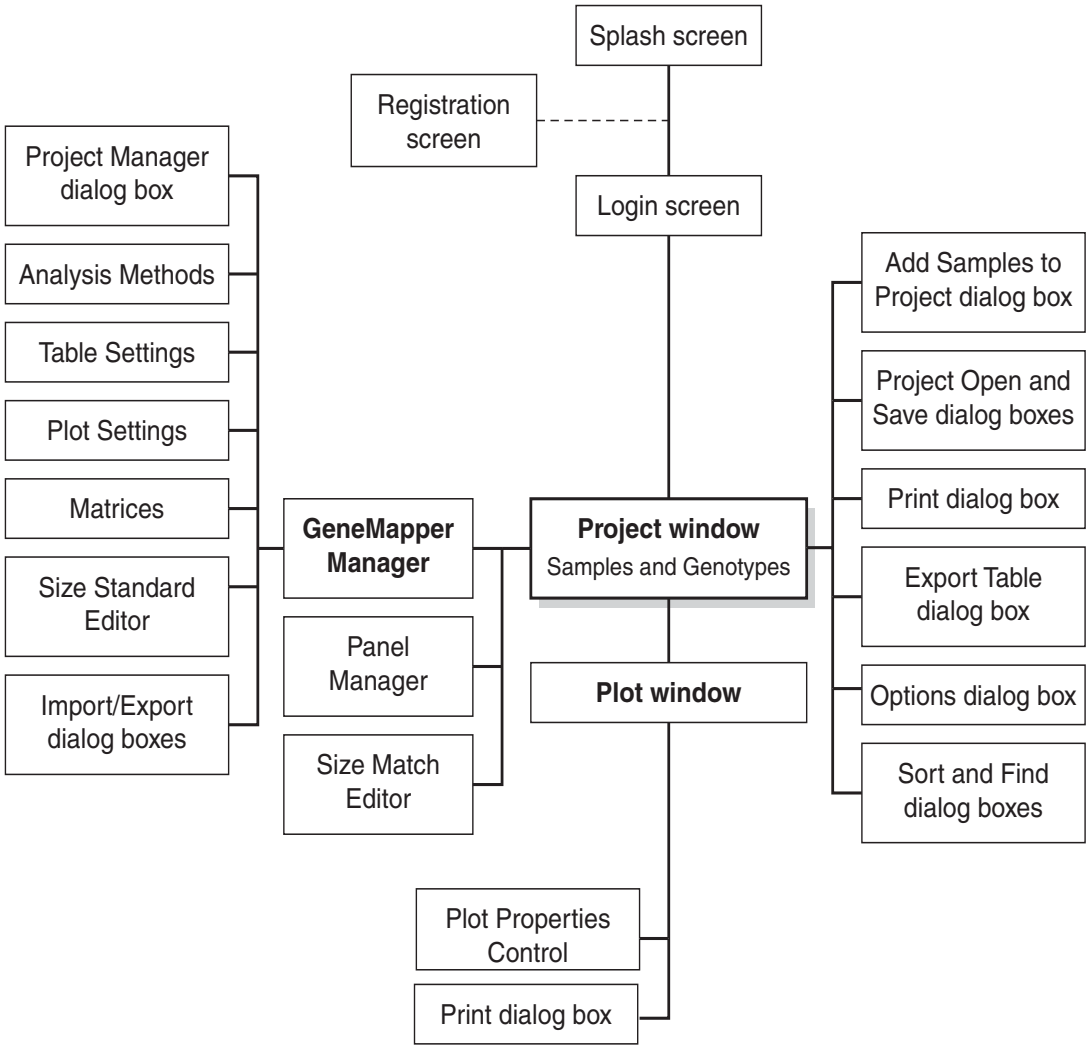
Introduction This chapter provides a general overview of the Project window, the central window of the ABI Prism® GeneMapper™ Software Version 3.0 interface.

In This Chapter This chapter contains the following topics:

Central GeneMapper Software Window

Importance of the Project Window

As shown by the figure below, the Project window is the most important element in the GeneMapper software user interface. The GeneMapper software is organized around the concept of the project to support its purpose of automatically calling alleles or performing genotyping.



The figure on page 3-2 shows the Project window as the main window and the manager utilities, editors, and dialog boxes as the subordinate windows. These windows are opened from and provide support for the project.

As the focal point of the GeneMapper application, the Project window makes it easy for you to:

- Add samples, initiate analysis, and export results
- View sample data and access data plots

  You can view the data associated with a particular sample file by:

  – Viewing the pre-analysis (raw data) version of the data in the Samples view of the Project window

  – Viewing the tabular results of analysis in the Genotypes view of the Project window, or

  – Viewing plot(s) of selected Project sample data in the Plot window
- Initiate the process of creating analysis methods and defining panels and bins

Windows and Utilities Supporting the GeneMapper Software Project

The following items support the information in the Project window and are available in the GeneMapper Manager in the Project window.

- **Table Setting Editor** (GeneMapper Manager) - This page enables you to change the way information is displayed in the Project window, including:

  – Hide/Show table columns in the Project window

  – Filter table entries

  – Set profiles to generate reports easily for downstream processing (*i.e.* export text files to other applications)

  For more details see “Table Settings Editor” on page 6-4.
- **Analysis Method Editor** (GeneMapper Manager) - This page enables you to create and edit analysis methods, including:

  – General properties such as type of analysis (Microsatellite, or SNP Genotyping)

  – Allele-calling properties such as the bin set to be used and marker repeat information (di-, tri-, or tetranucleotide type, cut-off value, PlusA distance, PlusA ratio, stutter distance, and stutter ratio, etc.)

  - Peak Detector parameters and Algorithm type (basic, advanced, or classic)
  - Marker Quality Values to specify various requirements for analysis
  - Peak Quality parameters, such as homozygous and heterozygous peak heights, maximum expected alleles, etc.
  - Quality flags settings and PQV threshold values (pass and fail ranges for sizing and genotype quality values)

  For more details see “Analysis Method Editor” on page 5-5.

- **Panel Manager** - This window enables you to create and edit panels or “reference data” and has the following components:
  - A tool for creating product or chemistry kit folders
  - A table for specifying panels
  - A table for defining markers within a panel
  - A graphical editor for defining bins

  For more details see Appendix C, “Project Window Software Interface.”

- **Size Standard Editor** (GeneMapper Manager) - This window enables you to create new size standard definitions to be used with new groups of samples and delete existing size standards.

  For more details see “Creating/Editing a Size Standard” on page 10-5.

- **Size Match Editor** - This window enables you to adjust an in-lane size standard to compensate for peak shift or a missing peak.

  For more details see “Size Match Editor” on page 10-12.

- **Display Plots** - This window enables you to display graphically the data associated with samples and markers, and visually assess the quality of the data.

  For more details see “Displaying Plot Windows” on page 7-2.

Details of the GeneMapper Project Window

Introduction

The following information is provided for the GeneMapper Project window.

Project Window Interface Conventions

The Project window enables you to:

- Minimize the window to an icon on the Microsoft Windows taskbar or to the title bar
- Maximize the window to fill the entire screen
- Resize the window by dragging any window edge or corner

Reformatting the Window

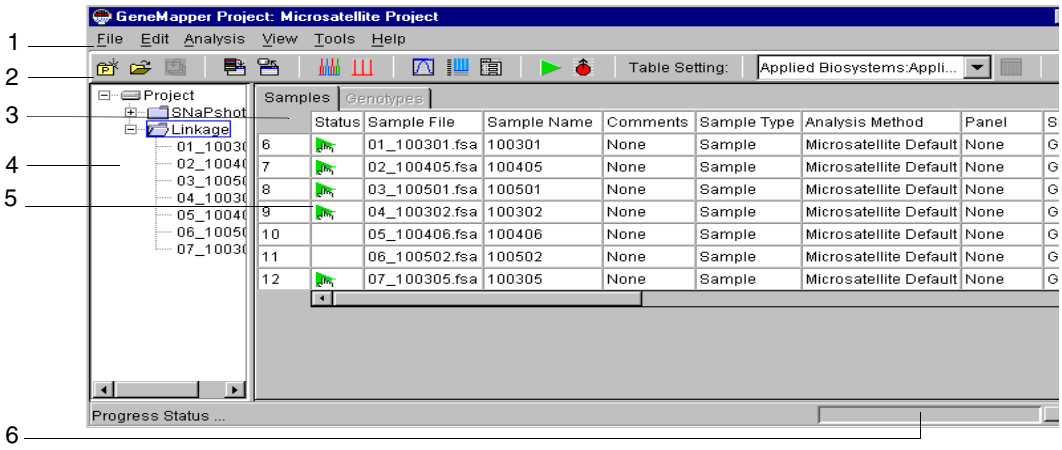
The following reformatting can be done in the Project window to change the presentation of information.

- The View menu provides a Show Navigator toggle command to show/hide the navigation pane for both the Samples and Genotypes views. When the navigation pane is hidden, the table occupies the entire width of the Project window.
- Shift-clicking a column header sorts the data by that column. The Sort dialog box under the Edit menu allows cumulative sorting over three columns; see “Sort Dialog Box” on page C-47.
- The first column of the table, displaying row numbers, is fixed during horizontal scrolling of the Samples and Genotypes tables.

- You can create Hide/Show columns as needed with different table settings created using the Table Settings in the GeneMapper Manager window.
- Resize columns by dragging the separating lines. When resizing is enabled, the cursor changes to a resize cursor (a double-headed arrow that looks like this <—>).

Parts of the Main Window

Once you have loaded Sample files into a Project window, the main window looks like the example below. For more information see Appendix C, “Project Window Software Interface.”



The following table describes the parts of the main window in the above figure.

Parts of the main window

Item	Name	Description
1	Drop-down menus	These menus are described in “Project Window Menus” on page C-9.
2	Toolbar	Toolbar icons are described in “Project Window File Menu Dialog Boxes” on page C-27.
3	Samples and Genotypes tabs	The use of these tabs is described in “Samples View” on page C-2 and “Genotypes View” on page C-6.
4	Navigation pane	The use of this pane is described in “Navigating in the Project Window” on page 3-7.

Parts of the main window *(continued)*

Item	Name	Description
5	Samples view columns	These columns are described in “Samples View Columns” on page C-3.
6	Progress indicator	The progress of analysis and import/export of projects is shown in this indicator.

Navigating in the Project Window

This section provides information on how to navigate as well as how to access different views for samples files.

- Navigation in Samples and Genotypes View - (See page 3-7)
- Info Tab - (See page 3-8)
- Raw Data Tab - (See page 3-9)
- EPT Tab - (See page 3-10)

Navigation in Samples and Genotypes Views

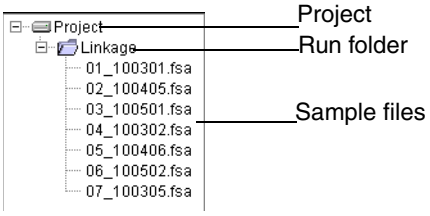
The left panel of the Project window has a tree view navigation device. In the Samples view, the tree viewer has three levels. Each sublevel of the tree-view is indented to the right.

In the Genotypes view, the tree viewer has three levels and each sublevel of the tree view is again indented to the right.

Samples View
● Project
● Run folder
● Sample

Genotypes View
● Panels (root level)
● Panels
● Markers

As soon as you click the tree view controller (indicated in the figure below), the tree view expands with the subordinate level indented. Clicking the controller a second time will collapse the level back to its original form.

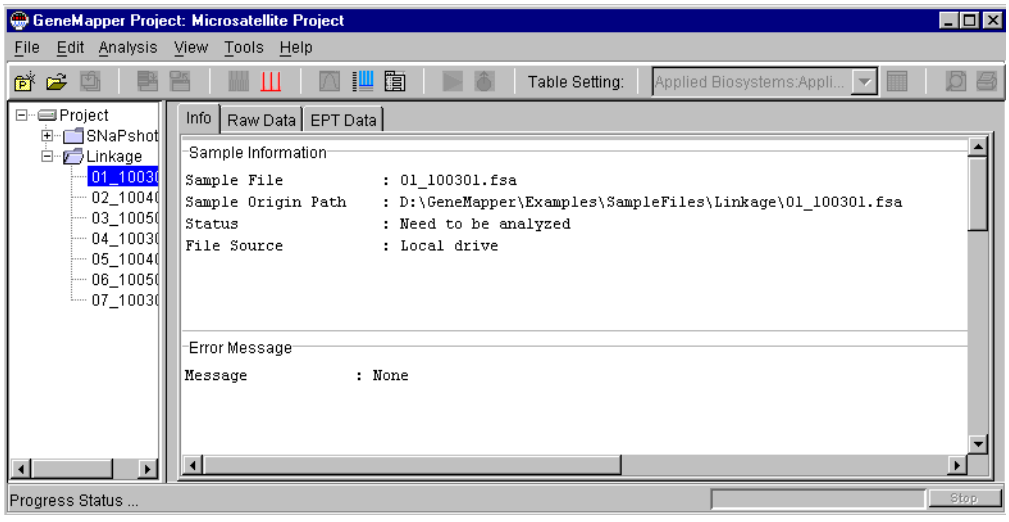


Once you have added files to the Project window, the following rules apply.

If you...	Then...
select the Project item in the Navigation pane	all samples in the project are displayed in the table.
select a Run folder in the Navigation pane	samples in the selected Run folder are displayed in the table.
select a Sample file in the Navigation pane	sample information associated with the selected sample is displayed in the right pane (See figure on page 3-8).
click the Raw Data tab (with the Info view displayed)	raw data associated with the selected sample is displayed in the right pane (See figure on page 3-9), replacing the Info view display.
click the EPT Data tab	sample information associated with voltage, power, current and run temperature is displayed (See page 3-10).

Info Tab

When a Sample file is selected from the navigation pane in the Samples view, a special information window like that shown below is presented and “Info” is the active tab.



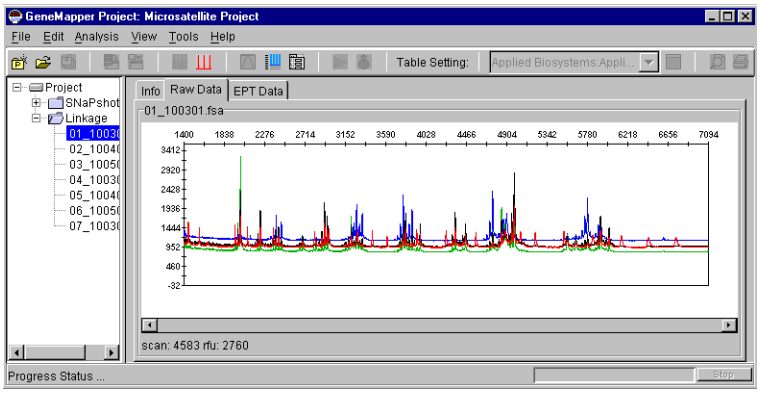
The Info tab displays sample file information such as:

- sample information
- run information
- data collection settings
- gel information
- capillary information
- error messages
- current settings

Sample information comes directly from the Instrument Data Collection and/or Sample sheet.

Raw Data Tab

Clicking the Raw Data tab in the Samples view, when the Sample file is selected, displays the Raw Data view for the selected Sample file.



There are several plot-scaling features in the Raw Data view:

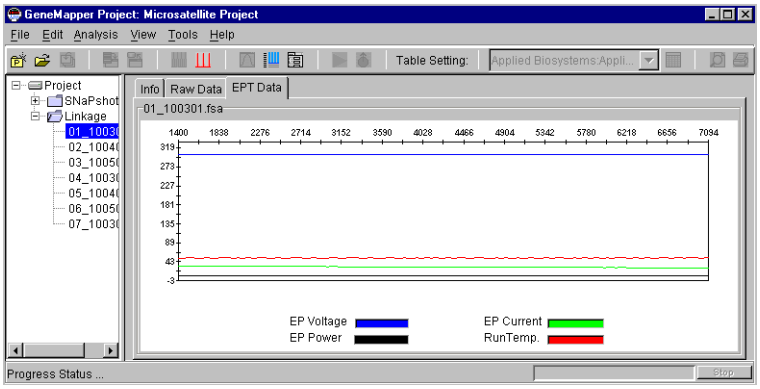
- Zoom in on one area by placing the cursor in either the X or Y axis label areas, and click and drag. The software zooms in on the area of selection.
- Return to a full scale plot by double-clicking in the label area again. The plot window is restored to full scale.
- You can scale the Y axis to maximum Y or a user defined value by selecting Y-Axis Scale from the View menu.

Use the Raw Data view to evaluate the following:

- problems or noise in the baseline that could result in poor size calling
- start and stop points for analysis

EPT Tab

Clicking the EPT (electrophoresis, power, and temperature) tab in the Samples view, when the Sample file is selected, displays a window that shows the voltage, power, current, and run temperature associated with that sample.



Line	Description
Blue	EP Voltage
Black	EP Power
Green	EP Current
Red	Run Temperature

There are several plot-sealing features in the EPT view:

- Zoom in on one area by placing the cursor in either the X or Y axis label areas, and click and drag. The software zooms in on the area of selection.
- Return to a full scale plot by double-clicking in the label area again. The plot window is restored to full scale.
- You can scale the Y-axis to maximum Y or a user defined value by selecting the Y-Axis Scale from the View menu or specify upper and lower limits in the Scale to dialog box.

Samples and Genotypes Views

The Samples and Genotypes tabs provide access to two separate tables, each of which is considered a different view of the same project data. Only one of these views is displayed in the Project window at any time.

- Samples View - (See page 3-12 and in detail on page C-2)

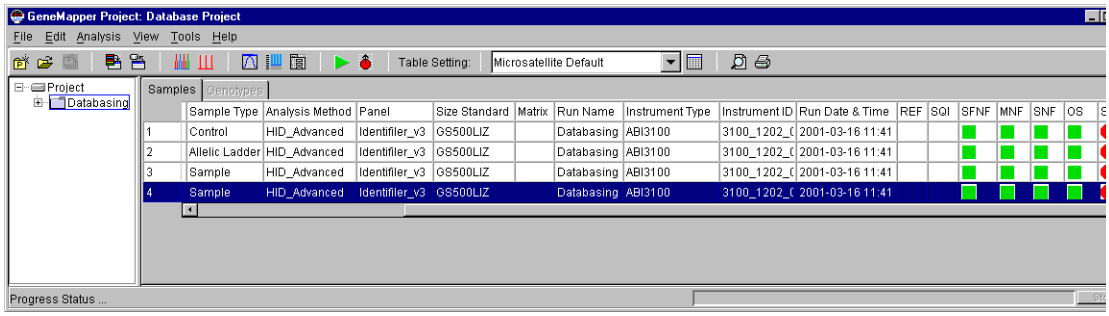
  This view is used to enter samples and its tab is always enabled.

- Genotypes View - (See page C-6)

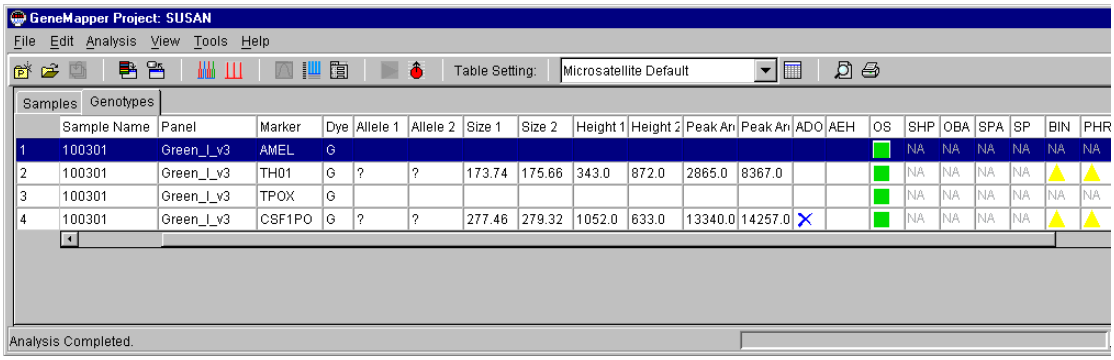
  The tab for this view is enabled when results are present. Samples that do not have a Panel attribute do not display in the Genotypes table.

Note Samples with no results do not display in the Genotypes tab.

Samples View



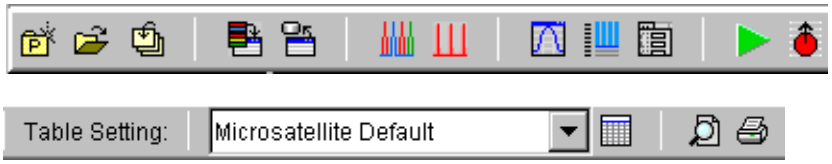
Genotypes View



Note Shown with navigator pane closed.

Project Window Toolbar

The table below is provided as a reference for the Project window toolbar commands.



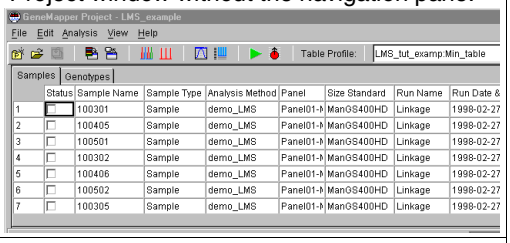
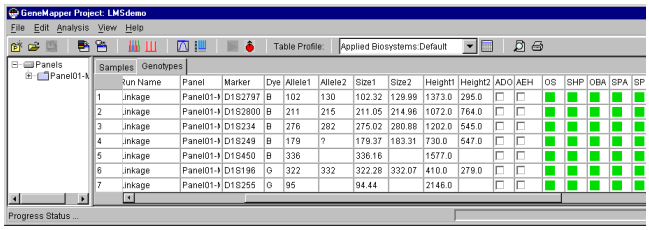
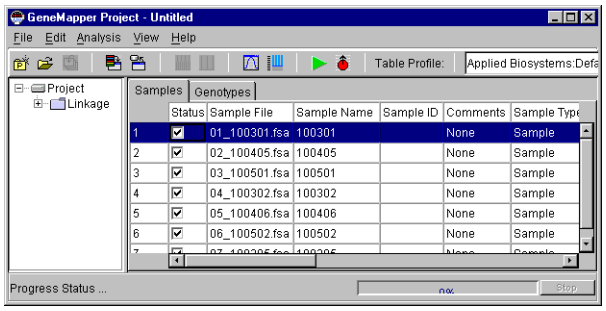
Icon	Description
	Creates a New Project Tooltip: **New Project**

Icon	Description
	Opens the Open Project dialog box Tooltip: **Open Project**
	Saves the Project Tooltip: **Save Project**
	Opens the Add Samples To Project dialog box Tooltip: **Add Samples To Project** (Ctrl+C)
	Opens the Export dialog box; exports the contents of the Samples or Genotypes tables in tab or comma delimited format (Ctrl+E) Tooltip: **Export Table**
	Opens the Plots Table window Tooltip: **Display Plots**
	Opens the Size Match Editor window Tooltip: **Size Match Editor**
	Opens Analysis Method Editor Tooltip: **Analysis Method Editor**
	Opens the Panel Manager window Tooltip: **Panel Manager**
	Opens GeneMapper Manager Tooltip: **GeneMapper Manager**
	Starts the analysis; displays progress bar and Stop button on the Status bar during analysis Tooltip: **Analyze** (Ctrl+R)
	Brings samples with errors to the top of the table; sorts by overall confidence value in the Samples or Genotypes table Tooltip: **Low Quality to Top** (Ctrl+B)
	Opens the Table Setting Editor for the currently selected table Tooltip: **Table Setting Editor** (Ctrl+T)
	Opens the Find dialog box Tooltip: **Find** (Ctrl+F)
	Opens the Print dialog box Tooltip: **Print** (Ctrl+P)

Viewing the Project Window Contents

The following table lists how to display information in a Project window.

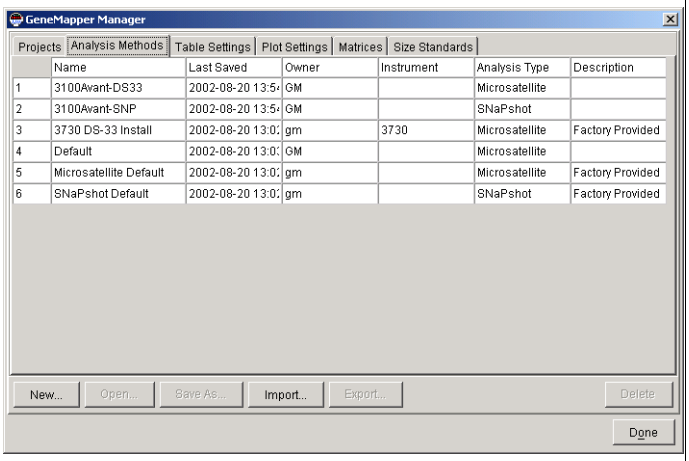
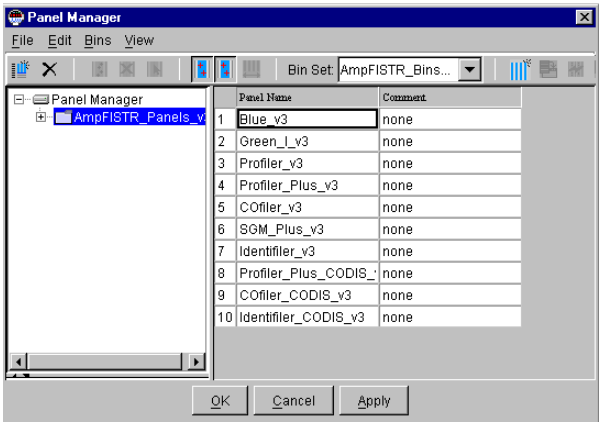
To display information in a Project window:

To hide the...	Open the...	Clear the...	And GeneMapper software displays the...
Navigation pane	**View** menu from the **Project** window Alternative = Ctrl+Shift+N	**Show Navigator** check mark	Project window without the navigation pane. 
To see the...	**Click the...**	**And GeneMapper software displays the...**	
Genotypes view	**Genotypes** tab Alternatives = Select **Genotypes** command from the **View** menu, or Ctrl+Shift+2 **Note** Samples must be analyzed to see genotypes.	Project in the Genotypes view. 	
To see the...	**Click the...**	**And GeneMapper software displays the...**	
Samples view again	**Samples** tab Alternatives = Select **View > Samples**, or Ctrl+Shift+1	Project in the Samples view. 	

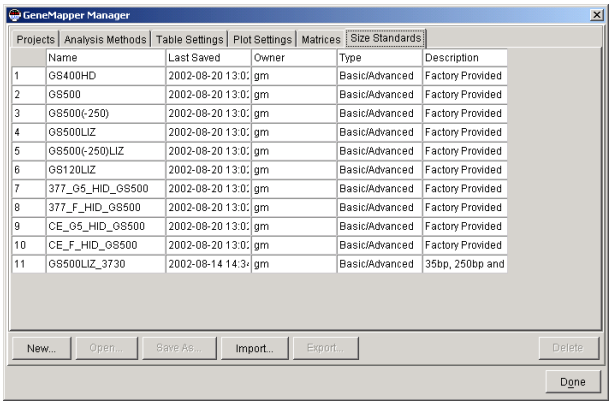
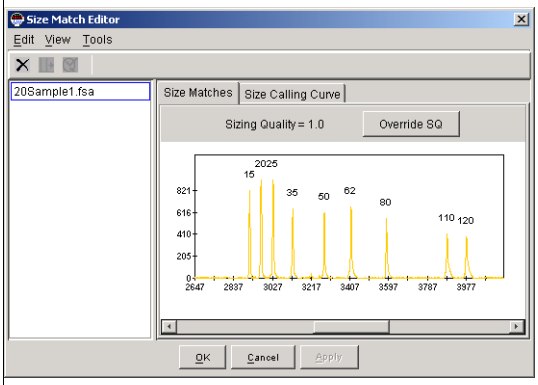
Access to Subordinate Application Windows

The following table lists how to access subordinate GeneMapper software windows.

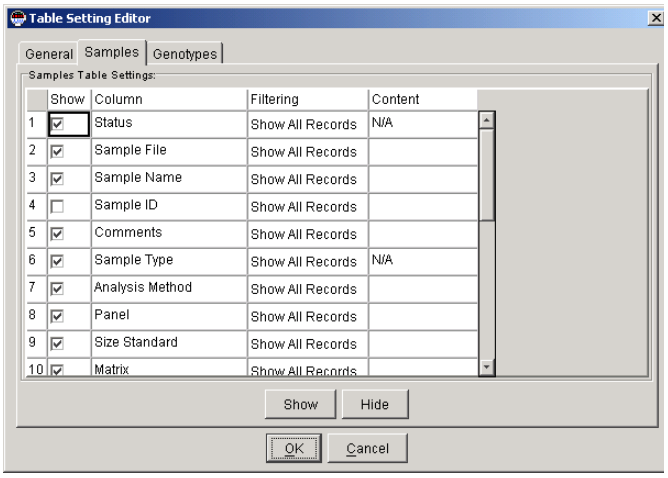
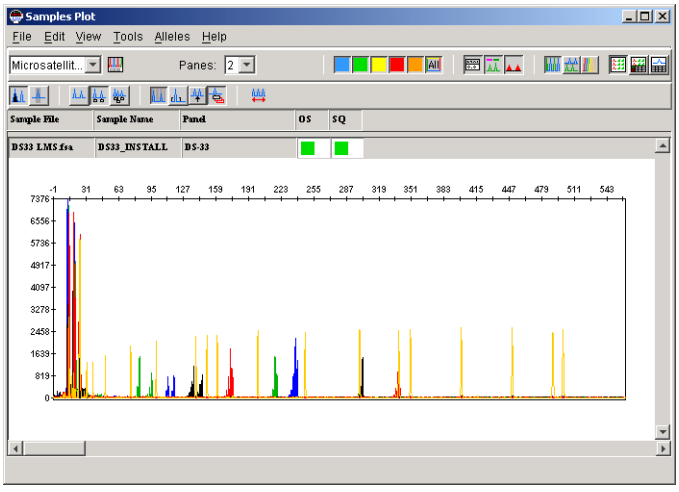
Access GeneMapper Application Windows

To see the...	Select...	And GeneMapper software displays the...
Analysis Methods	**Tools > GeneMapper Manager** Then select the **Analysis Methods** tab.	Analysis Methods page. 
Panel Manager	**Tools > Panel Manager**, or click the icon: 	Panel Manager window. 

Access GeneMapper Application Windows *(continued)*

To see the...	Select...	And GeneMapper software displays the...
Size Standards	**Tools > GeneMapper Manager** Then select the **Size Standards** tab.	Currently Defined Size Standards. 
Size Match Editor	**Analysis > Size Match Editor,** or click the icon: 	Size Match Editor. 

Access GeneMapper Application Windows *(continued)*

To see the...	Select...	And GeneMapper software displays the...
Table Setting Editor	**Tools > Table Setting Editor**, or press Ctrl+T. If samples have been imported, you can click the icon:  **Note** To create a new table, select **Tools > GeneMapper Manager**. Then select the **Table Settings** tab and click **New**.	Table Setting Editor. 
Plot window	a sample or results row, and then either select **Analysis > Display Plots**, press Ctrl+L, or click the icon: 	Plot Window 

Column Menus and Rules for Columns

Columns are used to set up analyses after you have added samples to a project.

Column Menus

Five columns in the Samples view of the Project window have drop-down menus to apply settings to selected cells in the column:

- Sample Type
- Analysis Method
- Panel
- Size Standard
- Matrix

Note The Panel column displays a pop-up window which contains a hierarchical list of kits and panels. A panel is required for any allele-calling analysis.

Each of these columns is used to apply needed analysis parameters to selected samples. Different list selections can be made to the individual column rows representing samples.

Rules for Columns

The following rules apply to column selections:

- Clicking the header for a column selects the entire column.
- Shift+clicking selects a continuous selection range.
- Ctrl+clicking on individual cells makes discontinuous selections.

Applying a List Selection to an Entire Column

To apply a Menu selection:

Step	Action
1	Apply the selection to the top cell in a column: a. Select the top cell in a column. This will display a drop-down list of items. b. Select and click an item to apply the selection to the cell.
2	Select all other cells in the column, either as a continuous or discontinuous selection, to which the list selection is to be applied.
3	Click **Edit > Fill Down** to apply the choice made for the first cell to all selected cells. (Ctrl+D)

Fill Down the Panel Column

To fill down the panel column:

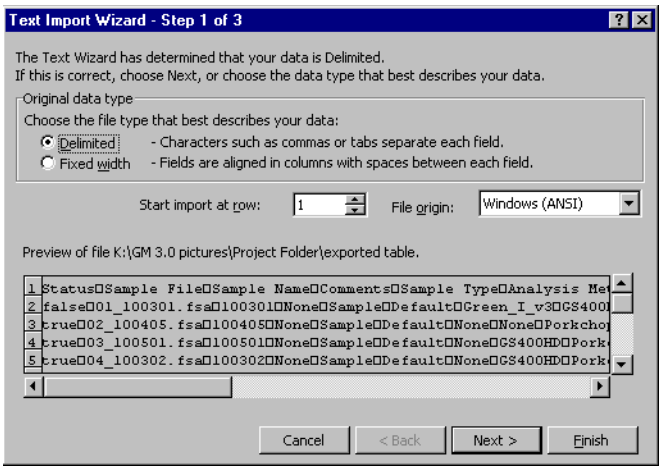
Step	Action
1	Select the top cell in the Panel column to open the Select a Panel window.
2	Expand the appropriate folder.
3	Double-click the appropriate panel.
4	Click **Edit > Fill Down**.

Exported Project Tables

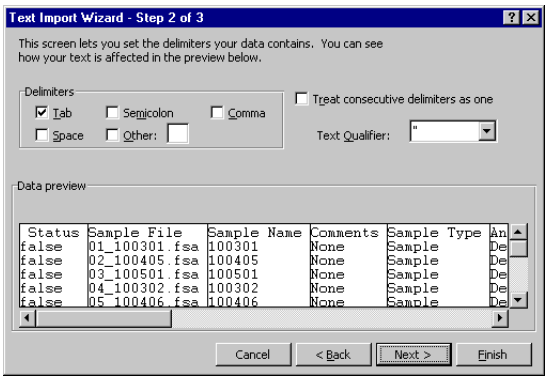
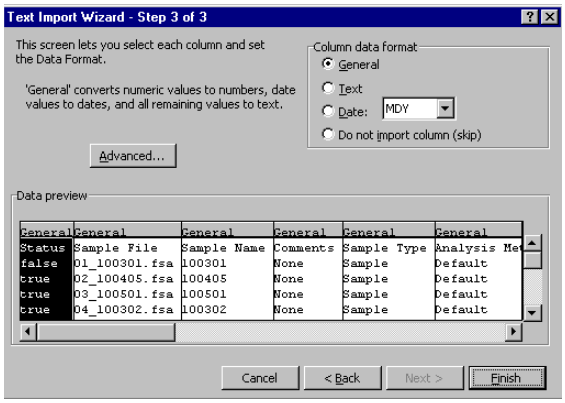
Opening Exported Project Tables in Microsoft Excel

Project tables are exported as text (.txt) files. When they are opened in Microsoft® Excel software, use the Text Import Wizard as described in the following procedure so that all columns of the table are interpreted correctly.

To open and save an exported text file in an Excel spreadsheet:

Step	Action
1	Start Excel software, and either: ♦ select **File > Open > Files of Type**, or ♦ select **Data > Get External Data > Import Text file**. Then select the .txt file and click **Import**.
2	Navigate to the folder containing your exported table files and double-click the table file you wish to open. The Text Import Wizard window opens. 

To open and save an exported text file in an Excel

Step	Action
3	Move through the steps to import text. a. Click the **Next** button to proceed to Step 2 of 3 in the Text Import Wizard window. b. Select either **Tab** or **Comma Delimiters** according to how you exported the table (if necessary). 
4	Click the **Next** button to continue to Step 3 of 3 in the Text Import Wizard window.  As you can see, the first column of the table is already selected.

To open and save an exported text file in an Excel

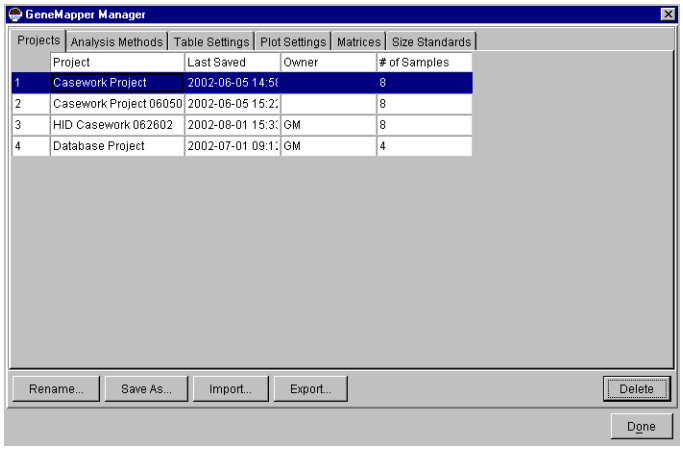
Step	Action
5	Open the table file by performing the following actions: a. Hold down the **Shift** key. b. Scroll to the last column of the table. c. Click the last column of the table and release the **Shift** key. d. Click the **Text** option button. e. Click the **Finish** button to open the table file with all columns in text format.
6	Save the text file as a Microsoft Excel Workbook file. The next time you open the file in Excel, the table columns will display correctly in text format.

Projects Page

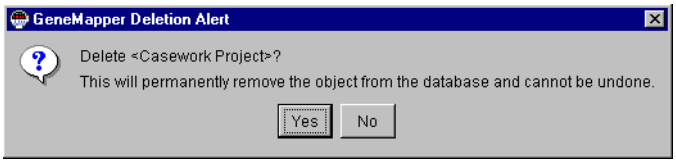
Using the Projects Page

The GeneMapper Manager Projects page, shown below, is a utility which is used to rename, save as, import, export, or delete projects.

To display the Projects page, select the GeneMapper Manager from the Tools menu and select the Projects tab.



Select the project you would like to change and click the appropriate button. If you click the Delete button, the Project Deletion dialog box displays. Click **No** if you do not want to delete the project. Click **Yes** only if you want to delete the project permanently.



Projects Tab

The Projects Tab allows you to view information about the projects stored within the GeneMapper database and perform certain functions such as renaming and exporting of projects.

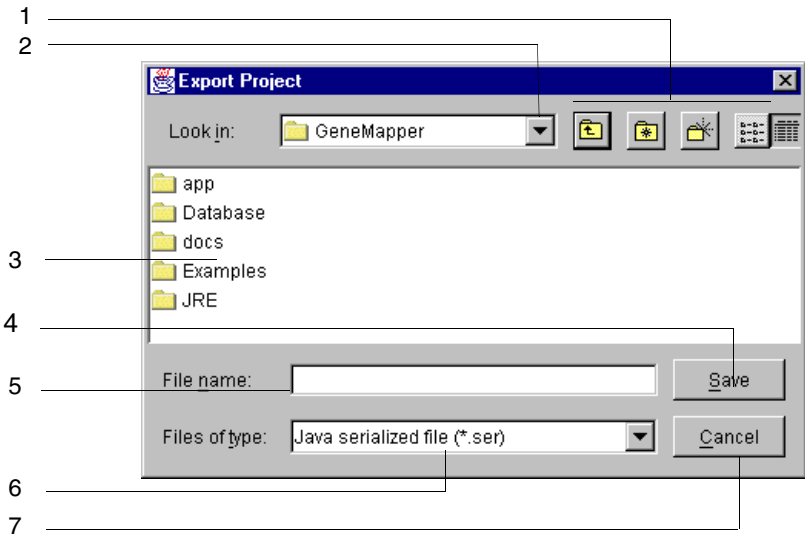
Note The only method of backing up your projects is to export them.

Description of items on the Projects tab:

Item	Description
Project column	Project name
Last Saved column	Date/Time stamp showing when the project was last saved
Owner column	User name of the person who created the project
Samples column	Number of samples contained in the project
Rename button	Opens a Rename dialog box for renaming the project Enabled when a project is selected
Save As button	Displays the Save Project As dialog box Enabled when a project is selected
Import button	Displays a dialog box for Importing projects
Export button	Displays a dialog box for Exporting selected projects Enabled when projects are selected **Note** Projects can be exported as a group by holding down the shift key and selecting multiple projects. This feature works with one or more selected projects.
Delete button	Deletes the selected projects Enabled when a project is selected **Note** This feature works with one or more selected projects.
Done button	Closes the GeneMapper Manager.

Export Project Window

The Export Project window enables you to export a project out of the GeneMapper database into a specified location. See an explanation of each area on this window in “Using the Export Project Window” on page 3-26.



Note Reference data and Size Standards used by exported Projects are not exported with the Projects.

Using the Export Project Window

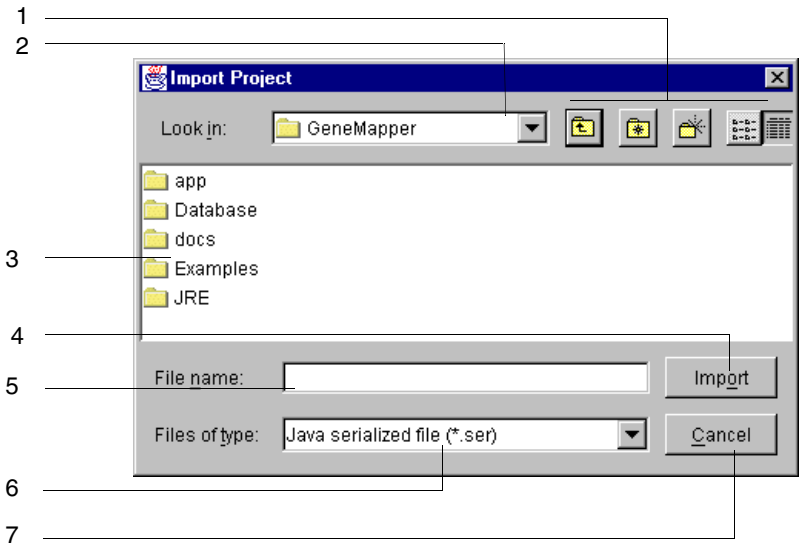
The elements called out in the figure in “Export Project Window” on page 3-25 are described in the table below.

Export Project Callouts

Item	Name	Description
1	Toolbar	These icons are used as follows:  - Clicking moves display up one level in main pane.  - Clicking moves display to Home level in main pane. This level is usually Profiles\<user>.  - Clicking creates a new folder at the present directory level.  - Clicking presents a list of the contents of the selected folder.  - Clicking presents details of the selected folder.
2	Drop-down directory menu	Select drive letter and/or folder.
3	Folder/file display pane	The contents of the disk or directory are selected in the toolbar and the directory menu is displayed here.
4	Save button	This button saves the exported project.
5	File name field	Enter a file name to use for saving your data.
6	Files of type field	This field is a display filter for files (folders are always shown). Use this drop-down menu to select the file extensions for display: ♦ All Files (*.*) shows all files ♦ Java Serialized file (*.ser) shows only files with the extension .ser, which is how GeneMapper projects are exported. **Note** There is only one preset file extension (.ser) that can be used.
7	Cancel button	Closes the Export Project window without exporting a project.

Import Project Window

The Import Project window enables you to import project data previously saved. See an explanation of each area in “Using the Import Project Window” on page 3-28.



Using the Import Project Window

The elements called out in the figure in “Import Project Window” on page 3-27 are described in the table below.

Import Project Callouts

Item	Name	Description
1	Toolbar	These icons are used as follows:  - Clicking moves display up one level in main pane.  - Clicking moves display to “Home” level in main pane. This is usually “Profiles\<user>”.  - Clicking creates a new folder at the present directory level.  - Clicking presents a list of the contents of the selected folder.  - Clicking presents details of the selected folder.
2	Drop-down directory menu	Select drive letter and/or folder.
3	Folder/file display pane	The contents of the disk or directory are selected in the toolbar and the directory menu is displayed here.
4	Import button	This button imports the selected project.
5	File name field	Enters name of selected project being imported.
6	Files of type field	This is a display filter for files (folders are always shown). Selects the file extensions for display: ♦ All Files (*.*) shows all files ♦ Java Serialized file (*.ser) shows only files with the extension .ser, which is how GeneMapper database projects are exported. **Note** There is only one preset file extension (**.ser**) that can be used.
7	Cancel button	Closes the Import Project window without importing a project.

Using Panel Manager 4

Chapter Overview

Introduction

This chapter describes the Panel Manager feature in the ABI Prism® GeneMapper™ Software Version 3.0, and provides instructions on how to use it for microsatellite data. Some information is provided regarding SNaPshot® specific features; however, additional information can be found in the tutorial guide (*SNP Genotyping with ABI PRISM® GeneMapper™ Software Version 3.0*, PN 4335524). Portions of this chapter are organized around the Panel Manager menu items to explain various features and actions that can be performed.

In This Chapter

This chapter contains the following topics:

Panel Manager Overview

Introduction

The Panel Manager utility allows you to manage all of the chemistry kits, panels, markers, and bin definitions required for automated allele calling.

There are two ways to get data for the Panel Manager:

- Creating kits, panels, markers, and bins manually.
- Importing preformatted text files that contain panels, markers, and bin definitions. (See “Formats of Panel and Bin Text Files” on page 4-23 for two examples.)

Purpose of the Panel Manager Window

The Panel Manager window enables you to create, edit, and import panels. It features a:

- Tool for creating kit folders
- Table for specifying panels
- Table for defining markers within a panel
- Graphical editor for creating and editing bins

Data for the panels is stored in the database.

- Clicking the Apply button after making changes to a panel sends all changes to the database but keeps the window open for further edits.
- Clicking **OK** sends all changes to the database and closes the Panel Manager window.

The changes in panel data are then reflected in the Project and Plot windows.

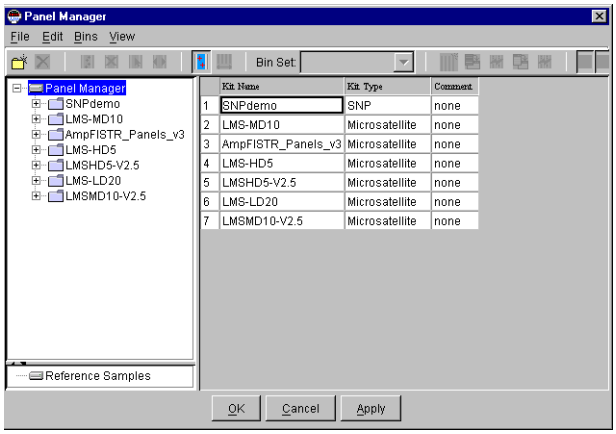
When to Click the Apply Button

- After deleting a kit, panel, marker, or bin set
- After making a number of edits
- After creating a bin set

Displaying the Panel Manager Window

To display the Panel Manager window, either:

- Click  (Panel Manager) from the Project window toolbar, or
- Select **Tools > Panel Manager** in the Project window.



Hierarchy of Panel Data

Conceptually, the hierarchy of panel data is as follows:

- Kit (chemistry kit or product)
- Panel
- Marker
- Bin

A kit is a collection of panels. A panel is a collection of markers. Bin sets are collections of the expected allele locations for markers contained within a kit. A different bin set should be used for each instrument type, as variations in run conditions and polymers affect allele positions.

The GeneMapper software requires kit, panel, and bin set names to be unique throughout the database and requires marker names to be unique within a panel. The Panel Manager enforces these requirements during user entry and editing of names as well as during data import.

Since the Panel Manager allows users to add comments as needed, no history or audit trail of panel data changes is kept. Such comments provide a way for users to track changes and the reasons for the changes. Each kit, panel, and marker has a comment field.

Panel Manager Toolbar

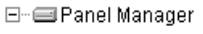
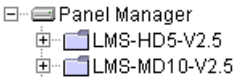
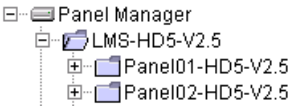
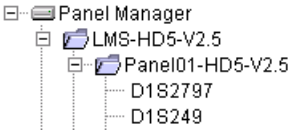
The toolbar icons enable specific Panel Manager actions similar to their associated menu items as described throughout this chapter. Position your mouse over an icon to view its tooltip description.

1 2 3 4 5 6 7 8

Item	Description
1 	New Kit/Panel/Marker icon automatically updates to reflect the current action depending on the item selected in the navigation pane (File menu).
2 	Clear icon performs kit/panel/marker deletion (Edit menu).
3 	Icons select bin options: Add Bin, Delete Bin, Edit Bin (Bins menu).
4 	Icons allow viewing of Project alleles (Bins menu).
5 	Icon switches view to full X-Axis and Y-Axis scales (View menu).
6 	Pull-down menu allows selection of a bin set for a selected kit.
7 	Icons select specific kit/panel/marker actions (Bins menu): New Bin Set,  Add Reference Data,  Auto Bin,  Panel Reference Data,  Auto Panel 
8 	Dye color toggle icons (View menu).

Panel Manager Navigation Pane

The Panel Manager navigation pane is the primary navigation interface in the Panel Manager window. Since it is found in all Panel Manager views, as shown in the table below, it enables moving back and forth between the views to view, edit, and create kit folders, panels, markers, and bins. The elements of this field are described below.

Element	Description
	The Panel Manager root node is the container for all panels. It is the top level of the hierarchy.
	Kits help users organize their panels in product- or project-specific folders. Clicking a kit folder displays the Panel table.
	The Panel folder icon represents a set of markers. Clicking a panel folder displays the Marker table. For SNP kits, clicking a panel folder also displays a plot view.
	The Marker icon represents an individual marker. Clicking a microsatellite marker displays the Bin view. Clicking an SNP marker displays the Bin table.

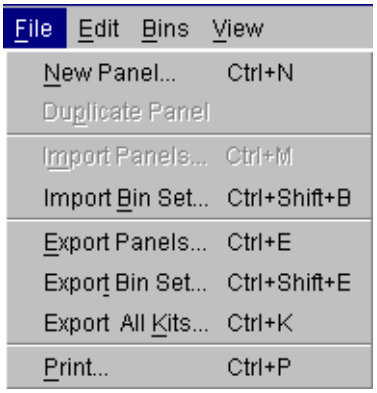
Panel Manager Window Commands

Introduction

The tables in this section describe the following Panel Manager views and menus.

File Menu

The File menu offers options to the user to create or export kits, duplicate, import and export panels, and import and export bin sets.



New Kit/Panel/Marker

The New Kit/Panel/Marker command changes its name and function depending on the item selected in the Panel Manager navigation pane as follows:

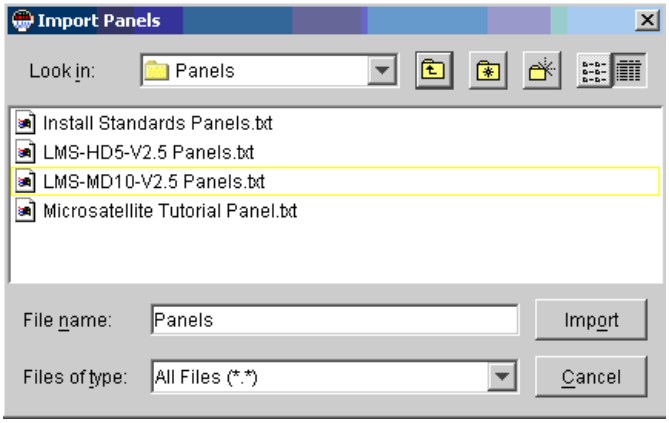
- **New Kit** – This menu item is enabled when the Panel Manager root node is selected in the navigation pane and allows creation of a new kit folder. When creating a new kit:
  - Enter in a unique kit name
  - Select the appropriate kit type (microsatellite or SNP) from the drop-down menu
  - Click **OK** to add kit to navigation pane

- **New Panel** – This menu item is enabled when a kit folder is selected in the navigation pane and allows creation of a new panel folder. When creating a new panel:
  - Select the “New Panel” name in the right hand window table and enter in the desired name. Names must be unique within the database.
  - Repeat to add multiple panels to a kit
  - Press [**Enter**], then click **Apply** after entering a Panel name to update the navigator pane
- **New Marker** – This menu item is enabled when a panel folder is selected in the navigation pane and allows creation of a new marker. When creating new markers:
  - Select the “New Marker” row in the right hand window table and enter in the appropriate information for each column. Refer to “Marker Table View” for more information.
  - Repeat to add multiple markers to a panel
  - Press [**Enter**], then click **Apply** to update the navigator pane

Duplicate Panel

The Duplicate Panel command creates a copy of a selected panel and places it in the same kit as the original with the name “[Selected Panel]-dup”. The panel and all associated bins with all bin sets are duplicated as well. This menu item is enabled when a panel folder is selected in the navigation pane.

Import/Export Panels



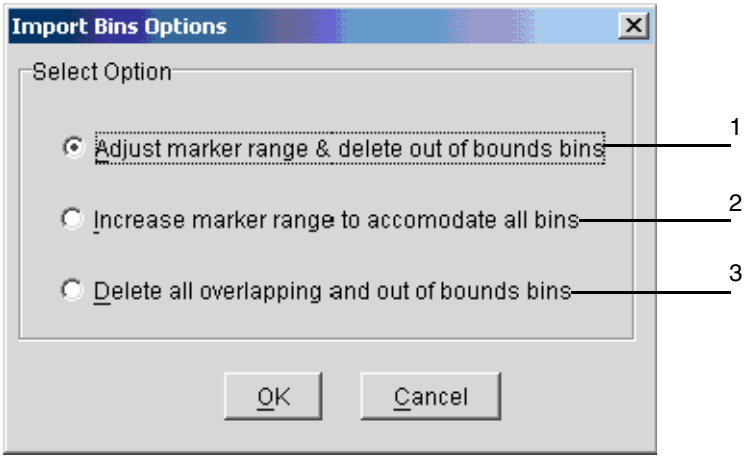
The Import Panels command and the Export Panels command open the corresponding dialog box to allow importing/exporting of kits and panels. This is enabled when the Panel Manager root node is selected in the navigation pane. See “Formats of Panel and Bin Text Files” for more information.

Export Panels is not available when the Panel Manager root node is selected; it is only active when a kit is selected.

Import/Export Bin Set

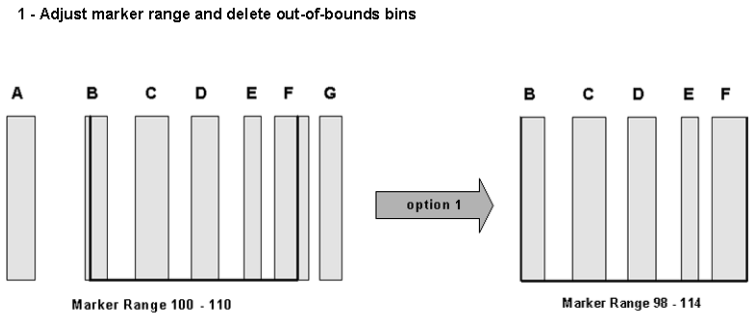
The Import Bin Set command and the Export Bin Set command open the corresponding dialog box to allow importing/exporting of bin sets. This is enabled when a kit name is selected in the navigation pane. See “Formats of Panel and Bin Text Files” for more information.

When bins are imported into a kit, GeneMapper software checks for bins that fall outside of their marker allele size ranges. If bins overlap or fall out of the marker range the Import Bins Options dialog box will open.

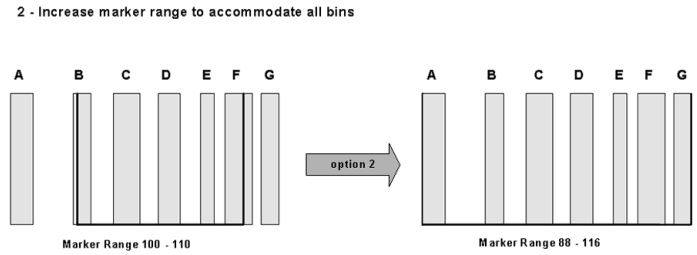


The Import Bins Options dialog box allows you to choose how GeneMapper software handles the bins being imported. The numbered lines in the graphic above refer to the individual numbered descriptions in the figures on .

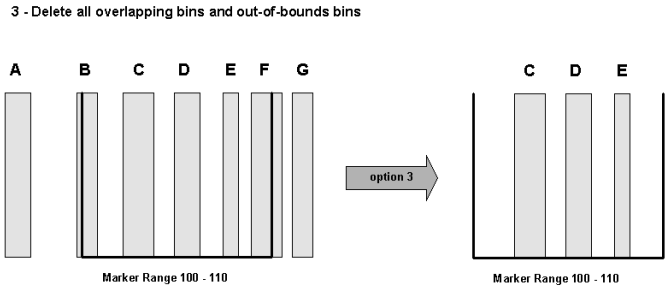
1.The option shown below adjusts the marker range to accommodate any bins that overlap the marker boundaries and will delete all bins that fall completely outside the marker size range.



– The option shown below increases the marker allele size range to accommodate all overlapping and out of bounds bins.



– The option shown below does not affect the marker allele size range, all overlapping and out of bounds bins are deleted.



If all the bins in an import are inside their marker ranges, the dialog box does not open.

Backing up Panels and Bin Sets

The only way to back up panels and bin sets is to export them. You will be prompted for a location and name for the exported item. See “Export All Kits” on page 4-11.

Export All Kits

The Export All Kits command opens the Export All Kits dialog to allow exporting all kits and their associated panels, markers, and bin sets to a selected location. When you use this option, panels retain their current panel name as listed in the Panel Manger. Bins are named as follows: *[Kit name]_[Bin set name]_bins.txt.*

Print

The Print command opens the Print dialog box to enable printing.

Edit Menu

The Edit menu contains the Undo command and the Clear command.

Undo

The undo command allows for the Clear command to be undone.

Clear

The Clear command deletes items selected in the navigation pane from the GeneMapper database, such as:

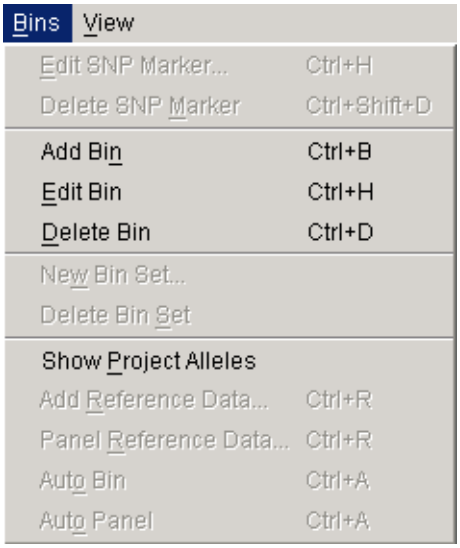
- Kits

  Deletes the kit and all bin sets associated with that kit.
- Panels
- Markers

Use the Bins Menu items to delete bins and bin sets.

Bins Menu

The Bins menu contains commands to edit markers and bins.



Edit SNP Marker

The Edit SNP Marker command opens the Edit SNP Marker dialog box allowing the user to edit the marker name and bin information for the selected marker. To enable this feature:

- Select the menu item when a SNP marker is selected in the Panel Manager Plot tab

OR

- Right-click on a SNP marker or bin in the Panel Manager Plot tab

Additionally, SNP markers and bins can quickly be edited by changing the data in the Panel Manager Table tab for a selected SNP Panel.

Delete SNP Marker

The Delete SNP Marker command deletes the SNP marker, and associated bins, selected in the Panel Manager Plot tab. To enable this feature:

♦ Select the menu item when a SNP marker or bin is selected in the Panel Manager Plot tab

OR

♦ Right-click on a SNP marker in the Panel Manager Plot tab

Add Bin

The Add Bin command creates a new bin associated with a panel as follows:

♦ For microsatellite data:

Step	Action
1	Select a marker in the Panel Manager navigation pane.
2	Select the Add Bin menu item OR right-click within the allele size range of the marker shown in the Panel Manager right-hand window to select the New Bin option.
3	Drag the length of the desired bin range with the mouse.
4	Edit the bin information in the Add Bin dialog box that opens.

♦ For SNaPshot data:

Step	Action
1	Select a SNaPshot panel in the Panel Manager navigation pane.
2	Select a marker in the Plot tab.
3	Select the Add Bin menu item OR right-click on a marker or bin in the Plot tab.
4	Edit the SNP Marker using the dialog box that opens.

Delete Bin

The Delete Bin command deletes a marker bin as follows:

♦ For microsatellite data:

Step	Action
1	Select a marker in the Panel Manager navigation pane.
2	Select a bin to be deleted.
3	Select the Delete Bin menu item OR right-click on the bin and select the Delete Bin option.

♦ For SNaPshot data:

Step	Action
1	Select a SNaPshot panel in the Panel Manager navigation pane.
2	Select a bin to be deleted in the Plot tab.
3	Select the Delete Bin menu item OR right-click on the bin and select the Delete Bin option.

Edit Bin

The Edit Bin command edits a marker bin as follows:

♦ For microsatellite data:

Step	Action
1	Select a marker in the Panel Manager navigation pane.
2	Select a bin to be edited.
3	Select the Edit Bin menu item, or right-click the bin and select the Edit Bin option.
4	Edit the bin information in the dialog box that opens.

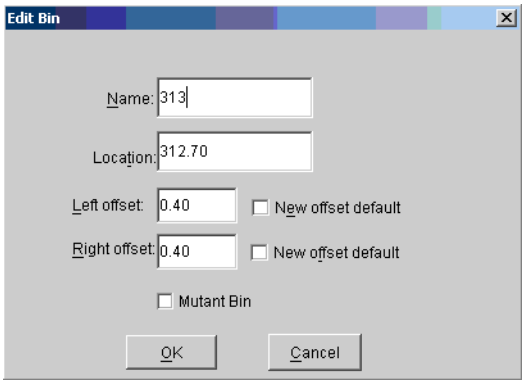
♦ For SNaPshot data:

Step	Action
1	Select a SNaPshot panel in the Panel Manager navigation pane.
2	Select a bin to be edited in the Plot tab.
3	Select the Edit Bin menu item, or right-click on the bin and select the Edit Bin option.
4	Edit the bin information in the Edit SNP Marker dialog box that opens.

To quickly resize a bin, select a bin and adjust the right and left handles to the desired size range.

To move a bin, select a bin and holding down the mouse button, move the bin to the desired location.

Edit Bin Dialog Box



Element	Description
Name	Editable; name of the bin can be alphanumeric.
Location	Editable; location of the bin center in base pairs.
Left Offset	Editable; the left boundary of the bin, expressed as the base pair distance to be subtracted from the location (default = 0.4).
Right Offset	Editable; the right boundary of the bin, expressed as the base pair distance to be added to the location (default = 0.4).
OK	Accepts the pending changes and closes the dialog box. Validation: the following criteria apply: ♦ The edited bin must have a name unique in its marker. ♦ The edited bin must have a location and offsets that do not overlap any other bins in the marker. If validation fails, an alert message will be displayed.
Cancel	Closes the dialog box without accepting the pending changes

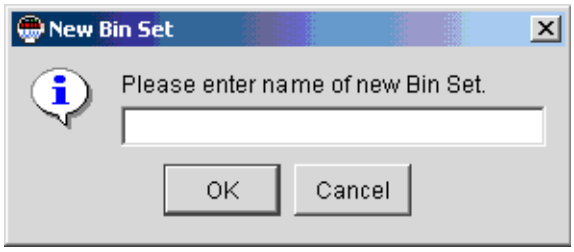
Element	Description
New Offset Default (checkbox)	Checking this box sets a left and right offset default, to be used for subsequent creation of bins.
Mutant Bin	Designates a bin as a mutant. Bin color is red.

New Bin Set

The New Bin Set command opens the New Bin Set dialog box, allowing the user to create a new bin set for the selected kit. Clicking **OK** checks the name for uniqueness; if the name is not unique, an alert is shown and the dialog box closes. The new bin set name then displays at the top of the Panel Manager window in the Bin Set pull-down menu.

Note Bin sets are always created at the kit level.

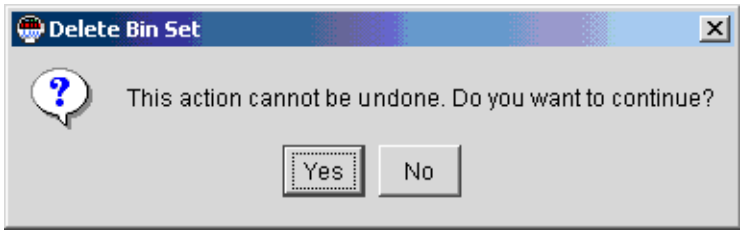
Note SNP kits can only contain one bin set.



Delete Bin Set

The Delete Bin Set command deletes the bin set currently listed for a kit in the Bin Set drop-down menu at the top of the Panel Manager window. If there are multiple bin sets associated with a kit, use the drop-down menu to select the appropriate bin set to be deleted.

Note This action cannot be undone.

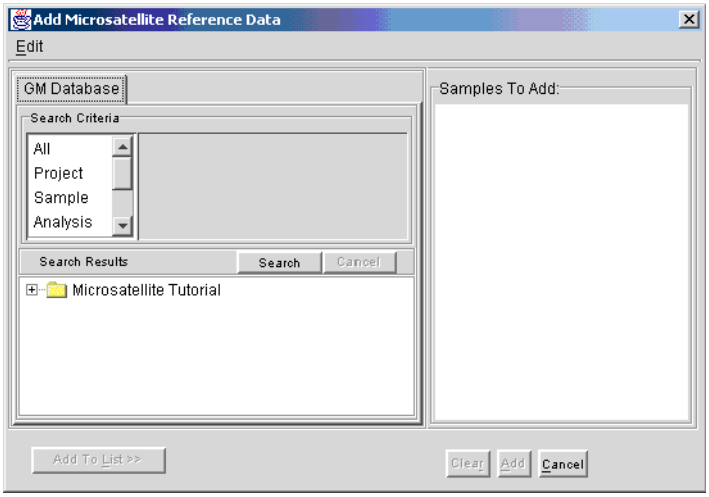


Show Project Alleles

The Show Project Alleles command displays all of the marker's allele calls that are present in the Genotypes table of the open project. Allele calls are displayed as blue asterisks. This option is enabled when a marker is selected in the Panel Manager navigation pane and genotypes exist for that marker. The option is for microsatellite data only.

Add Reference Data

The Add Reference Data command opens the Add Reference Data dialog box, allowing the user to associate data with a microsatellite panel or SNaPshot kit for creating markers and bins.



For microsatellite panel reference data, samples must have been analyzed using the panel for which the data serves as a reference and the appropriate size standard.

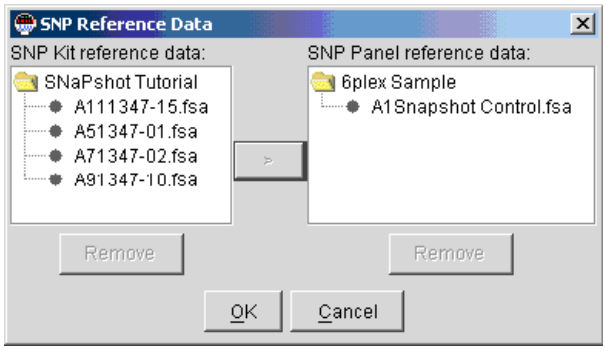
For SNaPshot kit reference data, samples must have been analyzed using the appropriate size standard and an Analysis Method with SNaPshot as the Analysis Type.

Panel Reference Data

This feature now works for both microsatellite and SNP data.

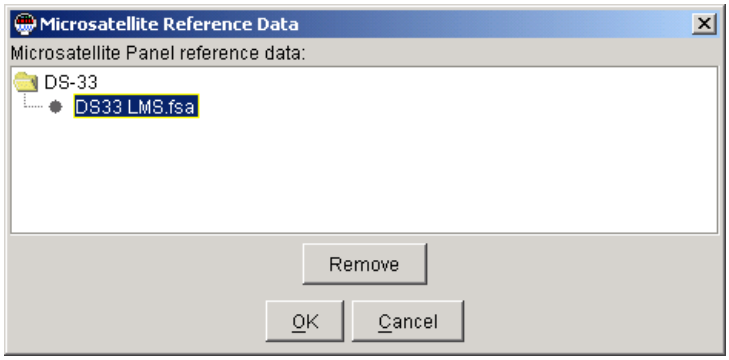
- SNP data

  The Panel SNP Reference Data command opens the SNP Reference Data dialog box, allowing the user to organize the reference data imported at the kit level into panels. This feature is only used when creating panels manually, not when using the Auto Panel feature. The panel is enabled when a SNaPshot panel is selected and reference data has already been added at the kit level.



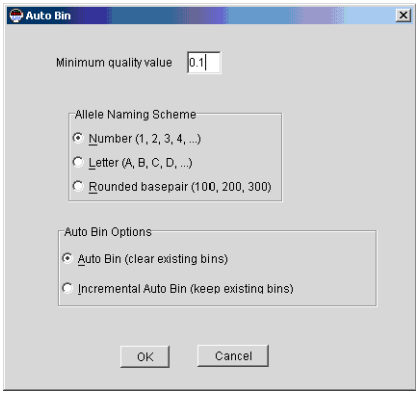
- Microsatellite data

  The Panel Reference Data command allows you to remove reference samples from the selected microsatellite panel.



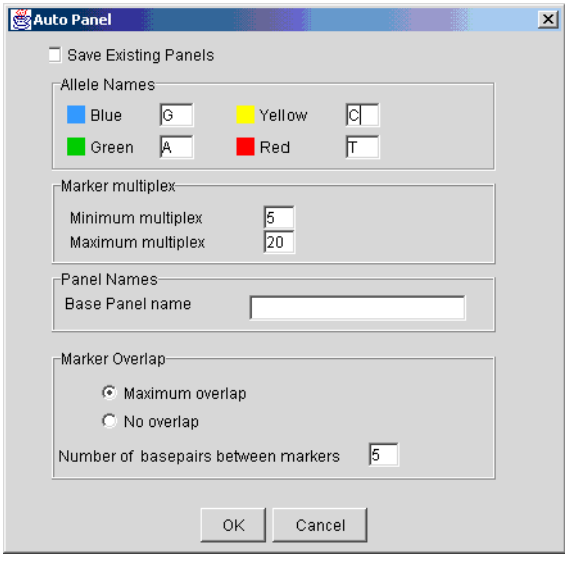
Auto Bin

The Auto Bin command opens the Auto Bin dialog box to automatically create bins for a microsatellite panel. This feature is enabled when a microsatellite panel is selected and reference data has been selected. Refer to the Microsatellite Tutorial for more information.

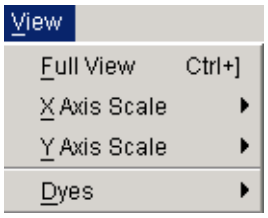


Auto Panel

The Auto Panel command opens the Auto Panel dialog box to automatically create panels, markers and bins for a SNaPshot Kit. This feature can only be used with reference data generated from the ABI PRISM® SNaPshot Primer Focus Kit. Refer to the SNaPshot Tutorial for more information.



View Menu

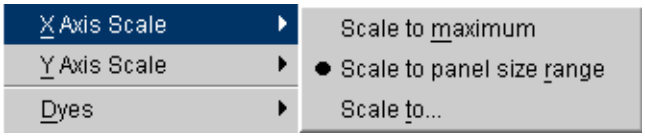


Full View

Depending on the X- and Y-axes mode, the Full View command resets the X- or Y-axis to the:

- maximum range
- panel size range
- most recent Scale To range

X Axis Scale for SNP data



The X Axis Scale menu command controls the horizontal scale of the reference data electropherogram being viewed in the Panel Manager as follows:

- **Scale to maximum** – The electropherogram being viewed is scaled to its maximum base pair length as collected from the instrument.
- **Scale to panel size range** – The electropherogram being viewed is scaled to the size range available for the selected panel.
- **Scale to** - The user can define the horizontal range being viewed.

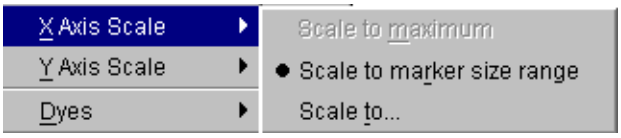
To zoom in to a specific region on the X-axis of the electropherogram:

- Click and drag with the left mouse button the desired region on the X-axis.

OR

- Right-click on the X-axis to open the X-Axis Scale menu command options.

X Axis for Microsatellite data



The X Axis Scale menu command controls the horizontal scale of the reference data electropherogram being viewed in the Panel Manager as follows:

- **Scale to maximum** – The electropherogram being viewed is scaled to its maximum base pair length as collected from the instrument.
- **Scale to marker size range** - The electropherogram being viewed is scaled to the size range available for the selected marker.
- **Scale to** - This setting allows the user to define the horizontal range being viewed.

To zoom in to a specific region on the X-axis of the electropherogram:

- Click and drag with the left mouse button the desired region on the X-axis.

OR

Right-click on the X-axis to open the X-Axis Scale menu command options.

Y Axis Scale

The Y-Axis Scale menu command controls the vertical scale of the reference data electropherogram being viewed as follows:

- **Scale to maximum** – The electropherogram being viewed is scaled to its maximum height as.
- **Scale to**- The user can set the Y-Axis scale value.

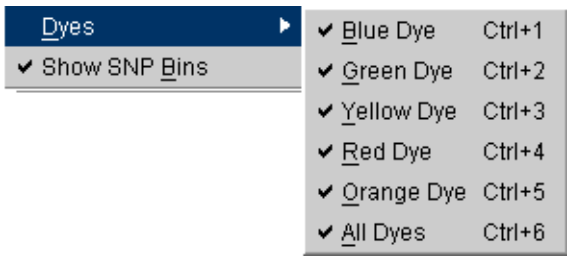
To zoom in to a specific region on the Y-axis of the electropherogram:

- Click and drag with the left mouse button the desired region on the Y-axis

OR

- Right-click on the Y-axis to open the Y-Axis Scale menu command options.

Dyes



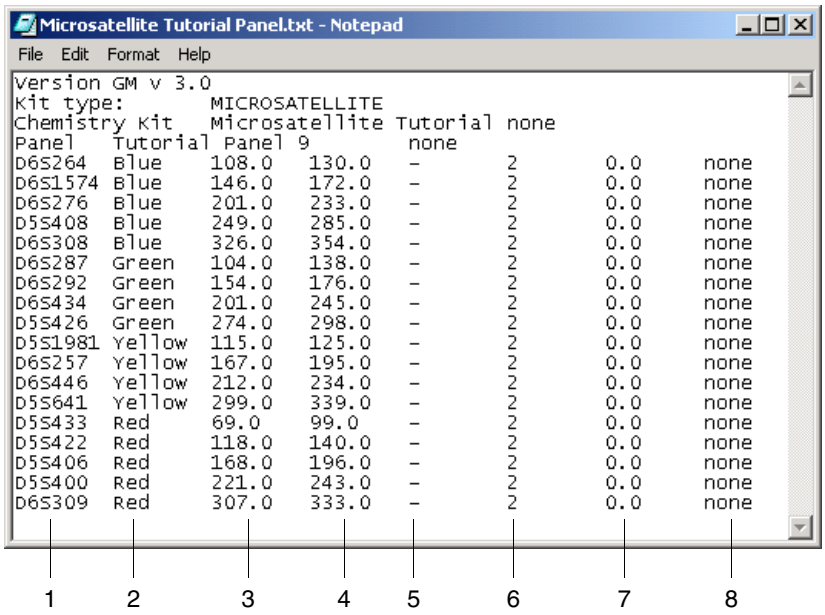
The Dyes menu item controls the dye colors shown when viewing SNaPshot reference data and microsatellite reference data electropherograms in the Panel Manager Plot view. Checked when active. Multiple dye colors may be selected.

Formats of Panel and Bin Text Files

This section provides an example of a file used to import panel definitions and a file used to import bin definitions. Files like these are created using Microsoft® Excel or other spreadsheet programs. Files must be saved as a tab-delimited text file.

Panel Definition Example

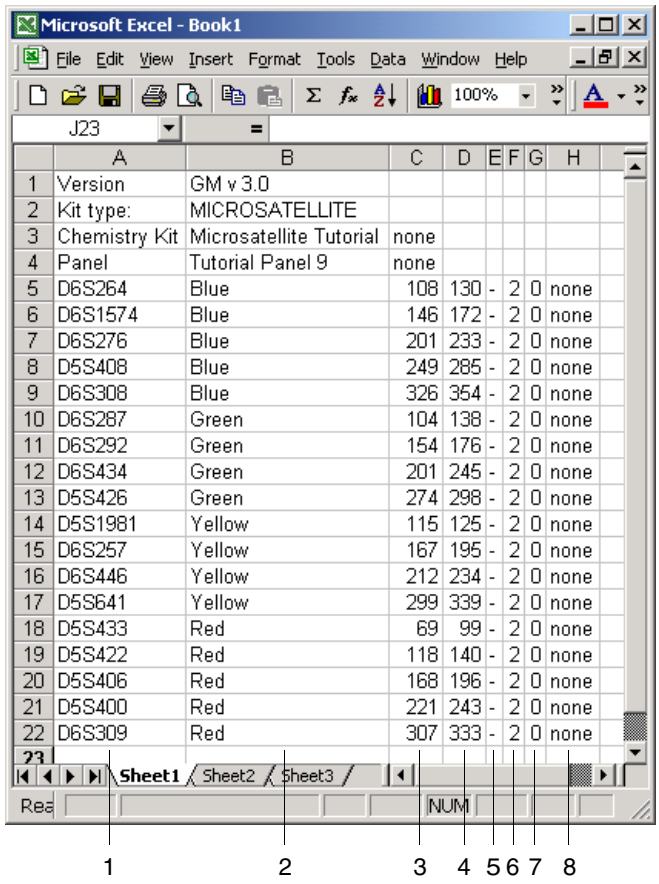
The examples below show panels defined in the format used by GeneMapper software v3.0 for a panel definition in a tab-delimited text file (.txt) using notepad or wordpad, or a Microsoft® Excel spreadsheet saved as a .txt file.



Columns:

1: marker name
2: dye color
3: min ASR (ASR = allele size range; actually the **marker** size range)
4: max ASR
5: control allele names as comma-separated bin names (1, 2)
(if none, there must be a dash "-")
6: type of repeat (2 = dinucleotide, 3 = trinucleotide, 4 = tetranucleotide, 5 = non-repeat)
7: marker-specific stutter ratio (if none, use a zero "0")
8: comments (if none, write "none")

Notice that there are no extra rows between markers and panels.



	A	B	C	D	E	F	G	H
1	Version	GM v 3.0						
2	Kit type:	MICROSATELLITE						
3	Chemistry Kit	Microsatellite Tutorial	none					
4	Panel	Tutorial Panel 9	none					
5	D6S264	Blue	108	130	-	2	0	none
6	D6S1574	Blue	146	172	-	2	0	none
7	D6S276	Blue	201	233	-	2	0	none
8	D5S408	Blue	249	285	-	2	0	none
9	D6S308	Blue	326	354	-	2	0	none
10	D6S287	Green	104	138	-	2	0	none
11	D6S292	Green	154	176	-	2	0	none
12	D6S434	Green	201	245	-	2	0	none
13	D5S426	Green	274	298	-	2	0	none
14	D5S1981	Yellow	115	125	-	2	0	none
15	D6S257	Yellow	167	195	-	2	0	none
16	D6S446	Yellow	212	234	-	2	0	none
17	D5S641	Yellow	299	339	-	2	0	none
18	D5S433	Red	69	99	-	2	0	none
19	D5S422	Red	118	140	-	2	0	none
20	D5S406	Red	168	196	-	2	0	none
21	D5S400	Red	221	243	-	2	0	none
22	D6S309	Red	307	333	-	2	0	none

Columns:

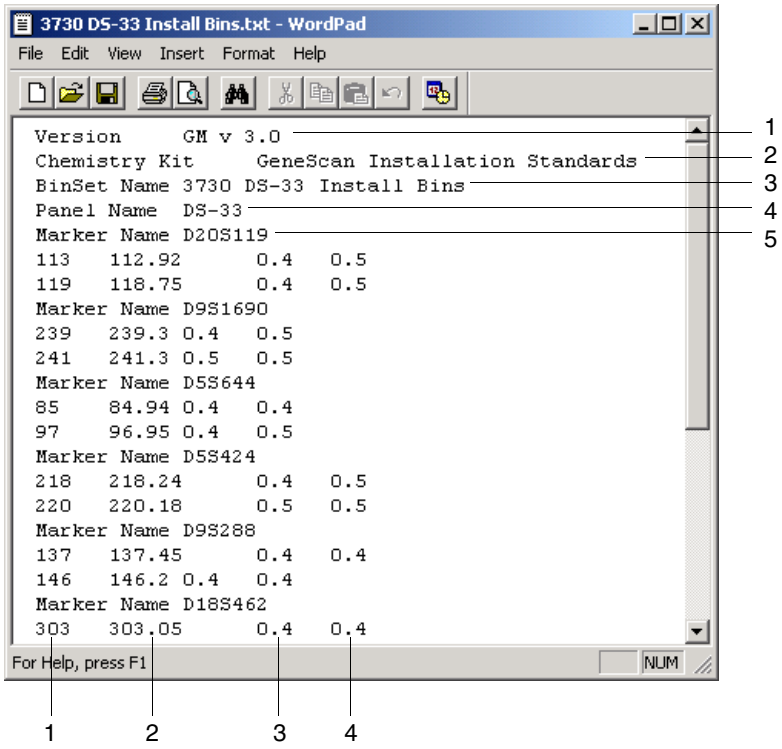
1: marker name
2: dye color
3: min ASR (ASR = allele size range; actually the **marker** size range)
4: max ASR
5: control allele names as comma-separated bin names (1, 2)
(if none, there must be a dash "-")
6: type of repeat (2 = dinucleotide, 3 = trinucleotide, 4 = tetranucleotide, 5 = non-repeat)
7: marker-specific stutter ratio (if none, use a zero "0")
8: comments (if none, write "none")

Notice that there are no extra rows between markers and panels.

Bins Definition Example

The examples below show bins defined in the format used by GeneMapper software v3.0 in a tab-delimited text file (.txt) or an Excel spreadsheet saved as a .txt file.

Note The ASR in the bin definitions file should match the ASR in the Panels definition file. Also, kit, panel, and marker names must be the same between the two files.

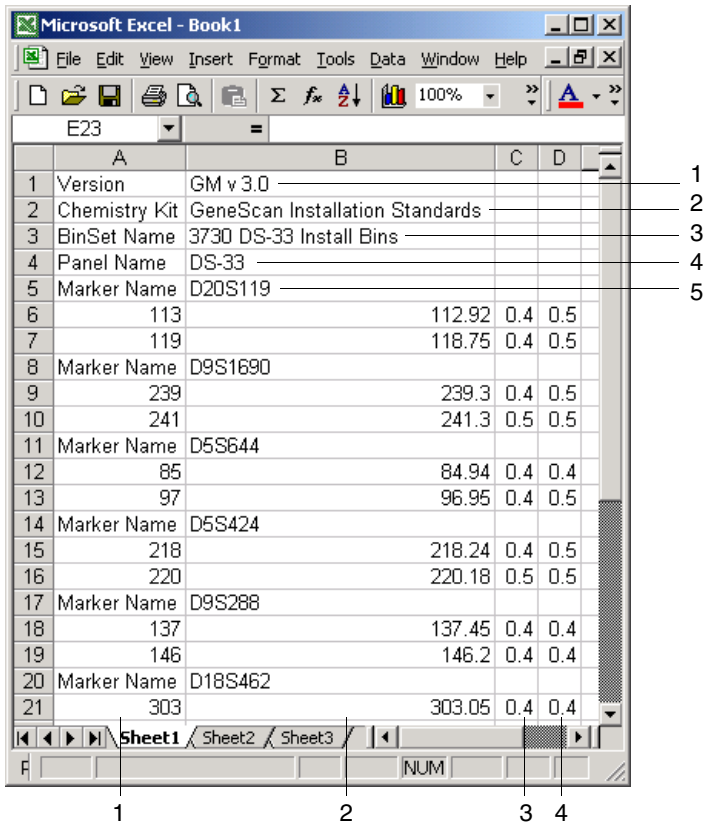


Columns:

1: bin name
2: bin center
3: bin left offset
4: bin right offset

Rows:

1: GM version number
2: Kit name
3: bin set name
4: panel name
5: marker name



	A	B	C	D
1	Version	GM v 3.0		
2	Chemistry Kit	GeneScan Installation Standards		
3	BinSet Name	3730 DS-33 Install Bins		
4	Panel Name	DS-33		
5	Marker Name	D20S119		
6	113	112.92	0.4	0.5
7	119	118.75	0.4	0.5
8	Marker Name	D9S1690		
9	239	239.3	0.4	0.5
10	241	241.3	0.5	0.5
11	Marker Name	D5S644		
12	85	84.94	0.4	0.4
13	97	96.95	0.4	0.5
14	Marker Name	D5S424		
15	218	218.24	0.4	0.5
16	220	220.18	0.5	0.5
17	Marker Name	D9S288		
18	137	137.45	0.4	0.4
19	146	146.2	0.4	0.4
20	Marker Name	D18S462		
21	303	303.05	0.4	0.4

Columns:

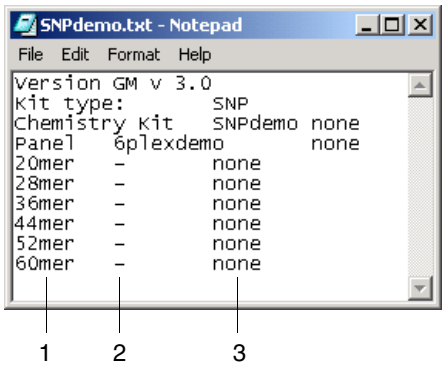
1: bin name
2: bin center
3: bin left offset
4: bin right offset

Rows:

1: GM version number
2: Kit name
3: bin set name
4: panel name
5: marker name

Panel Definitions for SNaPshot

The examples below show SNP panels defined in the format used by GeneMapper software v3.0 in a tab-delimited text file (.txt) or an Excel spreadsheet saved as a .txt file.

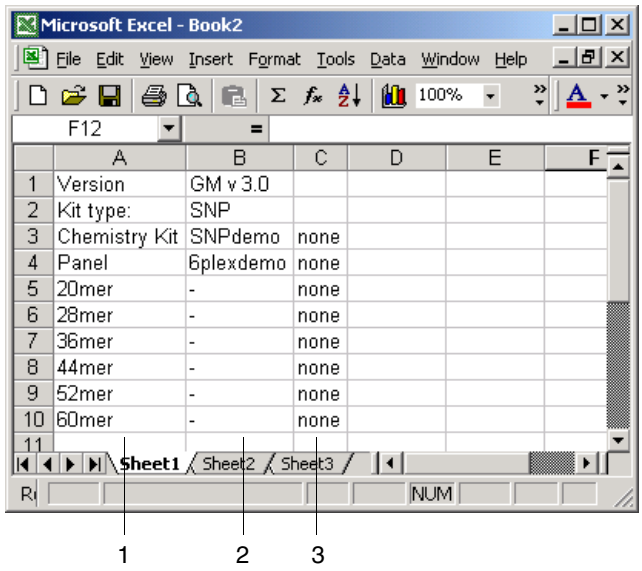


Columns:

1: marker name

2: control allele names as comma-separated bin names (A, G) (if none, there must be a “-”)

3: comments (if none, write “none”)



	A	B	C	D	E	F
1	Version	GM v 3.0				
2	Kit type:	SNP				
3	Chemistry Kit	SNPdemo	none			
4	Panel	6plexdemo	none			
5	20mer	-	none			
6	28mer	-	none			
7	36mer	-	none			
8	44mer	-	none			
9	52mer	-	none			
10	60mer	-	none			
11						

Columns:

1: marker name

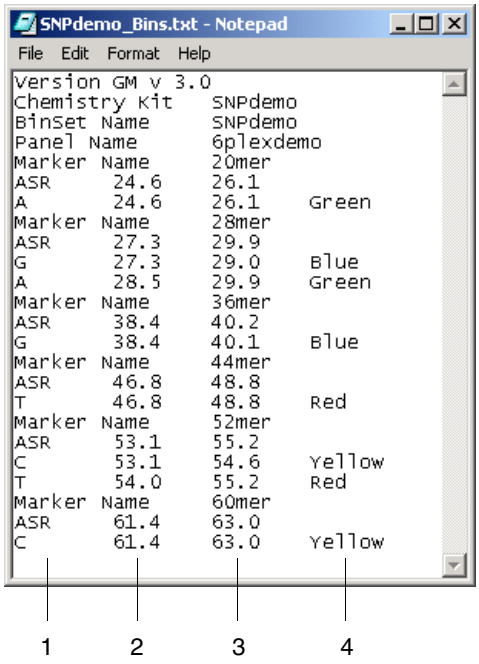
2: control allele names as comma-separated bin names (A, G) example (if none, there must be a “-”)

3: comments (if none, write “none”)

Bins Definitions for SNaPshot

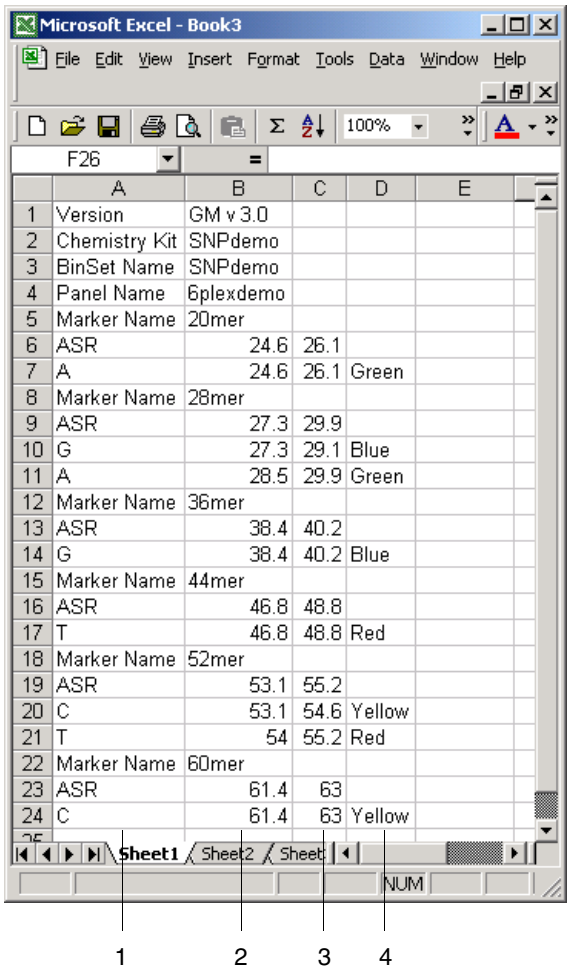
The examples below show SNP bin definitions in the format used by GeneMapper software v3.0 in a tab-delimited text file (.txt) or an Excel spreadsheet saved as a .txt file.

Note ASR = Total allele size range. The allele size data automatically exports and then the range is printed.



Columns:

1: bin name
2: bin size range min
3: bin size range max
4: bin color



Columns:

1: bin name
2: bin size range min
3: bin size range max
4: bin color

Import and Export Files

The Panel Manager imports and exports two text file data formats, panel data and bin set data. These files are tab-delimited and the following rules apply:

- Files to be imported must have the correct number of columns in the correct order.
- On import, panel and bin set names are checked for uniqueness in the GeneMapper™ database and markers are checked for uniqueness within the panel that will contain them. An alert is presented if a name is not unique and such a file cannot be imported.
- If any errors are found during import, no data from the file is accepted by the Panel Manager.
- Lines beginning with “#” are comments and are ignored on import.
- Both Panel and Bin set file formats have a header line with a GeneMapper software version string:

  Version GM v 3.0

  If the first line of a file to be imported does not begin with “Version GM,” then GeneMapper software handles the import as a GeneMapper software 1.x panel import. If the file is in GeneMapper software 1.x format, then it is imported without errors. If the file is in GeneMapper software 2.x or 3.x format, the file will not be imported and the following error alert will be presented: “The format of this file is wrong. Correct the file and try again.”

If the data type label does not match the type specified in the import command, the file is not imported and an alert is presented.

A PanelImportLog.txt file is generated during import of a panel or bin set and will list any errors encountered. This file is found in the *GMdistribution\app* folder on your hard drive. Panels and bin sets created in GeneMapper software v1.0.2 or GeneMapper software v2.0 are automatically updated when imported into GeneMapper software v3.0

Panel Table View

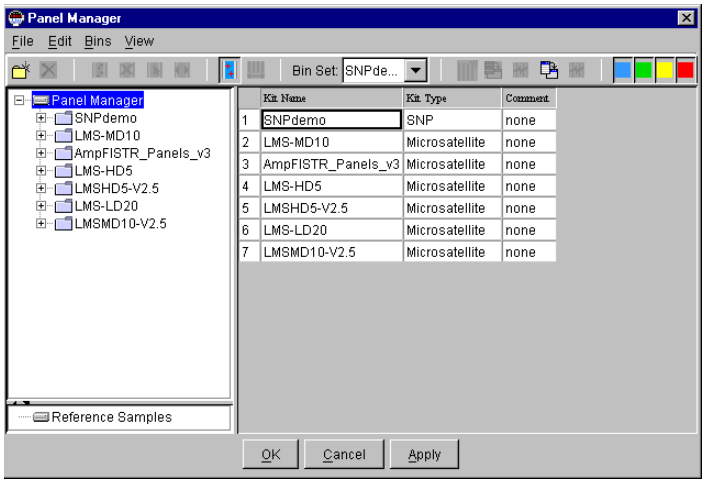
Introduction

Selecting the Panel Manager icon or text in the navigation pane (left pane shown below) shows a table of the current Panel Manager folders or kits in the right portion of the window. Selecting a particular kit produces a list of panels in the right portion of the window. This list is called the Panel Table view.

Tasks you can perform in the Panel Table view include:

- Editing panel names and entering comments in the panel table
- Creating new panels using the New Panel command
- Deleting an existing kit by selecting it in the Navigation pane and then choosing the Clear command
- Exporting panels and bin sets used for project analysis to backup

Note Editing or deleting kits, panels, and bin sets will prevent your from displaying analyzed projects. Export any panels and bin sets that you might use at a later date, or for reanalysis of old projects.



Note A Warning alert is shown whenever kits or panels are deleted to remind you that this reference data may have been used previously in Sample analysis.

Panel Table Columns

The table below lists the columns in the Panel table:

Note All columns are resizable.

Column Name	Description
Panel Name	Editable. Contains the name of the panel. Cell accepts alphanumeric characters (restricted for any invalid symbol characters – includes all Microsoft Windows invalid characters). **Note** When the cell is closed, the software must check that the name is unique. An alert message will display if the name is not unique.
Comments	Editable. Contains comments regarding the panel. Cell accepts alphanumeric characters.

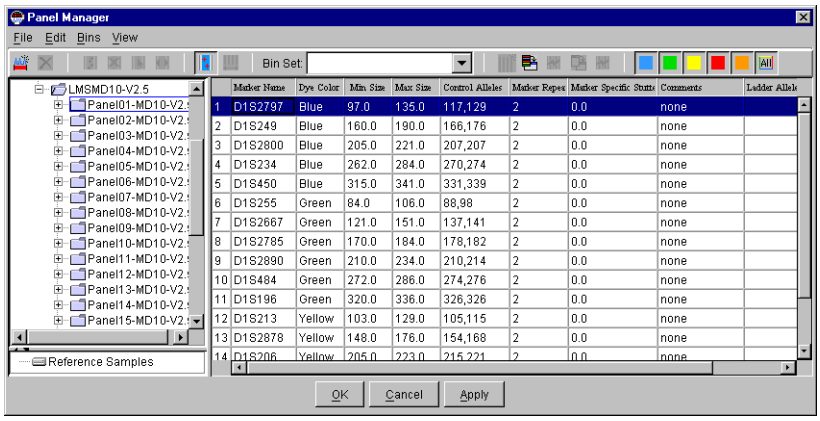
Name Rule for Panels

Panel names must be unique (within a kit and across kits).

Marker Table View

Introduction

Selecting a panel in the navigation pane displays the Marker table for that panel. The Marker table, shown below, enables you to view, create, and edit marker names and associated data.



Marker Table Columns

The figure on page 4-35 shows the columns in the microsatellite marker table. They are further described in the following table.

Marker table column descriptions:

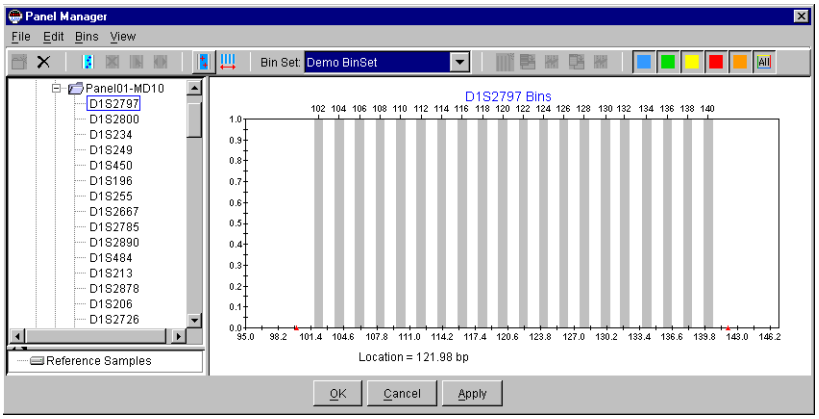
Column	Description
Marker Name	Editable. Contains the name of the Marker. Cell accepts alphanumeric characters from the keyboard. The field is validated if the marker name is unique in the panel. **Note** Marker names are case insensitive (*i.e.*, D1S83 is the same as d1s83).
Dye Color	Editable. Contains the color names blue, green, yellow, red, orange. The field is validated if it is one of these text strings, which are not case-sensitive.
Min Size	Editable. Contains the starting base pair number for the allele size range. Cell accepts numeric characters from the keyboard. The range defined by the Minimum Size parameter should include the stutter peaks for the smallest allele.
Max Size	Editable. Contains the ending base pair number for the allele size range. The cell accepts numeric characters from the keyboard.
Control Alleles	Editable. Contains a comma separated list of control allele names in the marker. No validation.
Marker Repeat	Editable. Indicates a di-, tri-, or tetra-nucleotide repeat. Contains a number for the number of repeats: 2, 3, 4. The field also accepts 9 for nonrepeat containing markers. The field is valid if it contains 2, 3, 4, or 9; any other number is invalid.
Marker Specific Stutter Ratio	Editable. Contains the ratio of stutter peak to main peak. Sets the maximum value of stutter for a specific marker, as opposed to using a global value for all markers.
Comments	Editable. Contains comments regarding the marker. Cell accepts alphanumeric characters from the keyboard.
Ladder Alleles	Editable. Comma-separated list of allele names in the allelic ladder. No validation. Applicable only to Human Identification (HID) genotyping or applications that use allelic ladders.

Bin View - (for Microsatellites Only)

Introduction

The Bin view enables you to view, create, and edit bins. The data displayed in the Bin view, imported into the Panel Manager using the Import BinSet command, is used to create the bins associated with the marker.

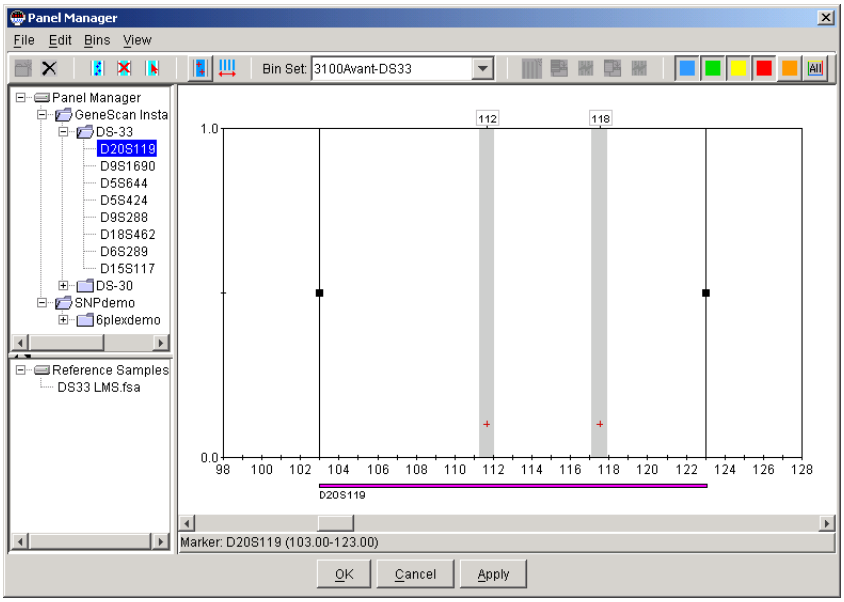
Bins allow the user to average the minor variations in size that occur from run to run and capillary to capillary.



Description of the Bin View

Reference Data, Bins, and Allele Calls

Reference data includes descriptions of bins (name, location, and boundaries) and the sample allele calls used to construct the bins. In the Bin view, the vertical bars are bins that are originally associated with the Markers imported as reference data. The “+” symbols shown in the figure above (marked in red on the monitor) are from the reference alleles used to build the bin set indicated in the drop-down menu at the top of the window. The red + s shown when those alleles are used in the autobinning process or in the manual process of bin creation.



If you analyze data using a bin set like that shown above, you will see the + s as shown above. Then, when you click the Show Project Alleles icon,

the + s will be overlaid by a set of "*" symbols (marked in blue on the monitor), which represent the data associated with a particular bin for the current project in the Project window and called according to the allele associated with the bin.

Bins may be used for calling alleles with or without allele calls in the reference data, but project alleles (* s) are required for automatic bin generation (the Auto Bin function). Reference alleles (+ s) are identified as a result of the Auto Bin command.

Selecting a reference data sample in the navigation pane displays that particular sample's electropherogram with bins overlaid to provide easy viewing while editing markers.

X- and Y-axes, Genotype Quality Symbols, and Cursor Location Information

The Bin view X-axis is the fragment size in base pairs; the Y-axis is quality, or signal height if a reference sample is selected. Vertical shaded bars are the bins that will be used to call alleles for the selected marker. The symbols (blue stars), described above as Allele Call

indicators, represent Genotype quality values on the Y-axis for the alleles of samples that have been analyzed and are currently displayed in the open project. The equivalent positions in the reference data are marked with red + s.

The Genotype quality values, representing analysis results, are not editable. Bin location, boundaries, and names are editable. Cursor location in base pairs for any selected bin are shown under the X-axis to the left. The Bin Sets drop-down menu, located just above the Bin view pane, selects the bin set that is applied to the marker.

Marker Allele Size Range (ASR)

Reference data for bins includes the marker ASR. This is the size range of bins in a marker and is set by the user to allow multiplexing of markers in a sample; an ASR should be changed only when a marker is under development.

ASR boundaries can be edited in the marker table or in the Bin view by adjusting the ASR handles on the right and left hand sides of the screen. The bins in a marker must not extend beyond the marker ASR. When bins are created and viewed in the Bin view, they are restricted so that their range does not extend beyond the marker ASR. The Show Alleles command may show new allele calls for a marker outside that marker's ASR, but bins cannot be created or extended to include alleles outside the ASR. To create bins for alleles that fall outside the ASR, edit that marker's ASR first and then click Apply. Then you can add a new bin.

Bins must not overlap in a microsatellite marker. When bins are created and edited, their locations and offsets are restricted to avoid overlap.

Import and export commands for panels and bin sets are enabled in the table views of the Panel Manager. Imported bin set reference data includes the bin boundaries but not sample reference data (the "+ s").

Automatic Bin Builder and Automatic Bin Assignment Algorithm

This section discusses the features of the Automatic Bin Builder (ABB) and Automatic Bin Assignment Algorithm (ABAA).

Combining Unique Features

The ABB process is used, along with the outcomes of the Multiple Allele Peak Determination Algorithms (MAPDA) and in conjunction with the ABAA to ensure that the called alleles are of the highest accuracy. The key benefit of combining these algorithms with the ABB is that, since only a small number of questionable alleles result from the integrated process, manual reexamination of the allele information is significantly minimized.

Automatic Bin Builder

The ABB is the first step in accurate allele assignment. The process of creating bins starts with a collection of Sample files. Bins are created by the ABB based on the chosen panel information and successive allele calls from the Sample file collection. As each Sample file in the collection is processed, the bin definitions are refined to reflect the actual data. The bin centers and widths continue to be refined until all Sample files in the collection are processed and GeneMapper software is ready for its final step of allele calling, Automatic Bin Assignment.

Automatic Bin Assignment Algorithms

Once bins are completely defined by the ABB, allele peaks are accurately and automatically assigned to their corresponding bins to complete the allele calls using the ABAA. The allele assignment algorithms calculate the certainty of each allele peak assignment, and determine if an allele should be called. Bin assignment quality values are assigned to the allele and become part of the overall PQVs.

Questionable alleles are appropriately marked with either a “Check ⚠” or “Low Quality ●” status marker. See the table under “Genotype View Columns” on page C-7 for more information on how this information is displayed in the Genotypes view.

Using Analysis Methods 5

Chapter Overview

Introduction This chapter provides information on how to use the Analysis Methods feature in the GeneMapper Manager window of the ABI Prism® GeneMapper™ Software Version 3.0, and describes how to use the features of the Analysis Method Editor.

In This Chapter This chapter contains the following topics:

Analysis Methods Tab

Introducton

The Analysis Methods Tab is used to create custom analysis methods for analyzing sample files. An analysis method is a set of algorithm parameters that are applied to data during analysis.

Default Analysis Methods

GeneMapper software v3.0 contains the following standard Applied Biosystems profiles:

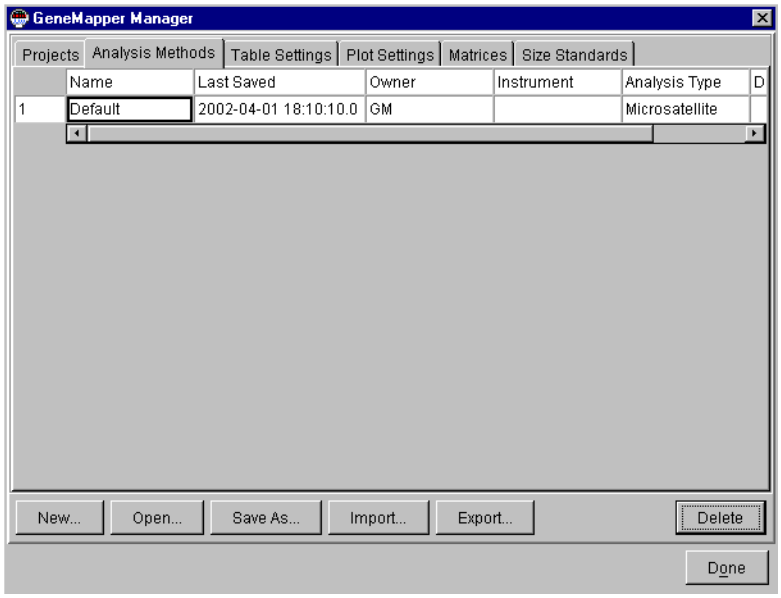
Default	Analysis Type	Bin Set	Detection Algorithm
Microsatellite	Microsatellite	None	Basic
SNP Genotyping	SNP Genotyping	None	Basic
3730 DS-33 Install	Microsatellite	3730 DS-33 Install Bins	Basic (For use with the Applied Biosystems 3730 DNA Analyzer only)

Note These profiles can be altered but should not be deleted. They are intended to be used as models for customizing new analysis methods.

About the Analysis Methods Tab

The Analysis Methods tab contains a list of analysis methods in the database. This tab is used to manage the contents of the project window.

To display the Analysis Methods tab, select **Tools > GeneMapper Manager > Analysis Methods**.



Parts of the Analysis Methods tab:

Item	Description
Name column	Analysis method name
Last Saved column	Date/Time stamp showing when the analysis method was last saved
Owner column	User name of the person who created the analysis method
Instrument column	The type of instrument used
Analysis Type column	Identifies the analysis mode of the analysis method (i.e., HID, Microsatellite, SNaPshot)

Parts of the Analysis Methods tab: *(continued)*

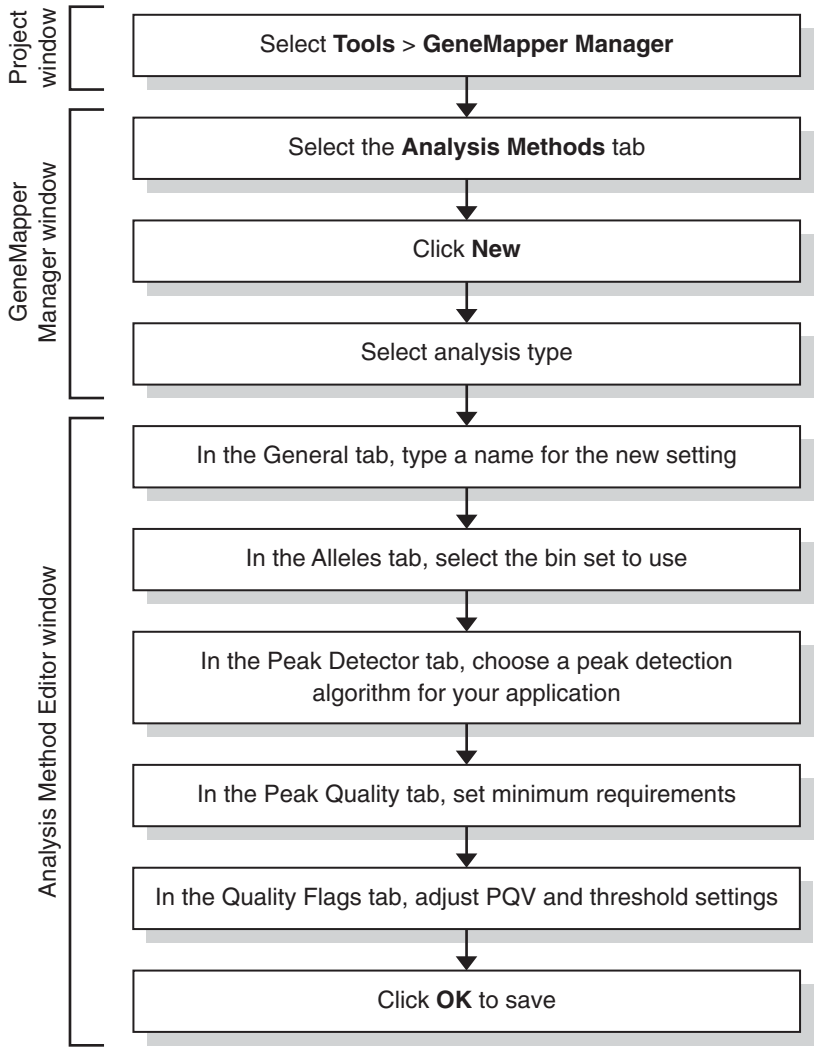
Item	Description
New button	Opens the New Analysis Method dialog box. Always enabled. This dialog box asks you to select the analysis type before opening the Analysis Method Editor. The analysis type you select: ♦ Sets the analysis algorithm ♦ Displays the appropriate fields for that type of analysis in the Analysis Method Editor dialog box.
Open button	Opens the editor for a selected analysis method. Enabled when a analysis method is selected.
Save As button	Displays the Save As dialog box. Enabled when an analysis method is selected.
Import button (.xml file type)	Displays a dialog box for Importing analysis methods. Always enabled.
Export button (.xml file type)	Displays a dialog box for Exporting selected analysis methods. Enabled when one or more analysis methods are selected.
Delete button	Deletes the selected analysis method(s)
Done button	Closes the GeneMapper Manager

Analysis Method Editor

Introduction

The Analysis Method Editor allows you to create or edit analysis methods to be used for analyzing your sample files. The analysis method determines the type of application being analyzed and allows for customization of the peak detection algorithm and quality value settings.

Flowchart



Accessing the Analysis Method Editor

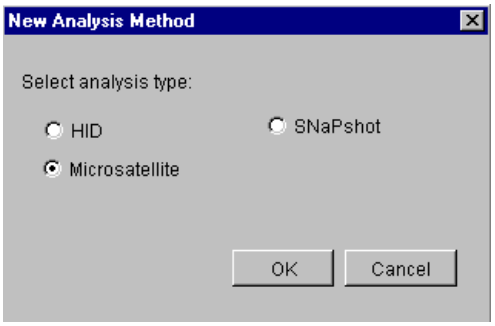
Access the Analysis Method Editor in the following ways:

- Click **Tools > GeneMapper Manager > Analysis Methods** Tab **> New** or **Open**
- In the Project Window, click the **Samples** tab. Click **New Analysis Method** to create a new method, or double click an existing method to edit it.
- Select a sample in the project window and click the Analysis Method Editor icon on the toolbar. Edit the currently selected samples' analysis method, if necessary.

Note The Analysis Method Editor icon is not active unless a sample with an analysis method is selected.

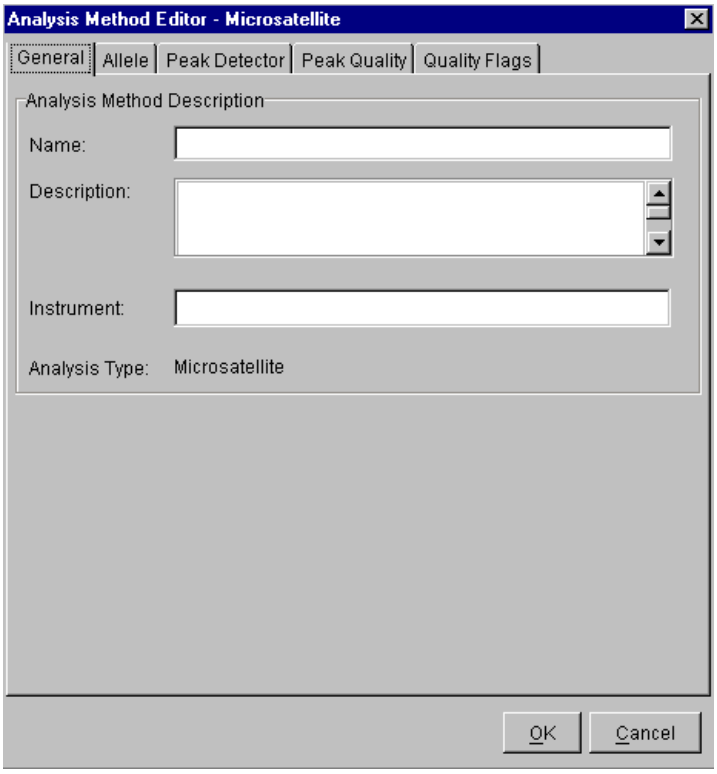
New Analysis Method Dialog Box

When you create a new analysis method, the following dialog box opens prior to opening the analysis method editor. This dialog box allows you to select the analysis type for your samples, and controls the items available for customization within the Analysis Method Editor tab.



General Tab

In the General tab, enter a name and description for the analysis method.

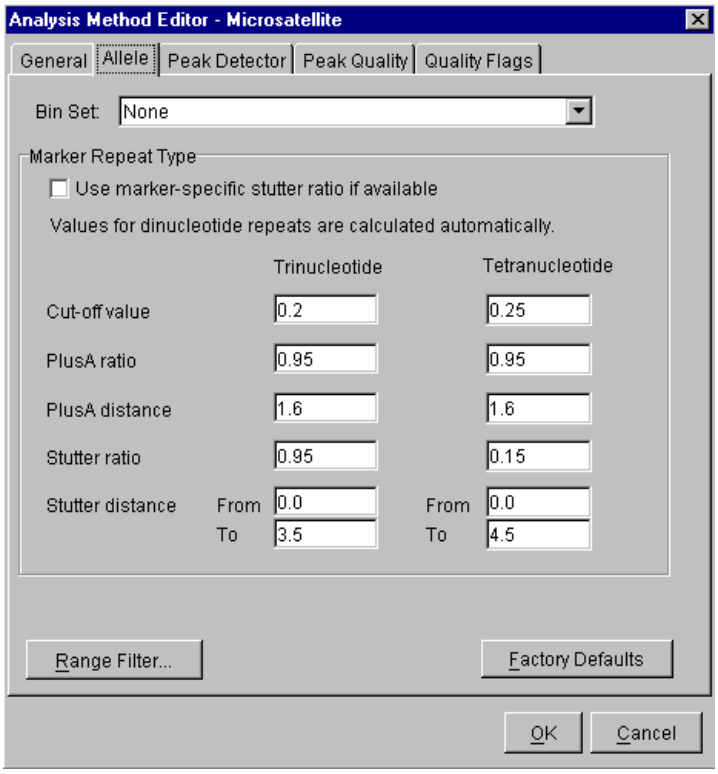


Note The Analysis Type reflects the choice selected in the New Analysis Method dialog box.

Allele Tab

In the Allele tab, set a number of parameters controlling allele calling, including:

- Bin Set
- Marker Repeat Type (for Microsatellites)
- SNP Cut-Off value (for SNaPshot)



Bin Set

Bins are a way of matching your data to the allele definitions contained in the panel assigned to the data. Bins are the locations in which you expect an allele to display. Alleles are defined in Markers as a size range (bin) centered around the average size in base pairs, for example 101.5 +/- 0.4 bp. If your sample has a peak that falls within a bin, the allele in your sample file is called with the identifier assigned to that allele bin.

A bin set must be chosen for your analysis in order to guide the algorithms in assigning allele calls. This drop-down list enables you to choose the bin set to be used for analysis. The bin set chosen for analysis must match the kit/panel chosen for analysis.

Marker Repeat Type (for Microsatellites)

As stated on the Allele tab view, no entries are required for dinucleotide repeats because the application calculates them automatically.

Check the “Use marker-specific stutter ratio if available” check box to insert stutter ratio information into the Panel Manager marker’s table. If you know the stutter ratio for an individual marker, you can set this number under the “Marker Specific Stutter%” column in the Panel Manager. Checking this box in the Alleles page causes the algorithm to use your defined stutter ratio and not the ones defined in the Stutter ratio box. Different values can be used for different markers.

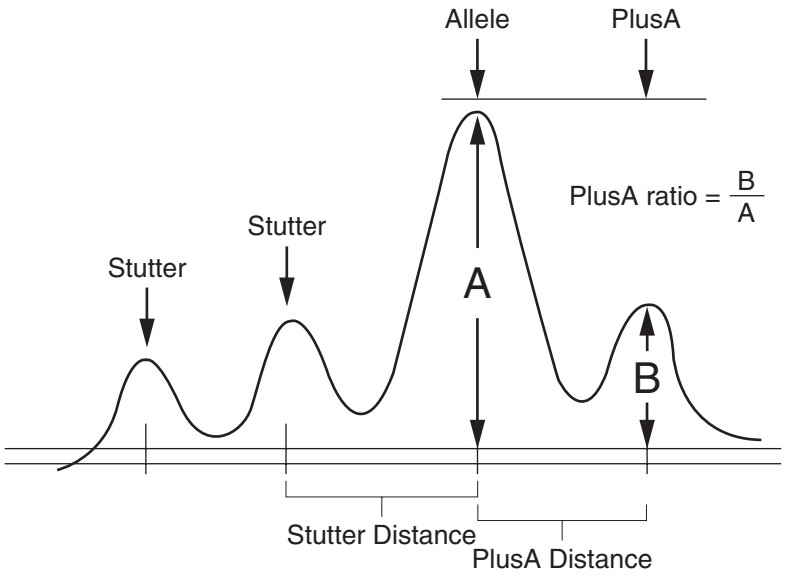
The “Cutoff value” ignores all peaks less than the cut-off ratio of the largest peak in the allele size range.

The “PlusA ratio” and “Stutter ratio” refer to the ratios you are expecting. For example, if you expect a stutter percentage of 20% for your markers, enter in 0.2.

The two columns labeled Trinucleotide and Tetranucleotide allow entry of all related parameters directly into fields, so that everything can be seen at once. The following table provides information about the ranges of possible values.

Parameter	Min	Max	Trinucleotide Default	Tetranucleotide Default
Cut-off value	0.0	1.0	0.2	0.25
PlusA ratio	0.0	1.0	0.95	0.95
PlusA distance	0.0	? (Infinite)	1.6 bp	1.6 bp
Stutter ratio	0.0	1.0	0.95	0.15
Stutter distance	0.0	? (Infinite)	from 0 to 3.5 bp	from 0 to 4.5 bp

The parameters in the table (except Cutoff value) are defined graphically in the following figure.



SNP Cutoff Value (for SNaPshot)

For heterozygous SNP genotyping alleles, if the highest peak height is X and a second, lower peak height is Y, then the ratio Y/X must be larger than the cutoff value in order for the second peak to be called. The default value is set at 0.3 and may need to be further optimized for your data.

Range Filter

The Range Filter allows you to remove labels from peaks within a specific size range for each color.

Peak Detector Tab

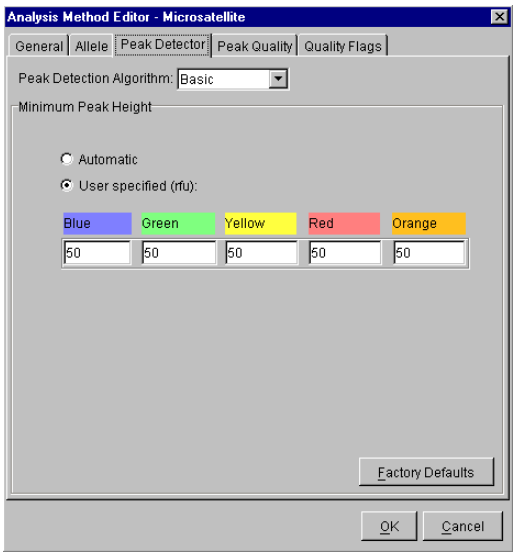
Use the Peak Detector tab to select the algorithm to use for detecting peaks:

- Basic
- Advanced
- Classic

See Appendix B, “Software Genotyping Algorithms,” for additional information on Basic, Advanced, and Classic peak detection algorithms.

Basic Peak Detection Algorithm

The default, Basic peak detection algorithm, is used for most applications.

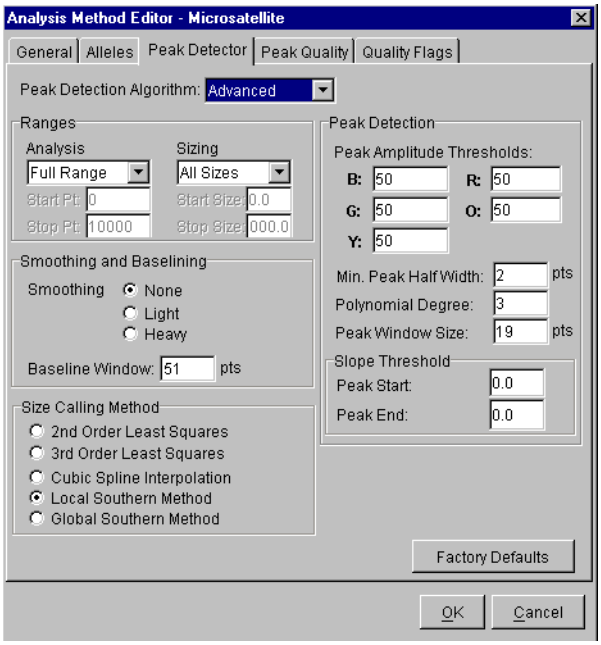


The application sets the level automatically for these five dye colors when “Automatic” is selected: blue, green, yellow, red, and orange. This level represents the minimum signal strength that will be identified as a peak for each dye (equivalent to 10 times the noise).

Selecting the User Specified option button enables the number entry fields for five dye colors. These numbers are the minimum signal strength that will be identified as a peak (in relative fluorescent units). For all dyes, the default is 50, minimum is 1, and the maximum is any number of 10 digits.

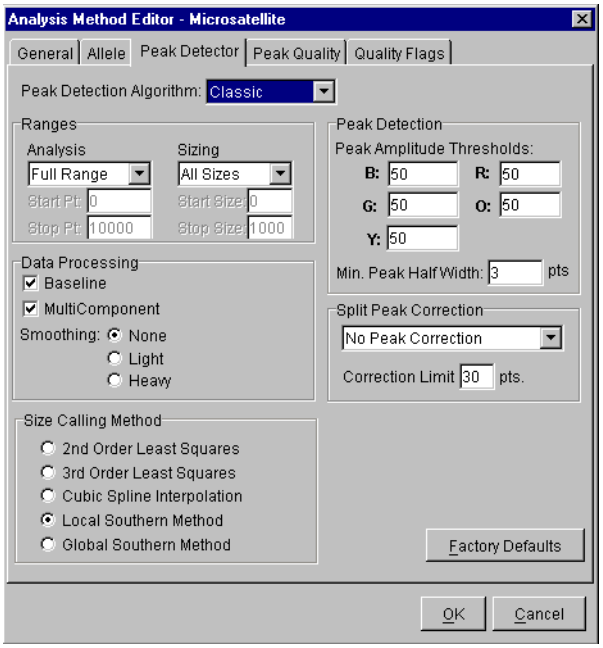
Advanced Peak Detection Algorithm

This algorithm is similar to the ABI PRISM® GeneScan® software on Windows NT operating system method of analyzing size standards and performing peak detection. Information on the individual parameters can be found in Appendix B, “Software Genotyping Algorithms.”



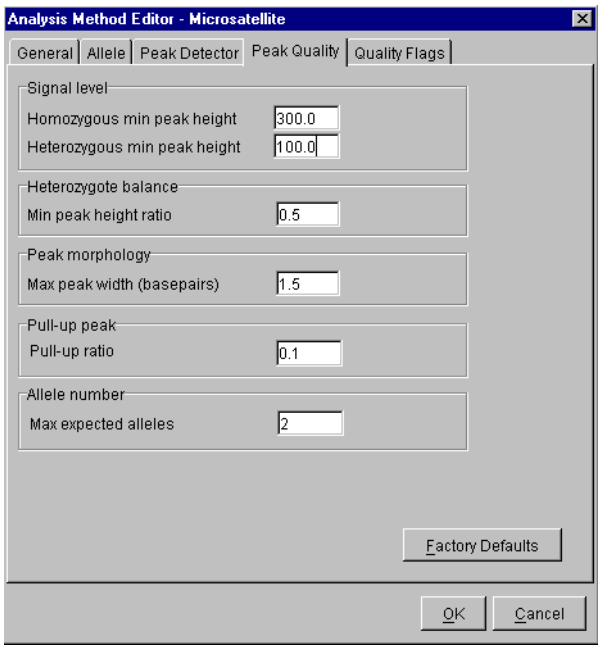
Classic Peak Detection Algorithm

This algorithm is similar to the GeneScan software on the MacIntosh operating system method of analyzing size standards and performing peak detection. Refer to Appendix B, “Software Genotyping Algorithms,” for additional information.



Peak Quality Tab

The Peak Quality page sets the thresholds for warning flags in the Genotypes view in the Project window. The PQV system uses these values to determine what types of warning flags should be set for each parameter. When you click the Peak Quality tab, the following page opens.



The settings include the following:

- Signal Level (in fluorescent units)
  - Homozygous min peak height (default = 200)
  - Heterozygous min peak height (default = 100)

  Peaks between the minimum peak height and the signal peak height setting (set in the Peak Detector page) will still be scored, but the peak height flag will be triggered.
- Heterozygote Balance
  - Min peak height ratio (default = 0.5)

  In a heterozygous allele, the higher peak height is X and the lower peak height is Y. If the ratio Y/X is less than the Heterozygote Balance, then the process quality value flag for Peak Height Ratio will be triggered and the GQ value will be lower.

- Peak Morphology
  - Max peak width (basepairs) (default = 1.5)
- Pull-up peak
  - Pull-up percentage (default = 0.1)

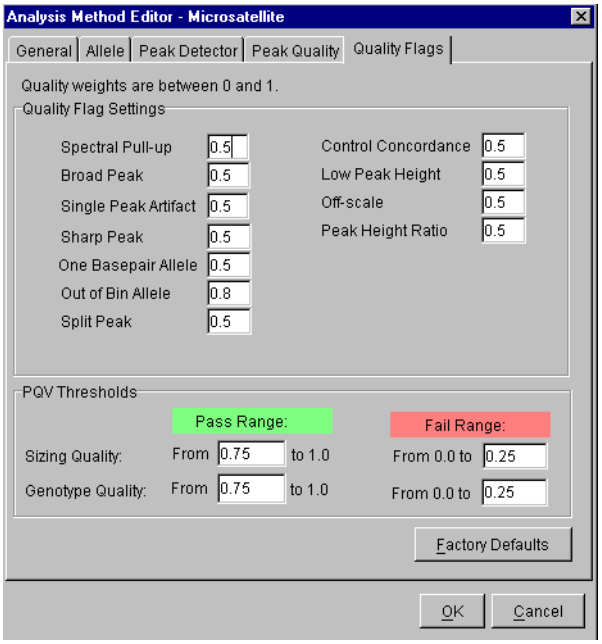
  Considers the ratio of any color peak directly under a major peak. Flags Spectral Pull-Up quality value as in the Genotypes table.
- Allele Number
  - Max expected allele number (default = 2)

  If analyzing polyploid alleles, enter in the maximum number of alleles you expect. You must also set the table profile to display the correct number of alleles.
- SNP (for SNaPshot application only)
  - Double peak (default = 0.5)

  (See “PQV Flags on the Genotypes Page” in Appendix A.)

Quality Flags Tab

Use the Quality Flags tab to set the importance level of the PQVs available for microsatellite and SNP analysis. The PQVs are weighted on a scale of 0 to 1, with 0 being of no importance and 1 being of the highest importance. Most defaults are set at 0.5, except for Out of Bin Allele, which is set at 0.8. These defaults emphasize the point that if a peak falls outside a bin, it should have a lower genotype call. The Default button will reset these calls to the default numbers discussed in the Peak Quality Tab section on page 5-14.



PQV Thresholds

Use the options under PQV Thresholds to set Pass/Check/Low Quality thresholds for the Sizing Quality (SQ) parameter for the Samples view and the Genotype Quality (GQ) parameter for the Genotypes view. (See the figure on page 5-16.) Anything that is not labeled Pass or Low Quality will be labeled as check.

Options

The Quality metric columns are set to display color symbols by default. However, the SQ column in the Samples view and the GQ column in the Genotypes view can be set to display the numerical equivalent on which the color symbols are based for these columns. This setting is made in the Analysis tab in the Options window by clicking the Numbers option button for the Quality Metrics Display setting.

Data entry restrictions for the PQV thresholds ensure that the lower limit of the Pass range is always greater than the upper limit of the Low Quality range. The Check range is the interval between Pass and Low Quality, if any. For example, if you use the default threshold settings shown in the figure above, any result over 0.75 passes, any result at 0.25 or below fails, and any result from 0.26 to 0.74 will be marked for you to check. (See “Process Component-Based Quality Values,” in Appendix A.)

When the default setting for the Quality Metrics Display (Analysis tab in Preferences window) is “Symbols,” a Pass for the SQ and GQ parameters is indicated by a green square  , a Check is indicated by a yellow triangle  , and a Low Quality is indicated by a red octagon  .

Some quality metrics are either Pass or Low Quality (for example, Background Peak), and some are warning flags with either Pass or Check values (for example, File Not Found). Such values are not adjustable by the Threshold parameter.

Using Table Settings 6

Chapter Overview

Introduction This chapter provides information about the Table Settings tab in the GeneMapper Manager window of the ABI Prism® GeneMapper™ Software Version 3.0, and describes how to use the features of the Table Settings Editor.

In This Chapter This chapter contains the following topics:

Table Settings Tab

Introduction

Use the Table Settings tab to perform the following tasks:

- Create new profiles
- Hide and show table columns
- Filter the table entries (rows) in Project windows

Default Table Settings

GeneMapper software v3.0 contains the following standard Applied Biosystems profiles:

- Default
- SNP Default
- Microsatellite Default

Default is a general profile, SNP Default is intended for SNaPshot samples,and Microsatellite Default is intended for Microsatellite samples. These profiles can be used as models for customizing new profiles.

Table Settings Tab

To display the Table Settings tab, select **Tools > GeneMapper Manager > Table Settings**.

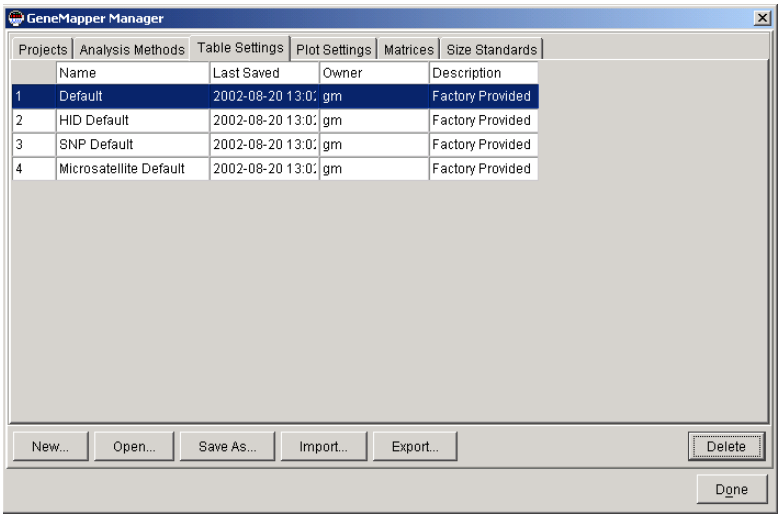


Table Settings allow you to determine what columns are viewed in the Samples and Genotypes tab, and to set filtering properties.

The Table Settings tab contains a list of Table Settings in the database. This tab is used to manage the contents of the Project window by providing the following menus and interface elements.

Parts of the Table Settings tab:

Item	Description
Name column	Table Setting name
Last Saved column	Date/Time stamp displaying when the object was last saved
Owner column	User name
Description column	Description of the object
New button	Opens the Table Setting Editor window Always enabled
Open button	Opens the editor for selected table settings Enabled when a single object is selected
Save As button	Displays the Save As dialog box Enabled when a single object is selected
Import button (.xml file)	Displays a dialog box for Importing objects Always enabled
Export button (.xml file)	Displays a dialog box for Exporting selected objects Enabled when one or more objects are selected
Delete button	Deletes the selected object(s)
Done button	Closes the GeneMapper Manager

Table Settings Editor

Introduction

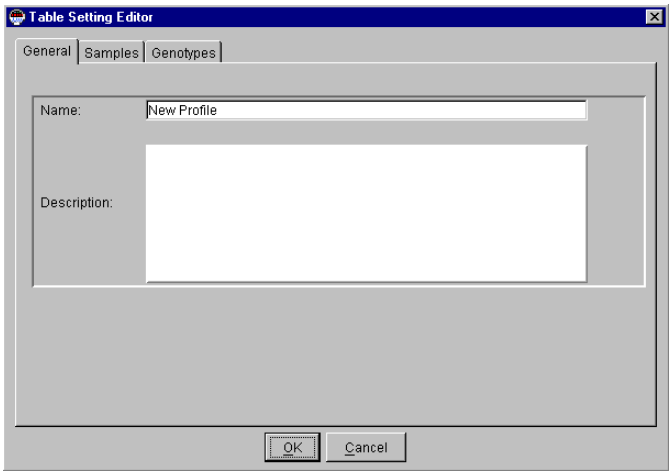
Use the Table Settings Editor to show or hide information specific to your sample, add filtering capabilities, and give content information for each column in the project window. The Table Settings Editor window contains the following three tabs:

- General
- Samples
- Genotypes

The General Tab

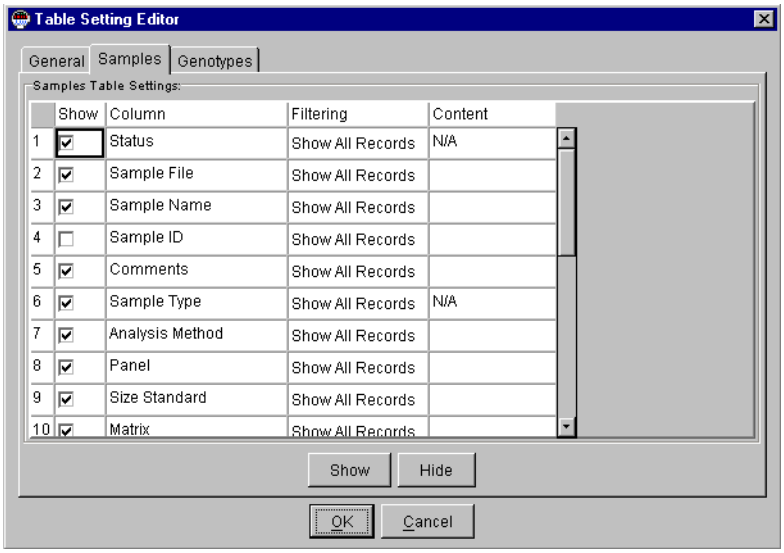
The General tab provides the capability to give a name and description to a new profile.

Note You cannot change the name of an existing profile, only the description.



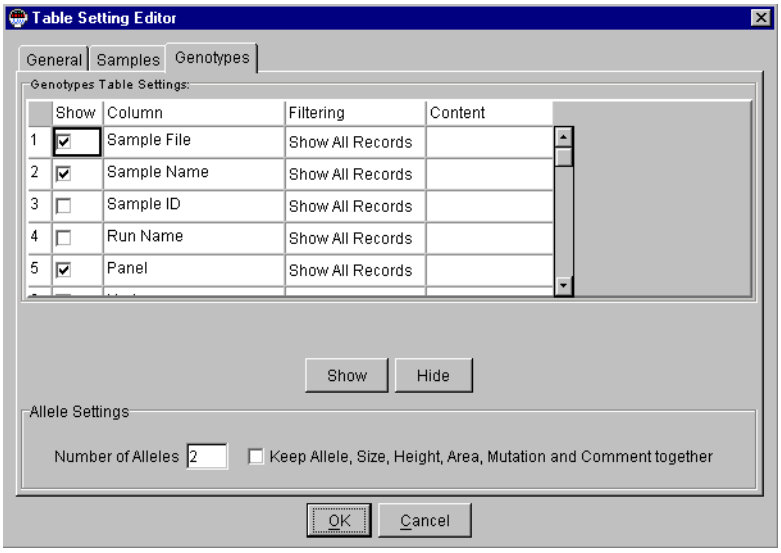
The Samples Tab

The Samples tab provides the capability to filter columns in the Samples table of the project window.



The Genotypes Tab

The Genotypes tab provides the capability to filter columns in the Genotypes table of the project window and the capability to control the allele settings.



The Sample and Genotypes Tab Elements

The Samples and Genotypes tabs display the Table Setting properties that will be applied to the corresponding tabs in the Project window. The properties or elements are described in the table below.

Samples and Genotypes Settings

Element	Description
Column settings	These settings contain the valid columns for either Samples or Genotypes data. ♦ **Show column** - List with an editable check box for each Project column, controlling whether the column is shown or hidden ♦ **Column column** - List of column headings for the Project window ♦ **Filtering column** - List of filtering properties for each Project window column ♦ **Content column** - Sets what information is displayed in each Project window column as selected by the appropriate filtering method. “N/A” indicates that no comments can be made for these rows.
Filtering Properties (Summary) (For more specific information, see “Filtering Controls” on page 6-10.)	The filtering properties for a Project table column are controlled in the Filtering and Content columns. Modifying these settings updates the property in the Column/Filter table. For more information, see “Filtering Controls” on page 6-10.
OK	Closes the Table Setting Editor window and applies any pending changes to the Project window. Contents of the Table Setting Editor are saved to the GeneMapper database.
Cancel	Closes the Table Setting Editor window without making any pending changes.
Show	Selects all highlighted rows as visible.
Hide	Selects all highlighted rows as hidden.

Allele Settings

The Allele Settings box on the Genotypes page of the Table Setting Editor window controls how the Allele, Size, Height, Area, Mutation and Comment columns are displayed in the Genotypes view.

Allele Settings	Description
Number of Alleles	The number of alleles for which genotypes are displayed. The default value is 2. This value is correct in most cases. However, when you are analyzing polyploid data you should change this value to the maximum expected allele number to view all calls.
Keep Allele, Size, Height, Area, Mutation and Comment together	If the box is unchecked: – then the columns display as: Allele 1, Allele 2, Size 1, Size 2, Height 1, Height 2, Area 1, Area 2, Mutation 1, Mutation 2, Comment 1, Comment 2 or If the box is checked: – then the columns display as: Allele 1, Size 1, Height 1, Area 1, Mutation 1, Comment 1, Allele 2, Size 2, Height 2, Area 2, Mutation 2, Comment 2

Creating a New Table Setting

To create a New Table Setting:

Step	Action
1	Select **GeneMapper Manager > Table Settings** and click **New** to open the Table Setting Editor window.
2	In the **General** tab, enter a name for the new setting and a description if necessary.
3	Open the **Samples** and/or **Genotypes** tabs and select the desired contents for the Samples and Genotypes tables in the corresponding tabs.
4	Click **OK** to save the new setting.

Editing a Table Setting

To edit a Table Profile:

Step	Action
1	Select **Tools > GeneMapper Manager** to open the GeneMapper Manager window. **Note** If the table is selected in the project window drop down menu, then you can click the Table Settings icon or **Tools > Table Setting Editor**.
2	Select the **Table Settings** tab.
3	Select a table setting name and click **Open**. The selected setting opens.
4	Perform edits in the table setting. **Note** See The Sample and Genotypes Tab Elements on page 6-7.
5	Click **OK** when you have completed editing. The changes you have made are saved to the Table Setting.

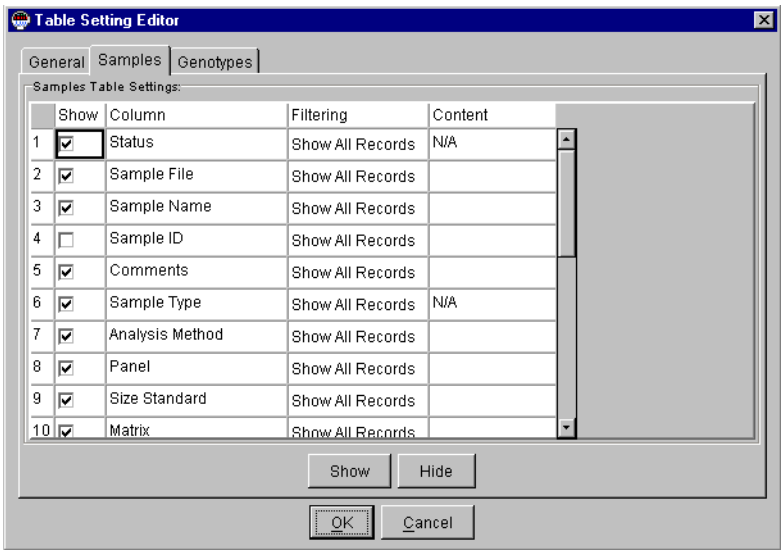
Deleting a Table Setting

To delete a Table Setting :

Step	Action
1	Select the **Table Settings** tab in the GeneMapper Manager window.
2	Select a table setting name and click **Delete**. A warning alert is shown. Click **OK** to remove the setting.

Filtering Controls

The Table Settings column labeled “Filtering”, shown in the figure below, is used to filter or set the type of record or display presented for a Project window column.



Samples View Column Filtering Settings

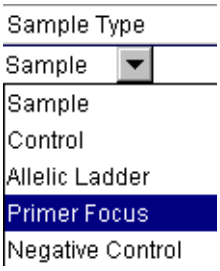
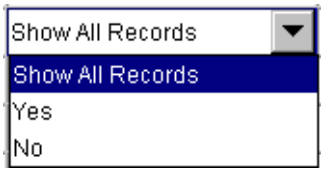
Note When you apply these settings to filter parameters, be aware that when no instance of the type specified exists, the Sample Table displays blank. If this occurs, return to the Table Manager and reset the filter.

The following information describes how to use the filter settings for the Samples Column Settings. The default “Show All Records” can be changed to one of the following:

Samples view filter settings:

Column Name	Filter Settings
Status Sample File	Analyzed or Not Analyzed 

Samples view filter settings: *(continued)*

Column Name	Filter Settings
Sample Name Sample ID Comments Analysis Method Panel Size Standard Matrix Run Name Instrument Type Instrument ID Run Date & Time User Defined Columns	Show Records Containing  **Note** This setting allows specification by a string. Input this information into the Content column.
Sample Type	Sample, Control, Allelic Ladder, Primer Focus, or Negative Control 
Reference Data Off-scale Sizing Quality Invalidated	Yes or No 
Sample File Not Found Matrix Not Found Size Standard Not Found	Show Pass (green squares) or Show Low Quality (red octagons) 

Samples view filter settings: *(continued)*

Column Name	Filter Settings
Sizing Quality (SQ)	Show Pass, Show Check, or Show Fail  **Note** These settings restrict the display to those records meeting these criteria.

Genotypes View Filter Settings

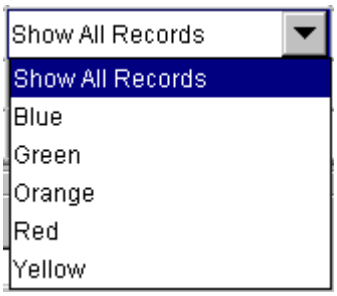
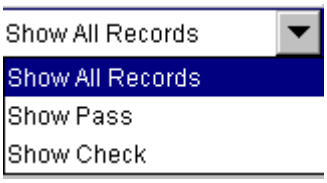
Note In applying these settings to filter parameters, be aware that when no instance of the type specified exists, the Genotypes table will be displayed blank. If this occurs, return to the Table Manager and reset the filter.

The following information describes how to use the filter settings for the Genotypes Column Settings. The default “Show All Records” can be changed to one of the following:

Genotypes view filter settings:

Column Name	Filter Settings
Sample File Sample Name Sample ID Run Name Panel Marker Allele Size Height Peak Area Mutation AE Comment Integration Comments User Defined Columns	Show Records Containing  **Note** This setting allows specification by a string. Input this information into the Content column.

Genotypes view filter settings: *(continued)*

Column Name	Filter Settings
Dye	Blue, Green, Orange, Red, or Yellow  **Note** This setting can be changed to display either all colors “Show All Records” or the choice of display of an individual color. SNaPshot data cannot be filtered by this selection.
Off-scale Sharp Peak (M) One Basepair Allele (M) Single Peak Artifact (M) Split Peak (M) Out of Bin Allele Peak Height Ratio Low Peak Height Spectral Pull-up Allele Number Broad Peak Double Peak (SNP) Narrow Bin (SNP) Control Concordance Overlap (HID)	Show Pass (green squares) or Show Check (yellow triangles) 
Allele Edit Allele Display Overflow	Yes or No 

Genotypes view filter settings: *(continued)*

Column Name	Filter Settings
Genotype Quality (GQ)	Show Pass, Show Check, or Show Low Quality 

Using Plot Windows - Samples and Genotypes 7

Chapter Overview

Introduction

This chapter describes how to examine and interpret electropherograms within the ABI Prism® GeneMapper™ Software Version 3.0 Samples and Genotypes plot windows, and explains the various electropherogram settings and interactions in the plot window menu items.

In This Chapter

This chapter contains the following topics:

About the Plot Window

Introduction

The Samples and Genotypes plot windows allow you to visually assess your data. If genotype calls were made, you can also view and edit allele calls assigned by the GeneMapper software algorithms.

Purpose of the Plot Window

Samples Plot Window

The Samples plot window allows you to:

- View electropherograms on a per sample basis
- View a sizing or genotypes table for sample(s) shown
- Overlay all samples to determine size standard quality
- Edit markers and bins
- View the relationship between controls and samples
- Edit allele calls

Genotypes Plot Window

The Genotypes plot window allows you to:

- View electropherograms on a per genotype basis
- View all genotype calls for a given marker
- Edit markers and bins
- View the relationship between controls and samples
- Edit allele calls

Displaying Plot Windows

Display the plot window from the GeneMapper Project window.

To open the plot window:

Step	Action
1	Highlight any number of samples or genotypes from the project window, by selecting the row number or sample file name.
2	Click  (Display Plots) on the toolbar, or **Analysis > Display Plots**.

When you select a sample, all of the corresponding genotypes are automatically selected in the Project Window Genotypes Tab. If a plot window is open, the view refreshes as you switch between tabs.

When you select a genotype, the corresponding sample(s) are automatically selected in the Project Window Samples Tab. If a plot window is open, the view refreshes as you switch between tabs. The genotype selection is maintained until additional samples or genotypes are selected or deselected.

Note Samples with a failed sizing quality (SQ) value cannot be displayed in the Plot window. Only raw data can be viewed for failed samples.

Note The Plot Window cannot contain electropherograms from both the Samples and Genotypes tabs at the same time.

Customizing Plot Windows

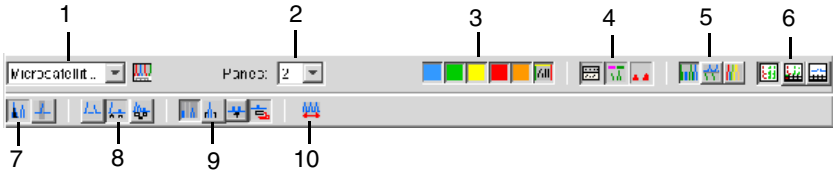
Certain features within the Plot Windows can be customized using the pull-down menu items or toolbar icons. Frequently used plot views can be saved as Plot Setting profiles using the Plot Settings Editor accessible from the GeneMapper Manager. See Chapter 8, “Using the Plot Settings Editor.”

- The last profile used in the Samples Plot is used to open the Genotype plot.
- The last profile used in the Genotypes Plot is used to open the Sample Plot.

Plot Window Toolbars

Toolbar Descriptions

The toolbar icons enable and disable plot features similar to their associated menu items as described throughout this chapter. Position your mouse over an icon to view its tooltip description.

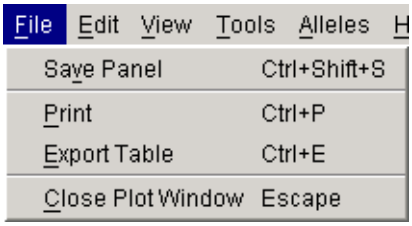


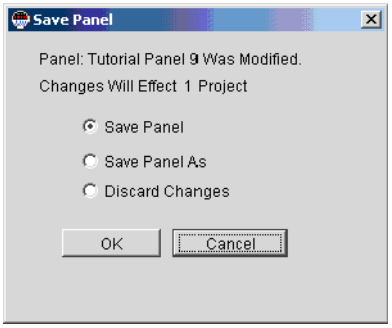
Item	Description
1	Pull-down menu that allows selection of a plot profile **Note** Click the Plot Settings Editor icon to edit the selected profile. (Tools menu)
2	Pull-down menu that controls the number of electropherogram panes shown
3	Dye color toggle icons (View menu)
4	Header, Marker Range and Marker Indicator toggle icons (View menu)
5	Icons that select the plot display: Combine Dyes, Separate Dyes, Overlay All (View menu)
6	Icons that select the Sample Plot window to be displayed: No Table, Sizing Table, Genotypes Table (View menu)
7	Icons that select either Peak Selection Mode or Binning Mode (Alleles menu)
8	Icons that select the Label display: No Labels, Horizontal Labels, Vertical Labels (View menu)
9	Icons toggle additional view features: Overlay Bins, Show/Hide Peak Position, Controls To Top, Show/Hide Allele History (View menu)
10	Icon switches view to full X-axis and Y-axis scales: Full View from the View menu.

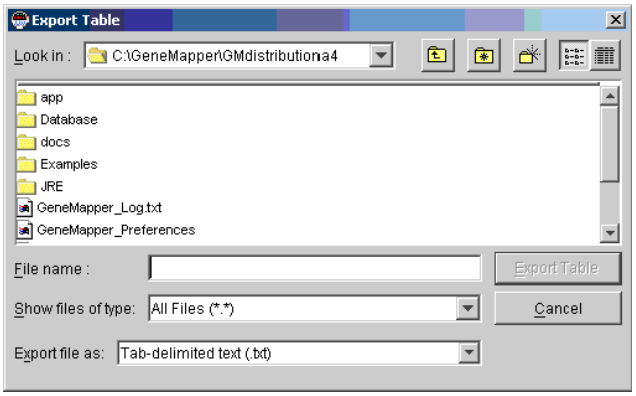
Plot Window Menus

File Menu

The File menu commands are used to perform the basic commands.



Command	Description
Save Panel (Ctrl+Shift+S)	Opens the Save Panel(s) dialog box informing the user of what panel(s) have been modified and how many projects currently in the GeneMapper database will be effected. The options are:  ♦ **Save Panel** - This option allows you to save any marker and bin changes made to the current panel name, affecting all projects analyzed using this panel. ♦ **Save Panel As** - This option allows you to save any marker and bin changes made to a new panel name, affecting only the current project being analyzed. **Note** When using Save Panel As feature, the application creates a duplicate panel with bins from the current bin set and all bins from associated bin sets. ♦ **Discard Changes** - This option allows you to discard any changes made to markers and bins.

Command	Description
Print (Ctrl+P)	Automatically prints the contents of the plot window in the following order: ♦ controls ♦ samples/genotypes ♦ table (if any) **Note** Customize the view prior to printing.
Export Table (Ctrl+E)	Opens the Export Table dialog box allowing the user to export either the sizing or genotypes table shown in the Samples Plot window.  **Note** This command is functional only when a table is currently being viewed. The exported table contains the same configuration as the currently viewed table.
Close Plot Window (Escape)	Prompts to save any pending changes and closes the plot window.

Edit Menu

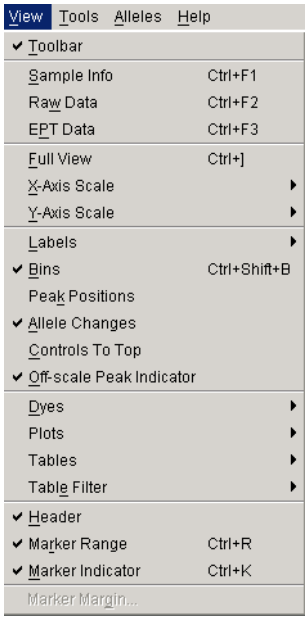
The Edit menu command provides basic editing for the Plot window menus described in this section.

Item	Description
Undo (Ctrl+Z)	The Undo command is enabled after a user action that adds, modifies, clears, or reorganizes. It allows you to undo certain actions, such as: ♦ Editing an Allele ♦ Editing a Bin ♦ Resizing Markers ♦ Zooming

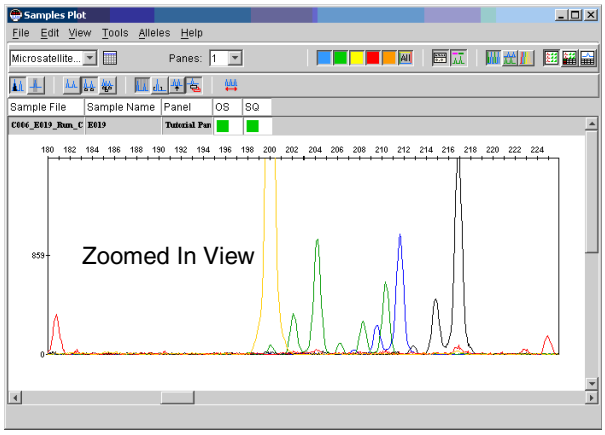
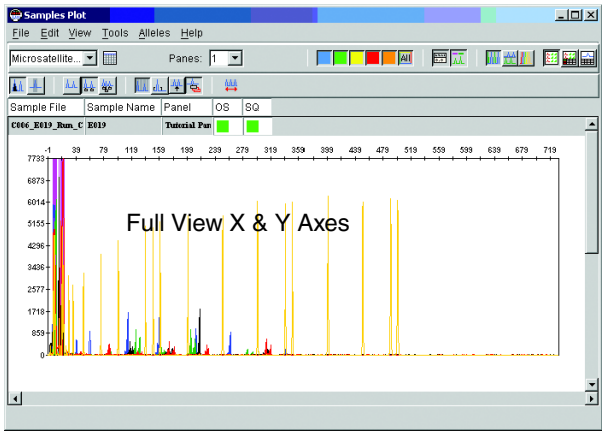
View Menu

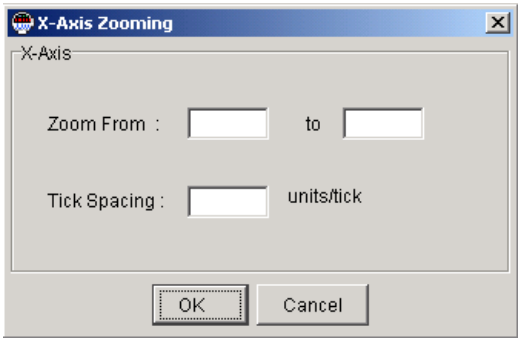
The View menu contains features you can use to control:

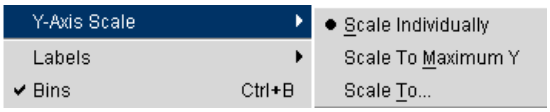
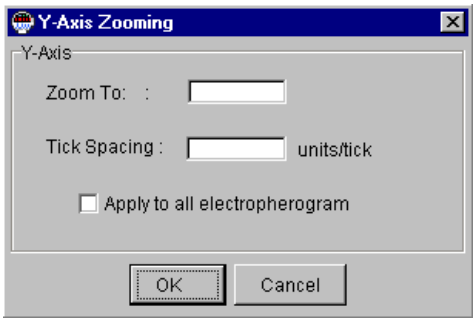
- zooming of electropherograms
- electropherogram lines/dye displays on the view
- a number of display activities

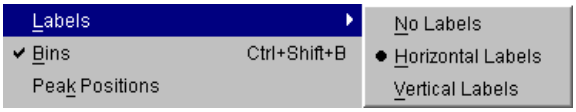
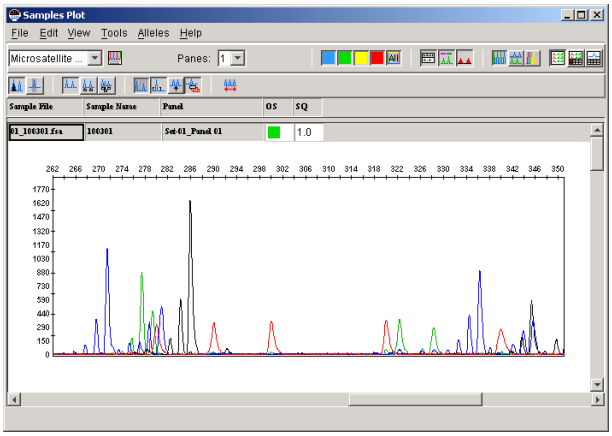
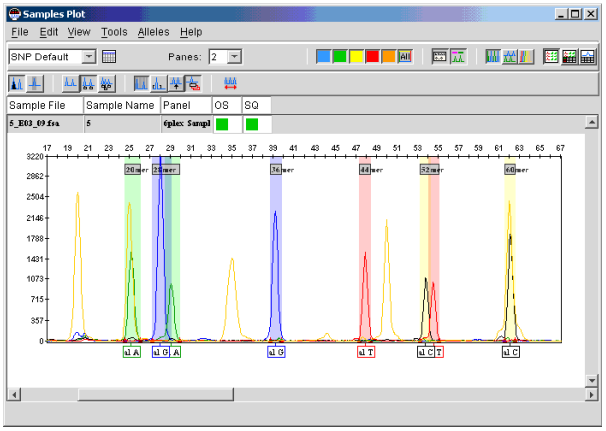


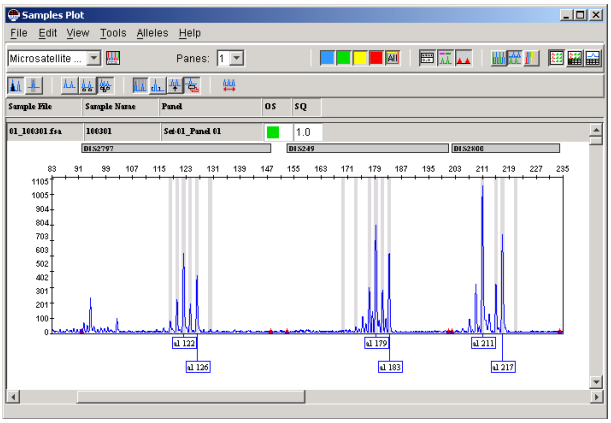
Item	Description
Toolbar	Controls whether or not the Plot toolbar is displayed at the top of the Plot window. This item is checked when active.
Sample Info (Ctrl+F1)	Provides quick access to the Sample Info data tab for the selected electropherogram. (See Chapter 3, “Using the Project Window.”)
Raw Data (Ctrl+F2)	Provides quick access to the Raw Data tab for the selected electropherogram. (See Chapter 3, “Using the Project Window.”)
EPT Data (Ctrl+F3)	Provides quick access to the EPT Data tab for the selected electropherogram. (See Chapter 3, “Using the Project Window.”)

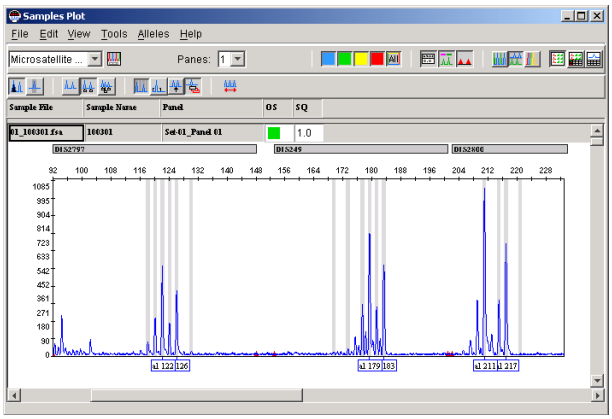
Item	Description
Full View (Ctrl+])	Automatically resets the X- and Y-axes of the electropherogram(s) currently viewed to their full scale size.  **Note** Double-clicking with the left mouse button on an axis automatically resets that axis to full view. 

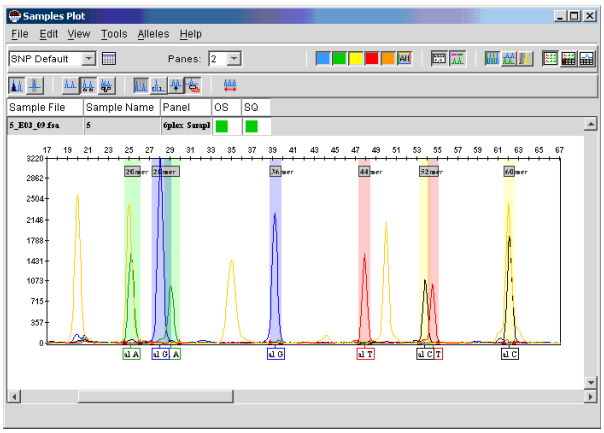
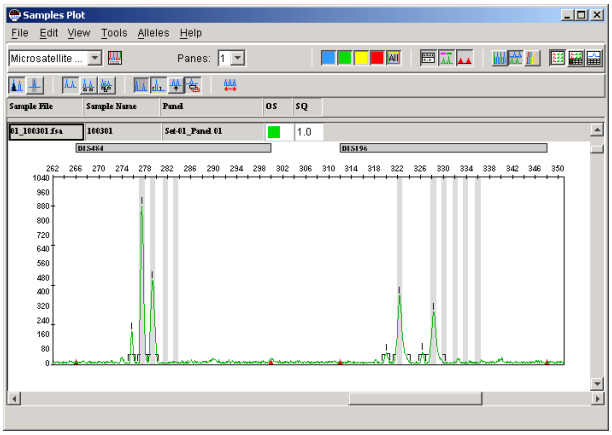
Item	Description
X-Axis Scale	Selects the horizontal scale units for the X-axis to be displayed in either base pairs (default) or data points.  Zooms in on a specific region on the X-axis of the electropherogram as follows: ♦ Click and drag, with the left mouse button, the desired region on the X-axis. In the Samples Plot, this affects all electropherograms. In the Genotypes Plot, this affects only the selected electropherogram. or, ♦ Right-click the X-axis to open the Zoom To dialog box, which enables specific zoom coordinates and tick spacing units to be set. Double-click the X-Axis Sample Plot and all the electropherograms return to Full View. Double-click the X-Axis Genotype Plot and only the selected electropherograms return to Full View. 

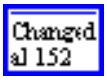
Item	Description
Y-Axis Scale	Controls the vertical scale of the electropherograms currently viewed as follows: ♦ **Scale Individually** - Each electropherogram being viewed is scaled to its maximum height. ♦ **Scale To Maximum Y** - All plots currently viewed are scaled to a global maximum value. ♦ **Scale To** - This setting allows a user to set the Y-Axis scale value. If multiple plots are currently viewed, all plots are scaled to this value.  Zooms in on a specific region on the Y-axis of the electropherogram as follows: ♦ Click and drag with the left mouse button the desired region on the Y-axis. This only affects the selected electropherogram. or ♦ Right-click the Y-axis to open the Zoom To dialog box, which enables specific zoom coordinates and tick spacing units to be set. Use the Apply To checkbox to apply the settings to all electropherograms (panes).  ♦ Double click on the Y-Axis to return selected electropherograms to Full View. ♦ Shift+double click on the Y-Axis to return all electropherograms to Full View.

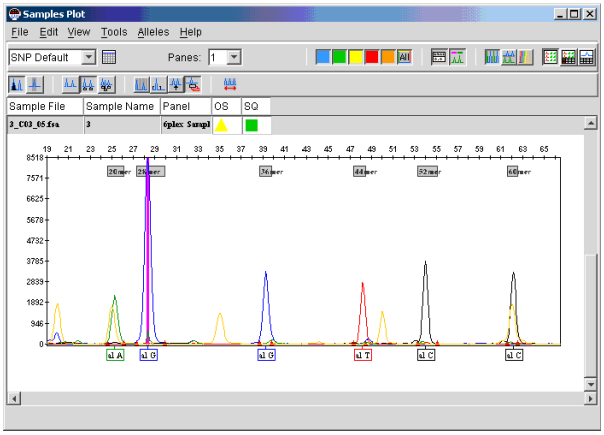
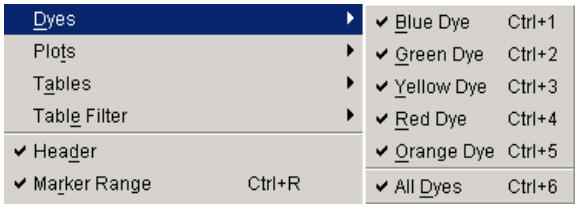
Item	Description
Labels	Controls how labels are viewed beneath an electropherogram.  ♦ **No Labels** - Labels are off.  ♦ **Horizontal Labels** - Labels are positioned horizontally across the screen. Labels in close proximity overlap with the left most label displayed on top. Click any label to bring it to the top for viewing. 

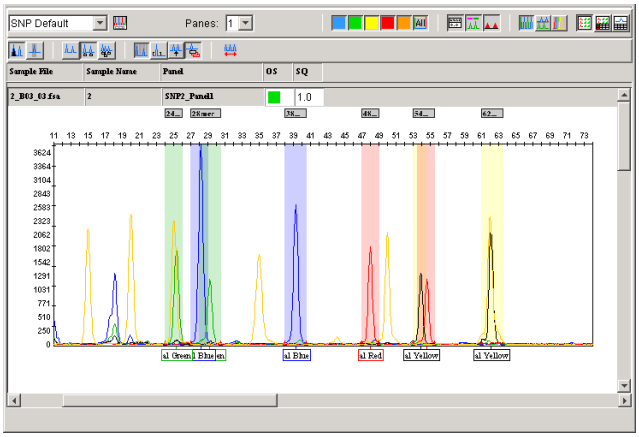
Item	Description
Labels (continued)	♦ **Vertical Labels** - Labels are positioned vertically across the screen to prevent overlap. This option is recommended for printing.  **Note** Labels are enabled in Separate Dyes mode only for Microsatellite samples, and are enabled in Combine Dyes mode only for SNaPshot® samples.

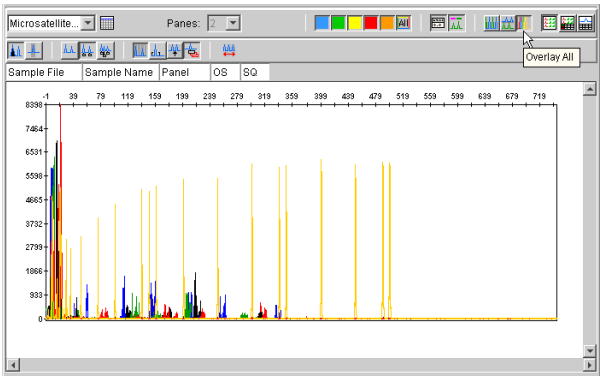
Item	Description
Bins (Ctrl+shift+B)	Controls whether or not transparent bins are shown or hidden on an electropherogram. In the Binning mode, bins are automatically displayed and the Bins menu item is checked. Separate Dyes mode  **Note** Bins are enabled in Separate Dyes mode only for Microsatellite samples, and are enabled in Combine Dyes mode only for SNaPshot samples. When you turn off a specific dye color for SNaPshot samples, the bins for that color turn off also.

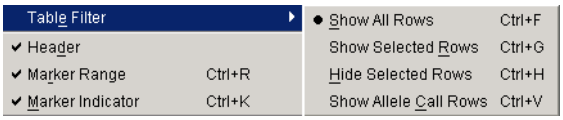
Item	Description
Bins (continued)	Combine Dyes Mode  **Note** Bins are enabled in Separate Dyes mode only for Microsatellite samples, and are enabled in Combine Dyes mode only for SNaPshot samples. When you turn off a specific dye color for SNaPshot samples, the bins for that color turn off also.
Peak Positions	Shows/hides the peak position indicators on the electropherogram currently viewed. Checked when active. Disabled for overlaid samples. 

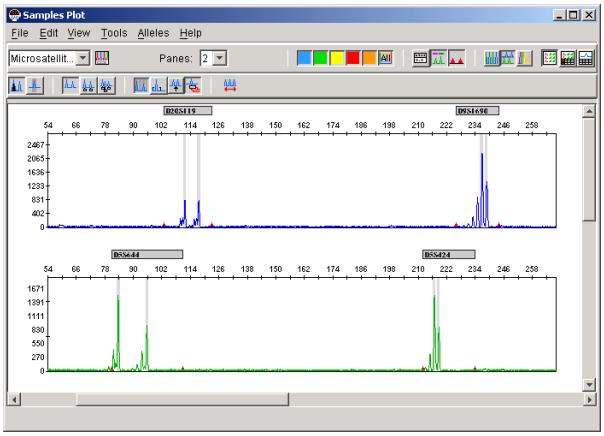
Item	Description
Allele Changes	Shows/hides label editing effects. ♦ Turned Off – Deleted label disappears – Edited/Added label looks normal ♦ Turned On, labels manually edited are shown with descriptors and the allele call. **Label deleted** - Allele label is shown with a diagonal slash through it.  **Label changed** - Allele label is shown with a double upper bar.  **Label added** - Allele label is shown with a double upper bar. 
Controls To Top	Controls whether or not control samples are displayed at the top of the Plot window. Checked when active. When active, a new pane is displayed at the top of the Plot window. Only samples and their corresponding genotypes designated as Control or Allelic Ladder in the Sample Type column of the Project window display in this pane. If multiple controls are shown, use the scroll bar to scroll through the different controls.

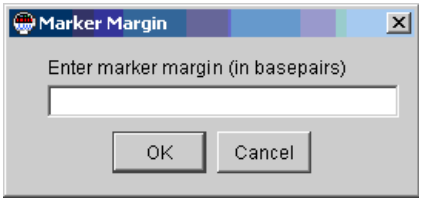
Item	Description
Off-scale Peak Indicator	Controls whether or not offscale peaks are highlighted in an electropherogram. Checked when active. When enabled, a magenta bar overlays any offscale peak in an electropherogram as shown in the figure below. An offscale peak is a peak whose height exceeds the dynamic range of the collection instrument. 
Dyes	Controls the dye colors shown in the Plot window. Checked when active. Multiple dye colors may be selected. **Note** This feature is not available when viewing microsatellite genotypes in the Genotype Plot window. 

Item	Description
Plots	Controls the way electropherograms of samples are shown in the Sample Plot window.  ♦ **Combine Dyes** - All dye colors from a sample are combined into a single source electropherogram. 

Item	Description
Plots (continued)	♦ **Separate Dyes** - Each dye color from a sample is separated into an individual electropherogram.  ♦ **Overlay All** - Multiple samples are combined into a single electropherogram. 
Plots (continued)	The Overlay All option can be used to verify size standards as follows: 1. Select any number of Samples in the Project window. 2. Click (Display Plots). 3. Turn off all dye color icons except the dye color used for the size standard. 4. Click (Overlay All). All size standard electropherograms are displayed; this allows the user to check for outliers.

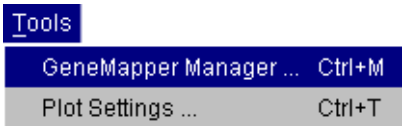
Item	Description
Tables	Controls which table, if any, is viewed at the bottom of the Sample Plot window.  ♦ **No Table [Ctrl+Q]** - Table is turned off. ♦ **Sizing Table [Ctrl+A]** - A sizing table is shown which displays information for all detected peaks in the selected samples. Use the Plot Settings Editor to configure the columns shown in the table. Use the Table Filter menu item command to control which rows of data are shown. ♦ **Genotypes Table [Ctrl+W]** - The genotypes table, as displayed in the Project window Genotypes tab, is shown for the selected samples. Use the Table Setting Editor to configure the columns shown in the table.
Table Filter	Controls which data rows are displayed in the Sizing or Genotypes Table viewed at the bottom of the Sample Plot window.  ♦ **Show All Rows** - All available data rows are shown. ♦ **Show Selected Rows** - Only peaks that are selected in the electropherogram(s) are shown. ♦ **Hide Selected Rows** - All peaks that are selected in the electropherogram(s) are hidden. ♦ **Show Allele Call Rows** - Only peaks that have allele calls assigned are shown. This is applicable for the Sizing Table only.

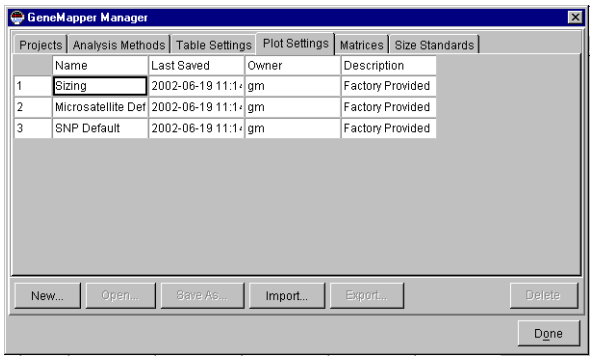
Item	Description
Header	Controls whether the individual Sample or Genotype Header is shown above an electropherogram. Checked when active. **Note** The information shown in the Header can be configured through the Plot Setting Editor. ♦ Headers Turned On  ♦ Headers Turned Off 
Marker Range (Ctrl+R)	Controls whether or not the colored marker bar is displayed at the top of an electropherogram.
Marker Indicator (Ctrl+K)	Controls whether or not the red triangles at the bottom of the electropherogram, which indicate the marker allele size range, are displayed.

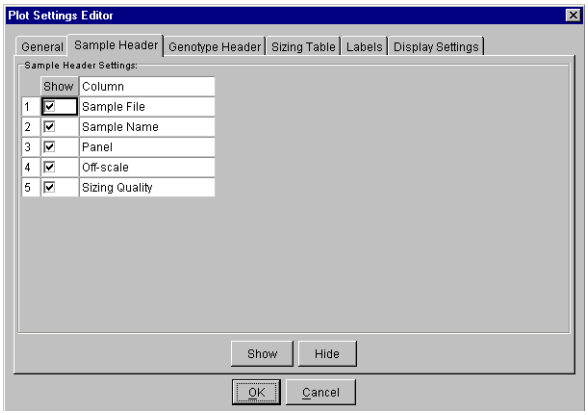
Item	Description
Marker Margin	Opens the Marker Margin dialog box to allow the user to determine how many base pairs beyond the allele size range are displayed in the electropherogram. **Note** This option is only enabled when viewing the Genotype plots. The value can be pre-configured through the Plot Setting Editor. 

Tools Menu

The Tools menu provides access to the GeneMapper Manager and the Plot Settings Editor.

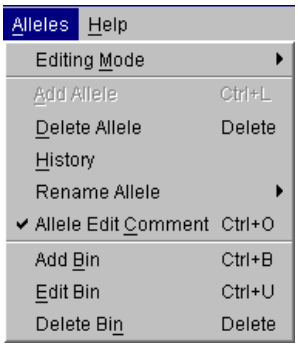


Item	Description
GeneMapper Manager (Ctrl+M)	Opens the GeneMapper Manager to allow creation or editing of various project settings. Used to create a new Plot Setting profile. See Chapter 2, “Using GeneMapper Software,” for more information.  **Note** New profiles can only be created through the GeneMapper Manager.

Item	Description
Plot Settings (Ctrl+T)	Opens the Plot Settings Editor to allow the currently selected Plot Setting profile to be edited. See Chapter 8, “Using the Plot Settings Editor,” for more information. 

Alleles Menu

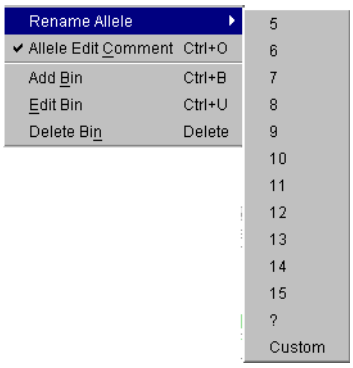
The Alleles menu is used to edit allele call labels on selected peaks and is only enabled when labels are displayed in the Plot window and one or more peaks are selected.

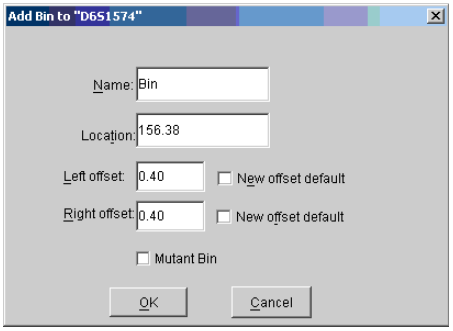


Item	Description
Editing Mode	Controls whether the action of the mouse within the electropherogram is in Peak Selection or Binning mode.  In Peak Selection mode (Ctrl+;): ♦ Peaks can be selected ♦ Allele calls can be edited Clicking on a peak automatically highlights that peak's data row within the Sizing Table or (Genotype Table, if an allele call has been made). ♦ Continuous peak selection is available Use the Shift key or Click+Drag inside the plot area. ♦ Discontinuous peak selection is available Use the CTRL key. The selected peak is maintained when switching between the Samples and Genotypes tabs in the Project window with the Plot view still visible.

Item	Description
Editing Mode (continued)	In Binning mode (Ctrl+\): ♦ Marker size ranges can be edited ♦ Bins can be edited When the Binning mode is selected, the Plot window automatically switches the display configuration to an appropriate view for editing bins. – Plots shown in Separate Dyes mode for microsatellite samples – Plots shown in Combine Dyes mode for SNaPshot samples – X-Axis displayed in base pairs – Marker Indicator and Marker Range displayed – Bins displayed To edit marker size ranges (for microsatellites only): a. Select **Binning** mode. b. Click the marker indicator of the marker to be edited. A red indicator displays. c. Drag the red indicator line to the desired size range. **Note** To edit allele calls, refer to Add Allele, Delete Allele, and Rename Allele rows in this table. To edit Bins, refer to Add Bin, Edit Bin, and Delete Bin rows in this table. To edit marker size ranges for SNPs, the bins need to be edited. Binning Mode is disabled when: a. There is no binset for the panel. b. The binset was deleted.

Item	Description
Add Allele (Ctrl+L)	Allows the user to add a new allele call to an unlabeled peak as follows: a. Select **Peak Selection** mode. b. Select an unlabeled peak using the left mouse button. c. Select the Add Allele menu item or right-click mouse button to open the Add Allele dialog box. If Allele Edit Comment is selected, the Add Allele Comment dialog box opens allowing the user to enter an allele comment for the selected peak. Labels must be turned on. A bin name is assigned to the label if the selected peak falls within a bin defined for the marker. Otherwise, the allele is designated with a question mark (“?”) until the user renames the allele. If the **Show type of edit** checkbox is selected in the plot profile, the word “Added” displays inside the box. **Note** For quick access to “Add Allele Call,” double-click an unlabeled peak.
Delete Allele (Delete)	Allows the user to delete an allele call from a labeled peak as follows: a. Select **Peak Selection** mode. b. Select a labeled peak using the left mouse button. c. Select the **Delete Allele** menu item or right-click mouse button to open the allele edit options and click **Delete**. If **Show Allele Edit Comment** is selected, the Delete Allele Comment dialog box opens allowing the user to enter an allele comment for the selected peak. The label is then deleted. If the **Show type of edit** checkbox is selected in the plot profile, the word “Removed” displays inside the label box (if Allele Changes is selected from the View menu). **Note** Multiple alleles may be deleted at the same time by selecting multiple peaks. This is constrained to the peaks in the plots currently shown.
History	Allows the user to view the allele history in columns labeled Basepair, Allele Name, User Name, Modification Date, Action, and Comments.

Item	Description
Rename Allele	 Allows the user to change the allele call on a labeled peak. a. Select **Peak Selection** mode. b. Select a labeled peak using the left mouse button. c. Select the **Rename Allele** menu item or click with the right mouse button to open the allele edit options and click **Rename Allele**. d. Select an allele call from the menu to rename the allele. If you select **Allele Edit Comment**, the Edit Allele Comment dialog box opens allowing the user to enter an allele comment for the selected peak. The allele is then renamed to the selected allele call. If **Show type of edit prefix** is turned on in the plot profile, the word “Changed” displays inside the label box.
Allele Edit Comment (Ctrl+O)	Controls whether or not the Allele Comment dialog box opens when making label changes. Checked when active. To view the comments entered: ♦ Show the AE Comment column in the Project Window Genotypes Table, or ♦ Select the History option when right-clicking a selected labeled peak, or ♦ Double click a label.

Item	Description
Add Bin (Ctrl+B)	Allows the user to add a marker bin as follows: a. Select the **Binning** mode. b. Click the **Marker Range** for the bin. A line and a cross hair opens on the electropherogram. c. Select the **Add Bin** menu item or right-click to open the Add Bin option. d. Add a bin by either dropping the cross hair at the center of the desired bin location or by dragging across the desired bin range. The Edit Bin dialog box opens allowing the user to add bin information. 
Edit Bin (Ctrl+U)	Allows the user to edit a marker bin as follows: a. Select **Binning** mode. b. Select the bin to be edited. c. Select the **Edit Bin** menu item, or right-click the selected bin to open the bin edit options, and select **Edit Bin**. The Edit Bin dialog box opens allowing the user to edit bin information. To quickly resize a bin, select a bin and adjust the right and left handles to the desired size range. To relocate or move the bin (mouse action only): a. Select **Binning** mode. b. Select the bin to be moved. c. Click and drag the center of the bin to a new location.

Item	Description
Delete Bin (Delete)	Allows the user to delete a marker bin as follows: a. Select **Binning** mode. b. Select the bin to be deleted. c. Select the **Delete Bin** menu item, or right-click the selected peak to open the bin edit options and then select **Delete Bin**. The bin will then be deleted.

Move Allele

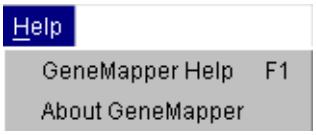
Using the mouse, you can move an allele label to a new peak.

To move an allele:

Step	Action
1	Left click a label to select it.
2	Hold down the Ctrl key and drag the label to the new peak. The old label will be marked “removed.” The new label will be marked “added.”

Help Menu

The Help menu provides access to online help and to the About GeneMapper software window.



Item	Description
GeneMapper Help (F1)	Opens the GeneMapper User Manual PDF file using Adobe Acrobat Reader.
About GeneMapper	Opens the About GeneMapper window to indicate registration information and software version number.

Special Feature

Invalidate Genotype Quality

This feature allows the user to quickly reset the Genotype Quality to 1.0 and all PQV icons will be greyed out. Since the allele call has not changed, the AE box will not be checked.

To use the Invalidate Genotype Quality feature in the sample plot:

Step	Action
1	Show the Genotype table.
2	Select the Genotype Quality cell of a genotype.
3	Right-click the selected cell to open the Override Genotype Quality dialog box.
4	Click **Yes**. The Genotype Quality now passes with a value set to 1.0, and the PQV icons are greyed out in the Genotypes table.

To use the Invalidate Genotype Quality feature in the genotype plot:

Step	Action
1	Select the sample header of a plot to select it.
2	Right-click on the header to open the Override Genotype Quality dialog box.
3	Click **Yes**. The Genotype Quality now passes with a value set to 1.0, and the PQV icons are greyed out in the Genotypes table.

Using the Plot Settings Editor 8

Chapter Overview

Introduction This chapter describes how to use the Plot Settings tab in the GeneMapper Manager window of the ABI Prism® GeneMapper™ Software Version 3.0, and describes how to use the features of the Plot Settings Editor.

In This Chapter This chapter contains the following topics:

Plot Settings Tab

Introduction

The Plot Settings tab enables you to create and edit plot setting profiles for viewing your data in the Samples and Genotypes plot views.

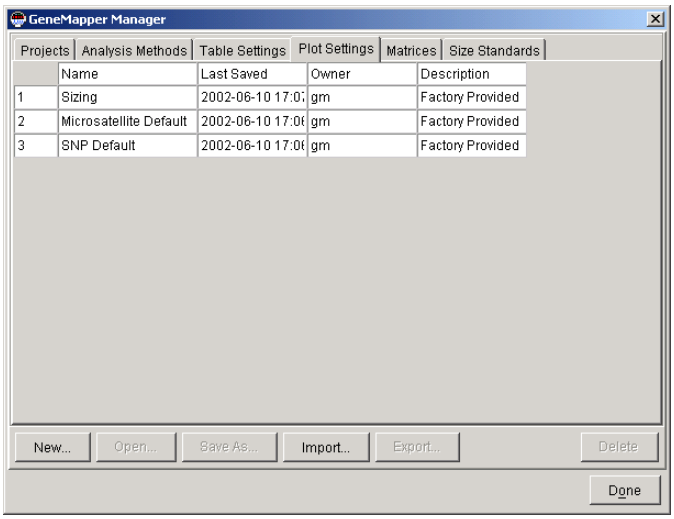
Default Profiles

GeneMapper software v3.0 contains the following standard Applied Biosystems profiles:

- Microsatellite Default
- SNP Default
- Sizing (view is similar to the view in the ABI PRISM® GeneScan® software)

Plot Settings Tab

The Plot Settings tab contains a list of Plot Setting profiles in the database and is used to manage the contents of the Samples and Genotypes Plot views by providing the following menus and interface elements. To display the Plot Settings tab, select **Tools > GeneMapper Manager > Plot Settings**.



The Plot Settings tab contains the following items:

Item	Description
Name column	Plot setting name
Last Saved column	Date/Time stamp showing when the plot setting was last saved
Owner column	User name of the person who created the plot setting
Description column	A description of the plot setting
New button	Opens the Plot Settings editor Always enabled
Open button	Opens the editor for a selected project Enabled when a single plot setting is selected
Save As button	Displays the Save As dialog box Enabled when one or more settings are selected
Import button (.xml file extension)	Displays a dialog box for Importing plot settings Always enabled.
Export button (.xml file extension)	Displays a dialog box for Exporting selected plot setting Enabled when one or more plot settings are selected
Delete button	Deletes the selected plot setting(s)
Done button	Closes the GeneMapper Manager

Plot Settings Editor

Introduction

The Plot Settings Editor allows you to customize your plot views by adjusting the following:

- The items displayed in the Sample headers
- The items displayed in the Genotype headers
- The columns displayed in the Sizing table
- How labels are shown
- Display settings

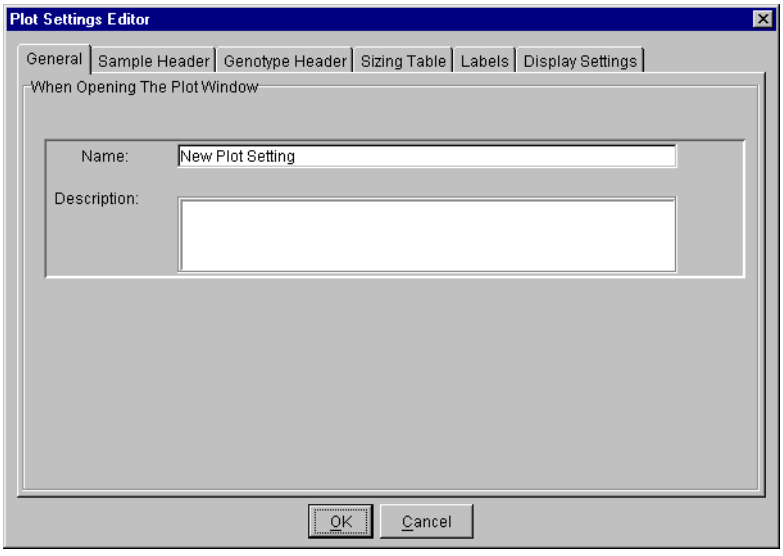
How to Access the Plot Settings Editor

The Plot Settings Editor can be accessed in the following ways:

- Select **Tools > GeneMapper Manager > Plot Settings Tab > New or Open**.
- In the Sample or Genotype Plot views, select a profile to edit and select **Tools > Plot Settings** or click the **Plot Setting Editor** icon on the toolbar.

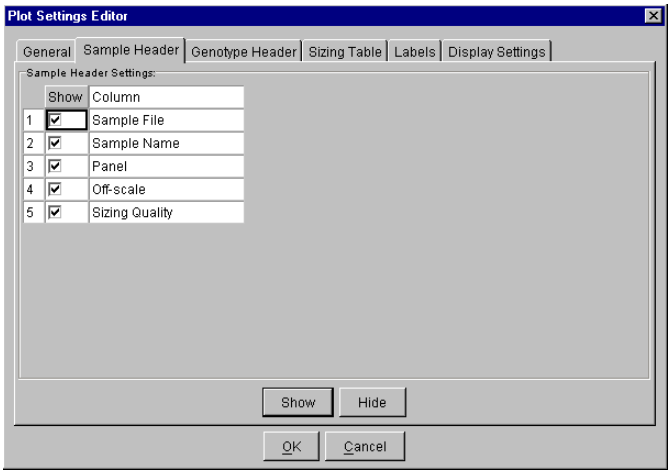
General Tab

The General tab allows you to enter in the name and description of the plot setting.



Sample Header Tab

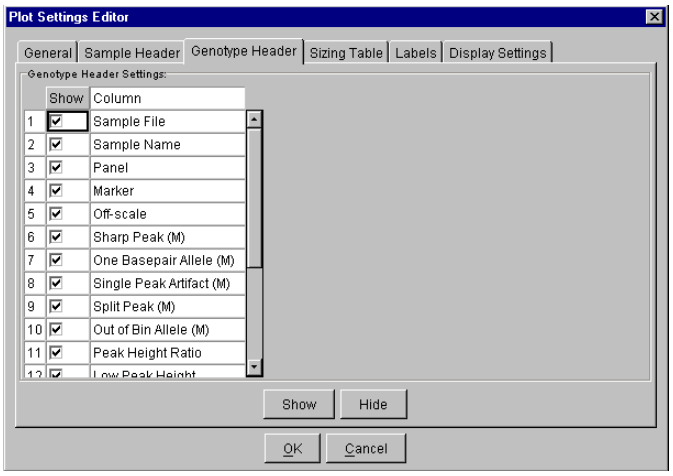
The Sample Header tab controls what is viewed in the header above each sample electropherogram in the Samples Plot view.



The Show button displays all selected rows as visible (checked), and the Hide button displays all selected rows as hidden (unchecked).

Genotype Header Tab

The Genotype Header tab controls what is viewed in the header above each Genotype electropherogram in the Genotypes Plot view.



The Show button displays all selected rows as visible (checked), and the Hide button displays all selected rows as hidden (unchecked).

Sizing Table Tab

The Sizing Table tab controls which columns are displayed in the sizing table as shown in the Samples Plot view.

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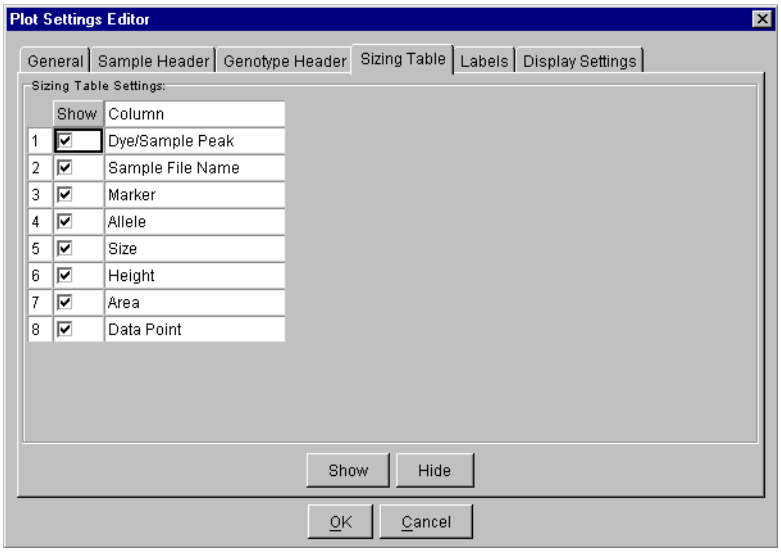
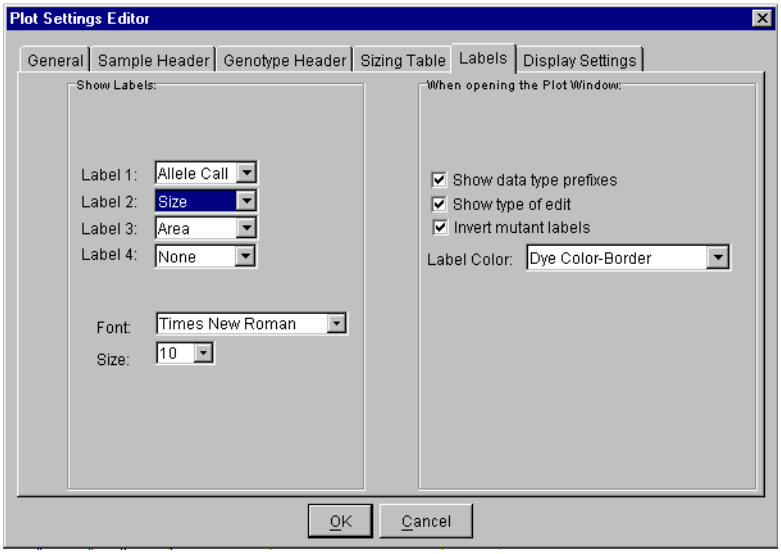


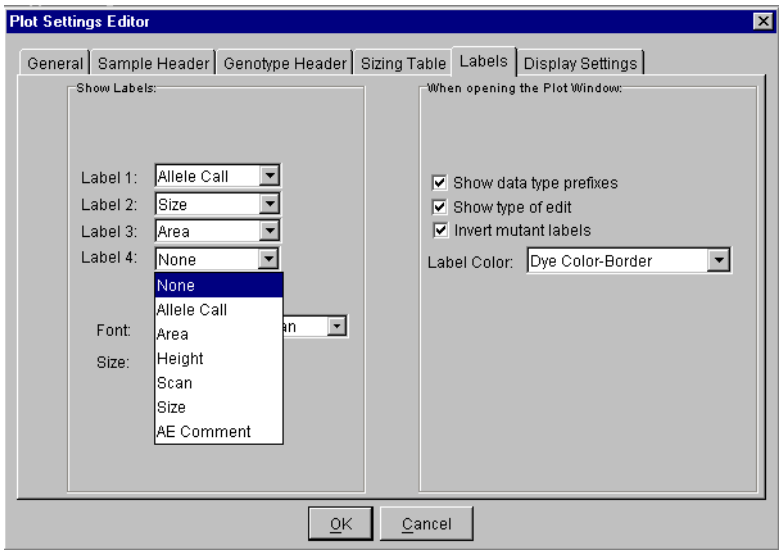
The Show button displays all selected rows as visible (checked), and the Hide button displays all selected rows as hidden (unchecked).

Labels Tab

The Labels tab controls how labels are viewed for each allele. You can display up to four labels per peak.



Use the drop-down menus to display the labels you want to use.

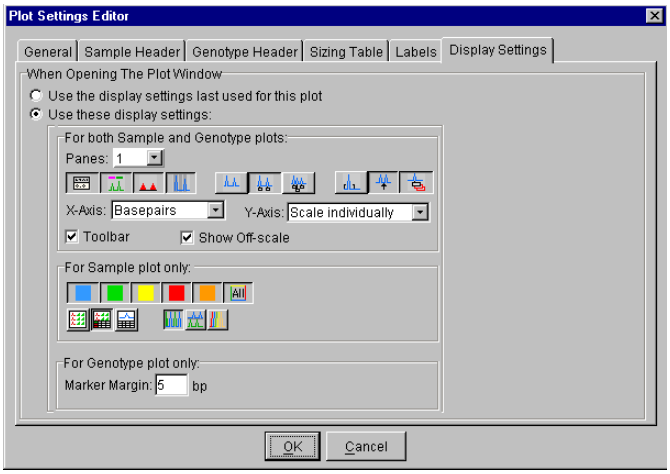


Description of items displayed on the Labels tab:

Name	Type	Description
Allele Call, Area, Height, Data point, and Size labels	Drop-down menu items in the label box	Labels are determined by the GeneMapper software v3.0.
AE Comment	Drop-down menu item in the label box	Shows the last allele edit comment
Show data type prefixes	Check box	Label prefixes such as “al” for allele call will be displayed.
Show type of edit	Check box	If an allele is manually edited, the label will display “changed” or “deleted.”
Invert mutant labels	Check box	If a bin is labeled as a mutant bin in the Panel Manager, then the label color will be inverted to distinguish it from normal bins.
Label Color	Drop-down menu	Controls the color of the labels. From the drop-down menu select one of the following: Black & White Dye Color - All (text & border in color) Dye Color - Border (text in black, border in color)

Display Settings Tab

The Display Settings tab controls how each plot setting will automatically open when a customer chooses that setting.



You can select **Use the display settings last used for this plot** button to have the Samples and Genotypes plot views display with your previous settings, or select **Use these display settings** to adjust the way these views display with the current settings on this page. These icons are the same as the ones in the Plot window. Move the mouse over each icon to view the tooltip description. The tooltip describes what action occurs if the button is pressed. For example, by default, all colors are shown. If you move your mouse over the All icon 

, the tooltip says “Hide All Dyes.” If you click the icon, all colors will be hidden.

Creating and Evaluating a Matrix 9

Chapter Overview

Introduction

This chapter describes the process of creating and evaluating a matrix. With the ABI Prism® GeneMapper™ Software Version 3.0, matrices are stored in the GeneMapper database. They become *.mtx* files on your hard drive only when you export them. You must export a file in order to run the 310/377 data collection. You have the option of selecting four or five dyes depending on the application when creating a new matrix for data collection.

Note This chapter applies only to the ABI PRISM® 310 Genetic Analyzer and the ABI PRISM® 377 DNA Sequencer instruments.

In This Chapter

Topics in this chapter include the following:

About the Matrices Tab

Purpose of the Matrices Tab

Matrices Tab

The Matrices Tab contains the list of Matrices in the database.

Item	Description
Name column	Matrix name
Last Saved column	Date/Time stamp showing when the matrix was last saved
Owner column	User name of the person who created the matrix
# of Dyes column	The number of dyes selected for the matrix
Description	A description of the matrix
New button	Opens the Matrix Editor dialog box Always enabled
Open button	Opens the editor for a selected matrix Enabled when a single matrix is selected
Save As button	Displays the Save As dialog box Enabled when a single matrix is selected
Import button	Displays a dialog box for Importing matrices Always enabled **Note** The extension must be *.mtx*.
Export button	Displays a dialog box for Exporting the selected matrix Enabled when one or more matrices are selected **Note** The extension must be *.mtx*.
Delete button	Deletes the selected matrix(s)
Done button	Closes the GeneMapper Manager

About Matrices

Introduction

There are two dye-labeling chemistries currently available to prepare nucleic acid samples to use the GeneMapper software on ABI PRISM® instruments:

- 5´ end labeling Dye Sets D, F, G5
- 3´ end labeling Dye Set E5

Each chemistry has a set of dye labels that fluoresce at different wavelengths when excited by a laser.

During data collection on the...	The wavelengths are separated...
ABI Prism® 310 Genetic Analyzer, ABI Prism® 377 DNA Sequencer, 377XL, or 96-lane upgrade instrument	by a spectrograph into a known spectral pattern across a detection system with the sequencer.

Matrix Definition

A Matrix is a mathematical formula that corrects for spectral overlap of fluorescent emission spectra data collected from ABI PRISM instruments.

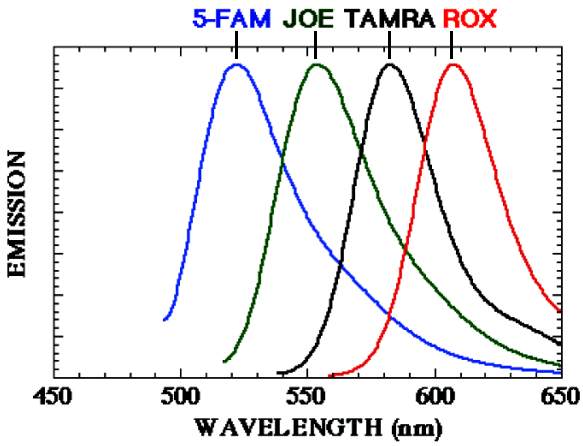
Multicomponent Definition

This process of eliminating the bleed-through caused by spectral overlaps is called multicomponenting.

Applying a matrix to raw data allows you to generate multicomponented data.

Why Is a Matrix Necessary

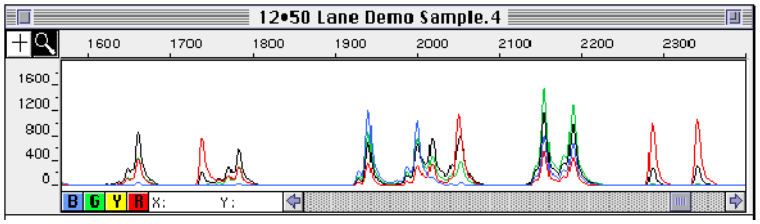
A matrix is necessary because the four or five dyes used to label the fragments fluoresce at different wavelengths and may have spectral overlaps:



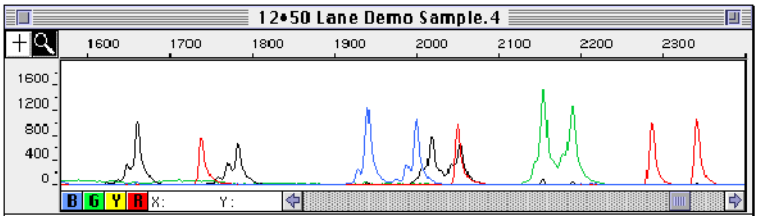
Sample Files Using A Matrix

The following figures show examples of data analyzed with and without a matrix.

You can see that peak data from a sample file analyzed without a matrix displays the expected peak, along with extra peaks in other dye colors, or bleed-through from other dye colors.



Sample file analyzed without a matrix



Sample file analyzed with a matrix

Assigning a Matrix to a Sample

Normally the matrix is assigned to a sample file automatically upon generation during or after a run. Additionally, a matrix can be manually assigned to a 377/310 sample file from within the GeneMapper software.

When to Assign a Matrix

Before you can successfully analyze 310/377 sample files using the GeneMapper software, you must make a new matrix or assign an existing one to a set of sample files.

Limitations to Matrices

The matrix is instrument-specific. You cannot apply a matrix you made on the ABI PRISM 377 DNA Sequencer to data you collected on an ABI PRISM 310 Genetic Analyzer, nor can you apply a matrix made on one ABI PRISM 377 to a sample or gel file made on another ABI PRISM 377. In other words, you cannot apply matrix created on one instrument to other instruments of the same model.

You can only assign a matrix to sample files generated on the same instrument, under the same electrophoresis, gel matrix and buffer conditions, and using the same dye set.

When to Create a New Matrix

Create a new matrix in the following conditions:

- For each dye set:
  - D, F, G5, E5
- When you change the dye set you use to label sample fragments, for example, if you are using the fifth dye.
- When you use gel materials or buffers with pH values that differ greatly from the pH value of the gel material or buffer on which the existing matrices were generated.
- When you see multiple unexpected peaks of different colors under an expected peak.
- When you recalibrate your CCD camera (ABI PRISM 310 Genetic Analyzer and ABI PRISM 377 DNA Sequencer) and the change is greater than 3 pixels from the original pixel position.
- When you replace the CCD camera (ABI PRISM 310 Genetic Analyzer and ABI PRISM 377 DNA Sequencer).
- When you see an increase in pull-up or pull-down.

Considerations Before Making a Matrix

The following table lists some of the considerations before making a matrix:

Consideration	Comment
How much dye matrix standard to load?	With the ABI PRISM 377 DNA Sequencer, loading more than 3 µL will overload most wells and may produce too much signal. Any amount that results in a signal over 4,000 RFUs is too strong.
Which lanes to load with the dye matrix standards?	For gel electrophoresis, load the matrix standards with an empty lane between each sample to avoid contamination of the individual dyes by residual material leaking adjacent samples.
Which gel data will be used for matrix creation?	After generating a gel image, for ABI PRISM 377 instrument, check that the tracking of the gel file is adequate.

Where to Store Exported Matrix Files

Store exported matrix files (*.mtx*) intended for use by Data Collection software in:

D:\AppliedBio\Shared\Analysis\Sizecaller\Matrix\

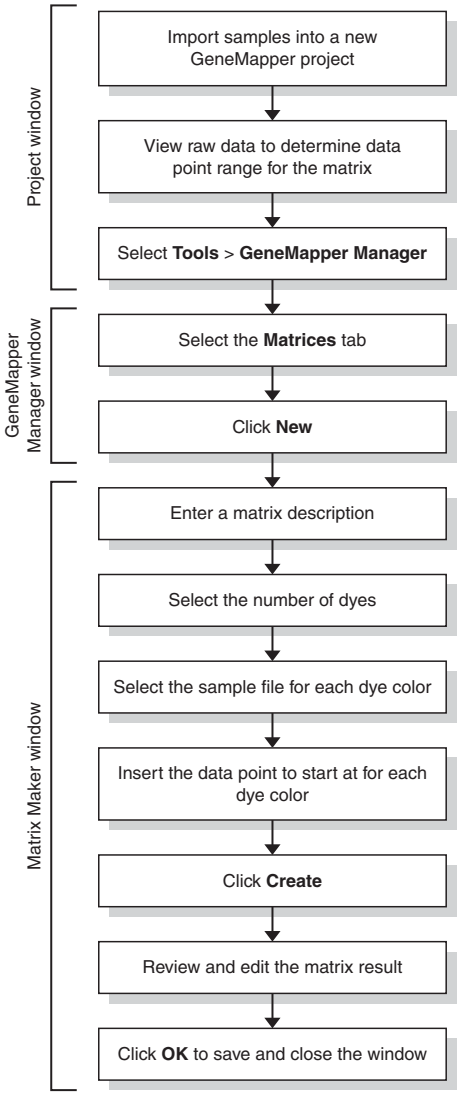
If Data Collection and Analysis are installed on different computers, the location is the same. Remember to copy the matrix from the analysis computer to the Data Collection computer.

Note GeneMapper software matrices are stored in the Oracle database.

Process of Creating a New Matrix

Process Diagram

The following diagram shows the procedure for making a new matrix:



For sample preparation and loading information, refer to the appropriate instrument user manual.

Steps to Create a New Matrix

The following table lists the steps to create a new matrix:

Step	Process	See Page
1	Choosing a Data Point Range for the Matrix Calculation	9-10
2	Generating a New Matrix	9-12
3	Assigning the Matrix to Samples	9-16
4	Evaluating the Matrix	9-17
5	Using the Matrix with Data Collection	9-18
6	Causes for Bad Matrices	9-19

Choosing a Data Point Range for the Matrix Calculation

Introduction

Depending on how well your Matrix Standards run, it may be necessary for you to choose a specific range of data points to be considered for your matrix calculation.

In order to choose appropriate values for the data point range, you must first view the sample file raw data from each of the matrix standard files, so you can decide where to choose the start point for the data point range.

Viewing the Raw Data

To view raw data:

Step	Action
1	Open a new GeneMapper Project.
2	Import Samples by selecting **File** > **Add Samples to Project**.
3	Select the files you want to view: a. Navigate to the disk/directory containing the samples for creating a matrix. b. Select the files you wish to use. c. Click **Add to List**.
4	Click **Add** to import the files into a new project and close the **Add Samples to Project** window. The Project window reopens with the imported files displayed in the **Samples** tab.
5	In the navigation pane, expand the folder containing sample files.
6	Select a sample file.
7	Click the **Raw Data** tab.

What to Look For in the Raw Data Display

In the raw data display of the sample files verify the following:

- Data peaks are present in all four or five of the matrix standards.
- There are no anomalies.
- The baseline is stable (flat between peaks).
- Peaks are on-scale—no more than 8191 relative fluorescent units—and the peaks of the dye of interest have a value of at least 200.

If peak data does not show these characteristics, refer to “Causes for Bad Matrices” on page 9-19, for possible interpretations of your peak data.

Choosing a Data Point Range

To choose a data point range:

Step	Action
1	Move the cursor well away from the primer peak, in a region at the beginning of the run and in a flat part of the baseline, and record the data point values for both the start and stop points in the flat part of the baseline of the data point range. **Note** When choosing the start point, do not include primer peaks in the data point range (refer to “Eliminating Primer Peaks”). You will need to enter these values in the next step when generating the new matrix (refer to page 9-12).
2	Return to the Project window by selecting the run folder in the navigation pane.

Eliminating Primer Peaks

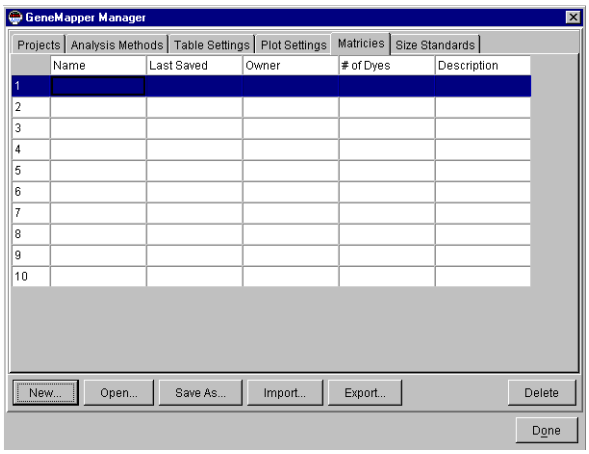
Both the primer peaks and the data peaks are displayed when viewing the raw data of your matrix standards. Any time you run dye-labeled samples on a gel (377 instrument), or capillary (310 instrument), you have excess dye-labeled primer in the reaction. The primer peak displays as the first peak, usually off-scale because it is in molar excess. Eliminate the primer peak when making a matrix, by choosing the start point after the primer peak in a flat area with a stable baseline.

Note To create a good matrix, you need at least five fragments in each color.

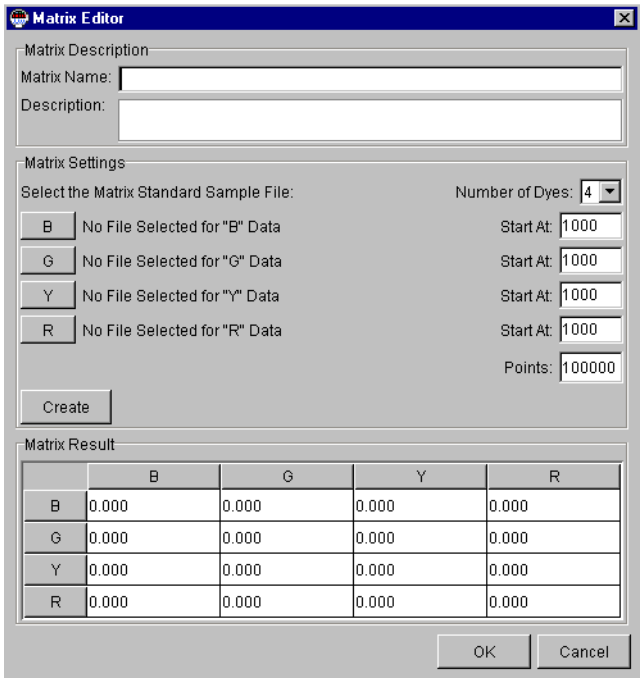
Generating a New Matrix

Creating a GeneMapper Software Matrix

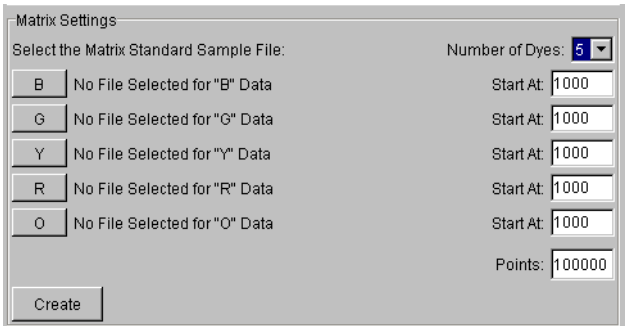
To create a GeneMapper software matrix:

Step	Action
1	Select **GeneMapper Manager > Tools** . Then click the **Matrices** tab. The **Matrices** page opens. 

To create a GeneMapper software matrix: *(continued)*

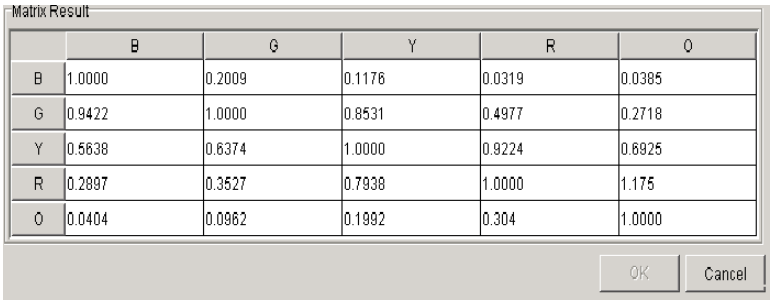
Step	Action
2	Click the **New** button on the Matrices tab. The **Matrix Editor** dialog box opens. 
3	Enter in a Matrix name and description, if desired.

To create a GeneMapper software matrix: *(continued)*

Step	Action
4	Choose the number of dyes from the **Number of Dyes** drop-down list. If 5 dyes are selected, a button for “O” Data is added to the bottom of the list. 
5	The B, G, Y, R, and O buttons represent dye colors. Choose the file associated with the appropriate dye color. a. Click a button to display a pop-up menu. b. Use the pop-up menu to access a sample file to link to each of the dye-labeled primers. c. Choose the sample file that represents the dye color for that button.
6	Enter the start point that you determined when choosing a data point range in the Start At field. Refer to “Choosing a Data Point Range” on page 9-11.
7	Enter the total number of data points to include to calculate the matrix in the Points field. In most cases, leave the default value, unless you must exclude a portion of your data because of artifacts or bleed-through. **Note** You must have at least five peaks to make a matrix.
8	Click **Create** to generates a new matrix.
9	Click **OK** to save and close the Matrix Editor dialog box.

Matrix Example

The following is an example of the matrix results showing the values used to calculate the overlap correction.



For each dye, the value where the dye fluorescence is read by the appropriate filter is 1.000. The adjacent colors show the amount of overlap for which the system must compensate. The adjacent values, in most cases, should be less than 1.000, but greater than or equal to 0.0000.

Assigning the Matrix to Samples

Introduction

After generating the new matrix, assign it to all the samples that you want to analyze.

IMPORTANT After assigning your matrix to samples, refer to “Evaluating the Matrix” on page 9-17.

Procedure

To assign a matrix to samples:

Step	Action
1	Open or create a GeneMapper Project containing the samples you wish to analyze.
2	Select the Matrix column in the project window.
3	Select the appropriate matrix from the drop-down list for each sample, or use the **Edit > Fill Down** feature.
4	Apply additional project settings prior to analyzing the sample files.

Evaluating the Matrix

Introduction

After creating a new matrix and assigning it to select sample files, evaluate the quality of the matrix. The quality of the matrix has a direct impact on the quality of the results data.

Procedure

To evaluate the matrix:

Step	Action
1	Analyze the sample files used to make the matrix.
2	Display results data for all the Dye Matrix Standard sample files in the plot window, showing only electropherogram data.

For each displayed sample file you should see the following:

You should see...	If not...
that the only visible peaks represent the color of the Dye Matrix Standard run in that lane, or for that injection (ABI PRISM 310).	you probably have a bad matrix. For instructions on how to identify and correct problems with bad matrices, see “Causes for Bad Matrices” on page 9-19.
all other lines should be relatively flat, indicating that the matrix properly compensated for the spectral overlap. For example, for the blue matrix standard sample file, you should only see blue.	
sharp, well-defined, singularly colored peak data.	

Using the Matrix with Data Collection

Using Different Computers for Data Collection and Analysis

Be sure to copy matrices generated and exported on the analysis computer (the computer running GeneMapper software) to the Data Collection computer, if different. Copying the matrices will ensure that the correct matrix name is stored in the sample file. The proper matrix is required for accurate analysis of 310 and 377 sample files.

To transfer the matrix from one computer to another:

Step	Action
1	Click the **Matrices** tab in the GeneMapper Manager window.
2	Select **Matrix to Export**.
3	Click **Export**.
4	Select a name and location for the matrix.
5	Transfer the *.mtx* file to the data collection computer.

Where to Store Exported Matrix Files

Store exported matrix files (*.mtx*) intended for use by Data Collection software in:

D:\AppliedBio\Shared\Analysis\Sizecaller\Matrix\

If Data Collection and Analysis are installed on different computers, the location is the same. Remember to copy the matrix from the analysis computer to the Data Collection computer.

Note GeneMapper software matrices are stored in the Oracle database.

Causes for Bad Matrices

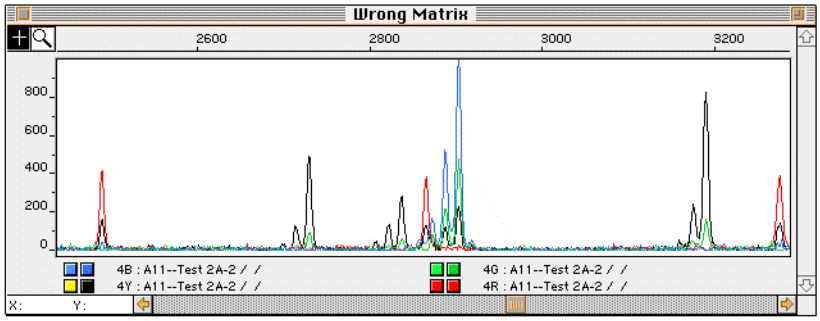
If an Error Message Diplays

There are two possible causes for the error messages shown in the following table:

If...	Take this action...
you designated the wrong files,	reassign the matrices. Refer to “Assigning the Matrix to Samples” on page 9-16.
the signal is too weak to make a matrix,	rerun the matrix standards.

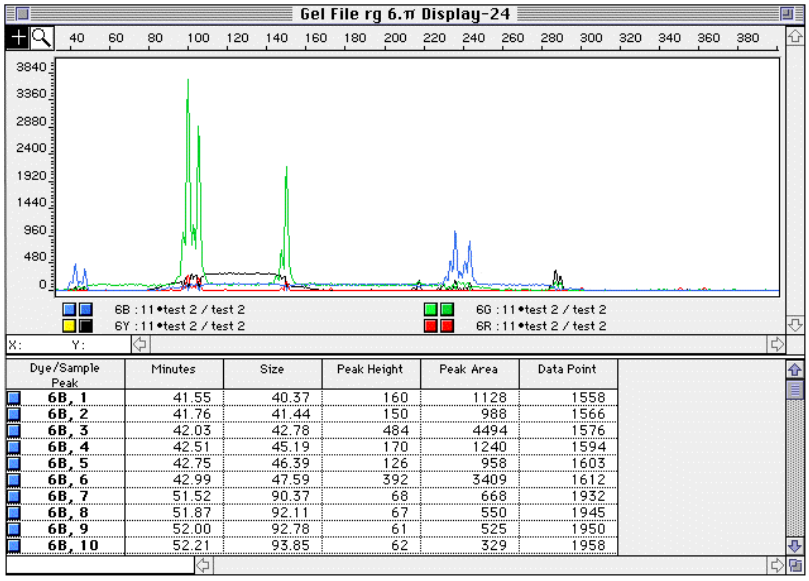
Two Causes of Bad Matrices

The following table lists two common causes of bad matrices:

Problem	Cause	What to do
Artifact peaks of different colors under the true peaks. Refer to the figure below.	Loading too much dye when running matrix standards, resulting in dye bleed-through.	Complete another run and recreate the matrix.
		
Noisy baseline.	If the matrix subtracts too much of a particular color from the sample, then the baseline may become too elevated, resulting in false peaks. Refer to the figure below.	Complete another matrix run and make sure you do not have any off-scale data. Or, choose a different set of fragments.

The following table lists two common causes of bad matrices: *(continued)*

Problem	Cause	What to do



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
6B, 1	41.55	40.37	160	1128	1558
6B, 2	41.76	41.44	150	988	1566
6B, 3	42.03	42.78	484	4494	1576
6B, 4	42.51	45.19	170	1240	1594
6B, 5	42.75	46.39	126	958	1603
6B, 6	42.99	47.59	392	3409	1612
6B, 7	51.52	90.37	68	668	1932
6B, 8	51.87	92.11	67	550	1945
6B, 9	52.00	92.78	61	525	1950
6B, 10	52.21	93.85	62	329	1958

Using the Size Standard Settings 10

Chapter Overview

Introduction This chapter describes the Size Standard and Size Match Editors in the ABI Prism® GeneMapper™ Software Version 3.0.

In This Chapter This chapter contains the following topics:

About Size Standards

What are Size Standards

Size standards are specific DNA fragments of known sizes. After defining the sizes of a size standard, the GeneMapper™ software matches this definition to the internal size standard included with the run. The software assigns the defined size values to the appropriate peaks of the internal size standard and uses this information with the selected size calling method to size all unknown fragments.

Applied Biosystems provides several fluorescently labeled size standards, which are described in Appendix D. If necessary, you can also define your own size standard.

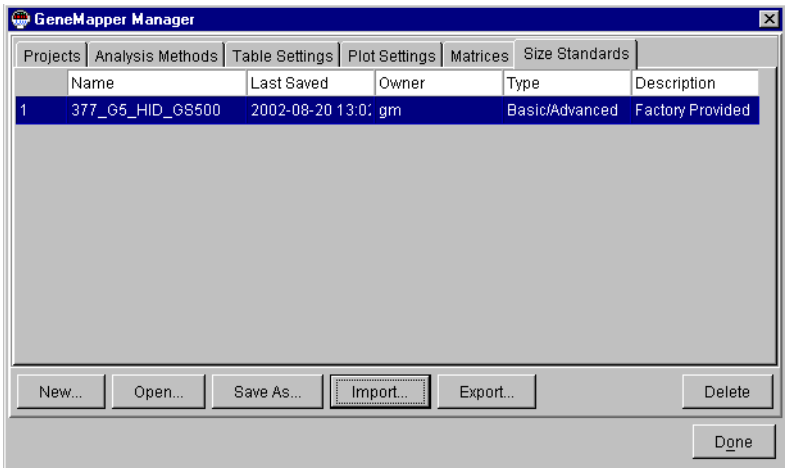
Advantages of Using a Size Standard

Running an internal size standard results in precise molecular length determination, because the internal size standard and the unknown fragments undergo exactly the same electrophoretic forces. GeneMapper software can then compensate for band-shift artifacts caused by variations in the gel and in the sample from lane to lane or injection to injection.

Size Standards Tab

Purpose of the Size Standards Tab

The Size Standards tab in the GeneMapper Manager window contains a table of size standards stored in the database.



Item	Description
Name column	Size Standard name
Last Saved column	Date/Time stamp showing when the size standard was last saved
Owner column	User name of the person who created the size standard
Type	Classic or Basic/Advanced
Description	A description of the size standard
New button	Opens the Specify parameters and Size Standard Editor dialog boxes to create a new size standard
Open button	Opens the editor for a selected size standard Enabled when a single size standard is selected
Save As button	Displays the Save As dialog box Enabled when a single size standard is selected
Import button	Displays a dialog box for Importing size standards Always enabled

Item	Description
Export button	Displays a dialog box for Exporting selected size standard(s) Enabled when one or more size standards are selected
Delete button	Deletes the selected size standard(s)
Done button	Closes the GeneMapper Manager

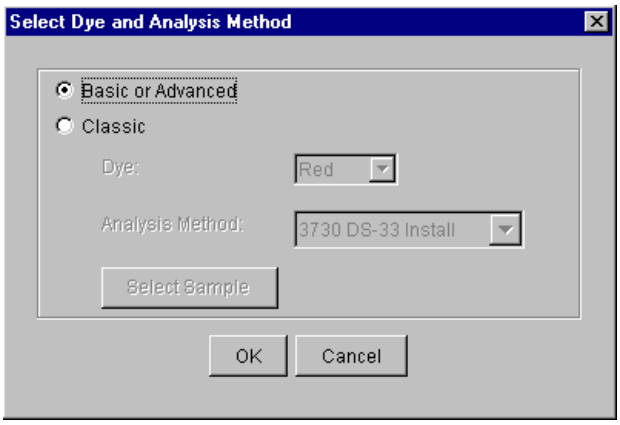
Creating/Editing a Size Standard

Overview

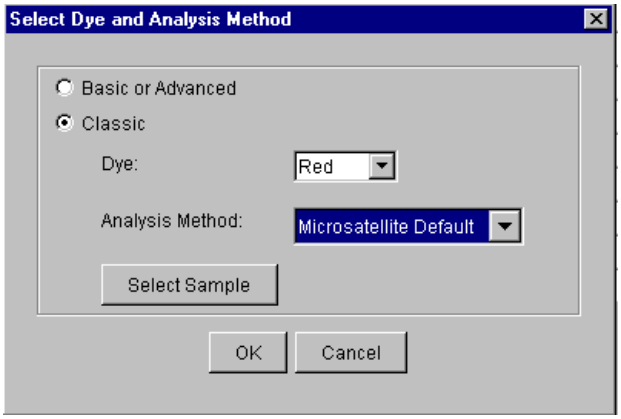
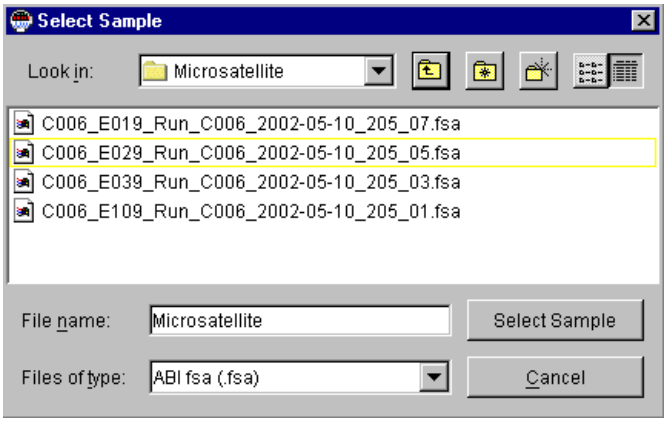
To create a size standard, you must first specify the parameters under which the size standard is created and then define the peaks using the Size Standard Editor. When editing a pre-defined size standard, only the Size Standard Editor dialog box will be shown.

Specifying Size Standard Parameters

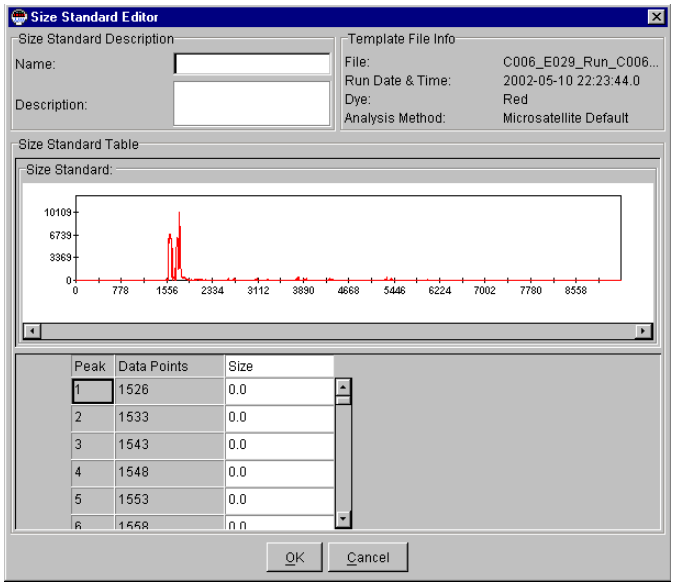
To specify size standard parameters:

Step	Action
1	Select **Tools** > **GeneMapper Manager**. Then click the **Size Standards** tab.
2	Click the **New** button to open the Select Dye and Analysis Method dialog box. 
3	Select the appropriate button, **Basic or Advanced** or **Classic**. The mode you select must be the same as the peak detection algorithm you use to analyze the samples in Analysis Method.

To specify size standard parameters:

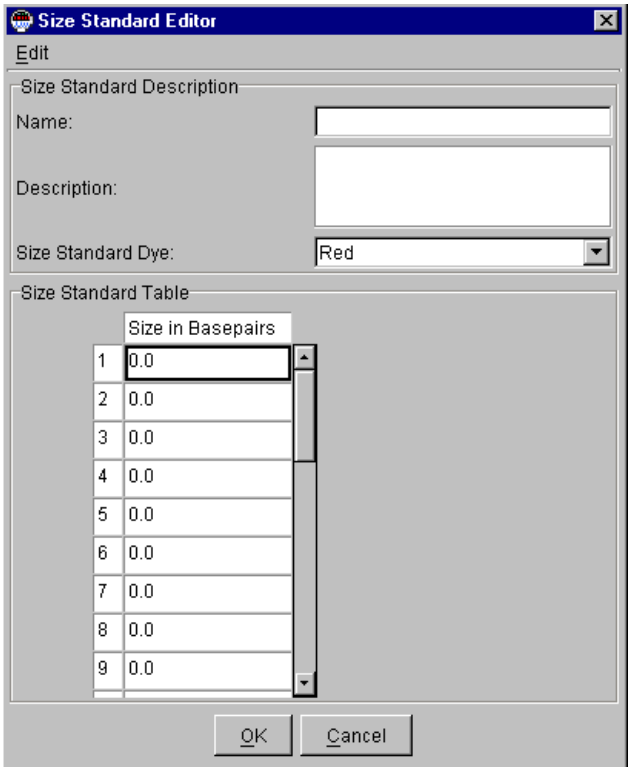
Step	Action
4	If using the Classic mode: a. Select a dye color from the drop-down list. b. Select an Analysis Method from the drop-down list.  c. Click the **Select Sample** button to open the Select Sample window, and select the sample file that contains the dye standard you want to use as the template. 

To specify size standard parameters:

Step	Action
5	Click **Select Sample** button to select the sample, and click **OK**. The Size Standard Editor opens. 

Using the Size Standard Editor in Basic or Advanced Mode

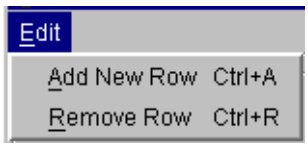
The Size Standard Editor window in Basic or Advanced mode allows you to define the peaks for each size standard dye you use.



To use the size standard editor in basic or advanced mode:

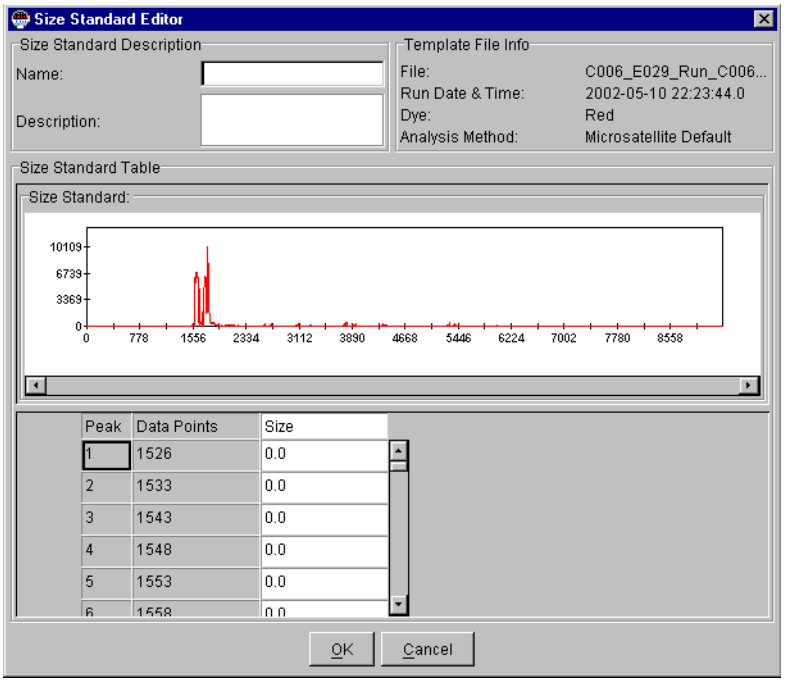
Step	Action
1	Choose a Size Standard Dye color from the drop-down list.
2	Enter a description for the size standard you are creating.

To use the size standard editor in basic or advanced mode: *(continued)*

Step	Action
3	Enter the base pair values to be defined in your size standard in the table provided. **Note** Use the Edit menu to undo certain actions or Add and Remove Rows as desired. 
4	Click **OK** to save and close the Size Standard Editor, or click **Cancel** to close without saving.

Using the Size Standard Editor in Classic Mode

The Size Standard Editor in Classic mode shows an electropherogram and a table of peaks for the dye color and sample selected. The software assigns a number to each peak found in the electropherogram in order, from left to right. You should be able to recognize the peak pattern of the standard in the electropherogram.



Note You can only change the peak size value in the right column of the table. You cannot change or rearrange the peak numbers.

Note If too many peaks display in the electropherogram or the baseline is too high, you might need to adjust the analysis parameters.

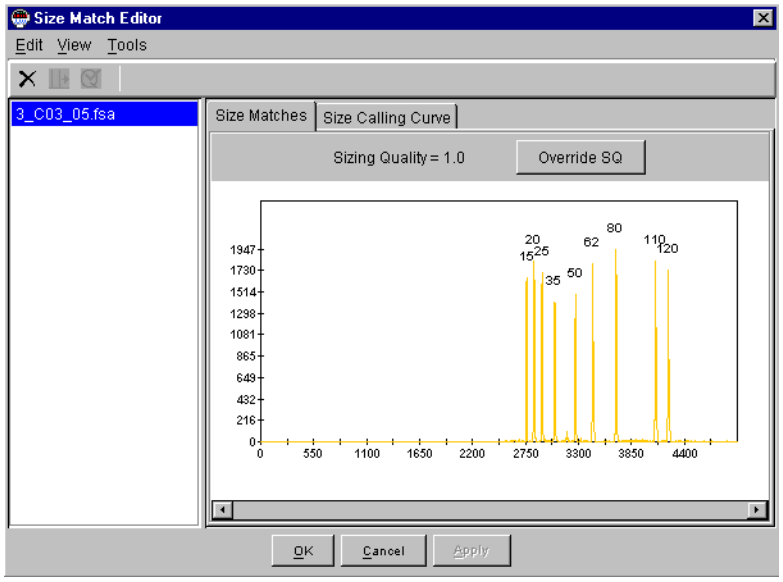
To use the size standard editor in classic mode:

Step	Action
1	Enter in a name and description for the size standard.
2	Specify the peaks of the standards and their sizes. Click the peak you want to define either in the electropherogram or in the table, or click on a row and the corresponding peak is highlighted. **Note** For easier viewing, you can zoom in on the electropherogram by click+drag on the X-Axis numbers.
3	Type the peak size in the corresponding column. **Note** Leave a zero in the Size column when you want to ignore a peak for the standard definition.
4	Press the tab key to automatically move to the next size standard peak.
5	Click **OK** to save and close the Size Standard Editor, or click **Cancel** to close without saving.

Size Match Editor

Introduction

The Size Match Editor window enables you to examine size standard electropherograms, edit the identification of size standard peaks, and view the size calling curve. To open the Size Match Editor window, click **Analysis** on the toolbar in the GeneMapper Project window and select **Size Match Editor**.



When the Size Match Quality indicator shows a marginal or fail state, you can use the Size Match Editor to help adjust the in-lane size standard to make it valid. Typical problems include the following conditions:

- **Peak shift** - The algorithm does not correctly identify a peak, resulting in invalid positions for other size standard peaks.
- **Missing peak** - The algorithm does not identify a peak.
- **Extra peak** - The algorithm identifies a peak that is not a size standard peak,but a spike or pull-up peak.

Window Elements

The Size Match Editor window elements are described in the following table below.

Element	Description
Sample navigation pane	Enables you to select a sample to view by scrolling through the Sample list. The list of samples displayed reflects the list of samples selected in the Project Sample view.
Size Quality indicator	Displays the quality of the currently displayed size standard (name listed above the plot). Value 0.0–1.0.
Size Matches electropherogram	Displays the size standard peaks. Clicking on a peak selects it (single selection only).
Size Calling curve	Displays the size calling curve for best fit.
OK button	Closes the Size Match Editor window, accepting the pending changes. For samples whose size standards are modified, the Project window is ready for reanalysis.
Cancel button	Closes the Size Match Editor window, disregarding any pending changes.
Apply button	Same as OK button, except the dialog box remains open.
Override SQ button	Allows the user to override the Sizing Quality value and automatically set the value to 1. This will cause the sizing quality to be invalidated and a check mark will display in the SQI column in the Project window Samples tab.

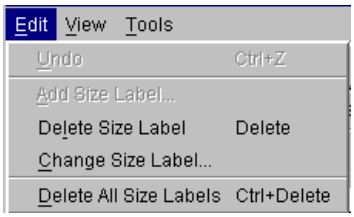
Editor Commands

The following Size Match Editor menus are described in this section:

- Edit
- View
- Tools

Edit Menu

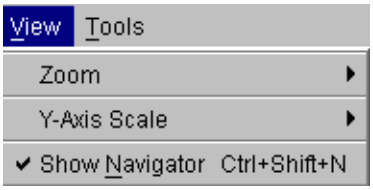
The Edit menu is used to delete or change size labels.



Item	Description	Enabling
Undo (Ctrl+Z)	Undoes the last user action.	Enabled after a user action that adds, modifies, or deletes.
Add Size Label	Adds a size label to the selected peak.	Enabled when an unlabeled peak is selected.
Delete Size Label (Delete)	Deletes the size label of the selected peak.	Enabled when a labeled peak is selected.
Change Size Label	Opens a dialog box for the selected peak, allowing the selection of a new size label.	Enabled when a labeled peak is selected.
Delete All Size Labels (Ctrl+Delete)	Removes/deletes all size labels for the selected size standard sample.	Always enabled.

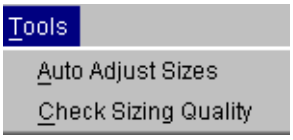
View Menu

The View menu is used to change the Size Match Editor window.



Item	Description	Enabling
Zoom	Submenu used to scale the plot to the following value: Full View (Ctrl+])	Always enabled.
Y-Axis Scale	♦ Scale to maximum Y (default) ♦ Scale to... user defined	Always enabled.
Show Navigator (Ctrl+Shift+N)	Switches the navigator pane (Removes the pane with the first use and restores it on the second use.)	Always enabled.

Tools Menu



Item	Description	Enabling
Auto Adjust Sizes	Auto adjusts size standard peaks. Tooltip: **Auto Adjust**	Always enabled.
Check Sizing Quality	Checks the quality of the current size matching. Tooltip: **Check Quality**	Always enabled.

Toolbar

The Size Match Editor toolbar contains the following icons:

Icon	Description	Enabling
	Autoadjusts size standard peaks. Adjusts the size of other peaks automatically to the right of the selected peak.[a] Tooltip: **Auto Adjust Sizes**	Enabled after a peak is changed.
	Checks the quality of the current size matching. Checks the quality of the revised peak positions and updates the Size Match Quality indicator.[b] Tooltip: **Check Sizing Quality**	Enabled after a peak label is changed.
	Deletes all size labels. Tooltip: **Delete All Size Labels**	Always enabled.

a. Only for Basic and Advanced modes.

b. Only for Basic and Advanced modes.

Using the Size Match Editor

The Size Match Editor window uses the mouse for editing.

Note All user edited size standard samples automatically have a sizing quality of 1.0, and display a check mark in the SQI column in the Samples window.

To edit a particular Size Standard peak:

Step	Action
1	Import and analyze samples in the Project window.
2	Sort failed samples to the top of the Samples tab view.
3	Select the samples whose size match you want to edit.
4	Select **Analysis > Size Match Editor**. The selected samples display in the navigation pane at the left side of the window. Selecting a sample name in this list displays its Size Standard electropherogram in the plot panel.
5	Click the peak to be edited.
6	Right-click the peak to open the editing pop-up window, and Add, Delete, or Change the selected peak.

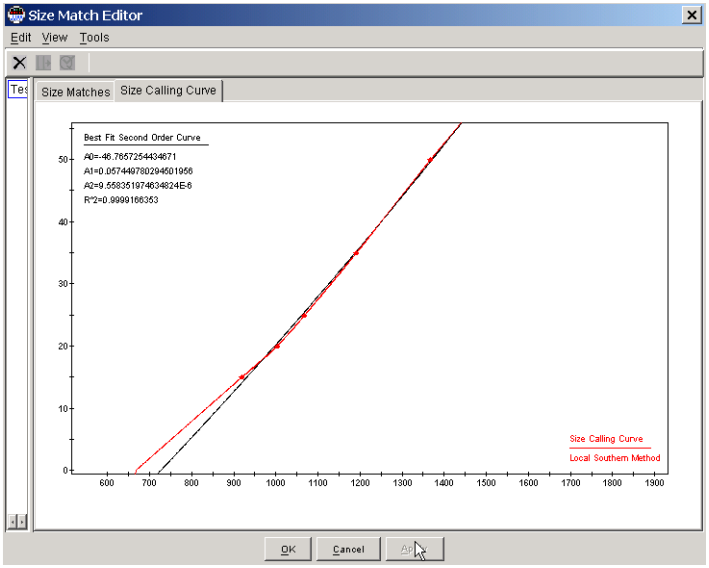
To edit a particular Size Standard peak: *(continued)*

Step	Action
7	If you choose Add or Change, move the cursor to the right to open the Select Size sub-menu. Choose the desired height value from this menu.
8	Check the sizing quality by selecting **Check Sizing Quality** under the Tools menu. If the peaks are sized correctly, but the quality score is still below passing, click the Override SQ button to set the SQ to 1.0.
9	Click **Apply** if you are going to edit a second size standard peak, or click **OK** if you are finished applying your changes to the edited peak.

Size Calling Curve

Overview The Size Calling Curve is used as a measure of how well the internal size standard matches the standard definition, and whether or not it is linear.

The Size Calling Curve displays two curves.



Red Curve If the size calling curve is a red curve, it is based on the sizecalling method used to analyze the data.

Black Curve If the size calling curve is a black curve, it is a best-fit, least squares curve, which the GeneMapper software calculates for all samples, regardless of the size calling method used. This curve is provided to help evaluate the linearity of the sizing curve. When the sizing curve and best-fit curve match, they overlap so you see only the size curve.

Note Sizing problems due to anomalous mobilities may be displayed as non-linear.

Performing a Sizing-Only Application

Introduction

Sizing-only applications quickly analyze data without generating genotype calls.

General Sizing-Only Application

To perform a general sizing-only application:

Step	Action
1	Import samples into the Project window.
2	In the Analysis Method column, select an analysis method with the following features: ♦ The appropriate analysis type selected for your data ♦ A Binset of *none* ♦ The appropriate Peak Detection Algorithm set for your data
3	In the Size Standard column, select the size standard used with your data.
4	Analyze your samples. ♦ Samples are analyzed for sizing only; no genotype calls will be made. ♦ When viewing samples in the Plot window, the Sizing Table displays information for all peaks detected.

Sizing Microsatellite Reference Data

To take advantage of the Auto Bin feature for generating marker bins for microsatellite data, reference data needs to be sized.

To size reference data:

Step	Action
1	Import microsatellite reference samples into the Project window.
2	In the Analysis Method column, select an Analysis Method with the following features: ♦ Analysis Type set to Microsatellite ♦ Binset set to *none* ♦ Peak Detection Algorithm set for your data
3	In the Panel column, select the panel that references this data. Panels must be created in advance of Auto Binning and should contain all marker information known.

To size reference data: *(continued)*

Step	Action
4	In the Size Standard column, select the size standard used with your data.
5	Analyze your samples. Samples are now ready to be used for Auto Binning in the Panel Manager.

Sizing SNaPshot Reference Data

Whether creating SNaPshot® bins manually or by using the Auto Panel feature with SNaPshot Primer Focus samples, reference data needs to be sized.

To size reference data:

Step	Action
1	Import SNaPshot or Primer Focus reference samples into the Project window.
2	In the Analysis Method column, select an Analysis Method with the following features: ♦ Analysis Type set to SNaPshot ♦ Binset set to *none* ♦ Peak Detection Algorithm set for your data
3	Set the Panel column to *none*.
4	In the Size Standard column, select the size standard GS120LIZ® or the appropriate size standard for your data.
5	If using Primer Focus samples, set the Sample Type column to Primer Focus.
6	Analyze your samples. Samples are now ready to be used for creating bins in the Panel Manager.

Process Quality Values

A

Appendix Overview

Introduction

This appendix describes how users can optimize the quality assurance level of their results by using the Process (component-based) Quality Values (PQV) in the ABI Prism® GeneMapper™ Software Version 3.0 to troubleshoot the data analysis process.

In This Appendix

This appendix contains the following topics:

Overview of Process (Component-Based) Quality Values (PQV)

What are Process Quality Values

Process Quality Values (PQVs), values reported by data analysis, aid in finding and fixing problems in sample preparation and analysis. These values are the end results reported by the PQV system.

The PQV Process

The PQV process includes the following :

- At the end of the process, quality values (also known as PQV or Process Component-Based Quality Values) are assigned to the size calling process and the allele calling process.
- On the Samples page and Genotypes page, the columns presenting PQV result data, other than SQ and GQ results, display the following samples after analysis:
  - "Pass  " (green square) symbol when no problem exists,
  - "Check  " (yellow triangle) symbol when there are problematic components such as missing size standards, or missing matrices
  - "Low Quality  " (red octagon) symbol when the result falls below an acceptable response

What are PQVs

PQVs reported by data analysis are an aid to finding and fixing problems in sample preparation and analysis. These values are the end results reported by the PQV system.

Editing PQVs

When you edit PQVs you must create an Analysis Setting and select the application mode to control which PQVs are used and available to the user for editing.

Using Process (Component-Based) Quality Values

The PQV System

Quality values reported by the GeneMapper software are an aid to finding and fixing problems in sample preparation and analysis. These values are the end results reported by the PQV system. For more information on the PQV system, see “Unique Features of GeneMapper Software v3.0” on page 1-3.

The color-coded, sortable PQV values on the Samples and Genotypes pages allow the user to isolate problem samples quickly and examine the electropherograms.

Two types of PQV values are presented on the Samples and Genotypes pages. On the Samples page:

- The SFNF, MNF, SNF, and OS parameters report results as Pass  or Check  .
- The second type, the Sizing Quality (SQ) parameter, reports results as Pass  , Check  , or Low Quality  .

On the Genotypes page:

- The SHP, AN, AE, OBA, SPA, Bin, PHR, LPH, SPU, BD, SP, OS, CC, OVL, NB, and DP parameters report results as Pass or Check  .
- The second type, the Genotype Quality (GQ) parameter, reports results as Pass  , Check  , or Low Quality  .

Note On the Genotypes page, the ADO and AE parameters are also PQV parameters but they report results as checks  instead of colored flags.

A Low Quality (red octagon) quality value for the Sample SQ indicates that the sizing standard has failed. A Low Quality value for the GQ parameter means the analysis has failed for that marker. You can set the thresholds for the SQ and GQ values in the Analysis Method Editor on the Quality Flags page.

PQV Values for Specific Applications

The PQV values (see page A-3 for more information) are used for specific analyses. The following table shows which values are used for each analysis.

LM = Linkage Mapping (Microsatellite),
HID = Human Identification, and
SNP = SNP Genotyping (SNaPshot®)

✔= Used with this analysis
No check mark = Not applicable.

	PQV Value	ADO	AE	SHP	AN	OBA	SPA	BIN	PHR	LPH	SPU	BD	SP	OS	CC	OVL	NB	DP	SFNF	MNF	SSNF	SQ
Analysis	LM (di-nucleotide)	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔				✔	✔	✔	✔
	L M (other)	✔	✔		✔			✔	✔	✔	✔	✔		✔	✔				✔	✔	✔	✔
	HID	✔	✔		✔			✔	✔**	✔	✔	✔		✔	✔	✔			✔	✔	✔	✔
	SNP	✔	✔		✔				✔	✔	✔	✔		✔	✔		✔	✔	✔	✔	✔	✔

**Not for ladder files

Rules for PQV Columns

In gaining familiarity with the PQV columns on the Samples page and Genotypes page, keep the following rules in mind:

- Quality metrics with Pass/Check values and no Low Quality value are warning flags. Analysis does not stop if problems are detected with these properties, but the user should examine results flagged with Check values.
- Holding the cursor over a column header displays a ToolTip identifying the full name of the column (the default names are often acronyms).
- PQV results are reported in the column labeled SQ on the Samples page and GQ on the Genotypes page.

  The SQ and GQ quality indicators (displayed in either symbols or in numerical values depending upon the Quality Metrics Display setting in the Tools-Options Analysis tab) have the meanings listed below:

  – Green = good data (default: numerical range of 0.75 to 1.0)
  – Yellow = questionable data (default: numerical range of 0.25 to 0.75)
  – Red = low quality data (default: numerical range of 0.0 to 0.25)

PQV Flags on the Samples Page

The PQV flags on the Samples page.

PQV Column	Description
SFNF	**Sample File Not Found** ♦ A flag (Pass  or Check) is automatically displayed when no sample file is found. ♦ Not editable.
MNF	**Matrix Not Found** ♦ A flag (Pass  or Check) is automatically displayed when no matrix file is found attached to the sample file. ♦ Not editable.
SNF	**Size Standard Not Found** ♦ A flag (Pass  or Check) is automatically displayed when no size standard is found. ♦ Not editable.
OS	**Offscale** ♦ A flag (Pass  or Check) is automatically displayed when there is offscale data in the signal. ♦ Not editable. ♦ Indicates an offscale result in the size standard region of the signal.
SQ	**Sizing Quality** ♦ A value (Pass  , Check , or Low Quality ) is automatically displayed to indicate the sizing process quality. ♦ The sizing quality is calculated based on the similarity between the size standard fragment pattern and the actual size standard peak distribution pattern in the sample. ♦ Not editable. ♦ This feature performs the following functions: – Identifies and eliminates the primer peaks based on peak shape – Performs size matching (ratio matching) – Makes a size calling curve using the Local Southern Method

PQV Flags on the Genotypes Page

The PQV flags on the Genotypes page:

Column	Description
AE	**Allele Edit** (check box) ♦ The check box displays unchecked immediately after analysis. ♦ The check box is checked when the marker allele calls have been edited by the user. **Note** The marker allele calls are edited in the Plot View page. The user can enter comments in the Allele History Comments column on the same page.
ADO	**Allele Display Overflow** (check box) ♦ The check box is checked when the number of alleles the marker calls exceeds the number to display previously set by the user. ♦ The user specifies how many alleles to display in the Table Settings Editor parameters. The default is two. ♦ There are six columns for each allele to indicate name, size, height, area, mutation and comments.
SHP	**Sharp Peak** ♦ A flag (Pass  or Check) is automatically displayed when there is a sharp peak present in the marker signal. ♦ Analysis does not stop if problems are detected with these properties, but the user should examine results flagged with Check values. ♦ The PQV system gives a label of SHP to indicate a cluster of peaks with a large, narrow peak in the middle whose width is 50% less than the neighboring peak. ♦ This flag is used for Linkage Mapping (Microsatellite, dinucleotide only) analysis. ♦ Not editable.

The PQV flags on the Genotypes page: *(continued)*

Column	Description
AN	**Allele Number** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when the number of alleles exceeds the maximum legal number for the organism, or no alleles are found. That number is specified in the analysis method. ♦ Triggering this flag reduces the final PQV Genotype value (GQ parameter) to zero (“0” multiplier). ♦ Not editable.
OBA	**One Basepair Allele** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when there is a one-base pair allele (a microvariant peak) present in the marker signal. ♦ Two allele peaks that are one base pair apart are flagged. This may indicate the presence of a microvariant and/or an invalid allele call. ♦ This flag is used only for LMS (Microsatellite, dinucleotide markers only). ♦ Not editable.
SPA	**Single Peak Artifact** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when the marker signal contains single peaks due to some problem in electrophoresis. ♦ The flag is triggered, when there are no peaks present within a two-base pair range prior to an allele peak. ♦ This feature detects the absence of stutter peaks, which indicates nonmicrosatellite peaks. ♦ This flag is used only for LMS (Microsatellite, dinucleotide markers only). ♦ Not editable.

The PQV flags on the Genotypes page: *(continued)*

Column	Description
Bin	**Out of bin allele** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when the called alleles' peak apex is out of the bin boundary. ♦ Triggering this flag reduces the final PQV Genotype value. The default is 20% (0.2 multiplier) of what it would be otherwise. ♦ This flag is used for LMS analysis. ♦ For HID, this is labeled OL (off ladder alleles). ♦ Not editable.
PHR	**Peak Height Ratio** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when there are two alleles present and the ratio between the lower allele height and the higher allele height is below a certain level. ♦ If there are more than two alleles present, the calculation iterates through all the peak pairs. ♦ PHR can be set in the Peak Quality tab of the analysis method (Analysis Manager). The default is 50%. ♦ For LMS markers, the ratio is calculated based on peak heights of the called allele peaks. ♦ For SNP, the ratios are calculated the same as for microsatellite markers except it goes across two different colors and only two peaks are used in the calculation. ♦ Not editable.
LPH	**Low Peak Height** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when the alleles are lower that the specified values and do not result in the proper intensity. ♦ The homozygous (default is 200) and heterozygous (default is 100) values can be set in the Peak Quality tab of the Analysis method (Analysis Manager). ♦ Setting this flag reduces the final PQV Genotype value (GQ parameter). The default is 50% (0.5 multiplier) of what it would be otherwise. ♦ Not editable.

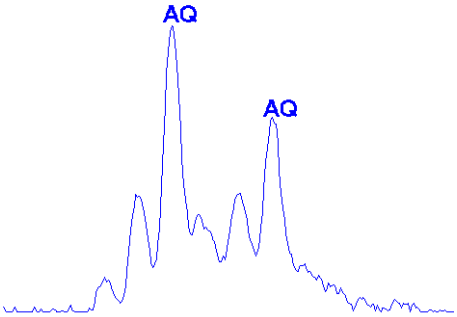
The PQV flags on the Genotypes page: *(continued)*

Column	Description
SPU	**Spectral Pull-Up** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when the marker signal contains bleed-through peaks (pull-up peaks). ♦ Pull-up is when the peak height of the called allele peak is less than X% of the larger peak within ±1 data point. ♦ The default is a conservative 10% to check all colors. The user can set this value. ♦ This flag is used for LMS (Microsatellite), SNP, and HID. ♦ Not editable.
BD	**Broad Peak** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when the called alleles' peak width is wider than a certain value. The default of this value is 1.5 basepair. ♦ This flag is used for SNP, HID, and LMS (Microsatellite, all markers). ♦ Not editable.
SP	**Split Peak** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when a split peak is found by GeneMapper software. ♦ A split peak is defined as overlapping peaks that are < .25 basepairs apart (the horizontal distance from two peak apexes). ♦ Not editable.
OS	**Offscale** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when there are offscale peaks present within the marker size range. ♦ This flag is used for all applications. ♦ Not editable.

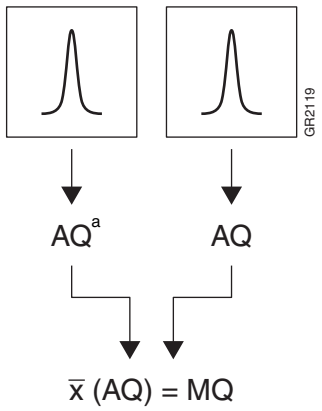
The PQV flags on the Genotypes page: *(continued)*

Column	Description
CC	**Control Concordance** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when the designated control sample does not exactly match the defined alleles for this marker in the panel being scored. ♦ This feature serves as an internal control for quality assurance, and allows you to see deviations in your controls. ♦ This flag is used for all applications. ♦ We recommend that you run the control sample at least once for every panel. ♦ Not editable.
OVL	**Overlap** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when a peak in the overlapped region is called twice. (It is possible to have two allele size ranges that overlap.) ♦ This feature serves as a warning for an allele calling error. ♦ This is used for HID only.
NB	**Narrow Bin** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when a peak is present at a position within 0.5 basepairs from a bin and no peak is present inside that bin. ♦ The benefit is to capture peaks that fall outside of bin boundaries due to experimental variations. ♦ This flag is used for SNP only.
DP	**Double Peak** ♦ A flag (Pass ■ or Check ▲) is automatically displayed when two peaks of the same color in the same bin have a ratio ≥0.5. (minor peak height/major peak height) ♦ This flag is used for SNP only. ♦ Not editable.

The PQV flags on the Genotypes page: *(continued)*

Column	Description
GQ	**Genotype Quality** ♦ A Low Quality or red octagon is shown when the analysis has failed for that sample. ♦ Pass, Check, or Low Quality flags are set by the Analysis Method. Not editable.
	Below is a representation of sample peaks with the allele quality (AQ) assigned.  See the following page for information about the mathematical formula used to calculate the Genotype Quality from a sample represented by the image above.

The PQV flags on the Genotypes page: *(continued)*

Column	Description
GQ (continued)	**Genotype Quality - Mathematical Formula** ♦ The mathematical formula used is:  $GQ = MQ^b \times (PQV_1 \times PQV_2 \times ...xPQV_N)$ a AQ (Allele Quality) is a function of quality value assignments for the following: sizing quality, allele calling quality, bin assignment quality, and bin quality. b MQ (Marker Quality) is modified by user defined PQVs to generate the final GQ value. ♦ PQVs are weighted from 0 to 1. ♦ The actual value of a PQV in the equation is: 1 minus the weight ♦ 0 (weight) = no effect on the final GQ calculation (1 minus 0 = 1, therefore no change to GQ value) ♦ 1 (weight) = full effect (1 minus 1 = 0) (If you multiply GQ by 0 and you get a GQ value of 0, then the sample analysis failed.) ♦ Between 0 and 1, the higher the value, the greater the impact on GQ. ♦ PQV filtering is controlled by the threshold set by GeneMapper software users, and remains fully functional irrespective of what weight is chosen.

Software Genotyping Algorithms B

Appendix Overview

Introduction This appendix provides a description of the new genotyping algorithms used in the ABI PRISM® GeneMapper™ Software version 3.0 and a detailed description of the Peak Detection Basic, Classic, and Advanced algorithm settings.

In this Appendix This appendix contains the following topics:

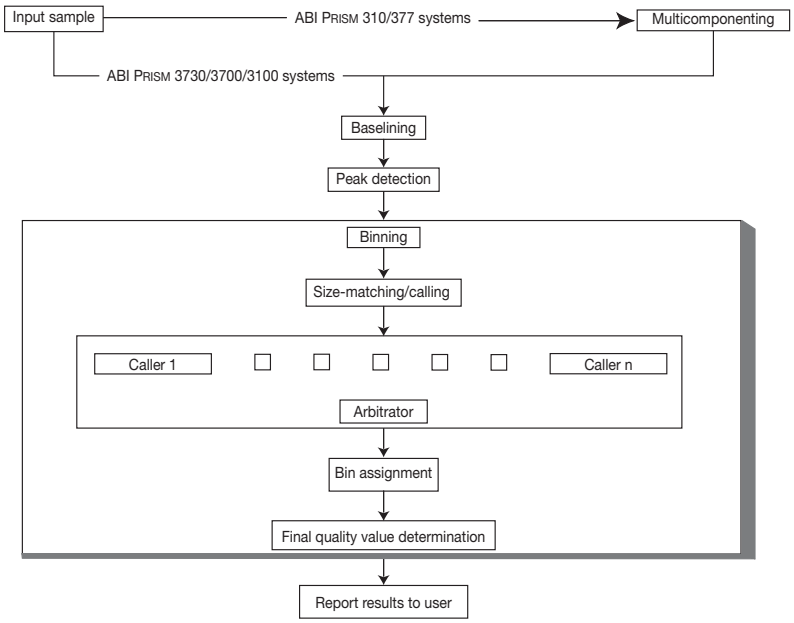
GeneMapper Software Genotyping Algorithms

Overview

Five algorithms used in the GeneMapper software are discussed in this appendix:

- **Peak Detection** - uses Basic, Advanced, or Classic mode to detect peaks and process data
- **Size-matching/calling**—matches found peaks to size standards
- **Binning**—determines bin centers for genotyping
- **Allele calling**—produces a consensus call based on several allele-calling algorithms
- **Quality value determination**—assigns a quality value to size- and allele-calling algorithms (See Appendix A, “Process Quality Values,” for more information.)

A flowchart of the data flow in GeneMapper software is shown below. Standard signal processing is applied to the data prior to being delivered to the GeneMapper algorithms.The algorithms discussed here are shown in the raised portion.



Peak Detection Algorithm

This algorithm uses Basic, Advanced, or Classic mode to detect peaks and process data. (See “Basic Mode - Peak Detection Algorithm Settings” on page B-8,“Classic Mode - Peak Detection Algorithm Settings” on page B-9, and “Advanced Mode - Peak Detection Algorithm Settings” on page B-14.)

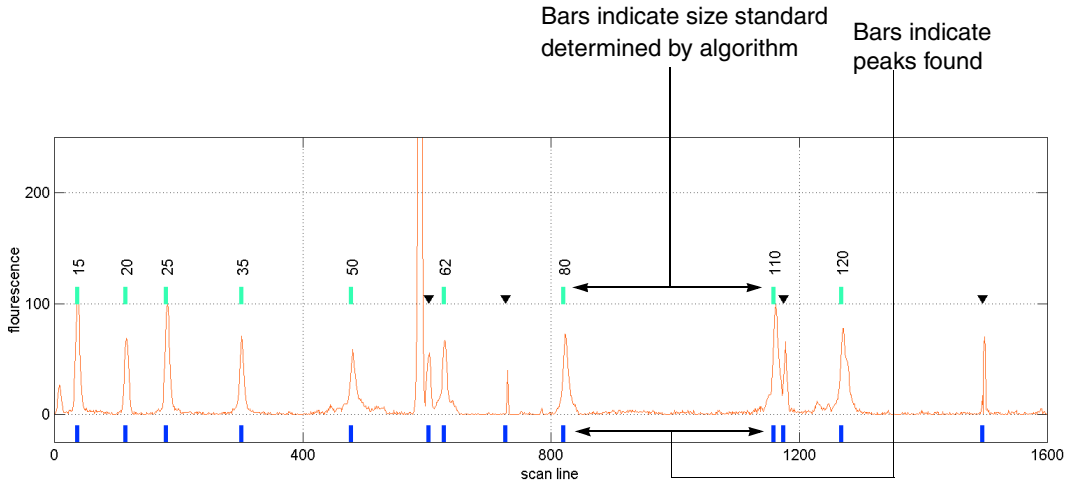
Size-Matching Size-Calling Algorithm

This algorithm uses a dynamic programming approach that is efficient (runs in low polynomial time and space) and guarantees an optimal solution. It first matches a list of peaks from the electropherogram to a list of fragment sizes from the size standard. It then statistically derives quality values determined by examining the similarity between the theoretical and actual distance between the fragments.

SizeSize-Matching Algorithm Example

An example of how the size-matching/calling algorithm works is shown below using a contaminated GeneScan™ 120 size standard data.

Peaks found (standard and contamination) are indicated by blue lower bars along the x-axis. The size standard fragments as determined by the algorithm (and their corresponding lengths in base pairs) are designated by the upper green bars. Note that there are more peaks than size standard locations because the standard was purposely contaminated to test the algorithm. The algorithm correctly identifies all of the size standard peaks and removes the contamination peaks (denoted by the black triangles) from consideration. The large peak is excluded from the candidate list by a filter that has identified it as being atypical with respect to the others.



Binning Algorithm

This algorithm estimates the bin centers using all found alleles. To determine true bin positions, the algorithm uses an iterative clustering technique and then reports a quality value proportional to the binning noise. To enhance the flexibility, bins may also be edited manually.

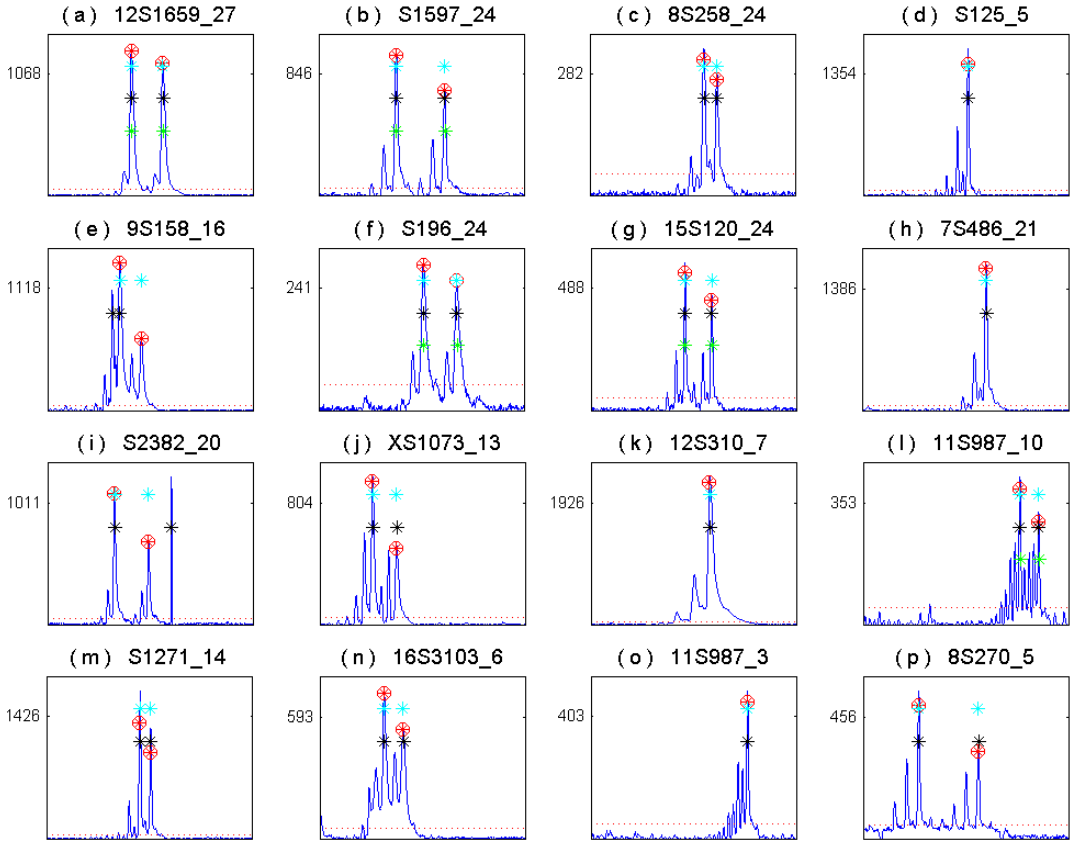
Note When the user creates bins manually, all alleles, regardless of their quality values, are treated as reference alleles as long as the alleles reside within the created bins' boundaries.

Allele-Calling Algorithm

Final allele calls are based on a consensus between several different allele-calling algorithms. Each caller has a different design philosophy such that it excels in a particular data regime. A variety of allele-calling algorithms are used. Allele-calling algorithms involve envelope detection, optimization of parametric models, and rule-based systems.

Example Output of Different Allele-Calling Algorithms

The following is an example of three different allele-calling algorithms for 16 samples. User annotations are denoted by the (red) circles, allele caller outputs are denoted by the (green, black, and blue) asterisks. Note that consensus between multiple callers virtually assures that the calls are correct. In certain cases some algorithms have not made a call since they have determined that the data is too complex to act on. Examples of this occur in (i) and (p). Here the blue asterisks show the calls transmitted to the user. Low quality values are reported because in both cases the first algorithm did not call and in (i) the black caller is not in agreement with the blue. However, despite these conditions, the calls are correct. The low quality values alert the user to potential problems such as the spurious peak in (i) and the high background in (p).



B-6 Software Genotyping Algorithms

Quality Value Determination Algorithm

The Quality Value Determination algorithm is used to create Process Quality Values (PQVs). PQVs are reported by data analysis and are an aid to finding and fixing problems in sample preparation and analysis. These values are the end results reported by the PQV system

The quality value determination algorithm equation is the following:

$$MQ^{1} = \bar{x} \ (AQ^{2}$$

$$GQ = MQ \times (PQV_1 \times PQV_2 \times ...xPQV_N)$$

- PQVs are weighted from 0 to 1.
- The actual value of a PQV in the equation is: 1 minus the weight
- 0 (weight) = no effect on the final GQ calculation
  (1 minus 0 = 1, therefore no change to GQ value)
- 1 (weight) = full effect (1 minus 1 = 0) (If you multiply GQ by 0 and you get a GQ value of 0, then the sample analysis failed.)
- Between 0 and 1, the higher the value, the greater the impact on GQ.

PQV filtering is controlled by the threshold set by GeneMapper software users, and remains fully functional irrespective of what weight is chosen.

1. MQ (Marker Quality) is modified by user defined PQVs to generate the final GQ value.
2. AQ (Allele Quality) is a function of quality value assignments for the following: sizing quality, allele calling quality, bin assignment quality, and bin quality.

Basic Mode - Peak Detection Algorithm Settings

Overview

The Basic peak detection algorithm is used as the default algorithm by GeneMapper software v3.0. Basic Mode uses the Local Southern size calling method which determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility.

In Basic mode there are two analysis parameter options.

- Minimum Peak Height - Automatic
- Minimum Peak Height - User specified (rfu)

Minimum Peak Height

The Minimum Peak Height group box provides two parameter options:

Item	Description
Automatic option button	This option button sets the level automatically for the five dye colors (blue, green, yellow, red, and orange). This level represents the minimum signal strength that will be identified as a peak for each dye (equivalent to 10 times the noise).
User specified (rfu) option button	This option button enables the number entry fields for the five dye colors (blue, green, yellow, red, and orange). These numbers are the minimum signal strength that will be identified as a peak in relative fluorescent units (rfu). For all dyes, the default is 50, the minimum is 1, and the maximum is any number of 10 digits.

Classic Mode - Peak Detection Algorithm Settings

Overview

In Classic mode there are five analysis parameter options. They are as follows:

- Ranges
- Data Processing
- Size Calling Method
- Peak Detection
- Split Peak Correction

Ranges Parameter Options

The following are the Ranges parameter options:

Item	Description
Analysis drop-down menu: Full Range Partial Range	Used to analyze all the data collected on the genetic analysis instrument for each sample. Enter Start and Stop data point numbers in the entry fields in order to specify only a limited range analyzed for each sample. The data point numbers affect what is displayed in the results display. Normally, set the analysis range to start after the primer peak. **Note** Sample files generated from the ABI PRISM® 377 DNA Sequencer may have already removed the primer peak by setting the data point range for gel image generation to exclude the primer peak.
Sizing drop-down menu: All Sizes Partial Sizes	Enter Start and Stop size numbers in the entry fields in order to specify only a limited range analyzed for each sample.

Data Processing Parameter Options

The Data Processing parameter options specify how the raw data is processed before peak detection and size calling.

Description of the data processing parameter options:

Item	Description
Baseline checkbox	Used to automatically adjust the baselines of all detected dye colors to the same level for a better comparison of relative signal intensity.
Multicomponent checkbox **Note** Do not check this box for multicapillary instruments. This user function was performed in Data Collection.	Used to specify that the GeneMapper software applies a predefined matrix to adjust for spectral overlap when it performs analysis. Although the dyes used to label DNA fluoresce at different wavelengths, the spectra overlap to some extent. Create a matrix file containing a mathematical matrix to correct for this overlap. For a description of matrix files and how to create them, see Chapter 9, Creating a Matrix File.
Smoothing option buttons	Used to help reduce the number of false peaks detected by the GeneMapper software. You have the following options: **Select...** / **To...** None — apply no smoothing. Select this option if the data has very sharp, narrow peaks of interest. Light — provide the best results for typical data. Heavy — apply to data from slower runs that has very broad peaks, or to avoid the detection of sharp edges. Choosing this option might reduce peak size or eliminate narrow peaks.

Size Calling Method Parameter Options

Click a radio button to select the desired size calling method. The GeneMapper software uses these methods to determine the molecular length of an unknown fragment.

Description of the size calling method parameter options:

Item	Description
2nd Order Least Squares and 3rd Order Least Squares	Both Least Squares methods use regression analysis to build a best-fit size calling curve.
Cubic Spline Interpolation	Forces the sizing curve through all the known points of the selected GeneScan size standard.
Local Southern method	Determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility. (Default method)
Global Southern method	Similar to the Least Squares method in that it compensates for standard fragments that may run anomalously.

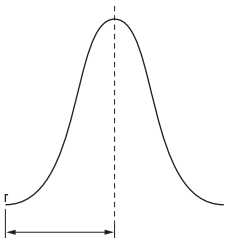
Peak Detection Parameter Options

Use the Peak Detection parameter options to specify the minimum peak height to be detected for analysis. This, in turn, controls the number of peaks analyzed. Peaks falling below the parameters specified display in the electropherogram, but are not analyzed, and no values display for them in the tabular data.

Description of the detection parameter options:

Item	Description	For example
Peak Amplitude Thresholds	Set the dye amplitude threshold at a level that allows the software to detect peaks, but eliminate noise. For each dye, the GeneMapper software detects peaks above the threshold entered in the entry field.	If you leave the default value of 50, peaks with amplitude above 50 are analyzed and display in the tabular data. Lower amplitude peaks still display in the electropherogram, but are not analyzed and do not display in the tabular data.

Description of the detection parameter options: *(continued)*

Item	Description	For example
Minimum Peak Half Width  	Defines what constitutes a peak. Use to specify the smallest half peak width for peak detection. The range is from 2 - 99. A typical number might be 3 for microsatellites, or 10 for SSCPs.	If this number is large, the software ignores noise spikes. If the peaks in the data are narrow, set the value to a low number. Experiment with this value to determine the best number for the data.

Split Peak Correction Parameter Options

Under denaturing conditions, certain fragments in the GeneScan 2500 standard display as doublets, or split peaks. This standard has labels on both stands of the DNA. Under poor denaturing conditions you see split peaks. One of the two fragments typically has normal mobility, while the other does not. The Split Peak Correction feature allows the software to correctly call each of the splits.

If you select split peak correction, you will also need to verify or change the correction limits. After you decide on a split peak correction method, use the same method for all projects to keep size calling consistent.

Description of the split peak correction parameter options:

Item	Description
No Peak Correction	No correction for doublets.
GS2500 Peak Correction	Makes the following peak size assignments for GeneScan 2500: ♦ The right peak for all fragments 222, 233, 238, 286, and 490. ♦ The left peak for all other splits.
Left Most Peak Correction	Chooses the left peak for every doublet.

Description of the split peak correction parameter options: *(continued)*

Item	Description
Right Most Peak Correction	Chooses the right peak for every doublet.
Correction Limit	Set a correction limit if correcting for doublets. Set this value slightly larger than the largest split observed. This value (set in data points) specifies the maximum width of split that should be corrected (the difference in data points of the positions of the two peaks).

Advanced Mode - Peak Detection Algorithm Settings

Overview

In Advanced mode, there are four main analysis parameter options. They are as follows:

- Ranges
- Smoothing and Baselining
- Size Calling Method
- Peak Detection

Ranges Options

The following are the Analysis Range options:

Item	Description
Full Range button	Use to analyze all the data collected on the genetic analysis instrument for each sample.
Partial Range (Data Points) button	Enter Start and Stop data point numbers in the entry fields in order to specify only a limited range to be analyzed for each sample. Data points affect what is displayed in the results. Normally, you set the analysis range to start after the primer peak.
Sizing drop-down menu: All Sizes Partial Sizes	Enter Start and Stop size numbers in the entry fields in order to specify only a limited range analyzed for each sample.

Smoothing and Baselining Options

The Smoothing and Baselining parameter options help to optimize peak size and eliminate noise from the baseline.

<table>
<tr><th>Item</th><th>Description</th></tr>
<tr><td>Smoothing option buttons</td><td>Used to help reduce the number of false peaks detected by the GeneMapper software.

You have the following options:

<table>
<tr><th>Select...</th><th>To...</th></tr>
<tr><td>None</td><td>apply no smoothing.

Select this option if the data has very sharp, narrow peaks of interest.</td></tr>
<tr><td>Light</td><td>provide the best results for typical data.</td></tr>
<tr><td>Heavy</td><td>apply to data from slower runs that has very broad peaks, or to avoid the detection of sharp edges.

Choosing this option might reduce peak size or eliminate narrow peaks.</td></tr>
</table>
</td></tr>
<tr><td>Baseline Window text field</td><td>Used to automatically adjust the baselines of all detected dye colors to the same level for a better comparison of relative signal intensity.</td></tr>
</table>

Baselining Option

The Baselining option controls the scope of the baseliner. Use this option to set the size *Beta* of the Baseline Window. GeneMapper software computes a baseline for the electropherogram of each dye independently.

How the Baselining Option Works

A baseline comprises a value at each data point *i*. Basically, the baseline value at each data point *i*, is the lowest electropherogram value in a window whose width *Beta* is set using the Baselining option, and centered at each data point *i*.

More accurately, the baseline computed in this manner is intermediate. The real baseline value at each data point *i*, is the highest intermediate value, again in a window whose width *Beta* is set using the Baselining options and centered at each data point *i*. GeneMapper software baselines an electropherogram by subtracting the baseline from the raw electropherogram

Troubleshooting the Baseline Window

The following table describes what happens if the baseline window is either too small or too large..

Using...	Causes...
a small baseline window size	the baseline to creep into the peaks, resulting in shorter peaks in the analyzed data.
a large baseline window size	the baseline to ride too low, resulting in elevated and possibly not baseline-resolved peaks.

Size Calling Method Options

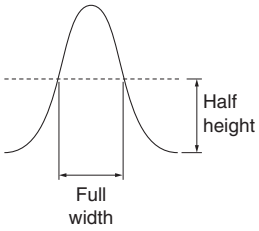
GeneMapper software uses these methods to determine the molecular length of an unknown fragment.

Description of the size calling method parameter options:

Item	Description
2nd Order Least Squares and 3rd Order Least Squares	Both Least Squares Methods use regression analysis to build a best-fit sizecalling curve.
Cubic Spline Interpolation	Forces the sizing curve through all the known points of the selected size standard.
Local Southern Method	Determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility.
Global Southern Method	Similar to the Least Squares Method in that it compensates for standard fragments that may run anomalously.

Peak Detection Options

The Peak Detection options locate peaks at the positive-to-negative zero crossings of the first derivative of the baselined electropherogram. The peak detector computes the first derivative at a data point *i* by fitting a polynomial to a window centered on *i*.

Item	Description	For example
Peak Amplitude Thresholds	GeneMapper software reports to the user only those peaks whose heights are at least the Peak Amplitude Threshold for that dye. Set the dye amplitude threshold at a level that allows the software to detect peaks, but eliminate noise.	If you leave the default value of 50, peaks with amplitude above 50 are analyzed and display in the tabular data. Lower amplitude peaks still display in the electropherogram, but are not analyzed and do not display in the tabular data.
Full Width Half Maximum 	Defines what constitutes a peak. Use to specify the smallest full width at half maximum for peak detection. The range is 2–99. A typical number might be 3 for microsatellites, or 10 for SSCPs.	If this number is large, the software ignores noise spikes. If the peaks in the data are narrow, set the value to a low number. Experiment with this value to determine the best number for the data.

<table>
<tr><th>Item</th><th>Description</th><th>For example</th></tr>
<tr><td>Polynomial Degree</td><td>Sets the degree of the polynomial.<table><tr><th>Min. setting</th><th>Max. setting</th></tr><tr><td>2</td><td>5</td></tr></table></td><td rowspan="3">These parameters control the sensitivity of this process. Sensitivity increases with the polynomial degree and decreases with the window size.

Use polynomials of degree 2 or 3 for well-isolated peaks, such as those from a size standard, and a degree 4 for finer control.

For degree 4, the Peak Window Size should be 1 to 2 times the full width at half maximum of the peaks that you wish to detect.

These parameters cannot be set for each color independently.</td></tr>
<tr><td>Peak Window Size</td><td>Sets the width of the window<table><tr><th>Min. setting</th><th>Max. setting</th></tr><tr><td>1 above the Degree of Polynomial differentiation setting.</td><td>Number of data points between peaks</td></tr></table></td></tr>
<tr><td>Slope Threshold for Peak Start

Slope Threshold for Peak End</td><td>Determines where a peak starts and stops</td><td>For example, a peak ends when the first derivative again exceeds the Slope Threshold for Peak End.

Slope Threshold for peak start must be non-negative and Slope Threshold for peak end must be nonpositive.

Values other than 0 will move the extent of the peak toward its center.</td></tr>
</table>

Peak Detection: Polynomial Degree and Peak Window Size Parameters

About These Parameters

Use the Polynomial Degree and the Peak Window Size settings to adjust the sensitivity of the peak detection. You can adjust these parameters to detect a single base pair difference while minimizing the detection of shoulder effects or noise.

Sensitivity increases with larger polynomial degree values and smaller window size values. Conversely, sensitivity decreases with smaller polynomial degree values and larger window size values.

How These Parameters Work

The peak window size functions with the polynomial degree to set the sensitivity of peak detection.

The peak detector computes the first derivative of a polynomial curve fitted to the data within a window that is centered on each data point in the analysis range.

Using curves with larger polynomial degree values allows the curve to more closely approximate the signal and, therefore, the peak detector captures more peak structure in the electropherogram.

The peak window size sets the width (in data points) of the window to which the polynomial curve is fitted to data. Higher peak window size values smooth out the polynomial curve, which limits the structure being detected. Smaller window size values allow a curve to better fit the underlying data.

How to Use These Parameters

Use the table below to adjust the sensitivity of detection.

To...	Polynomial Degree Value	Window Size Value
Increase sensitivity use...	Higher	Lower
Decrease sensitivity use...	Lower	Higher

Guidelines for Using These Parameters

To detect well-isolated, base-line-resolved peaks, use polynomial degree values of 2 or 3. For finer control, use a degree value of 4 or greater.

As a guideline, set the peak window size (in data points) to be about 1 to 2 times the full width at half maximum height of the peaks that you want to detect.

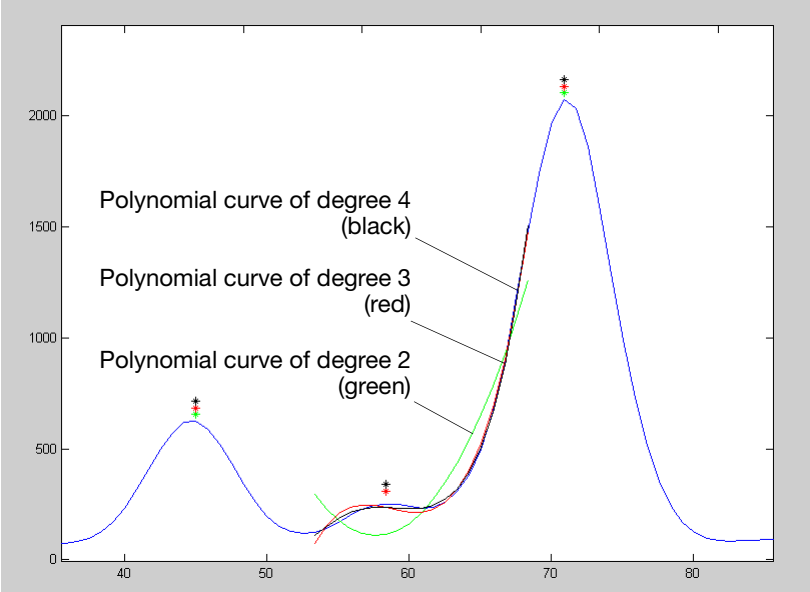
Examining Peak Definitions

To examine how GeneMapper software has defined a peak, select **View > Show Peak Positions**. The peak positions, including the beginning, apex, and end of each peak, are tick-marked in the electropherogram.

Effects of Varying the Polynomial Degree

The figure below depicts peaks detected with a window size of 15 data points and a polynomial curve of degree 2 (green); 3 (red); and 4 (black). The diamonds represent a detected peak using the respective polynomial curves.

Note that the smaller trailing peak is not detected using a degree of 2 (green). As the peak detection window is applied to each data point across the displayed region, a polynomial curve of degree 2 could not be fitted to the underlying data to detect its structure.

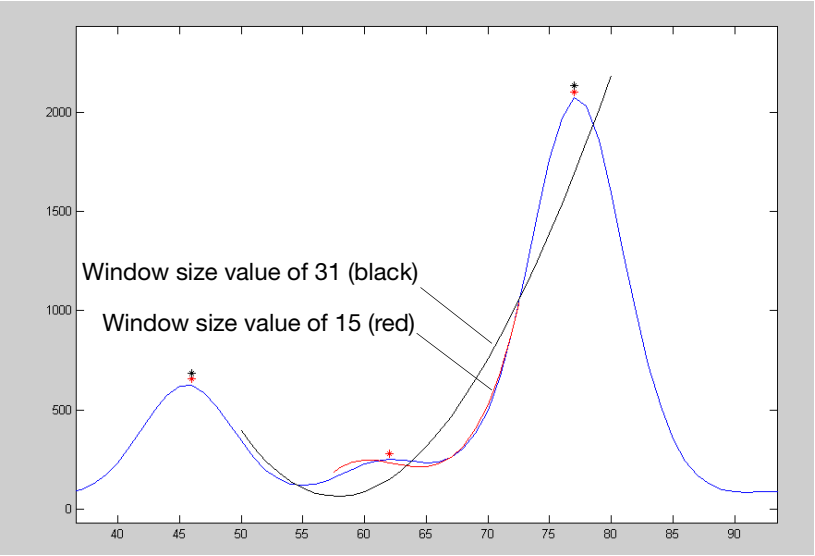


Electropherogram showing peaks detected with three different polynomial degrees

Effects of Increasing the Window Size Value

In the figure below, both polynomial curves have a degree of 3 and the window size value was increased from 15 (red) to 31(black) data points.

As the cubic polynomial is stretched to fit the data in the larger window size, the polynomial curve becomes smoother. Note that the structure of the smaller trailing peak is no longer detected as a distinct peak from the adjacent larger peak to the right.

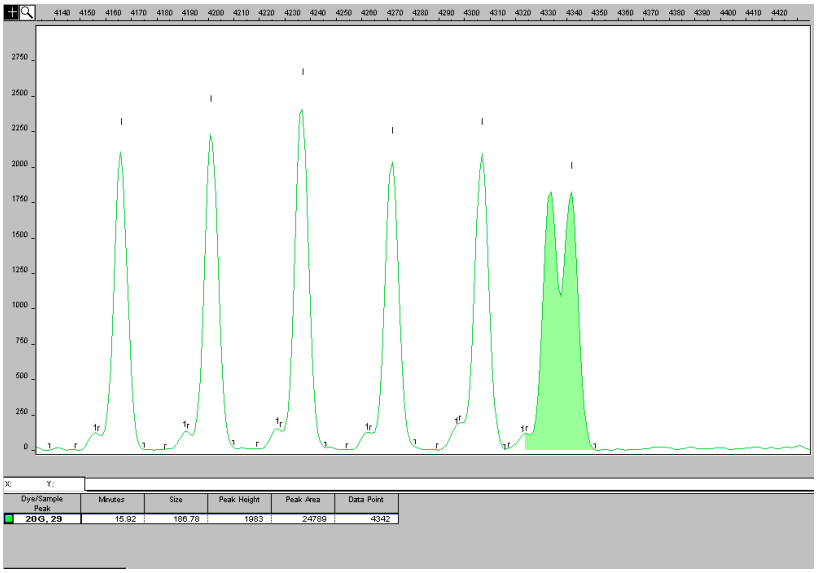


Electropherogram showing the same peaks as in the figure above (see page B-21) after increasing the window size value while keeping the polynomial degree the same

Optimizing Peak Detection Sensitivity: Example 1

Initial Electropherogram

The figure below shows two resolved alleles of known fragment lengths (that differ by one nucleotide) detected as a single peak. The analysis was performed using a polynomial degree of 3 and a peak window size of 19 data points.

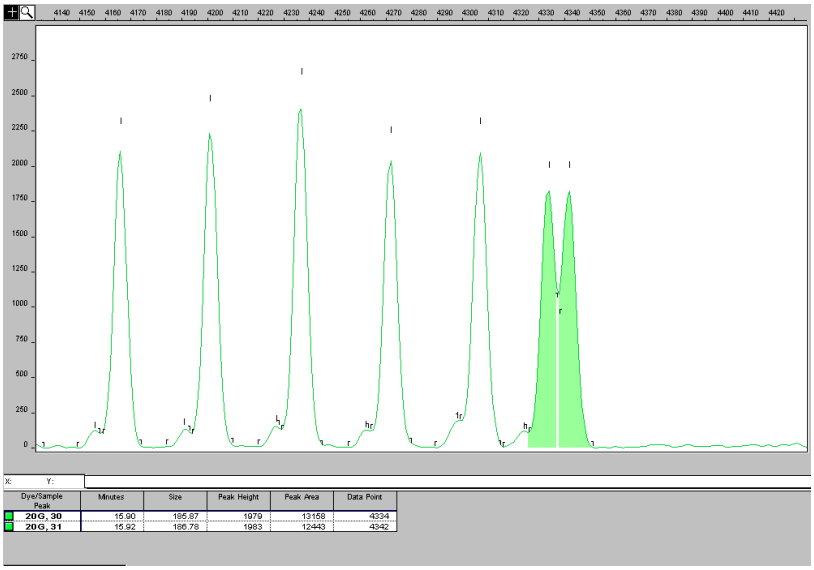


Electropherogram showing two resolved alleles detected as a single peak

Note For information on the tick marks displayed in the electropherogram see Examining Peak Definitions on page B-19.

Effects of Decreasing the Window Size Value

The figure below shows that both alleles are detected after re-analyzing with the polynomial degree set to 3 while decreasing the window size value to 15 (from 19) data points.

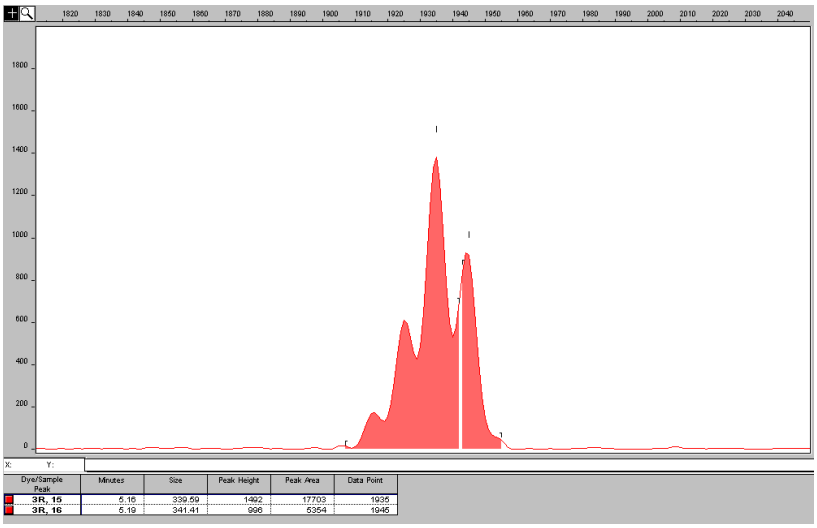


Electropherogram showing the alleles detected as two peaks after decreasing the window size value

Optimizing Peak Detection Sensitivity: Example 2

Initial Electropherogram

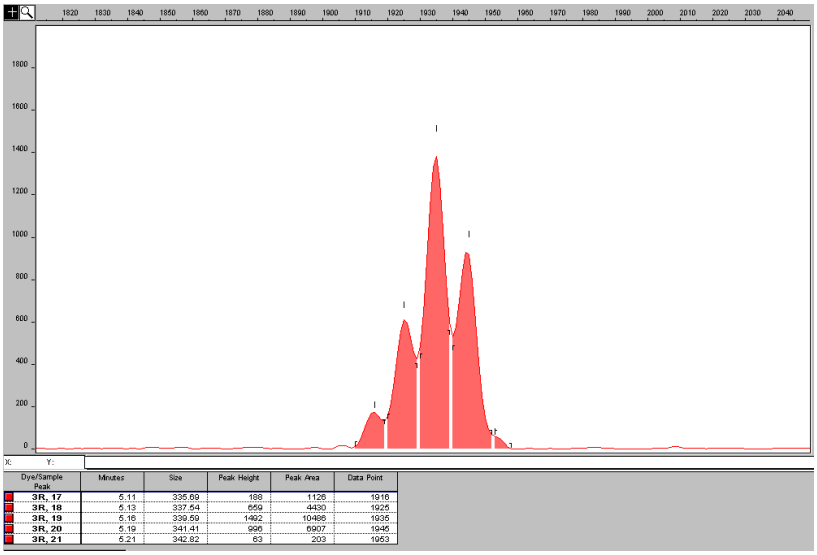
The figure below shows an analysis performed using a polynomial degree of 3 and a peak window size of 19 data points.



Electropherogram showing four resolved peaks detected as two peaks

Effects of Reducing the Window Size Value and Increasing the Polynomial Degree Value

The figure below shows the data presented in the figure above (see page B-25) re-analyzed with a window size value of 10 and polynomial degree value of 5.

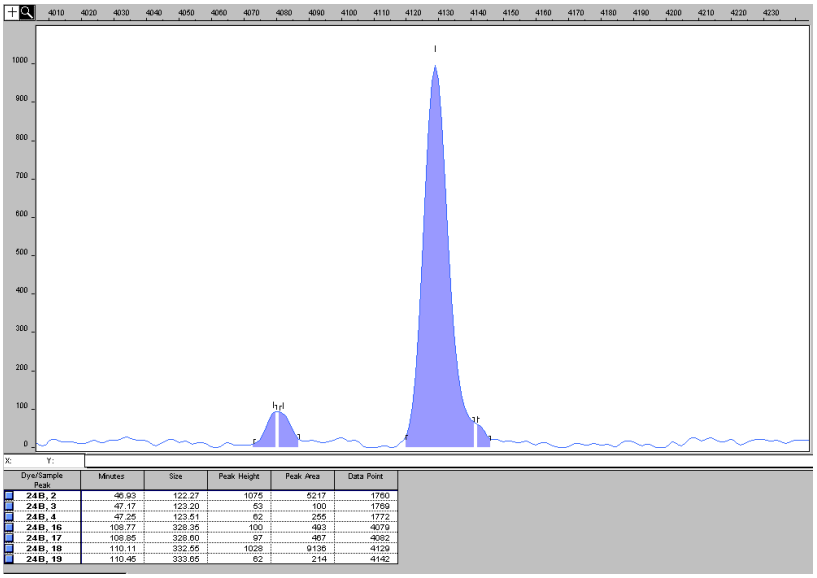


Electropherogram showing all four peaks detected after reducing the window size value and increasing the polynomial degree value

Optimizing Peak Detection Sensitivity: Example 3

Effects of Extreme Settings

The figure below shows the result of an analysis using a peak window size value set to 10 and a polynomial degree set to 9. This extreme setting for peak detection led to several peaks being split and detected as two separate peaks.



Electropherogram showing the result of an analysis using extreme settings for peak detection

Peak Detection: Slope Threshold for Peak Start and Slope Threshold for Peak End Parameters

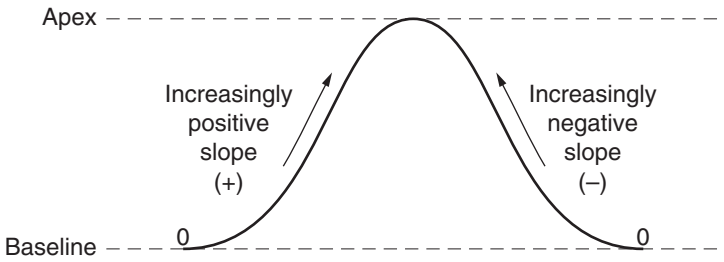
About These Parameters

Use the Slope Threshold for Peak Start and Slope Threshold for Peak End parameters to adjust the start and end points of a peak.

This parameter can be used to better position the start and end points of an asymmetrical peak, or a poorly resolved shouldering peak, to more accurately reflect the peak position and area.

How These Parameters Work

In general, from left to right, the slope of a peak increases from the baseline up to the apex. From the apex down to the baseline, the slope becomes decreasingly negative until it returns to zero at the baseline.



If either of the slope values you have entered exceeds the slope of the peak being detected, the software overrides your value and reverts to zero.

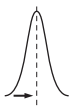
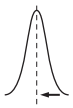
Guidelines for Using These Parameters

As a guideline, use a value of zero for typical or symmetrical peaks. Select values other than zero to better reflect the beginning and end points of asymmetrical peaks.

A value of zero will not affect the sizing accuracy or precision for an asymmetrical peak.

Using These Parameters

Use the table below to move the start or end point of a peak.

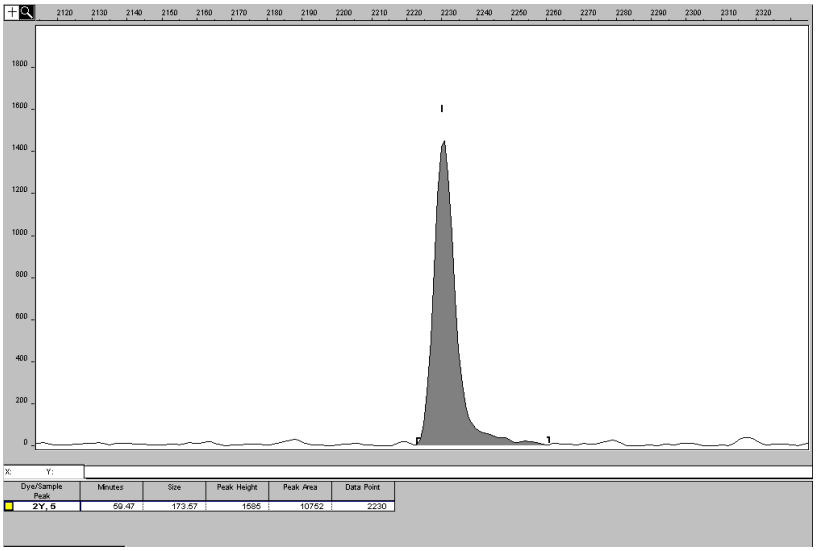
IF you want to move the...	THEN change the...
start point of a peak closer to its apex 	Slope Threshold for Peak Start value from zero to a positive number
end point of a peak closer to its apex 	Slope Threshold for Peak End value to an increasingly negative number

Note The size of a detected peak is the calculated apex between the start and end points of a peak and will not change based on your settings.

Slope Threshold Examples

Initial Electropherogram

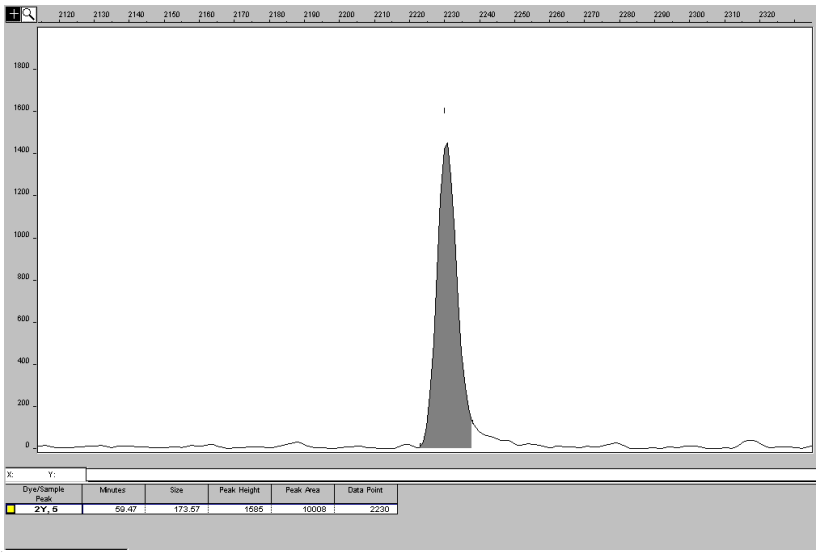
The initial analysis with a value of 0 for both the Slope Threshold for Peak Start and the Slope Threshold for Peak End value produced an asymmetrical peak with a noticeable tail on the right side.



Electropherogram showing an asymmetrical peak

Electropherogram After Adjustments

After re-analyzing with a value of –35.0 for the Slope Threshold for Peak End, the end point that defines the peak moves closer to its apex, thereby removing the tailing feature. Note that the only change to tabular data was the area (peak size and height are unchanged).



Electropherogram showing the effect of changing the slope threshold for peak end

Size Calling Methods for Classic and Advanced Modes

Overview

There are four size calling methods that you can use with the GeneMapper software v3.0. They are as follows:

- Least Square
- Cubic Spline Interpolation
- Local Southern
- Global Southern

Least Square Method

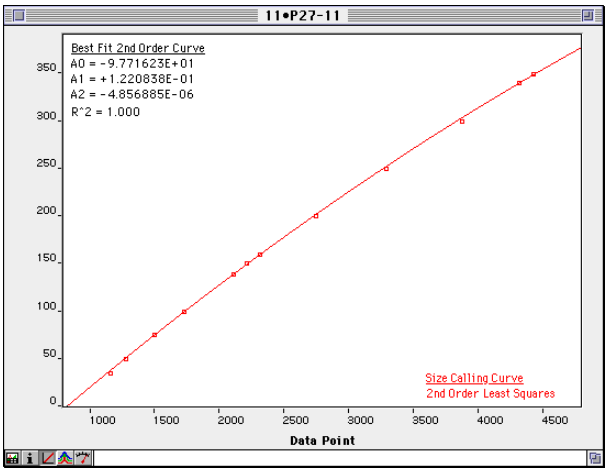
Both Least Squares methods (2nd Order and 3rd Order) use regression analysis to build a best-fit size calling curve. This curve compensates for any fragments that may run anomalously. As a result, this method normally results in the least amount of deviation for all the fragments, including the size standards and the samples.

Depending on whether you choose the 2nd or 3rd Order Least Squares Method in the Analysis Parameters dialog box, the resulting size curve is either a quadratic or a cubic function. The software uses the known standard fragments and the associated data points to produce a sizing curve based on Multiple Linear Regression.

Advantages

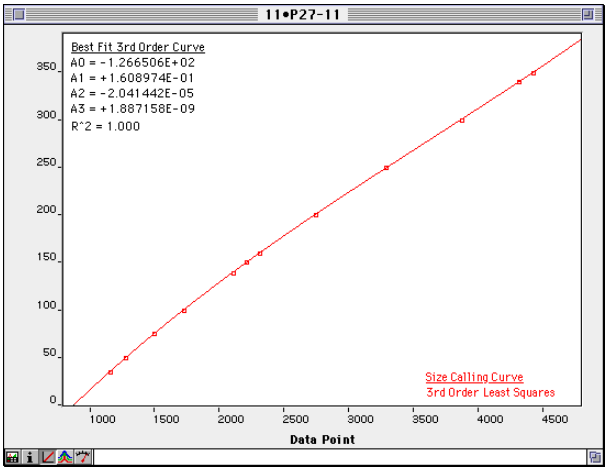
In the figures on page B-33, you can see that in nearly all instances the mobility of an individual DNA fragment is coincident with the best curve fit of the entire data set. Stated differently, the mobility of most DNA fragments is strictly length-dependent. This method automatically compensates for fragments that run anomalously.

GeneMapper software v3.0 calculates a best-fit least squares curve for all samples, regardless of the size calling method you choose. The curve is black in the Standard Sizing Curve window.



2nd Order Least Squares size calling curve

Note This graph was generated using GeneScan 3.7.1 software. These results are similar to results obtained when you use GeneMapper software v3.0.

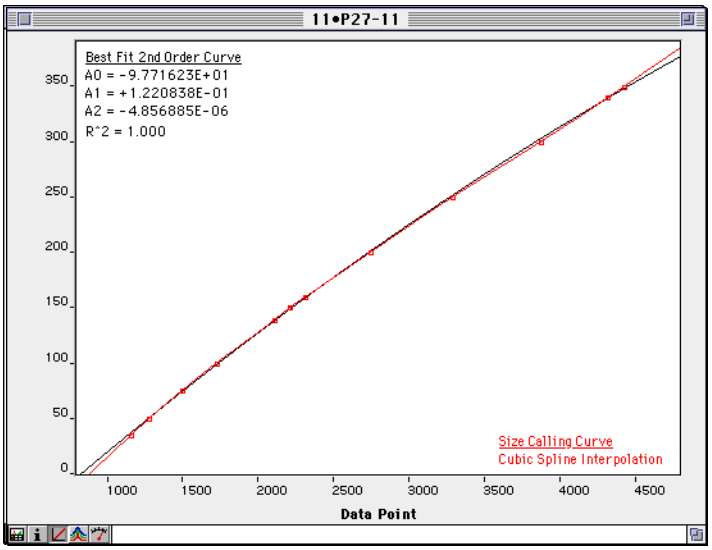


3rd Order Least Squares size calling curve

Note This graph was generated using GeneScan 3.7.1 software. These results are similar to results obtained when you use GeneMapper software v3.0.

Cubic Spline Interpolation Method

By definition, the Cubic Spline method forces the sizing curve through all the known points of the selected size standard. Although this enforement produces exact results for the values of the standards themselves, it does not compensate for standard fragments that may run anomalously.



Note This graph was generated using GeneScan 3.7.1 software. These results are similar to results obtained when you use GeneMapper software v3.0.

Possible Local Sizing Inaccuracy

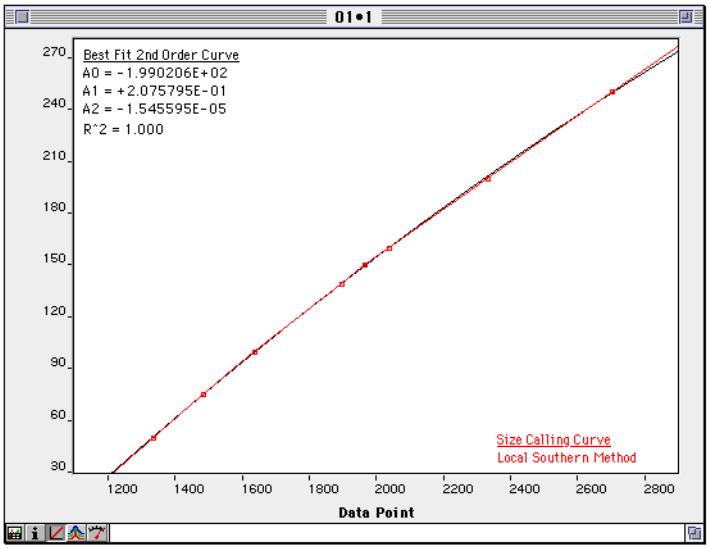
Mobility of any DNA fragment can be affected by its sequence, and by secondary and tertiary structure formation. If any internal size standard fragment has anomalous mobility, the Cubic Spline method may exhibit local sizing inaccuracy.

For example: Assume that a standard fragment is close in molecular length to an unknown sample fragment. Assume further that the standard fragment runs anomalously. The Cubic Spline method assigns the official value to this standard fragment, even though it may be slightly incorrect. The size of the unknown fragment is then likely to be calculated incorrectly as well.

Note This method does not determine the amount of sizing accuracy error.

Local Southern Method

The Local Southern method determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility, as described by E. M. Southern (1979).



Note This graph was generated using GeneScan 3.7.1 software. These results are similar to results obtained when you use GeneMapper software v3.0.

The Equation

$L = [c/(m-m0)] + L0$

The equation attempts to describe the reciprocal relationship between the mobility, m, and the length, L0, of the standard fragments.

How This Method Works

This method, which is similar to the Cubic Spline method, uses the four fragments closest in size to the unknown fragment to determine a best fit line value. Only the region of the size ladder near the fragment of unknown length is analyzed.

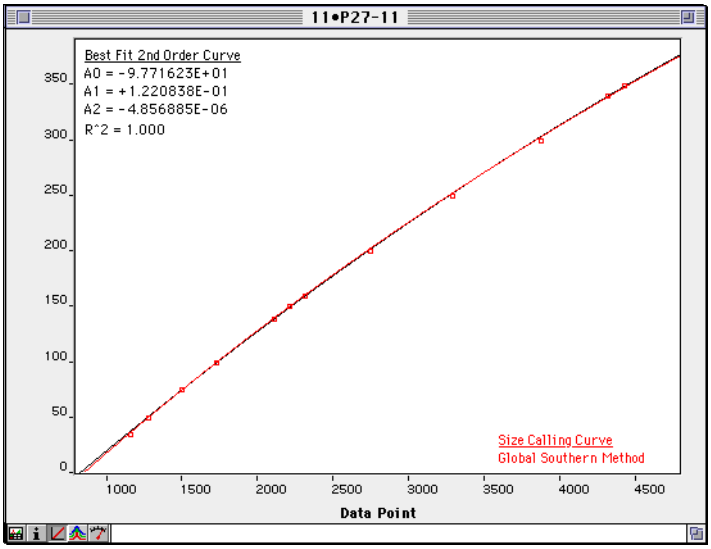
Note Size estimates may be off if any of the standard fragments run anomalously.

The following table lists how the Local Southern method works:

Step	Action
1	The fitting constants of the curve are calculated for each group of three neighboring points on the standard. A separate curve is created for each set of three points.
2	A curve is then created by using three standard points (two points below and one point above the fragment) and a fragment size is determined.
3	Another curve is created by looking at an additional set of three points (one point below and two points above the fragment) and another value is assigned.
4	The two size values are averaged to determine the unknown fragment length.

Global Southern Method

This method is similar to the Least Squares method in that it compensates for standard fragments that may run anomalously. The method creates a best-fit line through all the available points, and then uses values found on that line to calculate the fragment values.



Note This graph was generated using GeneScan 3.7.1 software. These results are similar to results obtained when you use GeneMapper software v3.0.

The Equations

The following table describes how the equations work:

Equation	Description
$L = [c/(m-m0)] + L0$	Attempts to describe the reciprocal relationship between the mobility, m, and the length, L0, of the standard fragments.
$\sum i(Li - (c/(mi-m0) + L0))^2$	The fitting constants L0, m0, and c are calculated by a least squares fit to minimize the left side quantity.

How This Method Works

All points in the standard are weighted equally and the curve is not constrained to go through any specific point. The software can analyze a large range of fragment sizes with this method.

DNA fragments that are...	Are sized using...
not bracketed within the size standard curve	a second order least squares curve extrapolation.
bracketed within the size standard curve	the method that was chosen.

For best results, use a standard that brackets all the fragments of interest.

Project Window Software Interface

C

Appendix Overview

Introduction

This appendix gives detailed descriptions of the features of the ABI Prism® GeneMapper™ Software Version 3.0 application, including details of each major application window.

In This Appendix

This appendix contains the following topics:

Overview of the Project Window

Menus and Dialog Boxes

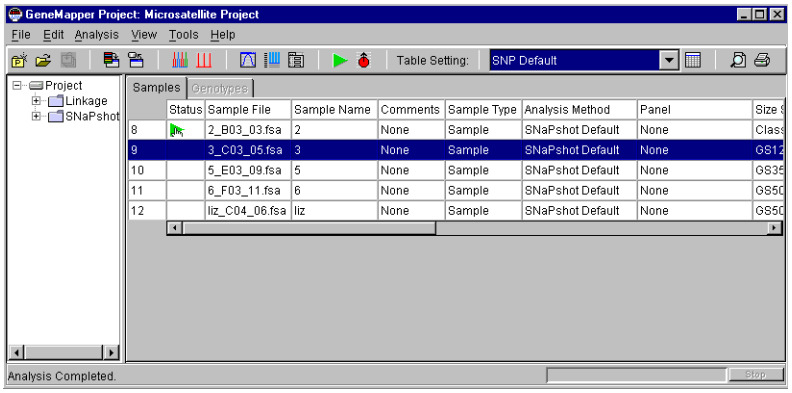
The Project window has a number of menus and dialog boxes associated with it. The drop-down menus available on each window (for example, File, Edit, View) provide a set of commands specific to the window. This section will describe the menu commands and dialog boxes associated with a given window.

Samples View

The following information is provided in this section for the Samples view.

Appearance of Samples View

The Samples view of the Project window displays the samples you want to analyze or have already analyzed. This view looks much like the following example when sample data has been imported.

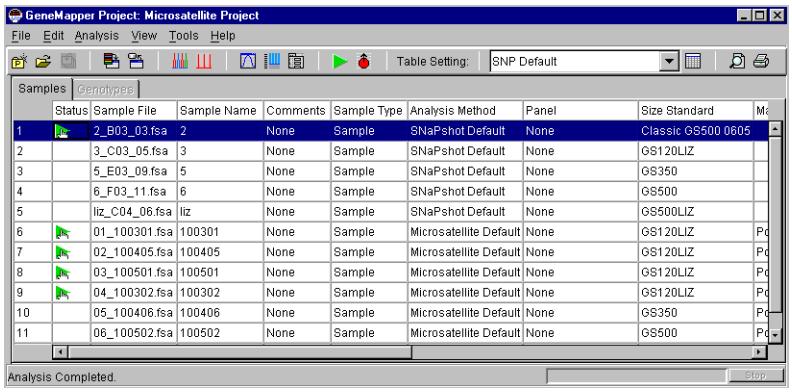


Note You can hide and show columns in the table using Table Setting.

Samples View Columns

The columns of the Samples view are explained in the table below.

Note The following image is shown with the navigation pane closed.



Samples View Column Descriptions

Column	Description
Sample or row number	From application; used to select a single entire row.
Status	A  symbol indicates that the sample needs to be analyzed or reanalyzed. **Note** These symbols indicate status of each sample, and they control which samples will be analyzed during the next analysis if the Analyze icon is selected. There are other analysis options which ignore the status field. ♦ The Status field next to each sample contains the  symbol prior to analysis. This field is empty after analysis, if analysis was successful. ♦ Resetting parameters such as the Panel, Size Standard, Sample Type, and the Analysis Method resets the symbol for a given sample, enabling reanalysis.
Sample File	From sample sheet; not editable.
Sample Name	From sample sheet; editable, free text.
Comments	Editable, free text.

Samples View Column Descriptions *(continued)*

Column	Description
Sample Type	Editable via Sample Type drop-down list box; valid types are Sample, Control, Allelic Ladder, and Primer Focus, Negative Control.
Analysis Method	Editable via a drop-down list box. The list is editable via the Analysis Method Editor in the Analysis Methods tab in the GeneMapper Manager window.
Panel	Editable via Panel window; Panel window contents come from Panel Manager.
Size Standard	Editable via a drop-down list box. The list is editable via the Size Standard Editor in the Size Standards tab in the GeneMapper Manager window.
Matrix	Editable via a drop-down list box. The list is editable via the Matrix Editor in the Matrices tab in the GeneMapper Manager. For the ABI PRISM® 377 DNA Sequencer and the ABI PRISM® 310 Genetic Analyzer instruments, the matrix file is created in the Matrices Tab in the GeneMapper Manager window.
Run Name	From sample sheet; not editable.
Instrument Type	From sample sheet; not editable.
Instrument ID	From sample sheet; not editable.
Run Date and Time	From sample sheet; not editable.
REF	Reference data;  indicates that the selected sample is defined as reference data in the Panel Manager.
SQI	Sizing quality invalidated; checkmark indicates that sizing quality value is 1.0.
User Defined 1-3	Editable free text. Also from the vertical bar enabled in the sample sheet fields. See Chapter 2, “Using GeneMapper Software.”

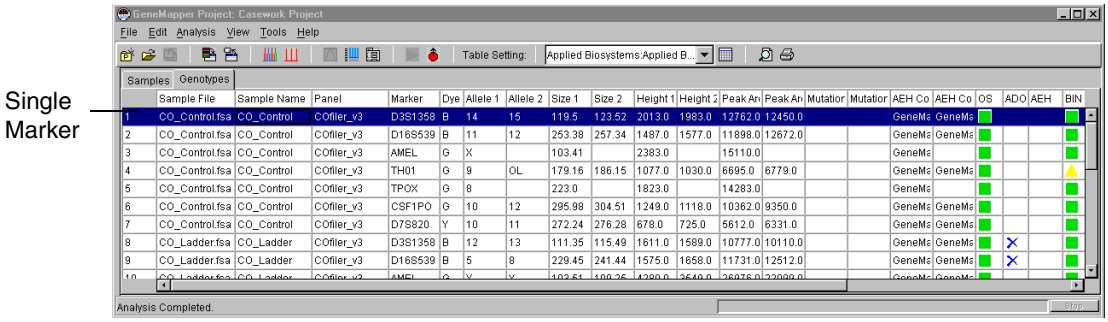
Note Refer to Appendix A for PQV information.

Samples View Features

Feature	Description
Select Row(s)	Click the Row Header box; drag to select a continuous range.
Select Column(s)	Click the Column Header box; drag to select a continuous range.
Resize columns	Click and drag between columns to change column width.
Quick sort column	Shift–click the column header to sort ascending; shift–click again to sort descending.
Deselect Row	Ctrl-click the Row Header box

Genotypes View

The Genotypes view, shown below, displays the results of allele calling analysis. Each row or “record” in the table is a single marker for a sample. For example, if a panel specified for a sample contains four markers, the Genotypes table contains four records for that sample (each in a separate row).



Genotype View Columns

The table in the Genotypes view can display some of the columns in the Samples view (see table on page C-3), plus these unique columns.

Genotypes View Columns

Column	Description
Marker	Not editable; name of the marker associated with the record; created in the Panel Manager.
Dye	From Panel Manager; not editable; indicates which dye is associated with the marker (B=blue, G=green, Y=yellow, R=red, or O=orange). **Note** Not used for SNP Genotyping (SNaPshot® analysis).
Allele 1, 2, etc.	Not directly editable in the table cell; allele calls are editable only in the plot windows. The call for Allele 1. Allele calls and names are defined in the Panel Manager as bin names. The Allele call text box displays a list of valid calls plus one of the following: ♦ ? (for unknown) for linkage and SNP (SNaPshot) applications ♦ OL (Off-ladder) for Human Identification (HID) applications ♦ blank for no allele calls. **Note** When alleles shown in the Genotypes table are edited in the Plot window, the allele call in the table also changes and confidence value indicators in the table turn to grey triangles.
Size 1, 2, etc.	Peak size for Allele. Not editable.
Height 1, 2, etc.	Peak height for Allele. Not editable.
Peak Area 1 & 2	Peak area for Allele. Not editable.
Mutation	Indicates allele falling within a mutant bin.
AE Comment	Displays last user-edited comment.

Genotypes View Columns *(continued)*

Column	Description
ADO (Allele Display Overflow)	♦ The box is labeled with an  when the number of alleles the marker calls exceeds the number to display previously set by the user. ♦ The user specifies how many alleles to display in the Table Settings parameters. (The default is two.) ♦ There are six columns for each allele to indicate name, size, height, area, mutation, and comment.
AE (Allele Edit)	♦ The box displays unlabeled immediately after analysis. ♦ The box is labeled with an  when the marker allele calls have been edited by the user. ♦ The marker allele calls can be edited in the Plot window. The user can enter comments in the Allele Edit column while editing allele calls.
User Defined 1-3	Editable; free text. Also from vertical bar enabled in sample sheet fields. See Chapter 2, “Using GeneMapper Software.”

Note Refer to Appendix A for PQV column information.

Project Window Menus

Introduction

The tables in this section describe the following Project Window menus.

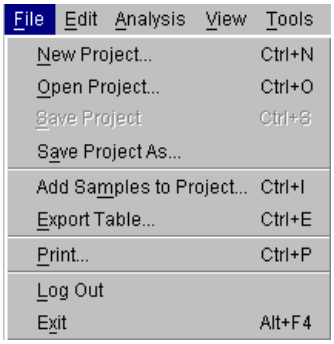
Command Activation and Special Terms

The commands in the Project Window menu can be activated in two ways:

- Clicking the command in the menu, or
- Using the special key combinations shown to the right of the name in the menu.

File Menu

The commands are described in the table below.



Project Window File Menu Commands

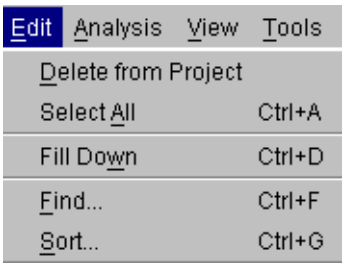
Item	Description	Enabling
New Project (Ctrl+N)	Clears sample files, results, and project name from the Project window, displaying a blank Project. If previous project has pending changes, the following alert message is displayed: *Do you want to save changes? [Yes] [No] [Cancel]*	Always enabled except when a blank Project is displayed.
Open Project (Ctrl+O)	Displays the Open Project dialog box. If previous Project has pending changes, the following alert message is displayed: *you want to save changes? [Yes] [No] [Cancel]*	Always enabled.
Save Project (Ctrl+S)	Saves Project to the file named at the start of analysis. Named projects are saved to the GeneMapper database without a dialog box.	Enabled when the Project table has pending changes.
Save Project As...	Displays the Save dialog box.	Always enabled.
Add Samples To Project (Ctrl+I)	Opens the Add Samples To Project dialog box.	Always enabled.
Export Table (Ctrl+E)	Displays the Export table dialog box. Exports table as tab or comma delimited text file.	Enabled when the Project table contains data.
Print (Ctrl+P)	Displays the Print dialog box. The standard Print Setup dialog is opened from the Print dialog.	Enabled when the project table contains data.
Log Out	Closes the Project window and displays the Login window. Displays a Save alert message if the Project has pending changes.	Always enabled.

Project Window File Menu Commands *(continued)*

Item	Description	Enabling
Exit (Alt+F4)	Exits the GeneMapper application; displays Save alert message if Project has pending changes.	Always enabled.

Edit Menu

The commands in the Edit menu are used to manage the contents of the Project window by performing standard actions like undo, delete, select, and by enabling access to settings for Preferences.



Project Window Edit Menu Commands

Item	Description	Enabling
Delete From Project	Deletes the selected sample(s) from the Project and the database and displays the following alert message: *Deleting the selected sample(s) will delete both samples and results from project. This action cannot be undone.* [OK] [Cancel]	Enabled when a Project sample is selected.
	Note Each use of the command removes a single run or sample. If you want to remove all samples and the associated folder, use the Select All command before using the Delete from Project command. This action does not delete the actual sample file or Sequence Collector sample data.	
Select All (Ctrl+A)	Selects all samples in the active view.	Always enabled.

Project Window Edit Menu Commands *(continued)*

Item	Description	Enabling
Fill Down (Ctrl+D)	Fills the selected column with the contents of the first cell. This command is applicable only to Analysis Method, Panel, Size Standard, and Matrix columns.	Enabled when a column is selected or multiple cells are selected.
Find (Ctrl+F)	Displays the Find dialog box.	Enabled when the table contains data.
Sort (Ctrl+G)	Displays the Sort dialog box.	Enabled when the table contains data.

Analysis Menu

The commands in this menu control analysis and display of the data in a Project and access to a number of other windows that support the Project window, such as the Analysis Manager and Panel Manager. The following table is provided as a reference for the Analysis menu.

Project Window Analysis menu commands:

Item	Description	Enabling
Display Plot (Ctrl+L)	Opens the Plot window.	Enabled when one or more samples or records are selected.
Size Match Editor	Opens the Size Match Editor window.	Enabled only when sized samples are present in the Project.

Project Window Analysis menu commands: *(continued)*

Item	Description	Enabling
Analyze (Ctrl+R)	Analyses all samples eligible for analysis. The symbol is shown in the Status column.	Enabled when Samples tab is visible and at least one sample in the table requires analysis.
Analyze Selected Samples	Analyzes selected samples only.	Enabled when Samples tab is visible.
Analyze All (Ctrl+Shift+R)	Analyzes all samples whether or not the Status column reports that the sample is eligible for analysis, including those previously analyzed. The following alert message is displayed: *Analyze all samples? This may change your existing results. This action cannot be undone. [OK] [Cancel]*	Enabled when Samples tab is visible.
Analyze Markers	Analyzes only the marker(s) you select within each sample. (“Analyze Markers Dialog Box” on page C-14.)	Enabled when Samples tab is visible.
Analysis Method Editor	Allows the user to change the settings for analysis.	Enabled when a sample with an analysis method is selected.
Size Standard Editor	Allows the user to edit base pair and dye settings.	Enabled when one or more samples or records are selected.
Low Quality To Top (Ctrl+B)	Sorts all Low Quality (SQ or GQ column) samples or results records to the top of the table.	Enabled when analyzed samples are present in the table.
Non-concordant Samples to Top	Brings non-concordant samples to the top of the analysis page.	

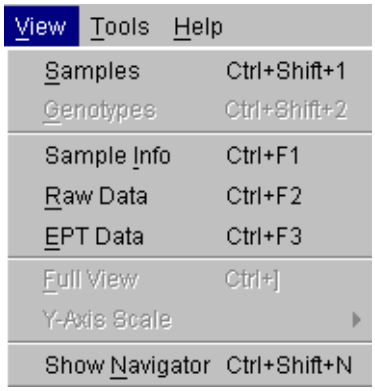
Analyze Markers Dialog Box

The Analyze Markers dialog box enables you to select which marker, or set of markers separated by commas, will be analyzed within each sample.

This option is only enabled when you are in the Project window Samples tab and samples are present.Does not work with HID samples.

View Menu

The View menu is used to hide/show the Project window navigation pane and switch between the two Project window tabs. The following table is provided as a reference for the View menu.



Project Window View Commands:

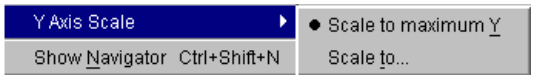
Item	Description	Enabling
Samples (Ctrl+Shift+1)	Switches to the Samples tab.	Always enabled.
Genotypes (Ctrl+Shift+2)	Switches to the Genotypes tab.	Enabled when results are present.
Sample Info (Ctrl+F1)	Switches to the Info tab for the selected sample in the project.	Always enabled.

Project Window View Commands: *(continued)*

Item	Description	Enabling
Raw Data (Ctrl+F2)	Switches to the Raw Data tab for the selected sample in the project.	Always enabled.
EPT Data (Ctrl+F3)	Switches to the EPT tab for the selected sample in the project.	Always enabled.
Full View (Ctrl+])	Shows the full view of the Raw Data or EPT graph.	Enabled only when viewing the Raw Data or EPT Data of a sample.
Y-Axis Scale	Changes Y-axis scale. (See "Y Axis Scale" on page C-15.)	Enabled only when viewing the Raw Data or EPT Data of a sample.
Show Navigator (Ctrl+ Shift+N)	Switches the navigator pane (shows/hides the pane).	Always enabled.

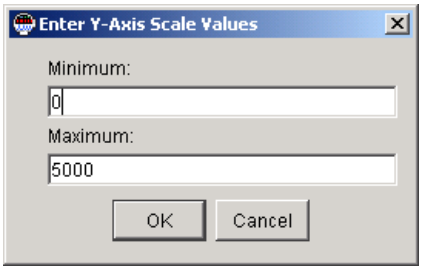
Y Axis Scale

The Y Axis Scale option is only enabled when viewing the raw data or EPT data of a sample file.



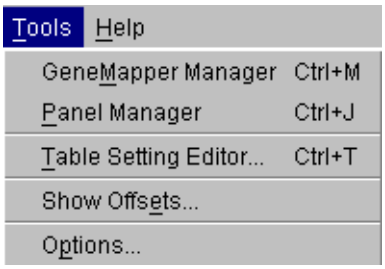
There are two options:

- **Scale to maximum Y** - scales to the maximum Y value for the selected sample.
- **Scale to** - opens a dialog box in which a user can define the value of the Y axis.



Tools Menu

The Tools menu provides access to the GeneMapper Manager, Panel Manager, and Table Setting Editor applications.



Item	Description	Enabling
GeneMapper Manager (Ctrl+M)	Opens the GeneMapper dialog box. (See “GeneMapper Manager” on page 2-11.)	Always enabled.
Panel Manager (Ctrl+J)	Opens the Panel Manager dialog box. (See Chapter 4, “Using Panel Manager.”)	Always enabled.
Table Setting Editor (Ctrl+T)	Opens the Table Setting Editor dialog box. (See “Table Settings Editor” on page 6-4.)	Only enabled when samples are present. Only allows editing of a currently selected Table Profile.
Show Offsets	Displays bin offsets for selected samples.	Always enabled.
Options	Opens the Options dialog box. (See “Options Dialog Box” on page C-17.)	Always enabled.

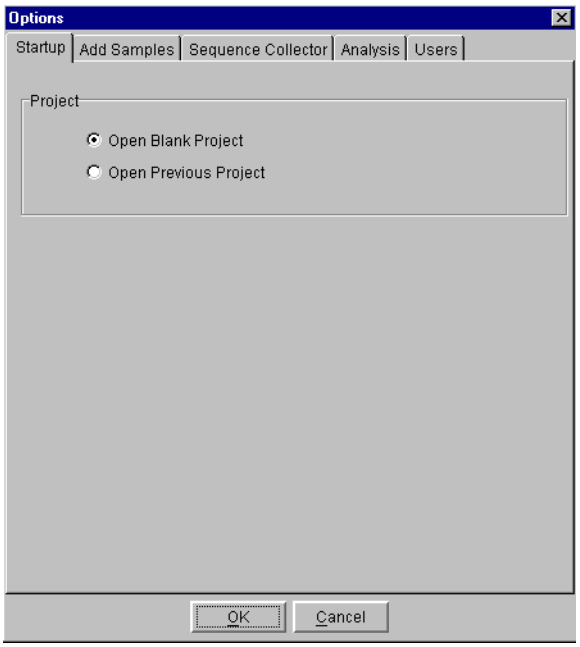
Options Dialog Box

The Options dialog box contains several tabs for setting preferences associated with various software features. The following tabs are described below:

Note Each registered user has his or her own set of preferences. When you set these options, it only affects the currently logged in user.

Startup Tab:

The Startup tab contains preferences associated with the startup of the software.

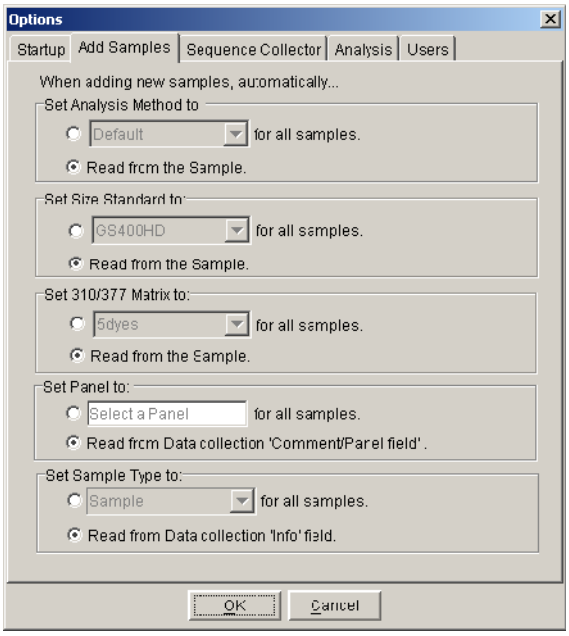


Startup Tab Elements

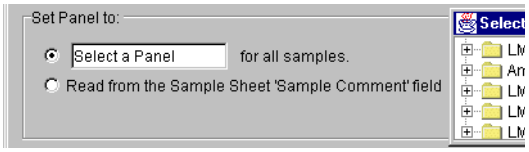
Element	Description
Open Blank Project option button	Sets the software so that upon launch, a blank Project window opens.
Open Previous Project option button	Sets the software so that upon launch, the last Project viewed will open.

Add Samples Tab:

The Add Samples tab contains preferences associated with the adding of samples.



Add Samples Tab Elements

Element	Description
Set Analysis Method to drop-down menu and **Read from the Sample** option button	When you add samples, the Analysis Method property is set to the value in the text box, which contains the list of analysis methods from the GeneMapper database.
Set Size Standard to drop-down menu and **Read from the Sample** option button	When you add samples, set the size standard property to the value in the text box, which contains the list of size standards from the GeneMapper database.
Set 310/377 Matrix to drop down menu **Read from the Sample** option button	Set the matrix file to the dye you are using or read from the sample file.
Set Panel to ♦ **Select a Panel for all samples** option button and window	When you add samples, the panel for all samples is set to the value in the text box. When the option button is selected, the following choices may be made: ♦ Leave the text alone, which selects the existing name, or ♦ Click the field to present the Select a Panel window (to select an existing panel) The Select a Panel window contains the list of panels from the GeneMapper database. 
♦ **Read from the Data collection ' Comment/Panel field'** option button	When you add samples, the panel for all samples is set to the value in the Sample Sheet "comment" column.

Add Samples Tab Elements *(continued)*

Element	Description
Set Sample Type to ♦ **Sample type for all samples** option button and drop-down menu ♦ **Read from Data collection 'Info' field** option button	When you add samples, the Sample Type for all samples is set to the value chosen from the Sample Type drop-down menu, contains choices for Sample, Control, Allelic Ladder, and Primer Focus. When you add samples, the GeneMapper software does a "best guess" match, comparing the contents of the field specified in the Sample type box with the list of sample types (Sample, Control, Allelic Ladder, Negative Control, and Primer Focus). ♦ If a match is found, then the Sample Type property for the sample is set to that sample type. ♦ If a match is not found, then the Sample Type property for the sample is set to "Sample."

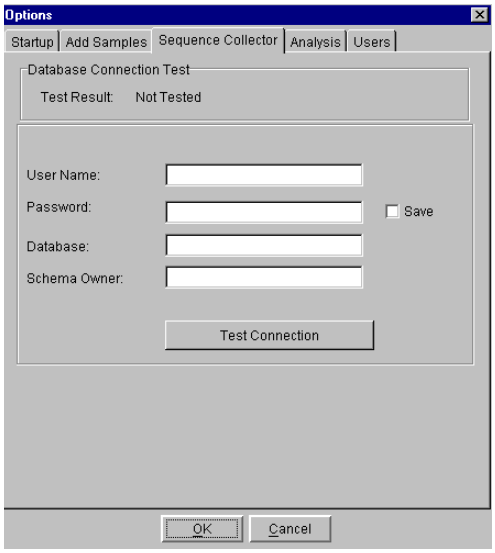
Note The Panel and Sample Type properties accounts for close spellings and abbreviations as shown in the following table.

Spellings and Abbreviations

Case	Result
A sample file field contains either Control, Ctrl, Crl, Cntrl, or a similar variation.	The sample type for the sample is set to Control.

Sequence Collector Tab:

The Sequence Collector tab enables access to a single Sequence Collector.

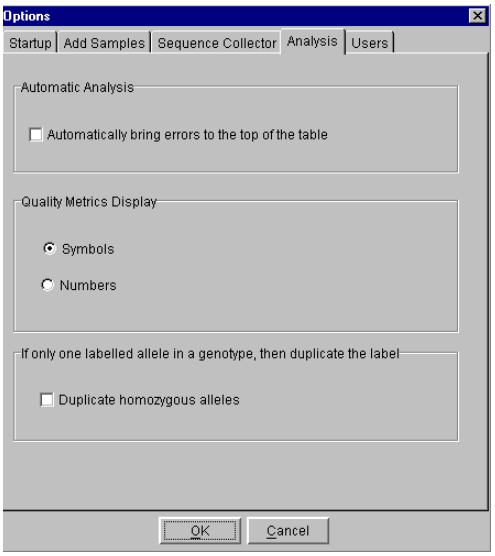


Sequence Collector Tab Elements

Element	Description
Test Result	The results returned are **Pass** or **Fail**. These results are displayed by clicking the Test Connection button. If the Test Connection button has not been pressed, the Test Result will display **Not Tested**.
User Name	Registered user name to access Sequence Collector.
Password	Password for the user name to access Sequence Collector; data entry into this field displays as bullets or asterisks.
Save (check box)	Save the Password; when set, the user does not have to enter a password to get access to Sequence Collector.
Database	Name of the Sequence Collector (Sequence Collector string); refer to Sequence Collector documentation for more information.
Schema Owner	The name of the owner who has access to desired sample collections.
Test Connection (button)	Tests the connection to the database specified; includes appropriate error checking per Sequence Collector.

Analysis Tab:

The Analysis tab contains preferences associated with the analysis of data.



Analysis Tab Elements

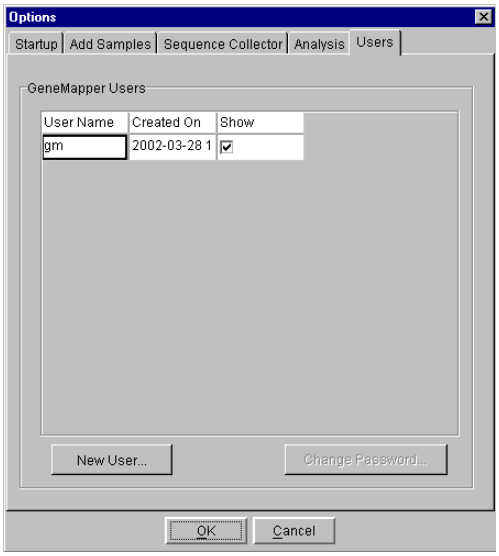
Element	Description
Automatic Analysis group box: **Automatically brings errors to the top of the table** check box	When samples are analyzed, the software sorts the table automatically to bring samples with errors to the top of the table. Same functionality as the Bring Errors To Top icon in the Project window.
Quality Metrics Display group box: **Symbols** button **Numbers** button	Display quality values in project tables as symbols (green square, yellow triangle, red octagon), or numerical values (0.0, ..., 1.0). **Note** If Symbols (default) is chosen, then all quality values are shown as symbols. If Numbers is chosen, then only SQ and GQ columns are shown as numbers; the other result columns are symbols.

Analysis Tab Elements *(continued)*

Element	Description
If only one allele is labeled in a genotype, then duplicate the label group box: **Duplicate homozygous alleles** checkbox	If this option is checked, single alleles will be displayed in the Genotypes table and in the Plot window as two labels to indicate presumed homozygotes.

Users Tab:

The Users tab contains preferences associated with managing registered users.

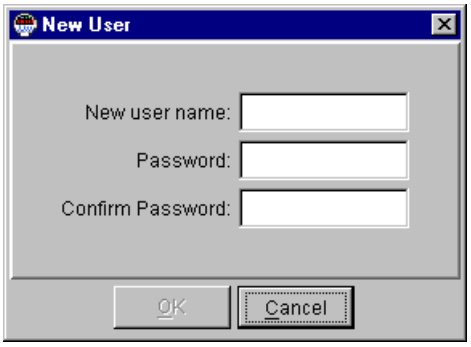


Note Once created, user names cannot be deleted.

Users Tab Elements

Element	Description
User Name field	Displays the list of registered users. ♦ **User Name column** = Name of the user ♦ **Created On column** = Date user was created ♦ **Show column** = Shows the user in User Name drop-down menu of the login window, if checked. Clearing a Show column check box for a user blocks login for that user (can be done by anyone running GeneMapper software). Shift–click the column label headers to sort the list by that column.

Users Tab Elements *(continued)*

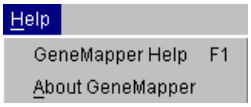
Element	Description
New User button	Opens the New User dialog box.  To add a user name for GeneMapper software, click **New User** to open a dialog box and then: 1. Type a user name into the dialog box. 2. Enter a password using only alphabetic characters. 3. Confirm the password. 4. Click **OK** to assign the user name and close the dialog. When you select this user name on launching GeneMapper software, the name is used to identify the preferences set by the user. **Note** User names must be unique. If the new user name is not unique, an alert message will be displayed. **Note** Users added here are for this GeneMapper software installation and is visible to all other users. The names are converted to lower case and passwords are not case sensitive.

Users Tab Elements *(continued)*

Element	Description
Change Password	Opens the Change Password dialog box, and allows you to change the password for the selected user. 

Help Menu

The Help menu provides access to online help and to the About GeneMapper software window.



Project Window Help Menu Commands

Item	Description	Enabling
GeneMapper Help (F1)	Opens the GeneMapper User Manual PDF file using Acrobat Reader. Does not open the tutorial guides.	Always enabled.
About GeneMapper	Opens the About GeneMapper window and displays the software version.	Always enabled.

Project Window File Menu Dialog Boxes

Introduction

The following dialog boxes are accessed from the Project window File menu. This section describes each of the dialog boxes.

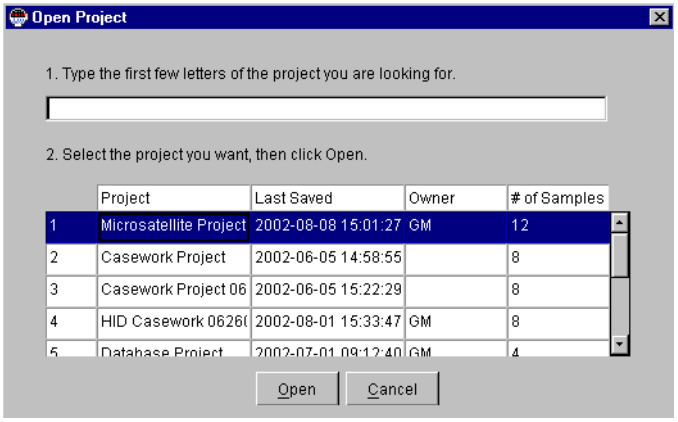
Note This section contains information on all application dialog boxes except for the “Add Samples to Project” dialog box, which is covered under its own section (See “Add Samples to Project Dialog” on page C-36).

Save Dialog Box

The Save dialog box, accessed from the New Project window, is presented when a project already exists in the Project window, and provides you with the option of saving the project.

Open Project Dialog Box

The Open Project dialog box displays the list of projects in the database. You use the first field to find a project quickly, then click the Open button to open the selected project (or double-click the project name).



Note Once you have opened a Project window, you may resize it horizontally and vertically to increase the width and depth of the Project table.

The following table describes the Open Project dialog box window elements.

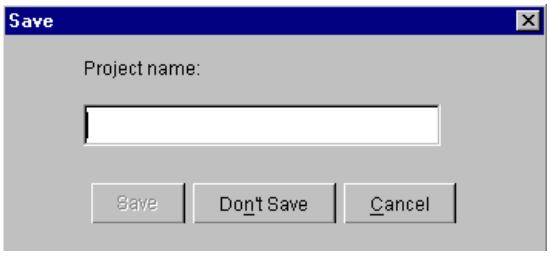
Open Project Window Elements

Element	Description
Search field	Typing in this field selects the first item in the project list whose name matches the characters.
Project table	Lists all the projects in the database. ♦ Project = Name of the project. ♦ Last Saved = Date and time stamp of when the project was last modified. ♦ Owner = Name of the user who created the project. ♦ # of Samples = the number of samples in the project. **Note** Columns may not be moved or hidden. You may resize the width of the columns by dragging between the column headers. Only one row in the table may be selected at a time. You may navigate up and down in the table using arrow keys. Shift-clicking a column header sorts the data by that column.
Open	Opens the selected project. If previous project has pending changes, the following alert message is displayed before the Open Project dialog box opens: *Do you want to save changes? [Yes] [No] [Cancel]*
Cancel	Closes the Project Manager dialog box.

Save Project and Save Dialog Boxes

The Save Project and Save boxes enable you to save projects to the database. The first dialog box is presented for the Save command if you want to save a project before it is analyzed. Using Save after analysis does not require a dialog box because you are required to name a Project before analysis.

The Save dialog box opens when you select the Save As command.



The elements of these dialog boxes are described in the table below.

Save Project Elements

Element	Description
Project name	Text field for the name of the project; accepts alphanumeric characters. The database requires standard Windows interface restrictions on length and symbol characters.
OK	Saves the project to the database. Saved projects are tagged with the Project Name, User Name, and Time/Date Stamp. If the name you entered already exists, an alert message is displayed. Cancel closes the alert dialog and displays the Save Project dialog box again.
Cancel	Closes the dialog box and does not save the project.

Save Project Elements *(continued)*

Element	Description
Save	Saves the project under the name entered in the dialog box.
Don't Save	Aborts the Save action.

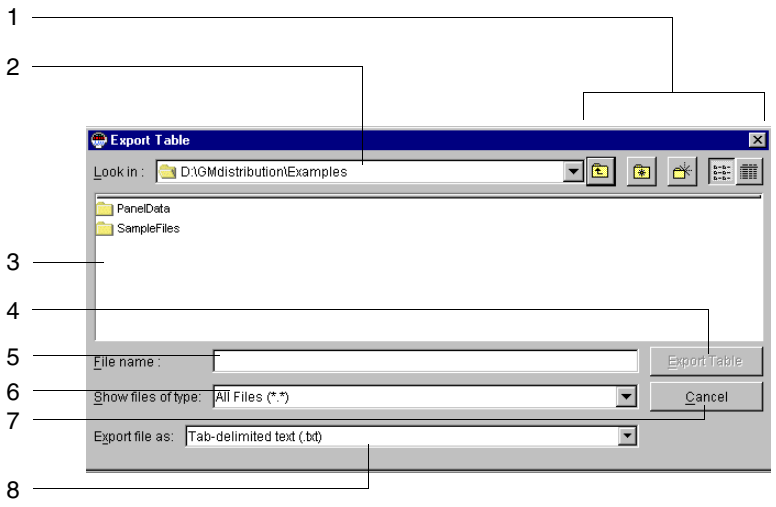
Export Table Dialog Box

The Export Table dialog box allows you to choose where to save the exported file.

The file types supported include:

- Tab-delimited text (.txt)
- Comma-separated values (.csv)

Note Export operates on the currently-displayed tab. For example, if the Samples tab is displayed, Export would export the tab-delimited data shown in the Samples table. Exported data includes column headers.



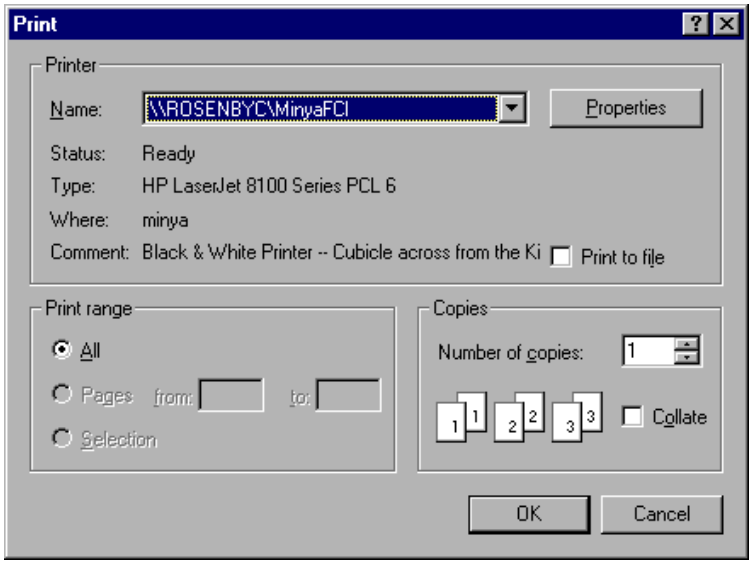
Export Table Callouts

Item	Name	Description
1	Toolbar	These icons are used as follows:  - Clicking moves display up one level in main pane.  - Clicking moves display to “Home” level in main pane. This is usually “Profiles\<user>”.  - Clicking creates a new folder at the present directory level.  - Clicking presents a list of the contents of the selected folder.  - Clicking presents details of the selected folder.
2	Drop-down directory menu	Select drive letter and/or folder.
3	Folder/file display pane	The contents of the drive/directory are selected in the toolbar and the directory menu is displayed here.
4	Export Table button	This button exports the selected table.
5	File name field	Enter a file name to use for exported table.
6	Show files of type field	This is a display filter for files (folders are always shown). Allows specification of file extension. Filters files for display in all files, .txt files, .csv files, .txt&.csv.
7	Cancel button	Closes the Export Table window without exporting a table.
8	Export file as field	Select the format of the file you want to export.

Note Exported text tables show PQV’s as numbers even if they are displayed as symbols.

Print Dialog Box

The Print dialog box, shown below, is a print file dialog box that controls printing. The Samples table, the Genotypes table, Panel Manager views, and plot windows may be printed.



The dialog box above contains the following items used for printing:

Item	Description
Properties button	Presents the dialog box used to set up the printer.
Name field	Shows the currently selected printer. **Note** The drop-down menu to the right allows selection of other available printers.
Status	Indicates status of selected printer.
Type	Shows type of printer.
Where	Shows the path name of the selected printer.
Comment	Check box enables printing the project to a file.
Page range	*All* is the only option available when working with tables.
Number of Copies	Select the number of copies to print.
Collate options	Check box enables collating of copies.

Printing Tables and Electropherograms

Introduction

All tables and electropherograms can be printed as what-you-see-is-what-you-get (WYSIWYG), except for these conditions:

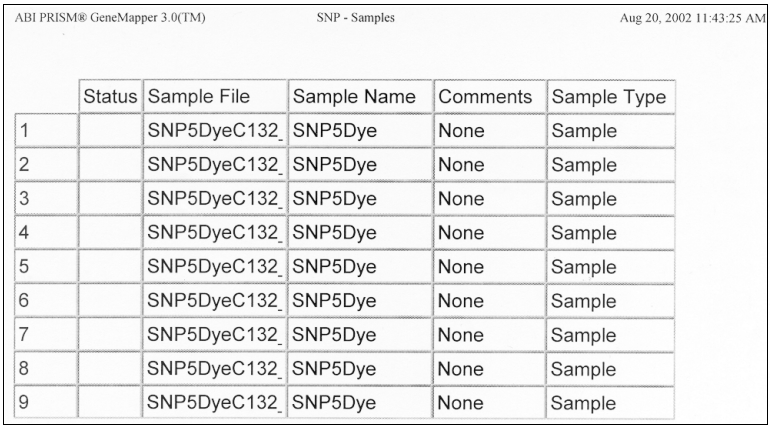
- Only the number of columns shown in the table printout below are printed out on a single sheet of paper in the Portrait mode. Additional columns to the right are printed out on additional sheets of paper. Change to the Landscape mode to minimize the number of sheets of paper used.

Note To reduce the number of sheets of paper printed for a table, hide columns you do not want to print using the Table Settings Editor.

- Printed plots are sized vertically to fill a single sheet of paper:
  - With four plots, the plots are sized close to the same size as the default size on your monitor.
  - Six plot panes are a practical limit for a single sheet.

Examples

Table Printout

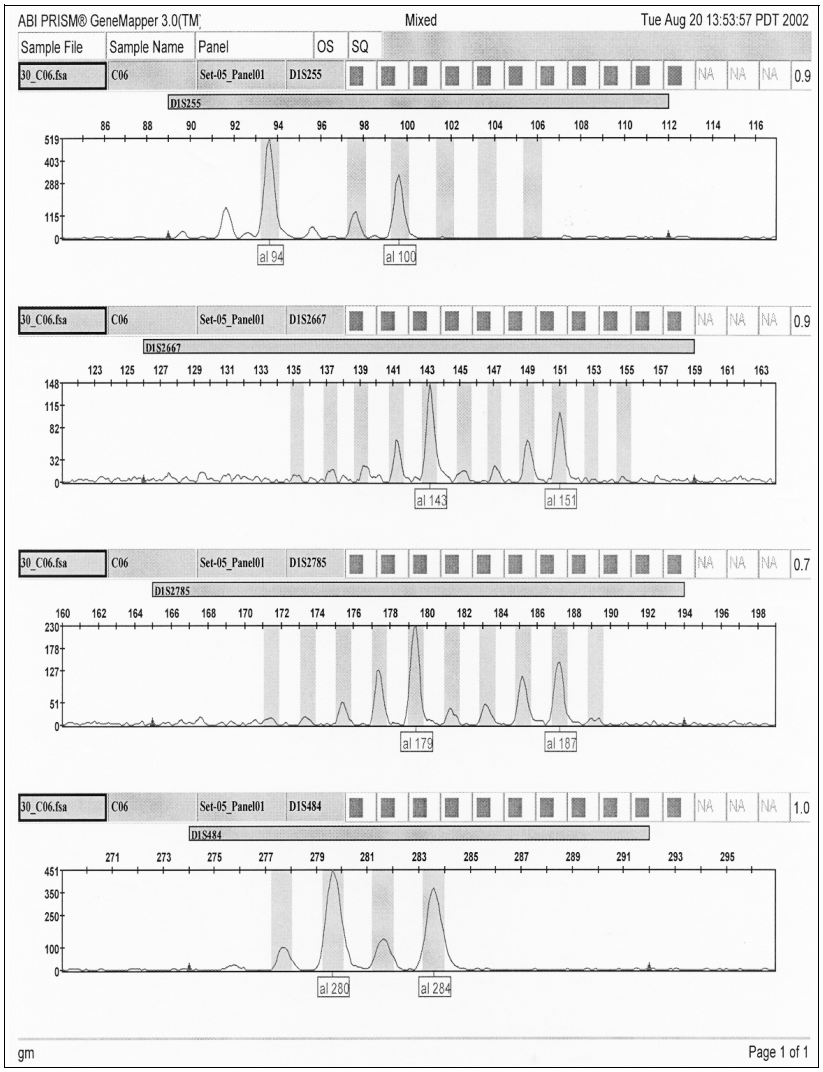


ABI PRISM® GeneMapper 3.0(TM) SNP - Samples Aug 20, 2002 11:43:25 AM

	Status	Sample File	Sample Name	Comments	Sample Type
1		SNP5DyeC132_	SNP5Dye	None	Sample
2		SNP5DyeC132_	SNP5Dye	None	Sample
3		SNP5DyeC132_	SNP5Dye	None	Sample
4		SNP5DyeC132_	SNP5Dye	None	Sample
5		SNP5DyeC132_	SNP5Dye	None	Sample
6		SNP5DyeC132_	SNP5Dye	None	Sample
7		SNP5DyeC132_	SNP5Dye	None	Sample
8		SNP5DyeC132_	SNP5Dye	None	Sample
9		SNP5DyeC132_	SNP5Dye	None	Sample

Note Additional pages are created to accommodate the number of rows in the table. Each additional page that continues the table to the right would have the same row numbers as shown in the left column.

Plot Printout



Note The number of plots per page is set by the Panes dialog box in the Plots window. The example above was scaled down vertically and would be printed on the whole height of the page on a printer.

Add Samples to Project Dialog

Introduction

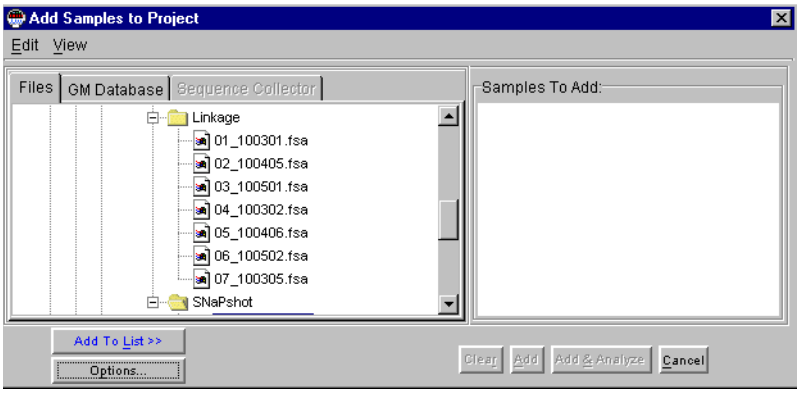
This section provides general information about:

- the Add Samples to Project dialog
- the Edit and View menus available in the window
- using the three tabs in the window.

This following topics are covered in this section.

General Information and Procedure

The Add Samples to Project window provides a navigation pane to enable you to add Samples to the project. You can also access the dialog box by clicking the Add Sample icon on the Project window toolbar.

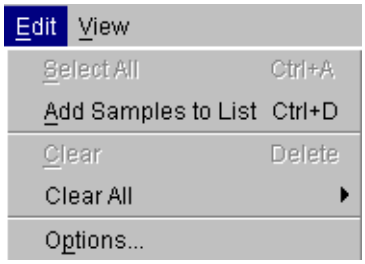


To add samples to the Project:

Step	Action
1	Click  (**Add Samples To Project**) on the Project window toolbar. Add Samples to Project window opens to the last tab viewed by the user (default is Files tab). Sample files can reside on the local hard drive(s), mapped network drive(s), and removable media drives. If Sequence Collector is configured and you are logged into Sequence Collector, the Sequence Collector tab is enabled.
2	In **Files** tab, navigate to folder containing sample files. Select the folder, then click the **Add to List** button. Folder is added to the **Samples To Add** field. Individual samples within a folder can be selected and added. Selecting the folder adds all samples.
3	Click the **Add** button**.** Add Samples to Project window closes and the project window becomes visible. Samples are added to the bottom of the Project window table.

Edit Menu

The commands in the Edit menu of the Add Samples window are used to manage the contents of the window. The commands are described in the table below.



Add Sample Files Edit Menu Commands

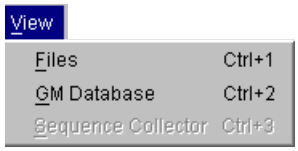
Item	Description	Enabling
Select All (Ctrl+A)	Selects all the items of a given type.	Enabled when a single valid selection has been made (*e.g.*, sample file).

Add Sample Files Edit Menu Commands *(continued)*

Item	Description	Enabling
Add Samples to List (Ctrl+D)	Adds the selected item(s) from the Files, GeneMapper database, or Sequence Collector to the Samples to Add list.	Enabled when a sample file or folder with files in the Files, GeneMapper database, or Sequence Collector is selected.
Clear (Delete)	Clears (deletes) the selected item(s) in the Samples to Add list.	Enabled when a valid selection is made.
Clear All	Hierarchical menu. Clears all sample files and folders from the specified field: ♦ Samples to add ♦ Criteria **Note** The Criteria subcommand functions in the GeneMapper Database tab but not in the Files tab and is used to clear all search criteria from the Criteria Selector List (see the figure on page C-41). If undo is not possible, the following alert message is displayed: *Clear all items? You cannot undo this action. [Yes] [No] [Cancel]*	Enabled when the field is present and an item exists in that field.
Options	Opens the Options dialog box in the Add Samples tab.	Always enabled.

View Menu

The View menu of the Add Samples window is used to switch between the three sources for samples and refresh the file directory. The commands are described in the table below.

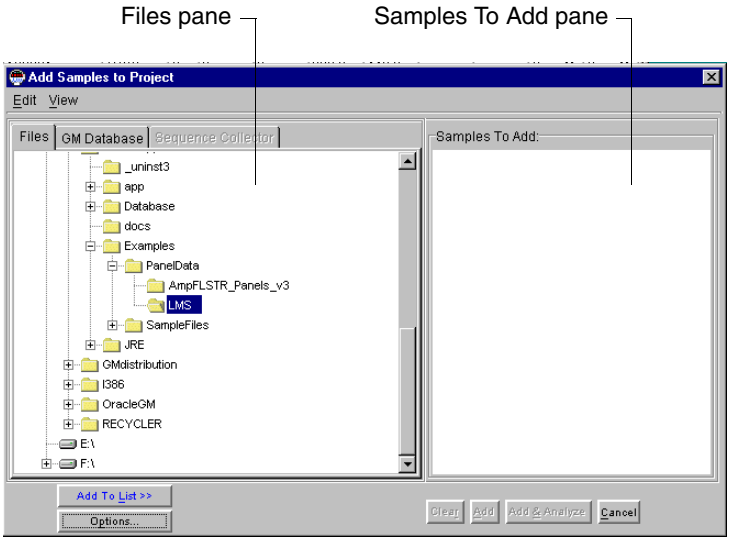


Add Samples View Menu Commands

Item	Description	Enabling
Files (Ctrl+1)	Opens the Files tab.	Always enabled.
GM Database (Ctrl+2)	Opens the GeneMapper Database tab.	Always enabled.
Sequence Collector (Ctrl+3)	Opens the Sequence Collector tab.	Enabled and visible if Sequence Collector is configured and you are logged into Sequence Collector. Configured through the Options menu.

Files Tab

The Files tab enables you to choose sample files from local or mapped networked storage devices.

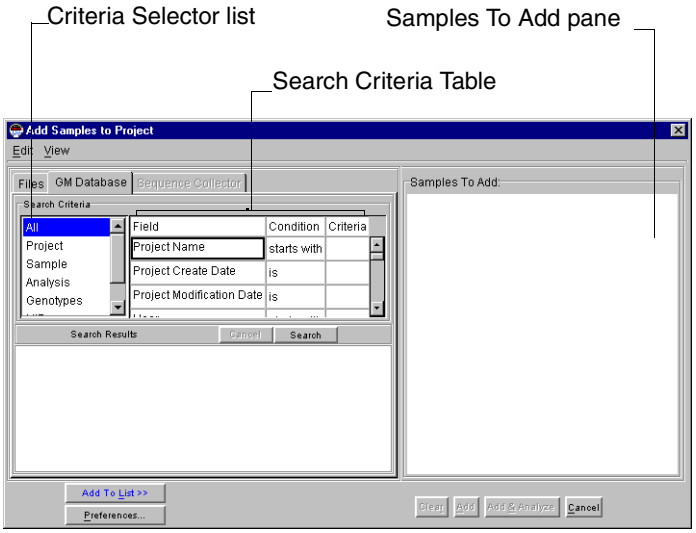


Add Samples to Project Window Elements

Element	Description
Files pane	Contains the directory structure configured for the computer running GeneMapper software. The navigation pane provides a tree viewer that works like the MS Windows Explorer interface. You can use Shift–click for continuous multiple selection and Ctrl–click for discontinuous multiple selections within a folder.
Samples To Add pane	Contains the folders and files that the system will add. This field also uses the tree viewer interface. You can use Shift–click and Ctrl–click as described above.
Add To List button	Adds the selected items from the files pane to the Sample to Add field.
Options button	Opens the Options window.
Clear button	Clears the selected files from the Samples To Add pane.
Add button	Adds the Samples To Add list into the Project window.
Cancel button	Closes the Add Samples to Project dialog box.

GM Database Tab

The GM (GeneMapper) Database tab enables you to choose sample files that have previously been added into a GeneMapper project. This database uses a search interface to extract samples that match your criteria.



This feature allows mixing and matching sample files. For example, you can merge sample files from a number of small projects into a single large project.

GM Database Search Rules

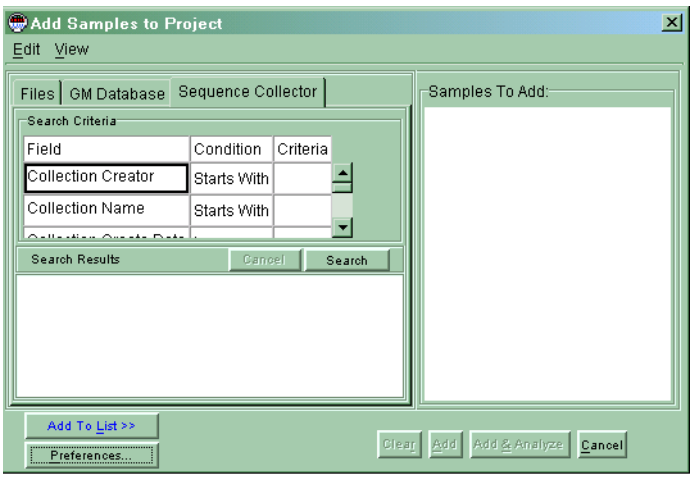
Criteria and Rules	Description
Search criteria	This list and table contains the search criteria from which to extract samples from the GeneMapper database. **Note** Locations of the list and table are pointed out in the figure on the previous page. ♦ **Criteria selector list** = list of properties (columns in the project window) grouped by topic. ♦ **Field column** = Text field that contains the properties (columns) in the selected group criterion. ♦ **Condition column** = Combo box that contains conditions that the system will use to search for samples. Search rules include: – Is – Contains – Starts With – Date criteria - Presents a calendar widget to specify the date ♦ **Criteria column** = Field for specifying the text string associated with the search rules which are case sensitive.
Rules for Search criteria	Criteria accumulate as they are entered in different rows and to different groups of criteria. Multiple items in the search criteria list use the natural language “and” for searching. For example, the natural language of the Criteria field is: “Display samples whose Panel property contains “XXX” and display samples whose Collection Instrument property contains “YYY”.”

GM Database Tab Elements

Element	Description
Search Criteria Cancel button	Enabled when a search is in progress; search stops when clicked. **Note** You may not stop the search during data transfer from the GeneMapper database.
Search button	Searches the database using the criteria list and displays the search results in the Search Results field.
Search Results field	Displays the results of the database search (sample files).
Add To List button	Adds the selected items from the Results field to the Samples to Add field.
Samples To Add field	Contains the folders and files that the system will add. This field uses the tree viewer interface.
Options button	Opens the Options window.
Clear button	Clears the selected files from the Samples To Add field.
Add button	Adds the Samples to Add list into the Project window.
Cancel button	Closes the Add Samples to Project dialog box.

Sequence Collector Tab

The Sequence Collector tab enables you to choose sample files that are stored in a Sequence Collector. The tab uses a search interface to extract samples that match your criteria. The Sequence Collector tab opens only when a connection to Sequence Collector exists.



The following table describes elements of the Sequence Collector tab.

Sequence Collector Tab Elements

Element	Description
Search Criteria	This table contains the search criteria from which to extract samples from the Sequence Collector. ♦ **Field column** = Text field that contains the properties (columns) in the selected group criterion. ♦ **Condition column** = Field that contains conditions that the system will use to search for samples. Search rules include: – Starts With – Contains – Equals – Date criteria - Presents a calendar widget to specify the date ♦ **Criteria column** = Field for specifying the text string associated with the search rules which are case sensitive.

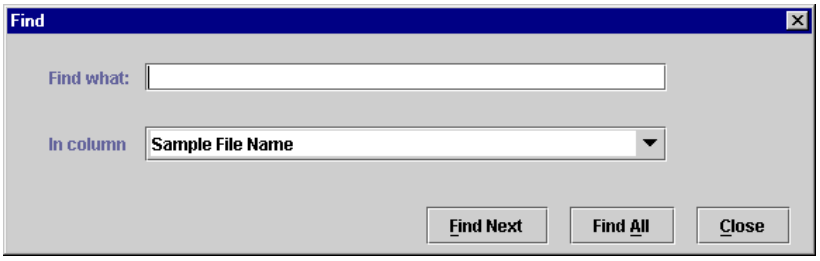
Sequence Collector Tab Elements *(continued)*

Element	Description
Rules for Search Criteria	Criteria accumulate as they are entered by the user. Multiple items in the search criteria list use the natural language “and” for searching. For example, the natural language of the Criteria field is: “Display samples whose Panel property contains “XXX” and display samples whose Collection Instrument property contains “YYY”.”
Search Criteria Cancel button	Enabled when a search is in progress; the search stops when clicked. **Note** May not stop the search during data transfer from the GeneMapper database.
Search button	Searches the database using the criteria list and displays the search results in the Search Results field.
Search Results	Displays the results of the database search (sample files). Columns in the table include Sample File plus others to match the fields specified in the search criteria.
Add To List button	Adds the selected items from the Results field to the Samples To Add field.
Samples To Add field	Contains the folders and files that the system will add. This field uses the tree viewer interface.
Options button	Opens the Options window.
Clear button	Clears the selected files from the Samples To Add field.
Add button	Adds the Samples To Add list into the Project window.
Cancel button	Closes the Add Samples to Project window.

Project Window Edit Menu Dialog Boxes

Find Dialog Box

The elements of the Find dialog box is described below.

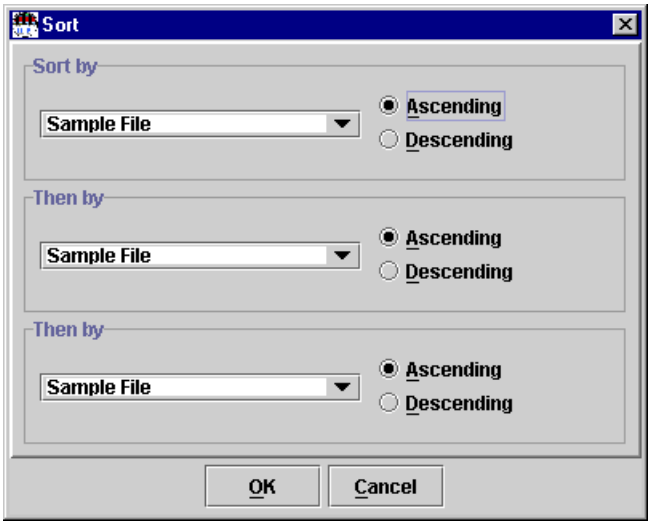


Project Window Find Elements

Element	Description
Find what	Field containing the search string.
In column	List box containing the names of columns in the table.
Find Next	Finds the next instance of the search string in the specified column and selects it.
Find All	Finds and selects all rows in the table that contain the search string in the specified column.
Close	Closes the Find dialog box. Table selections remain intact.

Sort Dialog Box

The Sort dialog box, shown below, allows sorting of sample files, results, etc., by ascending or descending order in up to three steps.



The Sort dialog box has three entry fields for entering sort criteria (the “Then by” fields allow successive sorting by two other criteria).

Project Window Sort Elements

Element	Description
Sort by (Then by)	Sort box containing the column which will be sorted.
Ascending and Descending buttons	Option buttons for choosing if the sort is ascending or descending in alphabetical/numerical order.
OK button	Closes the Sort dialog box and performs the sort.
Cancel button	Closes the Sort dialog box. Does not perform the sort.

GeneScan Size Standards

D

Appendix Overview

Introduction

The ABI Prism® GeneMapper™ Software Version 3.0 comes with several ready-to-use GeneScan® size standard definition files that you can choose from to analyze fragments run on ABI PRISM® genetic analyzers.

The following table lists the ready-to-use size standards stored in the GeneMapper database.

If using size standard...	Select this...
GeneScan™ 120 LIZ®	GS120LIZ
GeneScan™ 400HD	GS400HD
GeneScan™ 500	GS500
GeneScan™ 500(-250)	GS500(-250)
GeneScan™ 500 LIZ®	GS500LIZ
GeneScan™ 500(-250) LIZ®	GS500(-250)LIZ

Note The GeneScan™ 350 size standard is not automatically stored in the database; however, it is provided in the GeneMapper > Size Standards folder for import.

In This Appendix

This appendix contains the following topics:

GeneScan 120 Size Standard

About This Size Standard

The GeneScan™ 120 Size Standard is useful for sizing fragments between 15 and 120 base pairs. It is used when analyzing SNaPshot® samples.

How It Is Prepared

All aspects of the preparation of the GeneScan-120 LIZ size standard are proprietary. Each fragment contains a single LIZ fluorophore.

GeneScan 120 Molecular Lengths

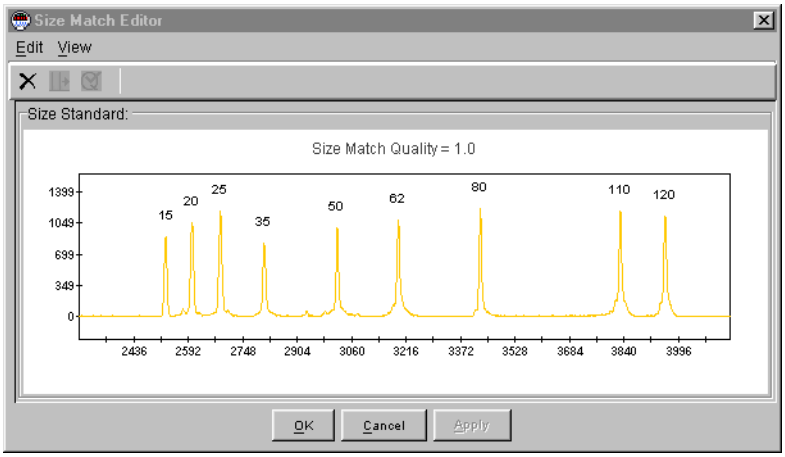
The following table lists the GeneScan-120 LIZ denatured molecular fragment lengths (nucleotides) for the nine fragments.

Fragment Length
15
20
25
35
50

Fragment Length
62
80
110
120

Electropherogram of GeneScan 120

The following screen shows an electropherogram of GeneScan-120 LIZ run under denaturing conditions.



GeneScan 350 Size Standard

About This Size Standard

The GeneScan™ 350 Size Standard is useful for sizing fragments between 35 and 350 base pairs. The native fragments are uniformly spaced to provide accurate size calling.

How It Is Prepared

The GeneScan 350 Size Standard is prepared by *Pst*I digestion of plasmid DNA, followed by ligation of a TAMRA™ or ROX™-labeled 22-mer oligodeoxynucleotide to the cut ends. A subsequent enzymatic digestion with *Bst*UI yields DNA fragments containing a single TAMRA or ROX dye (See “GeneScan 350 Molecular Lengths” below).

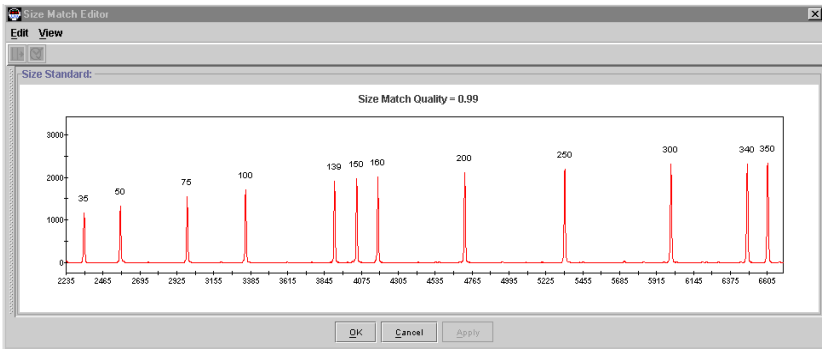
GeneScan 350 Molecular Lengths

The following table lists the GeneScan 350 Denatured Fragment Molecular Lengths (Nucleotides) for the 12 fragments.

Fragment Length	Fragment Length	Fragment Length
35	139	250
50	150	300
75	160	340
100	200	350

Electropherogram of GeneScan 350

The following screen shows an electropherogram of GeneScan 350 run under denaturing conditions.



GeneScan 400HD Size Standard

About This Size Standard

The GeneScan™ 400HD (High Density) Size Standard is used to determine fragment lengths between 50 and 400 base pairs.

Special Uses

The high density of marker bands in this standard makes it particularly useful for microsatellite analysis. All fragments have been checked for migration that is true to size under a wide variety of run conditions on all ABI PRISM® instruments.

How It Is Prepared

All aspects of the preparation of the GeneScan 400HD Size Standard are proprietary. Each fragment contains a single ROX fluorophore.

Fragment Lengths

The following table lists the lengths of the 21 fragments that make up the GeneScan 400HD Size Standard.

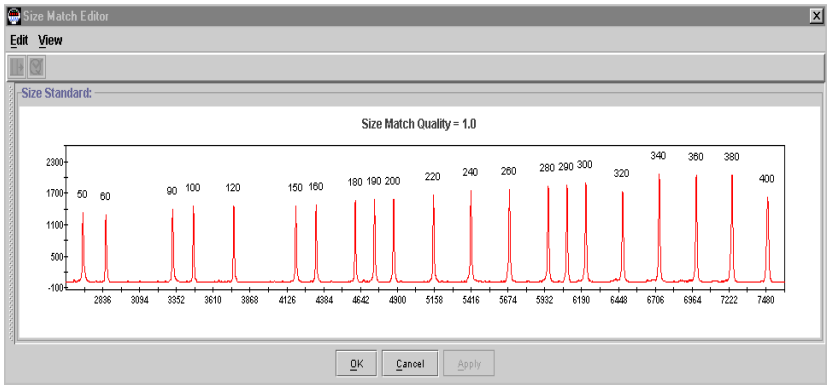
Fragment Length	Fragment Length	Fragment Length
50	180	290
60	190	300
90	200	320
100	220	340
120	240	360
150	260	380
160	280	400

Denaturing Electropherogram

Although the GeneScan 400HD Size Standard is made of double-stranded DNA fragments, only one of the strands is labeled. Consequently, even if the two strands migrate at different rates under denaturing conditions you will not need to worry about peak splitting. The following figure shows the peak patterns of GeneScan 400HD fragments run under denaturing conditions. Fragments were run using the 3700 POP-6™ polymer at 60 °C.

Electropherogram of GeneScan 400HD

The following screen shows an electropherogram of GeneScan 400HD.



GeneScan 500 and GeneScan 500(-250) Size Standards

About These Size Standards

The GeneScan™ 500, the GeneScan™ 500 LIZ®, the GeneScan™ 500(-250), and the GeneScan™ 500(-250) LIZ® Size Standards are useful for sizing fragments between 35 and 500 base pairs. The native fragments are uniformly spaced to provide accurate base calling.

The GeneScan 500(-250) contains all of the fragments in the GS 500 Size Standard except for the 250 bp fragment. Since it is identical in other respects, it is not discussed separately in this section.

How It Is Prepared

The GeneScan 500 all Size Standard is prepared by *Pst*I digestion of plasmid DNA, followed by ligation of a LIZ or ROX-labeled 22-mer oligodeoxynucleotide to the cut ends. A subsequent enzymatic digestion with *Bst*UI yields DNA fragments containing a single ROX or LIZ dye (see “GeneScan 500 Molecular Lengths” below).

GeneScan 500 Molecular Lengths

The following table lists the GeneScan 500 denatured fragment molecular lengths (nucleotides) for the 16 fragments.

Fragment Length
35
50
75
100
139
150

Fragment Length
160
200
250
300
340
350

Fragment Length
400
450
490
500

Electropherogram of GeneScan 500

The screen below shows an electropherogram of GeneScan 500 run under denaturing conditions.

Sample File Conversion

E

Appendix Overview

Introduction

This appendix describes how to use sample file conversion programs to prepare Macintosh® computer-generated fragment analysis sample files for transfer to a Microsoft® Windows®-based format and vice versa.

In This Appendix

This appendix contains the following topic:

Converting Macintosh Sample Files

About Converting Sample Files

Applied Biosystems created two conversion programs that prepare sample files for transfer from a Macintosh computer to computers running Microsoft Windows NT operating systems, and vice versa. These sample file conversion programs run only on a Macintosh computer.

The sample file conversion programs do not perform the file transfer from computer to computer. They set attributes of the files so that they can be used on the destination computer. For example, when transferring a fragment analysis sample file from a Macintosh computer to a computer running the Windows operating system, a file extension is required and the conversion program adds *.fsa* to the sample file name. For more detailed information on how these conversion programs function, refer to the SimpleText file entitled “About Conversion Programs” located in the same folder as the sample file conversion programs.

Installing Conversion Programs

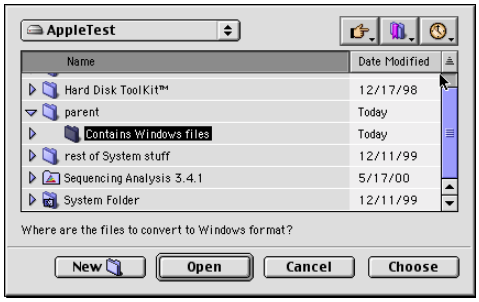
To install the sample file conversion programs on a Macintosh computer:

Step	Action
1	Insert the GeneMapper software CD-ROM into your Macintosh computer’s CD-ROM drive. An icon displays for the CD-ROM on the right-hand side of the screen.
2	Double-click the CD-ROM icon. A CD-ROM window displays containing files and folders.
3	Locate and double-click the *CONVFOLD* folder. Inside this folder are two files, *CONVPROG.HQX* and *README.TXT*, which contain the installation instructions.
4	Copy the *CONVPROG.HQX* file to your local hard drive by clicking on the file, dragging the file over to the local hard drive icon, and dropping it in.
5	Decompress the *CONVPROG.HQX* file by dragging and dropping it onto a program called “Stuffit Expander.” **Note** You can download a free version of Stuffit Expander from http://www.stuffit.com/expander.

Note Decompressing the *CONVPROG.HQX* file creates a folder on the local hard drive. This folder contains the conversion programs and the SimpleText file "About Conversion Programs." This file is a seven-page document that describes in detail how to use the conversion programs, why they are necessary, solutions to common problems, and possible alternative programs.

Converting Macintosh Sample Files to Micosoft Windows Files

To convert Macintosh computer sample files for use on a computer running Microsoft Windows operating system:

Step	Action
1	Double-click the  (**Sample File Mac to Win**) to start the program. The following dialog box opens.  **Note** On Macintosh computers running operating system 8.0 or less, this dialog box has a different appearance. For more information, refer to the SimpleText file "About Conversion Programs" mentioned above in "Installing Conversion Programs."
2	Using the triangle-shaped icons to the left of the folder names, navigate to the folder that contains the fragment analysis sample files you want to convert .

To convert Macintosh computer sample files for use on a computer running Microsoft Windows operating system: *(continued)*

Step	Action
3	Select the folder by single-clicking its name.
4	Click the **Choose** button at the bottom of the dialog box. If there are no problems, the program performs the task and quits automatically. When you open the folder, the sample files have the file extension *.fsa*. **Note** To convert sample files created on a computer running the Microsoft Windows operating system for use on a Macintosh computer, follow steps 1-3 above; in Step 1, double-click the **Sample File Win to Mac** icon.

Software Warranty Information

F

Appendix Overview

Introduction This appendix describes the software warranty provided by Applied Biosystems for the ABI PRISM® GeneMapper™ Software Version 3.0.

In This Appendix This appendix contains the following topics:

Computer Configuration

Configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited Product Warranty

Limited Warranty

Applied Biosystems warrants that for a period of ninety (90) days from the date the warranty period begins, its ABI PRISM® GeneMapper™ Software Version 3.0 will perform substantially in accordance with the functions and features described in its accompanying documentation when properly installed on the instrument system for which it is designated, and that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the software product will be free of defects in materials and workmanship under normal use. If buyer believes that it has discovered a failure of the software to satisfy the foregoing warranty, and if buyer notifies Applied Biosystems of such failure in writing during the ninety (90) day warranty period, and if Applied Biosystems is able to reliably reproduce such failure, then Applied Biosystems, at its sole option, will either (i) provide any software corrections or "bug-fixes" of the identified failure, if and when they become commercially available, to buyer free of charge, or (ii) notify buyer that Applied Biosystems will accept a return of the software from the buyer and, upon such return and removal of the software from buyer's systems, terminate the license to use the software and refund the buyer's purchase price for the software. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems within the ninety (90) day warranty period, Applied Biosystems will replace the defective media. Applied Biosystems does not warrant that the software will meet buyer's requirements or conform exactly to its documentation, or that operation of the software will be uninterrupted or error free.

Warranty Period Effective Date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for software installed by Applied Biosystems personnel. For all software installed by the buyer or anyone other than Applied Biosystems, the applicable warranty period begins the date the software is delivered to the buyer.

Warranty Claims

Warranty claims must be made within the applicable warranty period.

Warranty Exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation outside of the environmental or use specifications, or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems; and modification or repair of the product not authorized by Applied Biosystems.

The foregoing provisions set forth Applied Biosystems' sole and exclusive representations, warranties, and obligations with respect to its products, and Applied Biosystems makes no other warranty of any kind whatsoever, expressed or implied, including without limitation, warranties of merchantability and fitness for a particular purpose, whether arising from a statute or otherwise in law or from a course of dealing or usage of trade, all of which are expressly disclaimed.

Warranty Limitations

The remedies provided herein are the buyer's sole and exclusive remedies. Without limiting the generality of the foregoing, in no event shall Applied Biosystems be liable, whether in contract, tort, warranty**,** or under any statute (including without limitation any trade practice, unfair competition**,** or other statute of similar import) or on any other basis, for direct, indirect, punitive, incidental, multiple, consequential, or special damages sustained by the buyer or any other person or entity, whether or not foreseeable and whether or not Applied Biosystems is advised of the possibility of such damages, including without limitation, damages arising from or related to loss of use, loss of data, failure or interruption in the operation of any equipment or software, delay in repair or replacement, or for loss of revenue or profits, loss of good will, loss of business, or other financial loss or personal injury or property damage.

No agent, employee, or representative of Applied Biosystems has any authority to modify the terms of this Limited Warranty Statement or to bind Applied Biosystems to any affirmation, representation, or warranty concerning the product that is not contained in this Limited Warranty Statement, and any such modification, affirmation, representation, or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems unless in a writing signed by an executive officer of Applied Biosystems.

This warranty is limited to the buyer of the product from Applied Biosystems and is not transferable.

Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or wilful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Glossary

Allele	a variant of a marker
Allele calling	identification of alleles based on bin definitions; genotyping; GeneMapper software analysis
Bin	a fragment size or basepair range and dye color that define an allele
Bin set	a set of bin definitions for one source or set of experimental conditions, usually an instrument; bin sets are available inside a kit
Diploid, polyploid	having 2 or more alleles, respectively, per gene or locus
Genotype	the set of allele calls for a marker or genetic locus; usually 2 alleles
GM	GeneMapper software
HID	human identification and forensic applications
Kit	a set of panels; the grouping of panels in a kit is determined by the kit provider
LMS	Linkage Mapping Set; ABI chemistry using dinucleotide repeat microsatellite markers
Marker	a known microsatellite or SNP location
Microsatellite	short tandem repeat marker (di-, tri-, tetra-nucleotide repeat)
Panel	a set of bin definitions for one or more markers; the grouping of markers in panels is determined by the kit provider
Project	GeneMapper software project; a collection of samples
SNaPshot Multiplex Analysis	Primer extension-based chemistry for SNP validation
SNP	single-nucleotide polymorphism (used in this document to refer to SNaPshot® System markers)

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