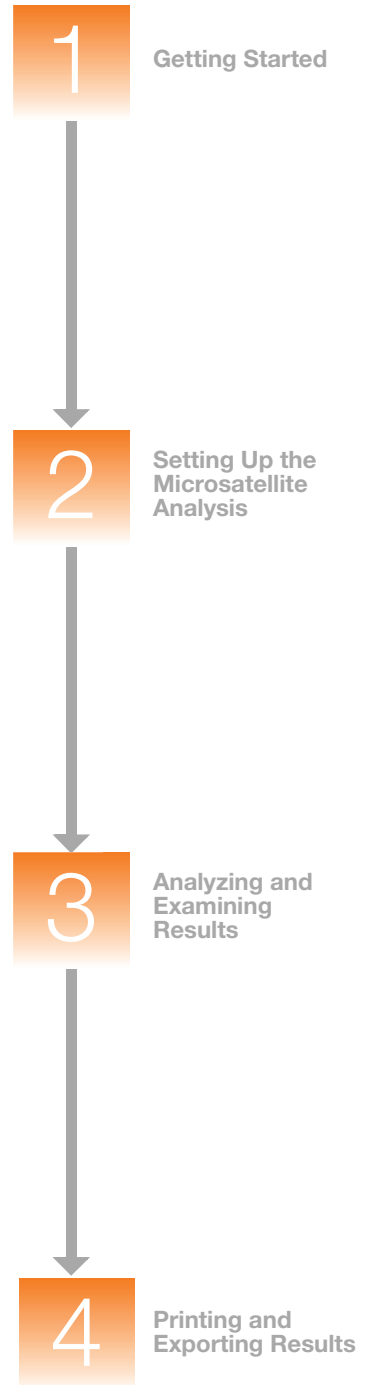
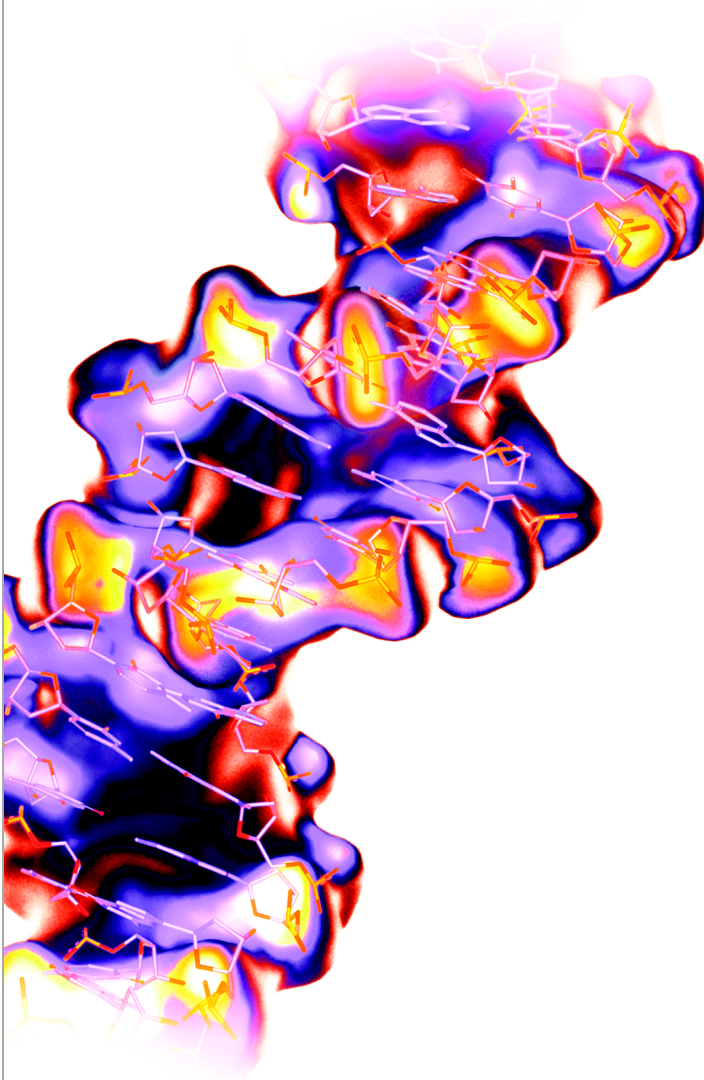


Microsatellite Analysis Getting Started Guide



Microsatellite Analysis Getting Started Guide

Getting Started

1

Setting Up the
Microsatellite
Analysis

2

Analyzing and
Examining
Results

3

Printing and
Exporting Results

4

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Contents

	Preface	v
	How to Use This Guide	v
	How to Obtain More Information	vi
	How to Obtain Support	viii
Chapter 1	Getting Started	1
	About Microsatellite Analyses	2
	About the Example Data	2
	Microsatellite Analysis Workflow	4
	GeneMapper® Software Terms	5
	Starting the Software and Logging In	5
	Using This Guide With Your Own Sample Files	6
	Using This Guide With GeneMapper Version 3.7 Software	6
	Alternatives to the Procedures in This Guide	7
Chapter 2	Setting Up the Microsatellite Analysis	9
	Overview	10
	Creating a Kit, Panel, and Markers	11
	Creating a New Project and Adding Sample Files	15
	Setting Analysis Parameters and Table Settings for the Project ..	18
	Performing the Initial Analysis on the Project	27
	Creating a Bin Set and Generating Bins Using the Auto Bin Feature	36

Chapter 3	Analyzing and Examining Results	45
	Editing the Analysis Method	46
	Analyzing the Project	48
	Examining the Results	48
Chapter 4	Printing and Exporting Results	53
	Printing Results	54
	Exporting Results	55
	Index	57

How to Use This Guide

Purpose of This Guide The *GeneMapper® Software Version 4.0 Microsatellite Analysis Getting Started Guide* provides brief, step-by-step instructions for sizing and genotyping microsatellite data generated using any of the compatible Applied Biosystems electrophoresis instruments and Data Collection Software. It describes how to troubleshoot, print and export data, and create reports. It is designed to help you quickly learn to use basic functions of the GeneMapper Software.

Audience This guide is intended for novice GeneMapper Software users.

Assumptions This guide assumes that:

- You have installed GeneMapper Software version 4.0 as described in the *GeneMapper® Software Version 4.0 Installation and Administration Guide* (PN 4363080).
- You have a working knowledge of the Microsoft® Windows® XP operating system.

Text Conventions This guide uses the following conventions:

- **Bold** indicates user action. For example:
Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:
Before analyzing, *always* prepare fresh matrix.
- A ▶ symbol separates successive commands you select from a drop-down or shortcut menu. For example:
Select **File ▶ Open ▶ Spot Set**.
Right-click the sample row, then select **View Filter ▶ View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The size of the column affects the run time.

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid Oracle user ID and password.

IMPORTANT! You must create a separate Sample Entry Spreadsheet for each 96-well plate.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see the *GeneMapper® Software Version 4.0 Installation and Administration Guide* (PN 4363080).

How to Obtain More Information

Safety Information


For safety information, see the *GeneMapper® Software Version 4.0 Installation and Administration Guide* (PN 4363080).

Software Warranty and License

For all warranty and licensing information, see the *GeneMapper® Software Version 4.0 Installation and Administration Guide* (PN 4363080).

Related Documentation


The following related documents are shipped with the software:

- ***GeneMapper® Software Version 4.0 Installation and Administration Guide*** – Provides procedures for installing, securing, and maintaining version 4.0 of the GeneMapper Software.
- ***GeneMapper® Software Version 4.0 Getting Started Guides*** – Five guides that explain how to analyze the application-specific example data provided with the GeneMapper Software. The guides provide brief, step-by-step procedures for the analysis of microsatellite, LOH, AFLP® system, SNaPshot® kit, and SNPlex™ system data generated by compatible Applied Biosystems electrophoresis instruments and Data Collection Software. The guides are designed to help you quickly learn to use basic functions of the GeneMapper Software.
- ***GeneMapper® Software Version 4.0 Online Help*** – Describes the GeneMapper Software and provides procedures for common tasks. Access online help by pressing **F1**, selecting **Help ▶ Contents and Index**, or clicking  in the toolbar of the GeneMapper window.
- ***GeneMapper® Software Version 4.0 Quick Reference Guide*** – Provides workflows for specific analysis types and lists instruments, software, and analysis applications compatible with the GeneMapper Software.
- ***GeneMapper® Software Version 4.0 Reference and Troubleshooting Guide*** – Provides reference information such as theory of operation and includes troubleshooting information.

Portable document format (PDF) versions of this guide and the other documents listed above are available on the *GeneMapper Software Version 4.0 Documentation CD*.

Note: For additional documentation, see “[How to Obtain Support](#)” on [page viii](#).

Obtaining Information from Online Help

The GeneMapper Software features an online help system that describes how to use each feature of the user interface. Access online help by pressing **F1**, selecting **Help ▶ Contents and Index**, or clicking  in the toolbar of the GeneMapper window.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

How to Obtain Support

For the latest services and support information for all locations, go to <http://www.appliedbiosystems.com>, then click the link for **Support**.

At the Support page, you can:

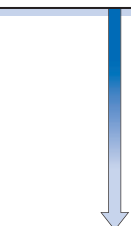
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates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

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

1

Getting Started

Chapter 1
Getting Started



Chapter 2
Setting Up the
Microsatellite
Analysis



Chapter 3
Analyzing and
Examining Results



Chapter 4
Printing and
Exporting Results

This chapter includes:

- About Microsatellite Analyses 2
- About the Example Data 2
- Microsatellite Analysis Workflow 4
- GeneMapper® Software Terms 5
- Starting the Software and Logging In 5
- Using This Guide With Your Own Sample Files 6
- Using This Guide With GeneMapper Version 3.7 Software . . 6
- Alternatives to the Procedures in This Guide. 7

About Microsatellite Analyses

Microsatellite Markers	Microsatellite markers, also known as short tandem repeats (STRs), are polymorphic DNA loci consisting of a repeated nucleotide sequence. The repeat sequence can be from 2 to 7 base pairs long. The number of repeat units varies in a population, thereby creating multiple alleles for a microsatellite locus.
Microsatellite Analysis	In a typical microsatellite analysis, microsatellite loci are amplified by PCR using fluorescently labeled forward and unlabeled reverse primers. The PCR amplicons are separated by size using electrophoresis; then the dye labeled products are identified by fluorescence detection. You can then use the GeneMapper® Software to size and genotype the alleles.
Custom Primers	Applied Biosystems provides custom primers for PCR amplification of microsatellite markers. For more information, visit the Applied Biosystems Web site at www.appliedbiosystems.com .
Compatible Kits and Instruments	For information about Applied Biosystems chemistry kits and electrophoresis instruments that are compatible with microsatellite analyses, see the <i>GeneMapper® Software Version 4.0 Quick Reference Guide</i> (PN 4362816).

About the Example Data

Sample File Location	To perform the exercise described in this guide, use the three sample files (.fsa) located on your computer at: <pre><drive>:\AppliedBiosystems\GeneMapper\Example Data\Microsatellite</pre>
	<hr/> Note: The above location will vary depending on the installation of the GeneMapper® Software. The default installation is the D drive. <hr/>
Instrument and Size Standard	Sample files were generated by running PCR-amplified and fluorescently tagged microsatellite samples on an ABI PRISM® 3100 Genetic Analyzer using the GeneScan™ 500 LIZ® size standard.

Marker Information

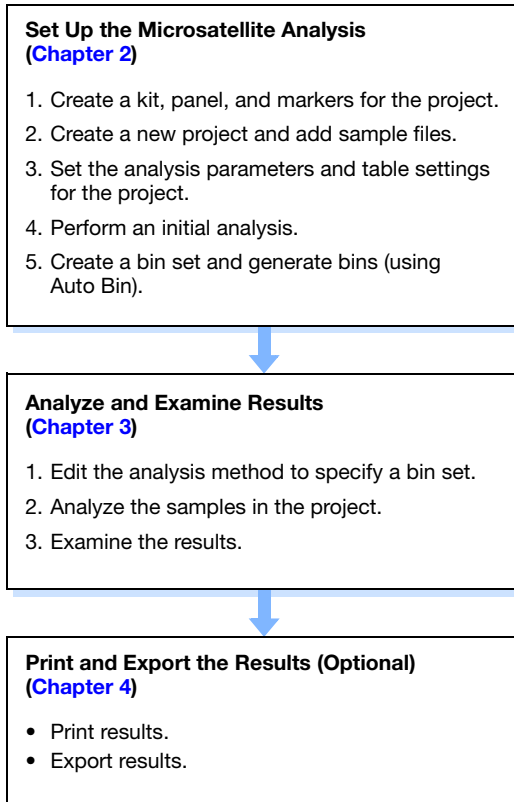
The sample files contain the following 17 fluorescently tagged markers.

Marker	Dye Label	Allele Size Range (bp)
D6S264	Blue	108 – 130
D6S1574	Blue	146 – 172
D6S276	Blue	201 – 233
D5S408	Blue	240 – 285
D6S308	Blue	326 – 354
D6S287	Green	104 – 138
D6S292	Green	154 – 176
D6S434	Green	201 – 245
D5S426	Green	274 – 298
D5S1981	Yellow	112 – 125
D6S257	Yellow	167 – 195
D6S446	Yellow	212 – 234
D5S641	Yellow	299 – 339
D5S433	Red	69 – 99
D5S406	Red	168 – 196
D5S400	Red	221 – 243
D6S309	Red	307 – 333

Note: You will use the information in the above table when creating a panel and markers in [Chapter 2, “Setting Up the Microsatellite Analysis.”](#)

Microsatellite Analysis Workflow

The following flowchart summarizes the steps for performing a microsatellite analysis using the GeneMapper® Software:



GeneMapper® Software Terms

Term	Definition
analysis parameters	A collection of user-defined settings (including an analysis method, size standard, and panel) that determine the sizing and genotyping algorithms used by the GeneMapper® Software to analyze all sample files in a project.
bin	A fragment size (bp) and dye color that define an allele within a marker. You create a bin for each possible allele associated with a marker.
bin set	A collection of bins (allele definitions), typically specific to a set of experimental conditions.
marker	A microsatellite marker is defined by a name, fragment size range (bp), dye color, and repeat length.
panel	A group of markers. In the GeneMapper Software, you associate a panel with a bin set to provide bin definitions for the markers.
kit	A group of panels.

Starting the Software and Logging In

To start the GeneMapper® Software and log in:

1. Select **Start ▶ All Programs ▶ Applied Biosystems ▶ GeneMapper ▶ GeneMapper 4.0**.
2. In the Login to GeneMapper dialog box:
 - a. Type the **User Name** and **Password** assigned by your system administrator.
 - b. Click **OK**.

Using This Guide With Your Own Sample Files

In addition to using this guide to analyze the example data provided with the software, you can use this guide to lead you through the general microsatellite analysis workflow when analyzing your own sample files. For information on advanced software features, see the *GeneMapper® Software Online Help*.

Using This Guide With GeneMapper Version 3.7 Software

The workflows and procedures presented in this guide are valid for the GeneMapper Version 3.7 Software also.

IMPORTANT! The GeneMapper Version 3.7 Software does *not* contain the correct example data used in the exercises of this guide. Therefore, if you have GeneMapper Version 3.7 Software, do *not* use this guide to analyze the example data that it references; instead, use it to lead you through the microsatellite analysis workflow when analyzing your own sample files.

Alternatives to the Procedures in This Guide

Overview This guide presents one of several possible solutions for analyzing microsatellite data using the GeneMapper® Software. Once you have completed the exercises in this document, you will most likely want to tailor the process to fit the requirements of your laboratory. This section provides you with a summary of several alternatives and where to go for further information.

Using Autoanalysis to Set Up Projects The GeneMapper Software includes an Autoanalysis feature that can eliminate most of the tasks leading up to the analysis of a microsatellite project. Much of [Chapter 2, “Setting Up the Microsatellite Analysis,”](#) explains how to manually create, add samples to, and analyze projects for use in microsatellite projects. When configured for Autoanalysis, the GeneMapper Software can automatically accomplish these tasks by coordinating with the Data Collection Software. For a more detailed explanation of how to use the Autoanalysis feature to set up microsatellite projects, see the *GeneMapper® Software Version 4.0 Installation and Administration Guide* (PN 4363080).

Using the Command Line Interface to Set Up Projects The GeneMapper Software features a command line interface that can perform most of the major functions of the software. The command line interface can be a useful tool when analyzing microsatellite projects because it automate many of the tasks explained in [Chapter 2, “Setting Up the Microsatellite Analysis.”](#) For a complete description of the command line interface and how it can be used to automate the functions of the GeneMapper Software, see the *GeneMapper® Software Version 4.0 Installation and Administration Guide* (PN 4363080).

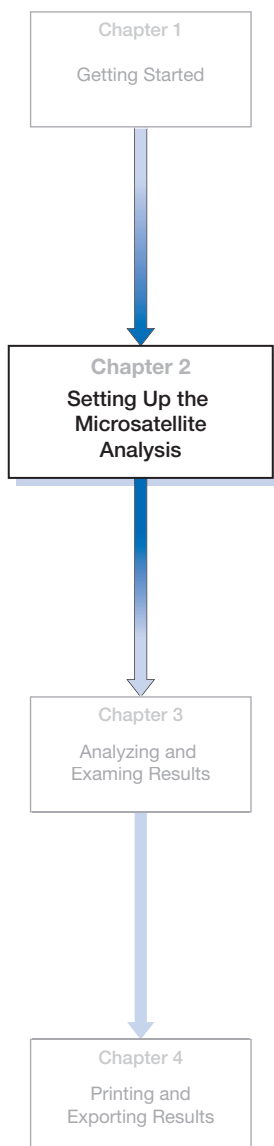


Chapter 1 Getting Started

Alternatives to the Procedures in This Guide

2

Setting Up the Microsatellite Analysis



This chapter includes:

- Overview 10
- Creating a Kit, Panel, and Markers 11
- Creating a New Project and Adding Sample Files 15
- Setting Analysis Parameters and Table Settings for the Project 18
- Performing the Initial Analysis on the Project 27
- Creating a Bin Set and Generating Bins Using the Auto Bin Feature 36

Overview

In This Chapter

In this chapter you will learn how to:

- Create a kit, panel, and markers
- Create a new project and add sample files
- Set analysis parameters and display settings for the project
- Perform the initial analysis on the project
- Create a bin set and generate bins using the Auto Bin feature

For More Information

This chapter contains basic procedures. It does not describe all features and parameters in the GeneMapper Software. For more detailed information on topics presented in this chapter, see the following topics in the *GeneMapper® Software Online Help*:

- Creating a New Kit
- Creating a Custom Panel
- Creating Markers
- Creating a Project
- Adding Samples
- Applying Analysis Settings
- Starting Analysis
- Creating a New Bin Set
- Using the Auto Bin Function

Online help is available from the Help menu, by clicking  , or by pressing F1.

Creating a Kit, Panel, and Markers



Overview You create the following hierarchical objects in the Panel Manager:

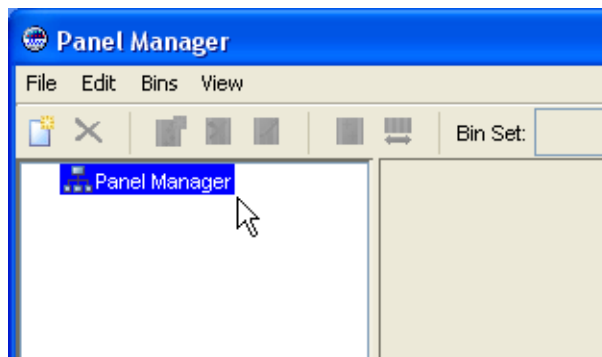
- **Kit** – A group of panels
- **Panel** – A group of markers
- **Marker** – A fragment size range (bp), dye color, and repeat length

Note: In this guide, you will learn how to create panels and markers. However, you can also import panels (text files) that contain marker information. For example, panel files are available in the GeneMapper® Software for some of the LMS kits available from Applied Biosystems. For more information on importing panels, see the *GeneMapper® Software Online Help*.

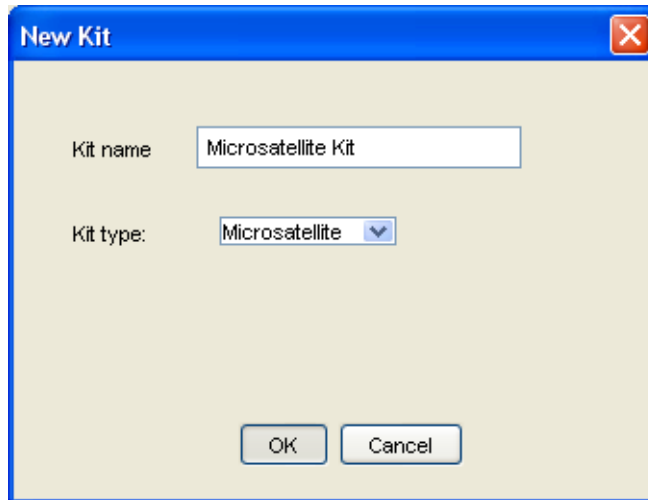
Creating a Kit, Panel, and Markers

To create a kit, panel, and markers:

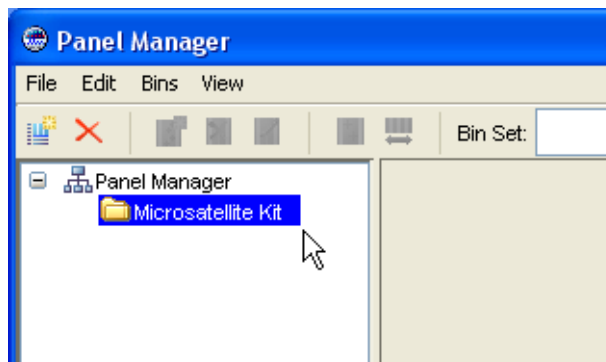
1. Open the Panel Manager by clicking  (**Tools ▶ Panel Manager**).
2. Select **Panel Manager** at the top of the Navigation Pane (left side), then click  (**File ▶ New Kit**).



3. In the New Kit dialog box, type **Microsatellite Kit** for the Kit Name, select **Microsatellite** for the Kit Type, then click **OK**.



Microsatellite Kit appears in the Navigation Pane (left side).

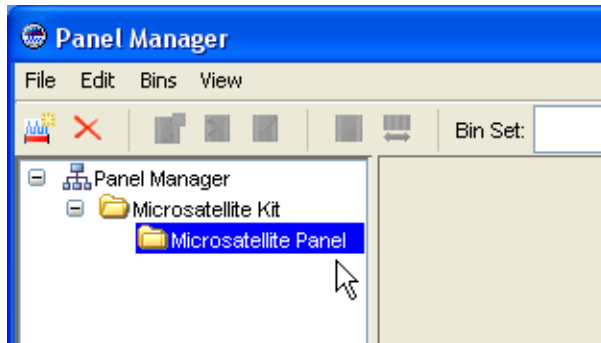



4. Select the **Microsatellite Kit** in the Navigation Pane, then click  (**File ▶ New Panel**).

- In the right pane of the Panel Manager, select **New Panel**, type **Microsatellite Panel** for the Panel Name, then press **Enter**.

	Panel Name	Comment
1	Microsatellite Panel	none

Microsatellite Panel appears under Microsatellite Kit in the Navigation Pane (left side).



- Select the **Microsatellite Panel** in the Navigation Pane, then click  (**File ▶ New Marker**).
- In the right pane of the Panel Manager, type the following marker information:

	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker Repeat
1	D6S264	blue	108.0	130.0		2

8. Repeat [steps 6 through 7](#) to create the following markers:

Marker	Dye Color	Minimum Size (bp)	Maximum Size (bp)	Marker Repeat
D6S1574	Blue	146	172	2
D6S276	Blue	201	233	2
D5S408	Blue	240	285	2
D6S308	Blue	326	354	2
D6S287	Green	104	138	2
D6S292	Green	154	176	2
D6S434	Green	201	245	2
D5S426	Green	274	298	2
D5S1981	Yellow	112	125	2
D6S257	Yellow	167	195	2
D6S446	Yellow	212	234	2
D5S641	Yellow	299	339	2
D5S433	Red	69	99	2
D5S406	Red	168	196	2
D5S400	Red	221	243	2
D6S309	Red	307	333	2

9. Click **OK** to apply your changes and close the Panel Manager.

Next Steps Create a new project and add sample files (.fsa) to it as described on [page 15](#).

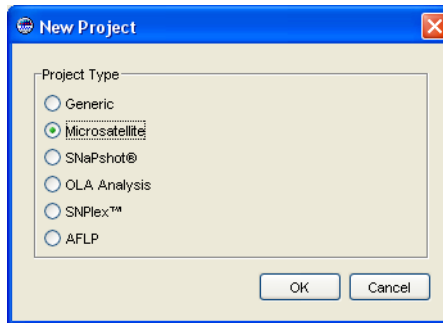
Creating a New Project and Adding Sample Files


Overview You create a project and add samples to the project in the GeneMapper window.

Creating a New Project and Adding Sample Files

To create a new project and add sample files:

1. Click  (**File ▶ New Project**).



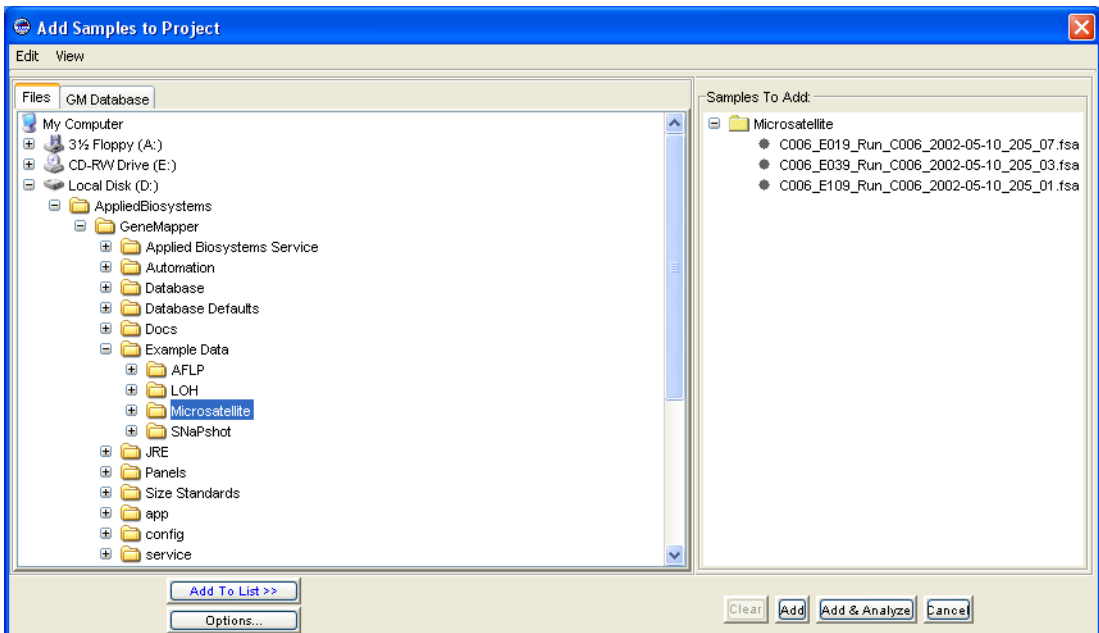
2. In the New Project dialog box, select **Microsatellite**, then click **OK**.
3. From the GeneMapper window, click  (**File ▶ Add Samples to Project**).
4. In the Add Samples to Project dialog box, in the Files tab, navigate to:

```
<drive>:\AppliedBiosystems\GeneMapper\Example  
Data
```

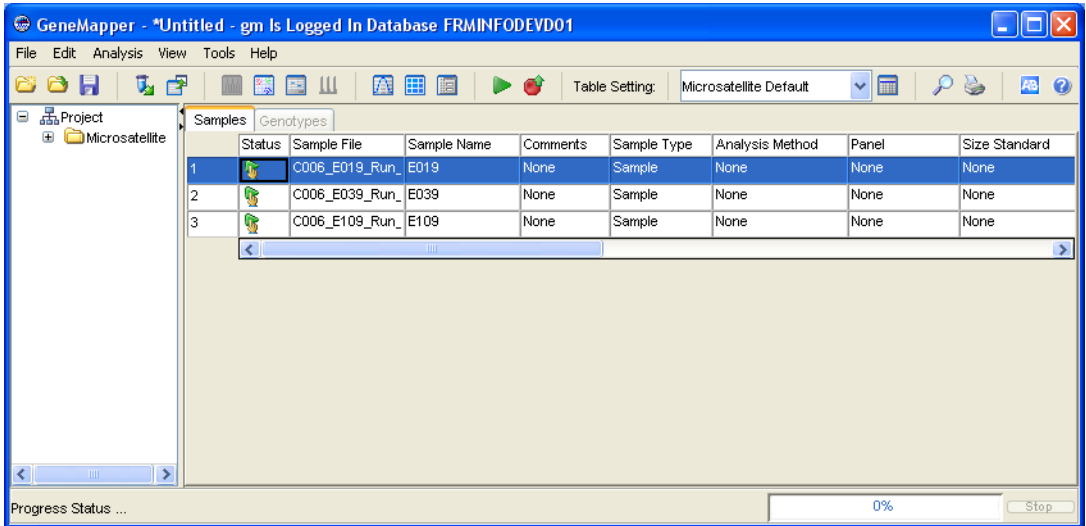
Note: The above location will vary depending on the installation of the GeneMapper® Software. The default installation is the D drive.

5. Select the **Microsatellite** folder, click **Add to List**, then click **Add**.

Note: For this guide you added all three sample files in the Microsatellite folder. However, you can add a subset of files from a folder by expanding the folder in the left pane, pressing and holding Ctrl, then selecting individual files before clicking Add To List.



The three sample files from the Microsatellite folder appear in the Samples tab, along with information entered in the Data Collection Software on the compatible Applied Biosystems electrophoresis instrument.



Next Steps Set analysis parameters and display settings for the project as described on [page 18](#).

Setting Analysis Parameters and Table Settings for the Project

Overview You set analysis parameters and display settings for the project in the GeneMapper window.

Analysis parameters include:

- Analysis method (including bin set)
- Panel (set of markers)
- Size standard

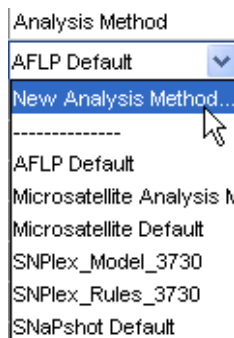
You set analysis parameters that determine the peak detection, sizing, and genotyping algorithms used by the GeneMapper® Software to analyze all sample files in a project.

Display settings include Table Settings and Plot Settings.

Setting Analysis Parameters

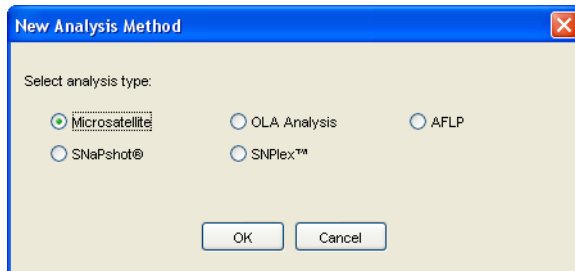
To set analysis parameters for the project:

1. Select the **Samples** tab in the GeneMapper window.
2. Click the first row in the **Analysis Method** column, then select **New Analysis Method** from the drop-down list.

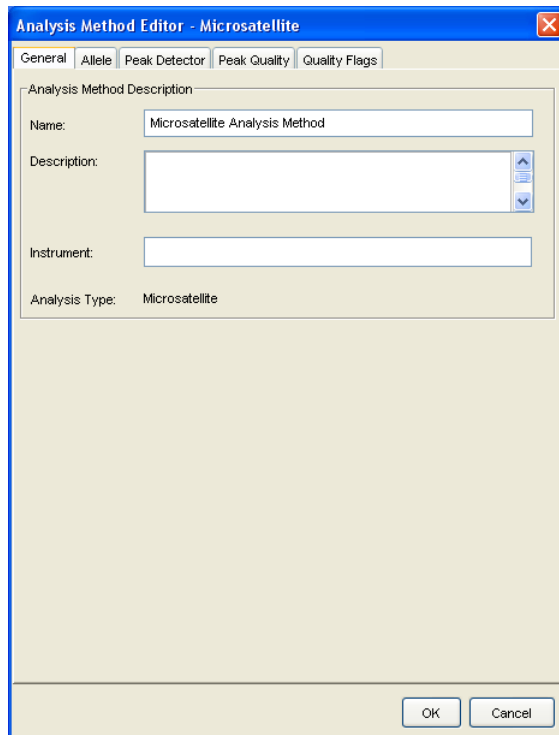


Note: You can also create a new analysis method from the Analysis Method tab in the GeneMapper Manager.

3. In the New Analysis Method dialog box, select **Microsatellite** for Analysis Type, then click **OK**.



4. In the Analysis Method Editor dialog box, select and edit the five tabs.
 - **General** – This tab includes reference information about the method. Type **Microsatellite Analysis Method** for the Name. Optionally, type a description and the instrument on which the data was generated.



- **Allele** – This tab includes settings that determine allele calling. Select **None** for the Bin Set. Leave the default values for all other settings.

Analysis Method Editor - Microsatellite

General | **Allele** | Peak Detector | Peak Quality | Quality Flags

Bin Set: None

Marker Repeat Type

Use marker-specific stutter ratio if available

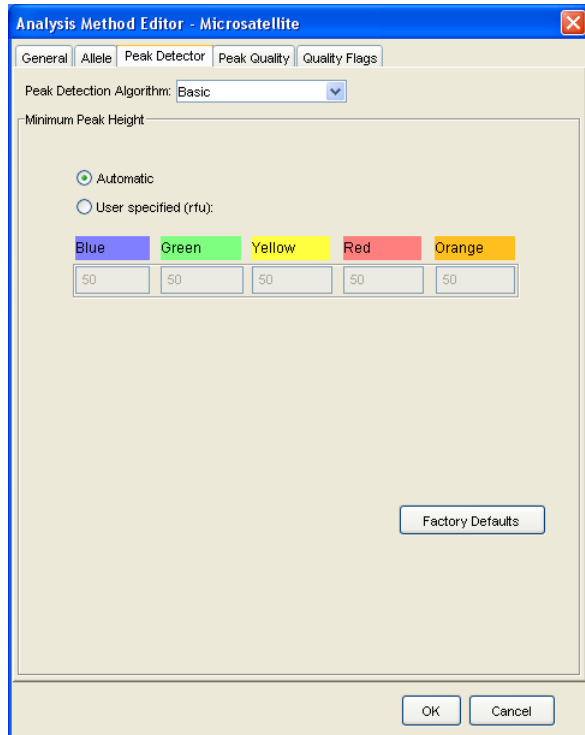
Values for dinucleotide repeats are calculated automatically.



	Mono	Tri	Tetra	Penta	Hexa
Cut-off value	0.25	0.2	0.25	0.25	0.25
PlusA ratio	0.0	0.95	0.95	0.95	0.95
PlusA distance	0.0	1.6	1.6	1.6	1.6
Stutter ratio	0.0	0.95	0.15	0.15	0.15
Stutter distance	From	0.0	0.0	0.0	0.0
	To	0.0	3.5	4.5	5.5

Range Filter... Factory Defaults

OK Cancel

- **Peak Detector** – This tab includes settings that determine peak detection and sizing of peaks. Select **Basic** for the Peak Detection Algorithm. Leave the default values for all other settings.



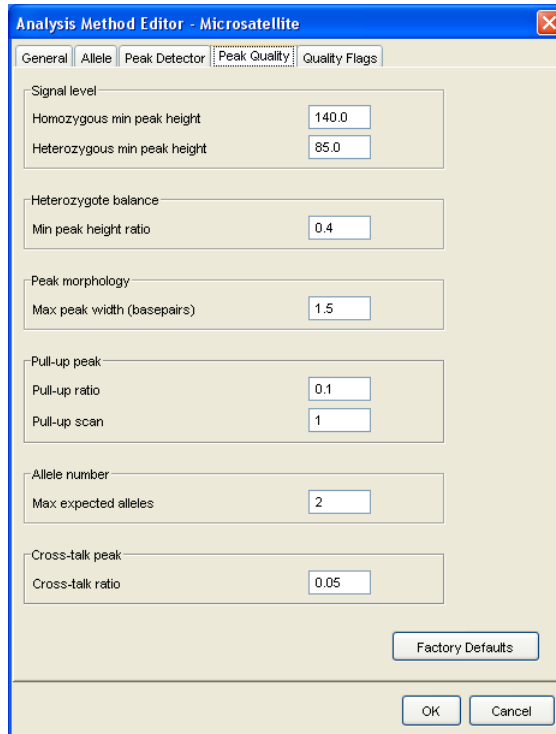
- **Peak Quality** – This tab includes settings that determine when specific PQVs are left green  (Pass) or flagged yellow  (Check).

Type **140.0** for the Homozygous min peak height.

Type **85.0** for the Heterozygous min peak height.

Type **0.4** for the Min peak height ratio.




Leave the default values for all other settings.



The screenshot shows the 'Analysis Method Editor - Microsatellite' dialog box with the 'Peak Quality' tab selected. The dialog has several sections with input fields:

- Signal level:**
 - Homozygous min peak height: 140.0
 - Heterozygous min peak height: 85.0
- Heterozygote balance:**
 - Min peak height ratio: 0.4
- Peak morphology:**
 - Max peak width (basepairs): 1.5
- Pull-up peak:**
 - Pull-up ratio: 0.1
 - Pull-up scan: 1
- Allele number:**
 - Max expected alleles: 2
- Cross-talk peak:**
 - Cross-talk ratio: 0.05

Buttons at the bottom include 'Factory Defaults', 'OK', and 'Cancel'.

- **Quality Flags** – This tab includes:
 - Settings that determine the importance of individual flagged Process Quality Values (PQVs) to the overall Genotype Quality (GQ). You can weight each PQV from 0 to 1, with 0 being of no importance and 1 meaning very important.
 - Threshold settings that determine when the SQ and GQ are flagged as Pass , Check , or Low Quality . The SQ and GQ are given initial scores of 1. The value of any flagged PQVs are then subtracted from 1 to give the final SQ and GQ scores.

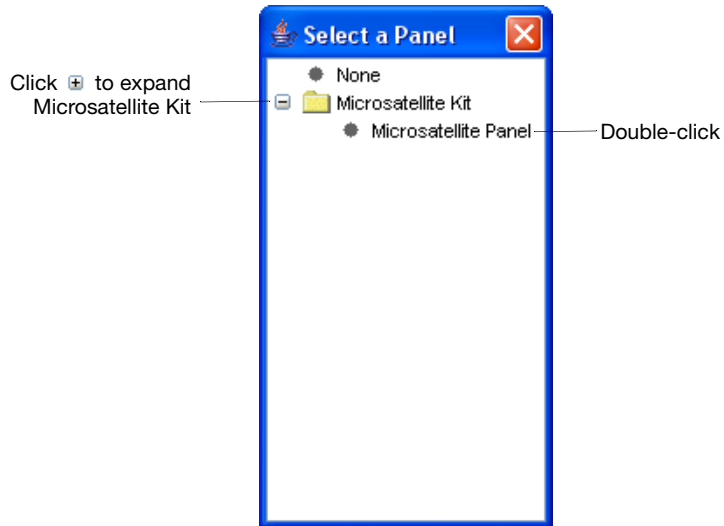
Leave the default values for all settings.



For details on analysis method parameters, see the *GeneMapper® Software Online Help*.

1. Click **OK** to save the method and close the Analysis Method Editor dialog box.

2. Select the first row in the **Panel** column. From the Select a Panel dialog box, expand the **Microsatellite Kit**, then double-click **Microsatellite Panel**. (This is the Panel you created on [page 11](#).)



3. Select the first row in the **Size Standard** column, then select **GS500(-250)LIZ** from the drop-down list.

Note: In the GeneMapper software, the following size standards are available for use with samples run with the GeneScan™ 500 LIZ® size standard:

- **GS500LIZ** – includes all peaks present in the actual GeneScan™ 500 LIZ® size standard
- **GS500(-250)LIZ** – excludes the 250-bp peak
- **GS500LIZ_3730** – excludes the 35-, 250-, and 340-bp peaks
- **GS500LIZ_3130** – excludes the 35-, 250-, and 340-bp peaks

Depending on your instrument, polymer type, and primer, it may be appropriate to choose one of the other size standards that omits peaks. Specifically, the 35-bp peak can be eclipsed by the neighboring primer peak, or the 250- and 340-bp peaks can migrate abnormally on the capillary electrophoresis instrument. Additionally, you can create your own custom size standards. For information on creating custom size standards, see the *GeneMapper® Software Online Help*.

4. Fill down your selections to all sample rows in the Samples tab:
 - a. Click-drag across the Analysis Method, Panel, and Size Standard column headers to highlight all rows in all three columns.

Analysis Method	Panel	Size Standard
Microsatellite Analysis Method	Microsatellite Panel	GS500(-250)LIZ
None	None	None
None	None	None

- b. Select **Edit ▶ Fill Down** (or press **Ctrl-D**).

Selecting Table Setting

At the top of the GeneMapper window, select **Microsatellite Default** from the Table Settings drop-down list.

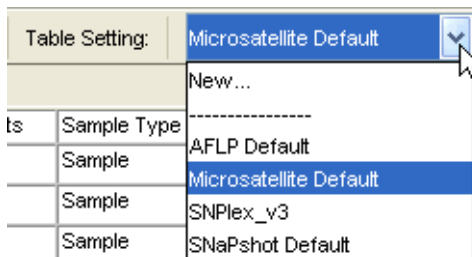



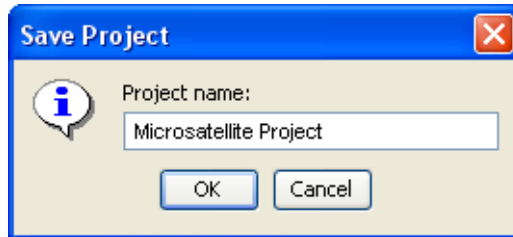
Table Settings control the information displayed in the Samples tab and Genotypes tab after analysis. Microsatellite Default is one of the default Table Settings provided with the GeneMapper Software.

You can also edit and create custom Table Settings in the GeneMapper Manager. For more information, see the *GeneMapper® Software Online Help*.

Saving the Project

To save the project:

1. Click  (**File ▶ Save Project**).
2. In the Save Project dialog box, type **Microsatellite Project**, then click **OK**.



Microsatellite Project appears in the title bar of the GeneMapper window.

Next Steps

Perform the initial analysis on the project as described on [page 27](#).

Performing the Initial Analysis on the Project

Overview Now that you have added sample files to and set analysis parameters for the project, perform an initial analysis to size the data so you have sample files available as reference data to create bins (allele definitions).

Note: Because you selected a Panel for the sample files in the project, the GeneMapper® Software will not only size the data but also try to genotype the data. However, because you did not specify a bin set in the analysis method, the Genotype Quality (GQ) will fail.

To perform the initial analysis:

- Analyze the project
- Review the SQ and contributing PQVs
- Examine the size standard
- View sample information (including raw data)
- Viewing samples plots

Analyzing the Project

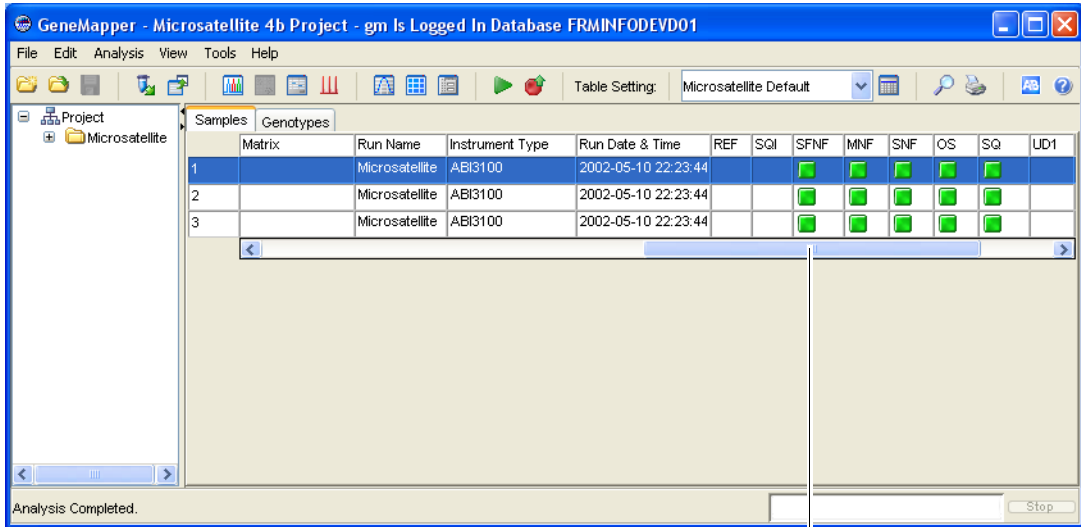
Click  (**Analysis ▶ Analyze**).

The GeneMapper Software analyzes each sample in the project, displaying its progress in the Status Bar (lower left) of the GeneMapper window.

Reviewing the SQ and PQVs

To review the Size Quality (SQ) and contributing PQVs:

1. Make sure “Analysis Completed” appears in the Status Bar (lower left) of the GeneMapper window.
2. Review the SQ by scrolling to the right in the Samples tab.



Click-drag scroll bar to right to view SQ column

If you followed the procedures and used the example data indicated in this guide, the SQ for each sample is ■ (Pass). The Process Quality Values (PQVs) that contribute to the SQ (SFNF, MNF, SNF, and OS) should also be ■.


Investigating Yellow ▲ and Red ● SQs

IMPORTANT! When analyzing your own data, you may find the SQ to be ▲ (Check) or ● (Low Quality) and associated PQVs (SFNF, MNF, SNF, and OS) to be ▲, indicating issues with the size standard, data, or analysis parameters. To investigate and correct these issues, see [“Examining the Size Standard” on page 29](#).

Note: Click to sort the samples by SQ score. Samples with a ● SQ will be listed at the top of the Samples tab.

Examining the Size Standard

To examine the size standard:

1. Select all samples in the Samples tab by selecting **Edit ▶ Select All**.
2. Open the Size Match Editor by clicking  (**Analysis ▶ Size Match Editor**).

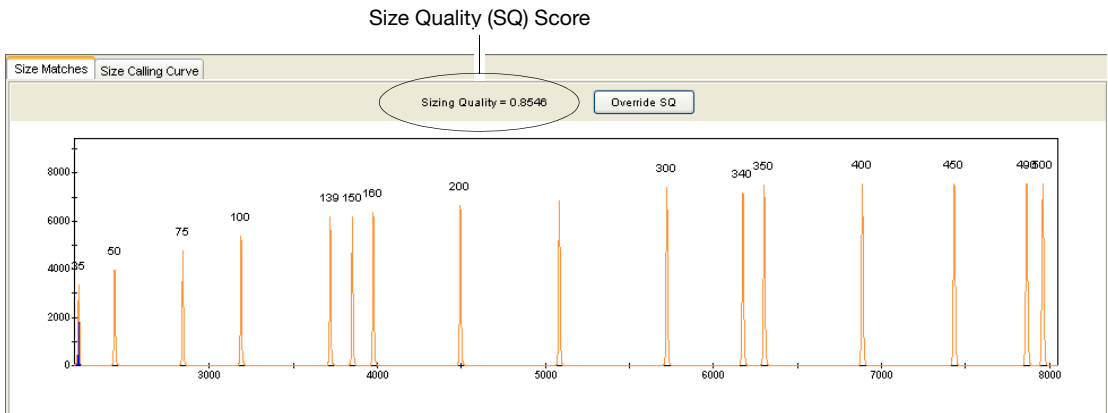








Figure 2-1 Size Match Editor – Size Matches tab

3. Click the **Size Matches** tab to view the following for the selected sample:
 - Size Quality (SQ) score
 - Size standard peaks
 - Size standard peak labels
4. Note the Sizing Quality score ([Figure 2-1](#)) for the sample. This score reflects how well the data from the size standard match the size standard you selected in the software. This score determines whether the SQ displays  (Pass),  (Check) or  (Low Quality).

If you followed the instructions in this guide, the Sizing Quality is > 0.75 and the SQ displays  (Pass).

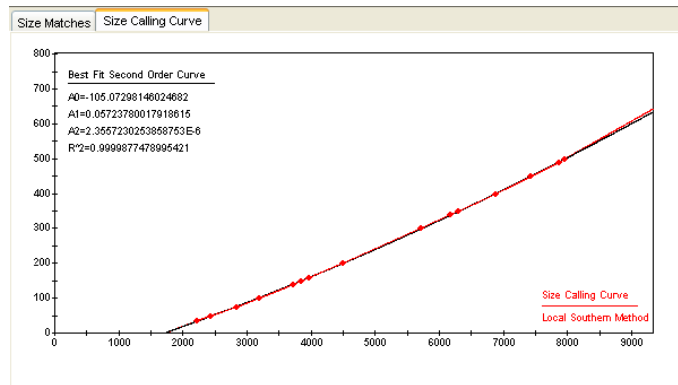
However, when analyzing your own data you may notice the Sizing Quality is less and the SQ displays  (Check) or  (Low Quality). For troubleshooting help, see [Table 2-1 on page 31](#).

- Determine if all peaks in the size standard are present and labeled correctly.

If you followed instructions in this guide, all peaks are present and labeled correctly as shown in [Figure 2-1](#).



However, when analyzing your own data you may find some size standards peaks to be incorrectly labeled or missing. For troubleshooting help, see [Table 2-1 on page 31](#).

- Click the **Size Calling Curve** tab to view the size standard curve for the selected sample. You will see red data points representing the fragments from the size standard and a black best-fit curve.



- Select another sample from the left pane of the Size Match Editor, then repeat [steps 3 through 6](#).
- Click **OK** to close the Size Match Editor.

Table 2-1 Troubleshooting the size standard

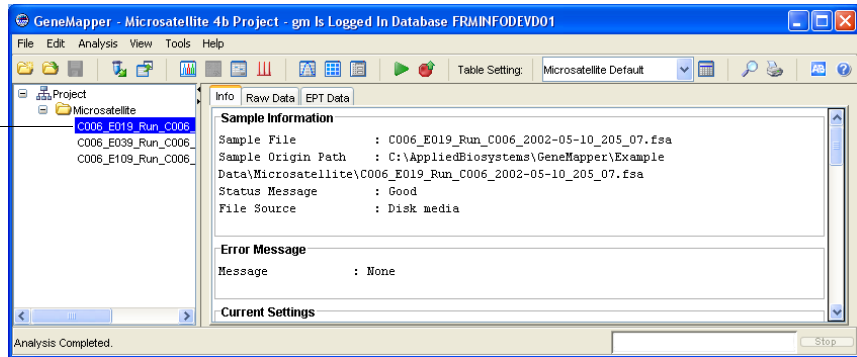
Problem	Action
Sizing Quality score is low and the SQ displays  (Check) or  (Low Quality), but all size standard peaks are present and labeled correctly.	Override the Sizing Quality by clicking Override SQ at the top of the Size Matches tab (Figure 2-1). Overriding changes the Sizing Quality score to 1.0, indicating the user verified the size standard.
Some size standard peaks are not labeled correctly.	Edit, delete, and add size labels in the Size Matches tab, then click Apply to reanalyze the data with the updated sizing information. For more information, see the <i>GeneMapper® Software Online Help</i> .
Some size standard peaks are not present.	Create a custom size standard in the software. For more information, see the <i>GeneMapper® Software Online Help</i> .

For additional help in troubleshooting sizing problems, see the *GeneMapper® Software Reference and Troubleshooting Guide*.

Viewing Sample Information


To view information and raw data associated with individual sample files, select a sample file in the Navigation Pane (left), then select the Info or Raw Data tabs.

Select sample file to view sample information and raw data



Viewing Sample Plots

To view the plots of the samples:

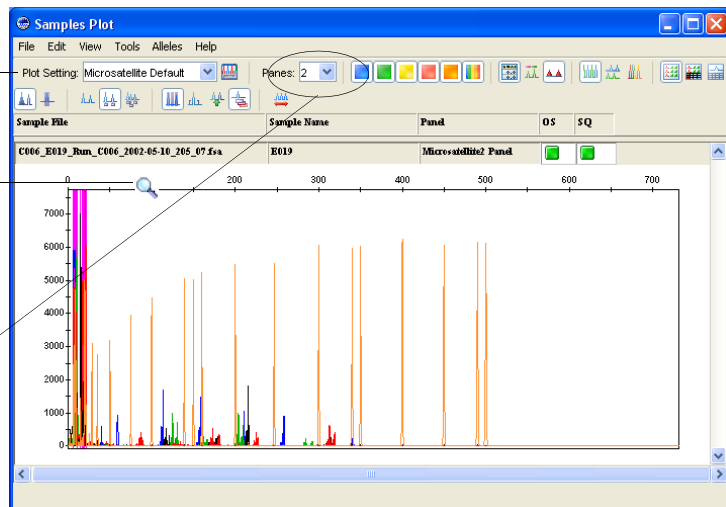
1. Select **View** ► **Samples** to display the Samples tab.
2. Select a sample (row) in the Samples tab. To select multiple samples, press and hold **Shift** or **Ctrl**. To select all samples, select **Edit** ► **Select All**.
3. Click  (**Analysis** ► **Display Plots**).

The Samples Plot window displays an electropherogram for each selected sample.

Select Microsatellite Default for the Plot Setting

Zoom by click-dragging on top x-axis



Select the number of plots to display



4. Select **Microsatellite Default** for the Plot Setting.

Note: Plot Settings control the information displayed in the Samples Plot window after analysis. Microsatellite Default is one of the default Plot Settings provided with the GeneMapper Software. You can also edit and create custom Plot Settings in the GeneMapper Manager. For more information, see the *GeneMapper® Software Online Help*.

5. Zoom on the x- and y-axes in the Samples Plot:

To ...	Then ...
Zoom on a specific region of the x-axis	Place the cursor on the top x-axis, then click-drag the  right or left to zoom all plots. Press and hold Shift while click-dragging to zoom only the selected plot. or Right-click the top x-axis, select Zoom To , type range, then click OK .
Zoom on a specific region of the y-axis	Place the cursor on the left y-axis, then click-drag the  up or down. or Right-click the left y-axis, select Zoom To , type maximum, optionally, select Apply to all electropherograms , then click OK .
Unzoom	Double-click the x-axis or y-axis. or Right-click the x-axis or y-axis, then select Full View .

Examining Data in the Samples Plot Window

Other tasks you can perform in the Samples Plot window include:

- Adjust the scale of the x-axes (basepairs or data points)
- Adjust the scale of the y-axes (scale to individual maximum, global maximum, or a specific value)
- Show and hide specific dye color peaks
- Display a status line for individual peaks
- Display a Sizing Table, which displays a row of sizing information for each detected peak
- Display a Genotypes Table, which displays a row of genotyping information for each detected peak
- Select peaks, which highlights a corresponding row of data in the Sizing Table

See [Figure 2-2 on page 35](#) for an illustration of some of the above features.

For more information on using the above features, press **F1**, then select the desired topic from the *GeneMapper® Software Online Help*.

When done viewing the Samples Plot, click  to close the window.

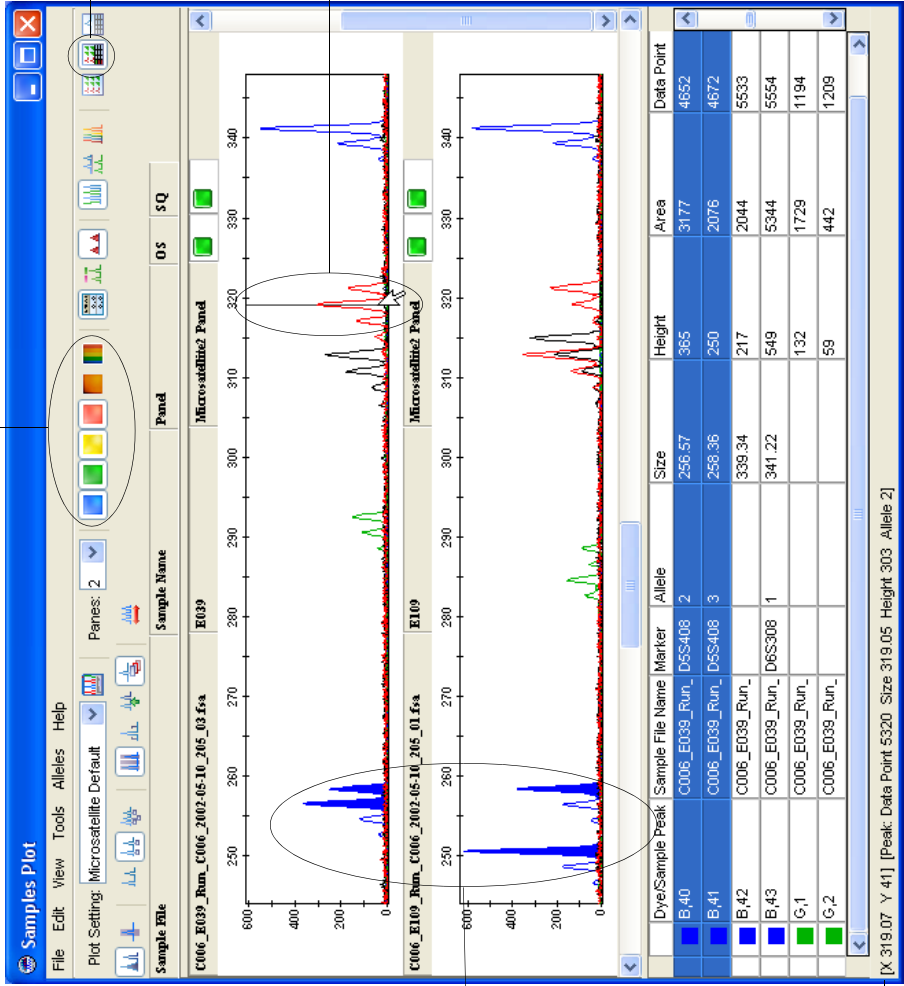
Next Steps

Create a bin set and generate bins using the Auto Bin feature and reference data as described on [page 36](#).

Click to show and hide dye colors

Click to display Sizing Table below

Place cursor over peak to display status line and data in Status Bar (lower left)



Click, Ctrl-click, or Shift-click to select them and highlight the corresponding rows of data in the Sizing Table below

Sizing Table

Status Bar

Hint: Press **Ctrl-G** to clear the Sizing Table, then click individual peaks to add their associated information to the Sizing Table.

Figure 2-2 Examining and comparing data from different sample files in the Samples Plot

Creating a Bin Set and Generating Bins Using the Auto Bin Feature

Overview Use the Panel Manager to create bin sets and generate bins (allele definitions).



Before you create a bin set, you must select a kit. You can then associate the bin set with any panels in that kit.

Before you generate bins, you must select a panel and a bin set. Only sample files in projects analyzed with that panel are available to add as reference data to the selected panel to generate the bins. The bins will be associated with markers in the selected panel and stored in the selected bin set.

Note: In this guide, you will learn how to create bins using reference data and the Auto Bin feature. However, you can also import bin sets (text files) that contain bin information. Or you can create bins manually. For more information on importing bin sets or creating bins manually, see the *GeneMapper® Software Online Help*.

Creating a Bin Set

To create a bin set:

1. Open the Panel Manager by clicking  (**Tools ▶ Panel Manager**).
2. In the Navigation Pane (left), select the **Microsatellite Kit** you created on [page 11](#).
3. Click  (**Bins ▶ New Bin Set**).
4. In the New Bin Set dialog box, type **Microsatellite Bin Set** for the Bin Set Name, then click **OK**.



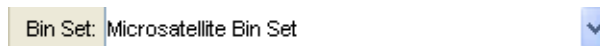
The Microsatellite Bin Set is added to the Bin Set drop-down list at the top of the Panel Manager. The Microsatellite Bin Set can now be associated with the Microsatellite Panel (or any other panels added to the Microsatellite Kit).

Adding Reference Data to a Panel and Bin Set

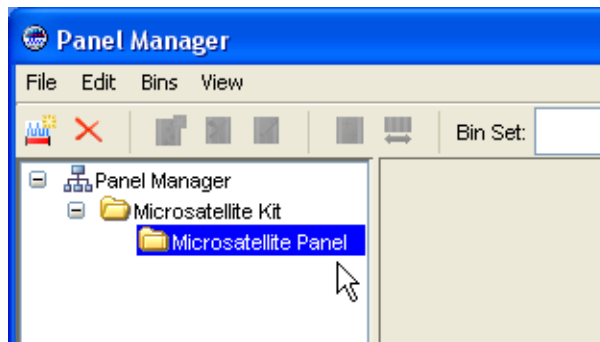
Note: You can add all or only a subset of the sample files in a project as reference data. Because there are a limited number of sample files, you will use all of them for reference data. If you have a large number of sample files, you could pick a subset of sample files that you believe to contain all of the alleles that exist in the sample files.

To add reference data to the Microsatellite Panel and Microsatellite Bin Set:

1. Make sure the **Microsatellite Bin Set** is selected in the Bin Set drop-down list.



2. In the Navigation Pane (left), expand the **Microsatellite Kit**, then select the **Microsatellite Panel** you created on [page 11](#).

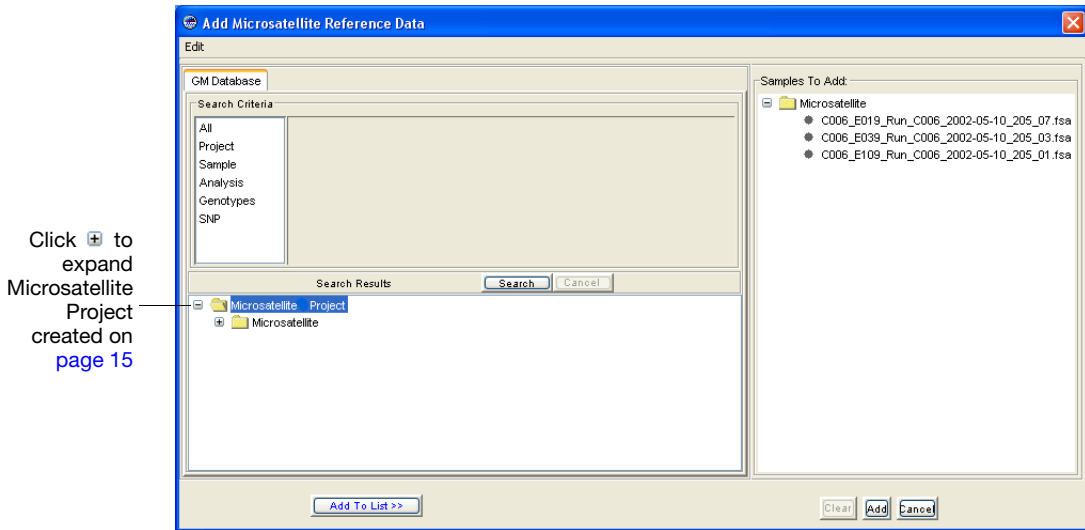


All the markers you created display on the right pane of the Panel Manager ([Figure 2-3 on page 39](#)).

3. Click  (**Bins ▶ Add Reference Data**).

The Add Microsatellite Reference Data dialog box opens displaying the Microsatellite Project you created in the lower left pane.

Note: The lower left pane always displays all projects that have been analyzed using the selected panel.



4. Expand the **Microsatellite Project**, select the **Microsatellite** folder, click **Add to List**, then click **Add**.

All the sample files in the Microsatellite Project are added as reference samples to the Microsatellite Panel and appear in the lower half of the Navigation Pane in the Panel Manager (Figure 2-3 on page 39).

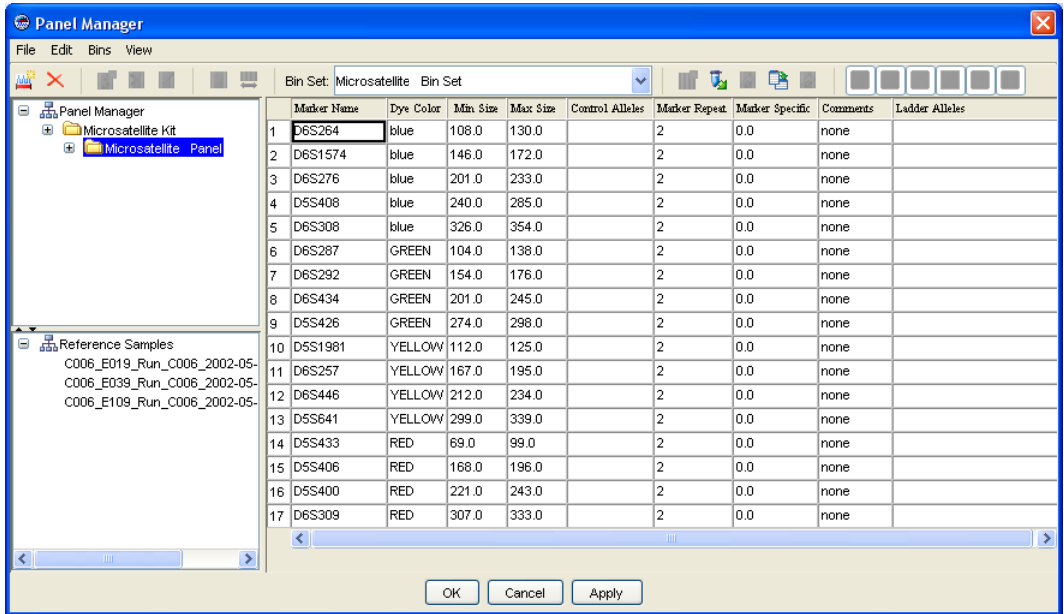

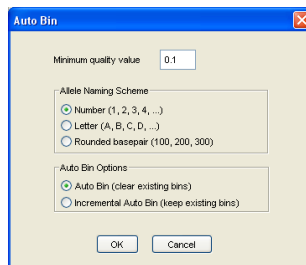


Figure 2-3 Panel Manager displaying markers and reference sample files for selected panel

Generating Bins using Auto Bin

To generate bins using the Auto Bin feature:


1. Make sure the Microsatellite Panel is selected in the Navigation Pane and Microsatellite Bin Set is selected in the Bin Set drop-down list, then click  (**Bins ▶ Auto Bin**).
2. In the Auto Bin dialog box:
 - Leave Minimum quality value set to **0.1**
 - Select **Number** for Allele Naming Scheme
 - Select **Auto Bin (clear existing bins)** for Auto Bin Options



3. Click **OK**.
4. When “Autobinning completed” displays, click **OK**.

Reviewing the Markers and Bins

To review the markers and bins generated from the reference data:

1. Expand the Microsatellite Panel by clicking , then select a marker in the upper half of the Navigation Pane.

A plot ([Figure 2-4](#)) displays:

- Marker (pink line)
 - Bins (grey columns) for that marker
 - Reference alleles (red cross hatches) for each bin
2. With a marker selected in the upper half of the Navigation Pane, select a reference sample in the lower half of the Navigation Pane.

The plot updates ([Figure 2-5](#)) to show the electropherogram peaks for the selected sample.

Note: Applied Biosystems recommends selecting each marker to confirm that bins were created for it. If no bins are present, investigate why. See the *GeneMapper® Software Reference and Troubleshooting Guide*.

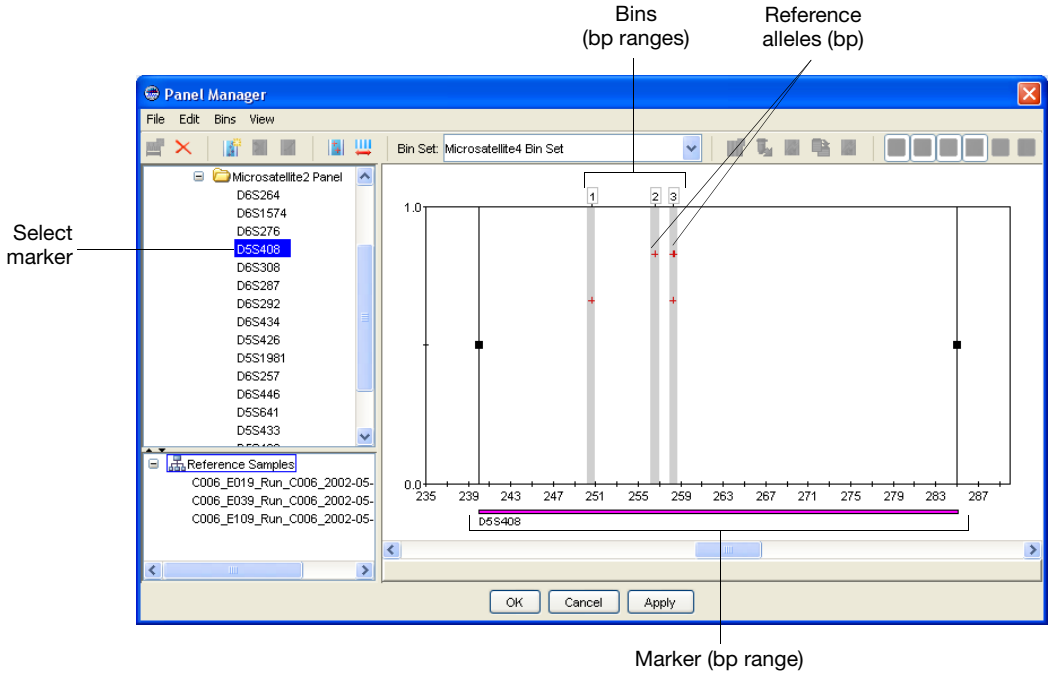


Figure 2-4 Selecting a marker

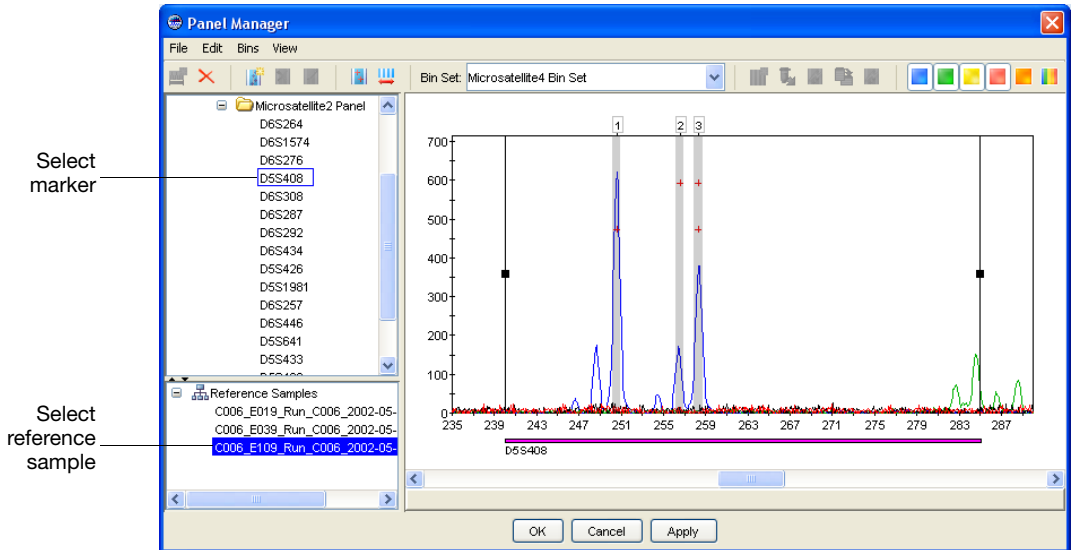


Figure 2-5 Selecting a marker and reference sample

Accepting the Bin Set


Click **OK** to accept the new bin set and close the Panel Manager.

Adding, Editing, and Deleting Bins and Markers (Optional)


To complete the experiment in this guide, you do *not* need to add, edit, or delete any bins or markers. However, you may wish to test these functions by opening the Panel Manager, then selecting the Microsatellite Kit and Microsatellite Panel.

IMPORTANT! If you edit or delete any bins or markers, make sure you click **Cancel** at the bottom of the Panel Manager. Clicking **OK** or **Apply** can adversely affect the results of the analysis.

Adding a Bin to a Marker

1. Select the marker in the upper Navigation Pane, then click  (**Bins ▶ Add Bin**).
2. Click in the plot at the location where you want to add the bin.
3. In the Add Bin dialog box, type a **Name**, **Location**, and **Offsets** for the bin, then click **OK**.

Editing a Bin

1. Click the bin (grey vertical bar) to select it.
2. Click  (**Bins ▶ Edit Bin**) or right-click the bin, then select **Edit Bin**.
3. In the Edit Bin dialog box, edit the **Name**, **Location**, and **Offsets** for the bin, then click **OK**.

Editing a Bin Graphically

1. Click the bin (grey vertical bar) to select it (Figure 2-6).
2. Click-drag the blue center line that defines the bin location.
3. Click-drag the left or right handles that define the bin offsets (range).

Note: To correct any undesired change, select **Edit ▶ Undo**.

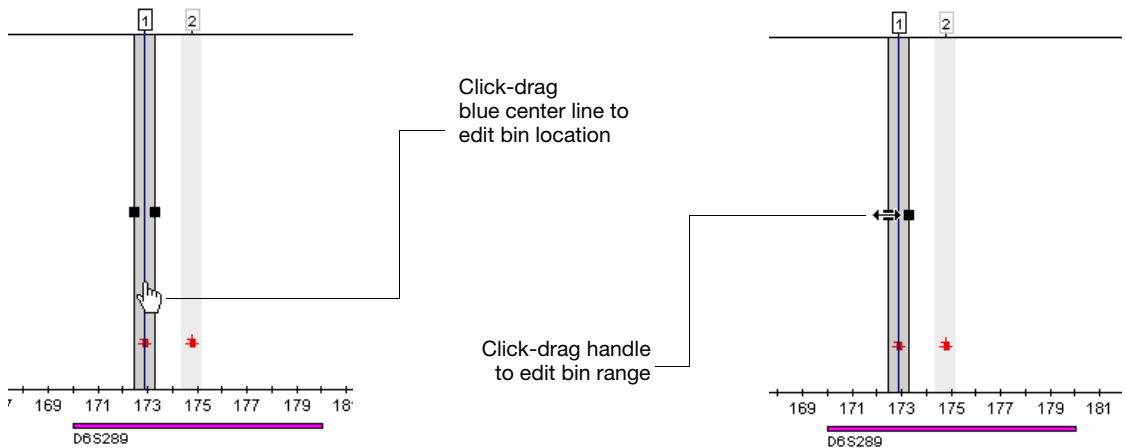



Figure 2-6 Editing a bin graphically

Deleting a Bin

To delete a bin from a marker:

- Select the bin, then click  (**Bins ▶ Delete Bin**)
or
- Select the bin, right-click the bin, then select **Delete Bin**.

Editing a Marker

1. Select the marker in the upper Navigation Pane ([Figure 2-7 on page 44](#)).
2. Click-drag the left or right handles that define the marker range.

Note: To correct any undesired change, select **Edit ▶ Undo**.

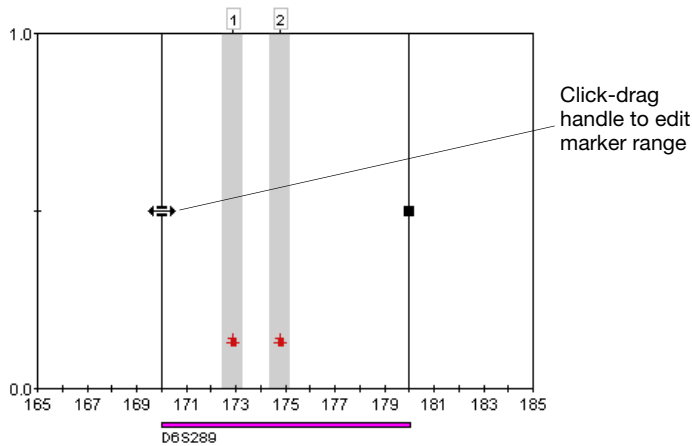



Figure 2-7 Editing a marker

Deleting a Marker From a Panel

1. Select the marker in the upper Navigation Pane.
2. Click  (**Edit ▶ Clear Marker**).

Next Steps Analyze and examine the data in the microsatellite project as described in [Chapter 3](#).

3

Analyzing and Examining Results

Chapter 1
Getting Started



Chapter 2
Setting Up the
Microsatellite
Analysis



Chapter 5
**Analyzing and
Examining Results**



Chapter 4
Printing and
Exporting Results


This chapter includes:

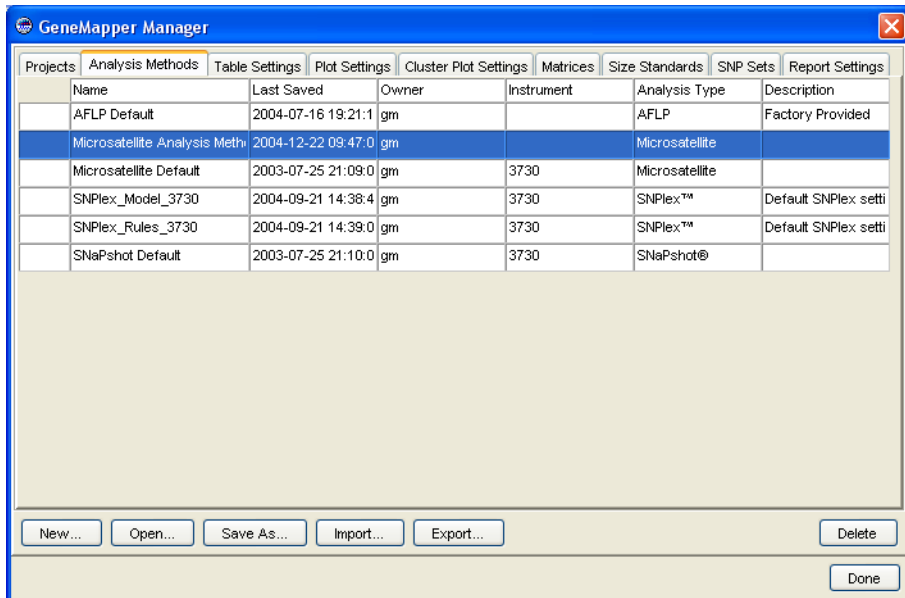
- Editing the Analysis Method 46
- Analyzing the Project 48
- Examining the Results 48

Editing the Analysis Method

Overview When you created the Microsatellite Analysis Method on [page 18](#), for the initial analysis, you did not select a bin set in the method. Now that you have created a bin set, select that bin set in the analysis method before analyzing the data. The bin set will allow the GeneMapper® Software to make allele calls when you analyze the sample files in the project.

Editing the Analysis Method To edit the Microsatellite Analysis Method:

1. Open the GeneMapper Manager by clicking  (Tools ► GeneMapper Manager).
2. Select the **Analysis Methods** tab.



3. Select the **Microsatellite Analysis Method**, then click **Open**.

- In the Analysis Method Editor, select the **Allele** tab, select **Microsatellite Bin Set** for the Bin Set, then click **OK**.

Analysis Method Editor - Microsatellite

General | **Allele** | Peak Detector | Peak Quality | Quality Flags

Bin Set: Microsatellite Bin Set

Marker Repeat Type

Use marker-specific stutter ratio if available

Values for dinucleotide repeats are calculated automatically.

		Mono	Tri	Tetra	Penta	Hexa
Cut-off value		0.25	0.2	0.25	0.25	0.25
PlusA ratio		0.0	0.95	0.95	0.95	0.95
PlusA distance		0.0	1.6	1.6	1.6	1.6
Stutter ratio		0.0	0.95	0.15	0.15	0.15
Stutter distance	From	0.0	0.0	0.0	0.0	0.0
	To	0.0	3.5	4.5	5.5	6.5

Range Filter... Factory Defaults

OK Cancel

- Click **Done** to close the GeneMapper Manager.



Note: You can also access the Analysis Method Editor by double-clicking any row in the Analysis Method column in the Samples tab of the GeneMapper window (see [page 18](#)).

Next Steps Analyze the project as described on [page 48](#).

Analyzing the Project

Overview Now that the analysis method specifies a bin set, when you analyze your samples files, the GeneMapper® Software will size and genotype the data.

Note the following in the Samples tab of the GeneMapper window:

- The  icon displays in the Status column, indicating that the samples are ready to be analyzed and have not been analyzed with the current analysis parameters selected in the Samples tab. This icon displays because you modified the Microsatellite Analysis Method.
- The  icon displays in the REF column, indicating these samples were used as reference data for creating a bin set.

Analyzing Click  (**Analysis ▶ Analyze**).

The GeneMapper Software analyzes each sample in the project, displaying its progress in the Status Bar (lower left) of the GeneMapper window.

Next Steps Examine the results as described below.

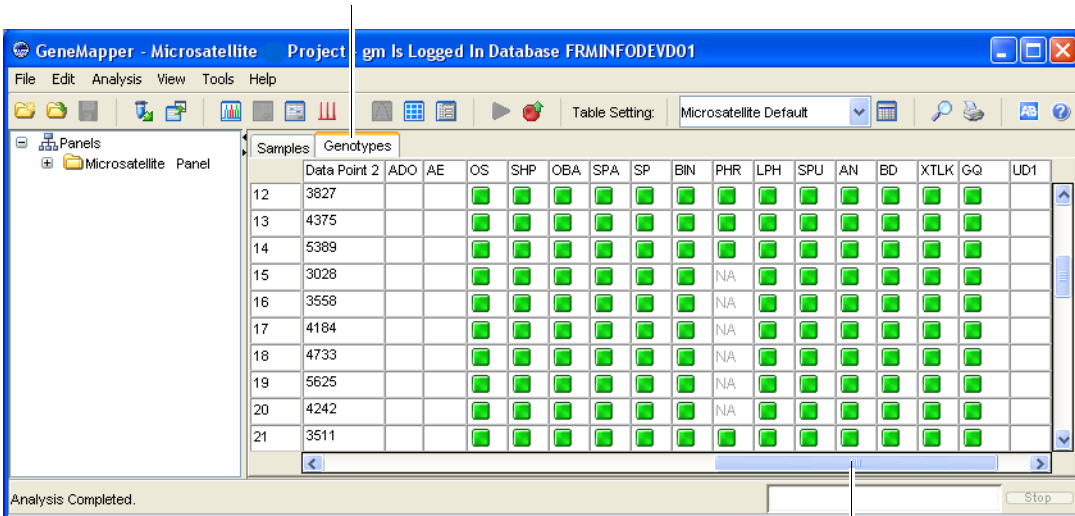
Examining the Results

Overview To examine the sizing and genotyping results:

- Review the SQ, associated PQVs, size standard, sample information, and samples plots (described on [pages 28 through 34](#))
- Review the GQ and contributing PQVs ([page 49](#))
- Review the allele calls for each sample ([page 50](#))
- View genotype plots ([page 50](#))
- Examine data in the Genotypes Plot window ([page 52](#))
- View project alleles ([page 52](#))

Reviewing the GQ and PQVs To review the Genotype Quality (GQ) of the data, select the **Genotypes** tab and scroll to the right.

Select Genotypes tab



Click-drag scroll bar to right to view GQ column

If you followed the procedures and used the example data indicated in this guide, the GQ for most samples should be (Pass). The Process Quality Values (PQVs) that contribute the GQ (AN, BD, BIN, LPH, OBA, OS, PHR, SHP, SP, SPA, SPU, and XTLK) should also be .

Investigating Yellow and Red GQs



IMPORTANT! When analyzing your own data, you may find the GQ to be (Check) or (Low Quality) and the contributing PQVs (AN, BD, BIN, CC, LPH, OBA, OS, PHR, SHP, SP, SPA, SPU, and XTLK) to be , indicating issues with the data, marker or bin definitions, or analysis parameters. To investigate and correct these issues, see the *GeneMapper® Software Reference and Troubleshooting Guide*.

Note: Click to sort the samples by GQ score. Samples with a red GQ will be listed at the top of the Genotypes tab.

3. Select **Microsatellite Default** for the Plot Setting.

Note: Plot Settings control the information displayed in the Genotypes Plot window after analysis. Microsatellite Default is one of the default Plot Settings provided with the GeneMapper Software. You can also edit and create custom Plot Settings in the GeneMapper Manager. For more information, see the *GeneMapper® Software Online Help*.

4. Zoom on the x- and y-axes in the Genotypes Plot:


To ...	Then ...
Zoom on a specific region of the x-axis	Place the cursor on the top x-axis, then click-drag the  right or left to zoom that plot. or Right-click the top x-axis, select Zoom To , type range, then click OK .
Zoom on a specific region of the y-axis	Place the cursor on the left y-axis, then click-drag the  up or down. or Right-click the left y-axis, select Zoom To , type maximum, optionally, select Apply to all electropherograms , then click OK .
Unzoom	Double-click the x-axis or y-axis. or Right-click the x-axis or y-axis, then select Full View .

Examining Data in the Genotypes Plot Window

Other tasks you can perform in the Genotypes Plot window include:


- Adjust the scale of the x-axes (basepairs or data points)
- Adjust the scale of the y-axes (scale to individual maximum, global maximum, or a specific value)
- Show and hide specific dye color peaks
- Display a status line for individual peaks
- Add, rename, and delete allele calls
- Edit and delete markers and bins



For more information on using the above features, press **F1**, then select the desired topic from the *GeneMapper® Software Online Help*.

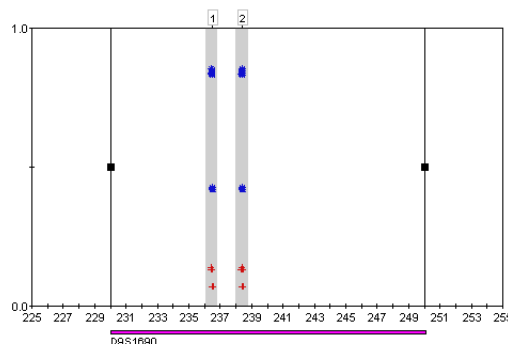
When done viewing the Genotypes Plot, click  to close the window.

Viewing All Project Alleles

To view all the alleles detected in the sample data for each marker:

1. Open the Panel Manager by clicking  (**Tools ▶ Panel Manager**).
2. In Navigation Pane, expand the **Microsatellite Kit**, expand the **Microsatellite Panel**, then select a marker in the Navigation Pane (left).
3. Select **Bins ▶ Show Project Alleles**.

The project alleles (alleles detected in the sample data) appear as blue asterisks  in each bin. The y-axis position of each  indicates the GQ score for that marker and sample.



4

Printing and Exporting Results

Chapter 1
Getting Started



Chapter 2
Setting Up the
Microsatellite
Analysis



Chapter 3
Analyzing and
Examining Results



Chapter 4
**Printing and
Exporting Results**

This chapter includes:

- Printing Results 54
- Exporting Results 55

Printing Results

You can print results from the following windows and tabs by selecting **File ▶ Print**:


Window/Tab	Access From GeneMapper Project Window by selecting
GeneMapper window – Samples tab	View ▶ Samples
GeneMapper window – Genotypes tab	View ▶ Genotypes
GeneMapper window – Info tab	View ▶ Sample Info
GeneMapper window – Raw Data tab	View ▶ Raw Data
GeneMapper window – EPT Data tab	View ▶ EPT Data
Samples Plot window	The Samples tab, then Analysis ▶ Display Plots
Genotypes Plot window	The Genotypes tab, then Analysis ▶ Display Plots
Report Manager window	Analysis ▶ Report Manager

Note: You can also print reports. For information on creating report settings and generating reports, see the *GeneMapper® Software Online Help*.

Exporting Results

Exporting Samples Tab and Genotypes Tab

To export the results displayed in the **Samples** tab and **Genotypes** tab of the GeneMapper window:


1. Prepare the content and format of the data to export:
 - a. Select the desired Table Setting from the drop-down list at the top of the GeneMapper window. The Table Setting controls which columns display and the sorting order for the samples.
 - b. Optionally, sort the data to determine the order that the samples appear. Select **Edit ▶ Sort** or Shift-click the column header in the Samples tab or Genotypes tab. You can also click  (**Analysis ▶ Low Quality on Top**) to sort the samples by GQ score.

Note: For more information on editing or creating Table Settings and sorting data, see the *GeneMapper® Software Online Help*.

2. Select one of the following commands:
 - **File ▶ Export Table** – Exports information displayed in the selected tab.
 - **File ▶ Export Combined Table** – Exports information displayed in both tabs. (This command is available only when the Samples tab is selected.)


Exporting Kits

To export all kits in the Panel Manager:

1. Open the Panel Manager by clicking  (**Tools ▶ Panel Manager**).
2. Select **File ▶ Export All Kits**.


Exporting Panels

To export all panels in a kit:

1. Open the Panel Manager by clicking  (**Tools ▶ Panel Manager**).
2. Select the kit in the Navigation Pane (left).
3. Select **File ▶ Export Panels**.


**Exporting
Bin Sets**

To export a bin set:

1. Open the Panel Manager by clicking  (**Tools ▶ Panel Manager**).
2. In the Navigation Pane, select the kit with which the bin set is associated.
3. Select the bin set from the Bin Set drop-down list.
4. Select **File ▶ Export Bin Set**.

**Exporting
Projects,
Methods,
Settings, and
Size Standards**

To export projects, analysis methods, table settings, plot settings, reports settings, and size standards:

1. Open the GeneMapper Manager by clicking  (**Tools ▶ GeneMapper Manager**).
2. Select one of the following tabs:
 - Projects
 - Analysis Methods
 - Table Settings
 - Plot Settings
 - Report Settings
 - Size Standards
3. Select the object(s) you want to export. Press and hold **Shift** or **Ctrl** to select multiple objects.
4. Click **Export**.

**Exporting
Reports**

You can also export reports. For information on creating report settings and generating reports, see the *GeneMapper® Software Online Help*.

A

- adding
 - bins to markers 42
 - sample files to project 15
 - size standard labels 31
- Advanced peak detection algorithm 21
- allele calls, reviewing 50
- Allele tab of Analysis Method Editor 20, 47
- alleles, project 52
- analysis method
 - creating 18
 - editing 46
 - exporting 56
 - saving 23
 - selecting bin set 20, 47
- Analysis Method Editor
 - Allele tab 20, 47
 - General tab 19
 - Peak Detector tab 21
 - Peak Quality tab 22
 - Quality Flags tab 23
- analysis parameters
 - definition 5, 18
 - setting 18
- analysis. *See* microsatellite analysis
- analyzing a project 27, 48
- Applied Biosystems
 - contacting viii
 - customer feedback on documentation viii
 - Information Development
 - department viii
 - Technical Support viii
- assumptions for using this guide v
- Auto Bin 40

B

- bin
 - adding to a marker 42
 - creating manually 36
 - definition 5, 36
 - deleting 43
 - editing 42, 43
 - generating using Auto Bin 40
 - reviewing 40
 - bin set
 - accepting 42
 - creating 36
 - definition 5, 36
 - exporting 56
 - importing 36
 - selecting in analysis method 20, 47
 - bold text, when to use v
- ## C
- conventions
 - bold text v
 - for describing menu commands v
 - IMPORTANT! vi
 - in this guide v
 - italic text v
 - Notes vi
 - user attention words vi
 - creating
 - analysis method 18
 - bin set 36
 - bins 40
 - kit 11
 - markers 11
 - panel 11
 - project 15
 - reports 56
 - customer feedback, on Applied Biosystems
 - documents viii

D

- data
 - See* example data
 - See* reference data
 - See* sample files
- deleting
 - bins 43
 - markers 44
 - size standard labels 31
- disclaimer, license ii
- documentation, related vii

E

- editing
 - analysis method 46
 - bins 42, 43
 - markers 44
 - size standard labels 31
- example data
 - instrument used 2
 - marker information 3, 14
 - overview 2
 - size standard for 2
- exporting
 - analysis methods 56
 - bin sets 56
 - Genotypes Plot window 55
 - kits 55
 - panels 55
 - plot settings 56
 - projects 56
 - report settings 56
 - reports 56
 - results 55
 - Samples Plot window 55
 - size standards 56
 - table settings 56

F

- files. *See* sample files
- fill down 25

G

- General tab of Analysis Method Editor 19
- genotype quality. *See* GQ
- Genotypes Plot window
 - displaying 50
 - examining data in 52
 - exporting 55
 - printing 54
 - zooming 51
- Genotypes tab 49
- Genotypes Table 34
- GQ
 - investigating 49
 - reviewing 49

H

- help, online, accessing vii
- heterozygous min peak height 22
- hiding dye color peaks 34, 52
- homozygous min peak height 22

I

- importing
 - bin set 36
 - panels 11
- Information Development department,
 - contacting viii
- instruments
 - compatible with microsatellite analyses 2
 - for example data 2
- italic text, when to use v

K

- kits
 - compatible with microsatellite analyses 2
 - creating 11
 - definition 5, 11
 - exporting 55
 - type 11

L

license disclaimer [ii](#)

M

markers

- adding bins to [42](#)
- creating [11](#)
- definition [2, 5, 11](#)
- deleting from a panel [44](#)
- editing [44](#)
- for example data [3, 14](#)
- plots, viewing [50](#)
- reviewing [40](#)
- viewing plots [50](#)

menu commands, conventions for
describing [v](#)

method. *See* analysis method

microsatellite analysis

- compatible instruments [2](#)
- compatible kits [2](#)
- definition [2](#)
- flowchart [4](#)
- markers [2](#)
- setting up [9](#)

min peak height ratio [22](#)

MSDSs, obtaining [viii](#)

O

online help, accessing [vii](#)

overriding SQ [31](#)

P

Panel Manager

opening [11](#)

panels

- adding reference data [37](#)
- creating [11](#)
- definition [5, 11](#)
- exporting [55](#)
- importing [11](#)
- selecting for analysis [24](#)

peak detection algorithm [21](#)

Peak Detector tab of Analysis Method
Editor [21](#)

Peak Quality tab of Analysis Method
Editor [22](#)

plot setting [33, 51, 56](#)

Plot window

- See* Genotypes Plot window
- See* Samples Plot window

plots. *See* sample plots

primers, custom [2](#)

printing

- Genotypes Plot window [54](#)
- reports [54](#)
- results [54](#)
- Samples Plot window [54](#)

project

- adding sample files to [15](#)
- analyzing [27, 48](#)
- creating [15](#)
- exporting [56](#)
- saving [26](#)
- setting analysis parameters [18](#)
- setting table settings [18](#)

project alleles

viewing [52](#)

Q

Quality Flags tab of Analysis Method
Editor [23](#)

R

reference data

- adding to a panel [37](#)
- icon [48](#)

report settings, exporting [56](#)

reports

- creating [56](#)
- exporting [56](#)
- printing [54](#)

results

- exporting [55](#)
- printing [54](#)

- S**
- sample files
 - adding to project 15
 - location 2
 - plots, viewing 32, 50
 - sorting 28, 49
 - viewing information on 32
 - viewing plots 32, 50
 - zooming 33, 51
 - sample plots
 - viewing 32, 50
 - zooming 33, 51
 - samples
 - sorting 28, 49
 - zooming 33, 51
 - Samples Plot window
 - displaying 32
 - examining data in 34
 - exporting 55
 - printing 54
 - zooming 33
 - Samples tab 18
 - saving
 - analysis method 23
 - project 26
 - setting up analysis 9
 - showing and hiding dye color peaks 34, 52
 - size calling curve 30
 - Size Match Editor 29
 - size quality. *See* SQ
 - size standard
 - custom 31
 - examining 29
 - exporting 56
 - for example data 2
 - labels, adding 31
 - labels, deleting 31
 - labels, editing 31
 - selecting for analysis 24
 - troubleshooting 31
 - Sizing Table 34
 - software
 - starting and logging in 5
 - terms defined 5
 - sorting samples 28, 49
- SQ**
- investigating 28
 - overriding 31
 - reviewing 28
 - score 29
- Status Bar 27
- T**
- table settings 18, 25, 56
 - Technical Support, contacting viii
 - text conventions v
 - training, information on viii
 - troubleshooting the size standard 31
- U**
- unzooming 33, 51
 - user attention words, described vi
- X**
- x-axis
 - scale 34, 52
 - zooming 33, 51
- Y**
- y-axis
 - scale 34, 52
 - zooming 33, 51
- Z**
- zooming
 - Genotypes Plot window 51
 - Samples Plot window 33
 - unzooming 33, 51
 - x-axis 33, 51
 - y-axis 33, 51

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