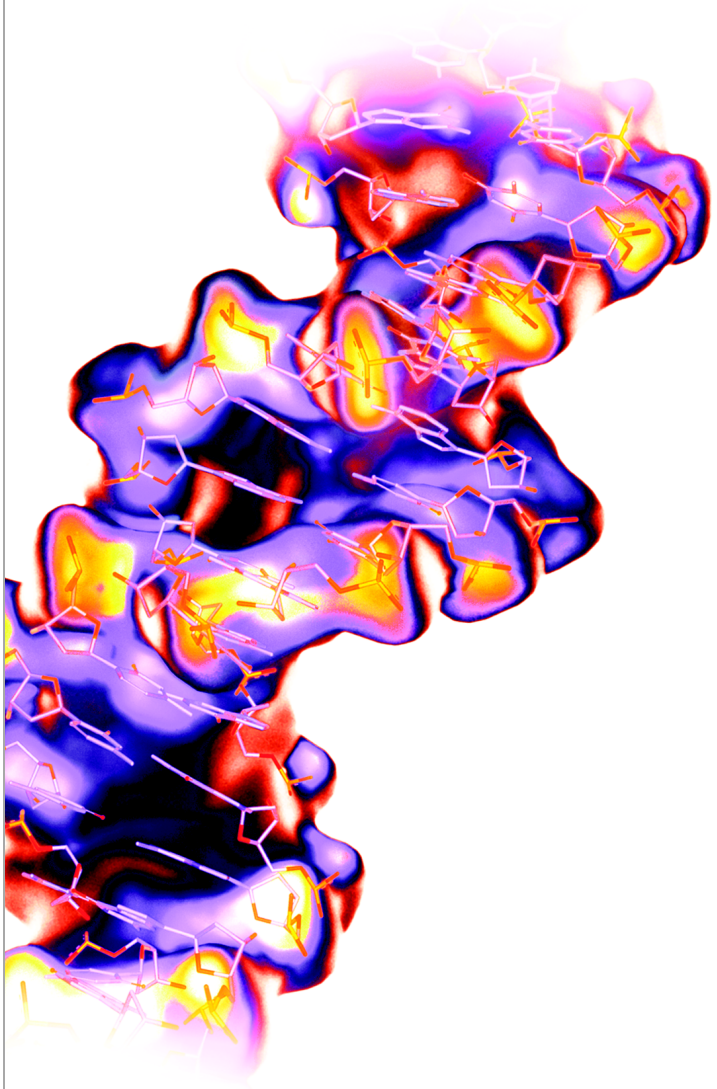
Applied Biosystems

# SNaPshot® Kit Analysis Getting Started Guide

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2 Creating Panels and Bin Sets Using Reference Data

3 Creating Panels and Bin Sets Using Primer Focus® Kit Data

4 Setting Up the SNaPshot® Kit Analysis

5 Analyzing and Examining Results

6 Printing and Exporting Results

Applied Biosystems

# SNaPshot® Kit Analysis Getting Started Guide

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# Preface

## How to Use This Guide

### Purpose of This Guide

The *GeneMapper® Software Version 4.0 SNapshot® Kit Analysis Getting Started Guide* provides brief, step-by-step instructions for sizing and genotyping SNaPshot kit 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*.

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**Note:** For additional documentation, see "How to Obtain Support" on page viii.

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## 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

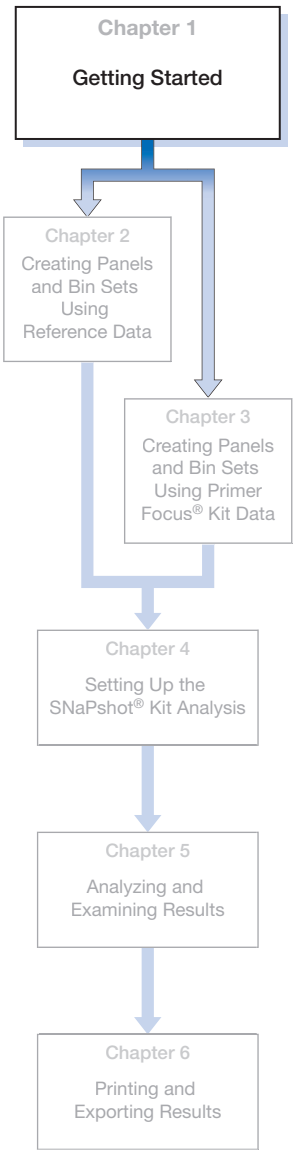
For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

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




This chapter includes: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- About the Example Data . . . . . 5
- SNaPshot® Kit Analysis Workflow . . . . . 6
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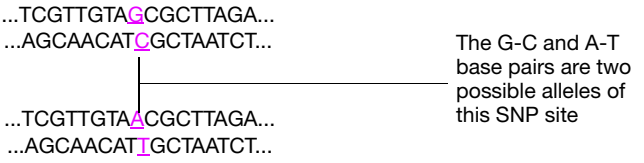
# About SNaPshot® Kit Analyses

## SNP Markers

A Single Nucleotide Polymorphism (SNP) marker consists of a single base pair that varies in the known DNA sequence, thereby creating up to four alleles or variations of the marker.




## SNaPshot® Kit Analysis

### SNaPshot® Multiplex Kit

The SNaPshot Multiplex kit investigates up to ten SNP markers simultaneously by employing PCR amplification followed by dideoxy single-base extension of an unlabeled primer. The primer is designed to anneal to the sequence adjacent to the SNP site. Once the primer anneals, the single-base extension occurs by the addition of the complementary dye-labeled ddNTP (dye terminator) to the annealed primer. Each of the four ddNTPs is fluorescently labeled with a different color dye. The result is marker fragments for the different SNP alleles that are all the same length, but vary by color. After electrophoresis and fluorescence detection, the alleles of a single marker appear as different colored peaks at roughly the same size in the electropherogram plot. The size of the different allele peaks will vary slightly due to differences in molecular weight of the dyes. You then use the GeneMapper Software to size and genotype the data. (Figure 1-1)

The SNaPshot Multiplex kit can investigate up to ten SNP markers simultaneously by using primers of different lengths. It may be necessary to add a non-annealing tail to a primer to make its length sufficiently different from other primers. This step prevents the SNP markers from overlapping. (Figure 1-2)

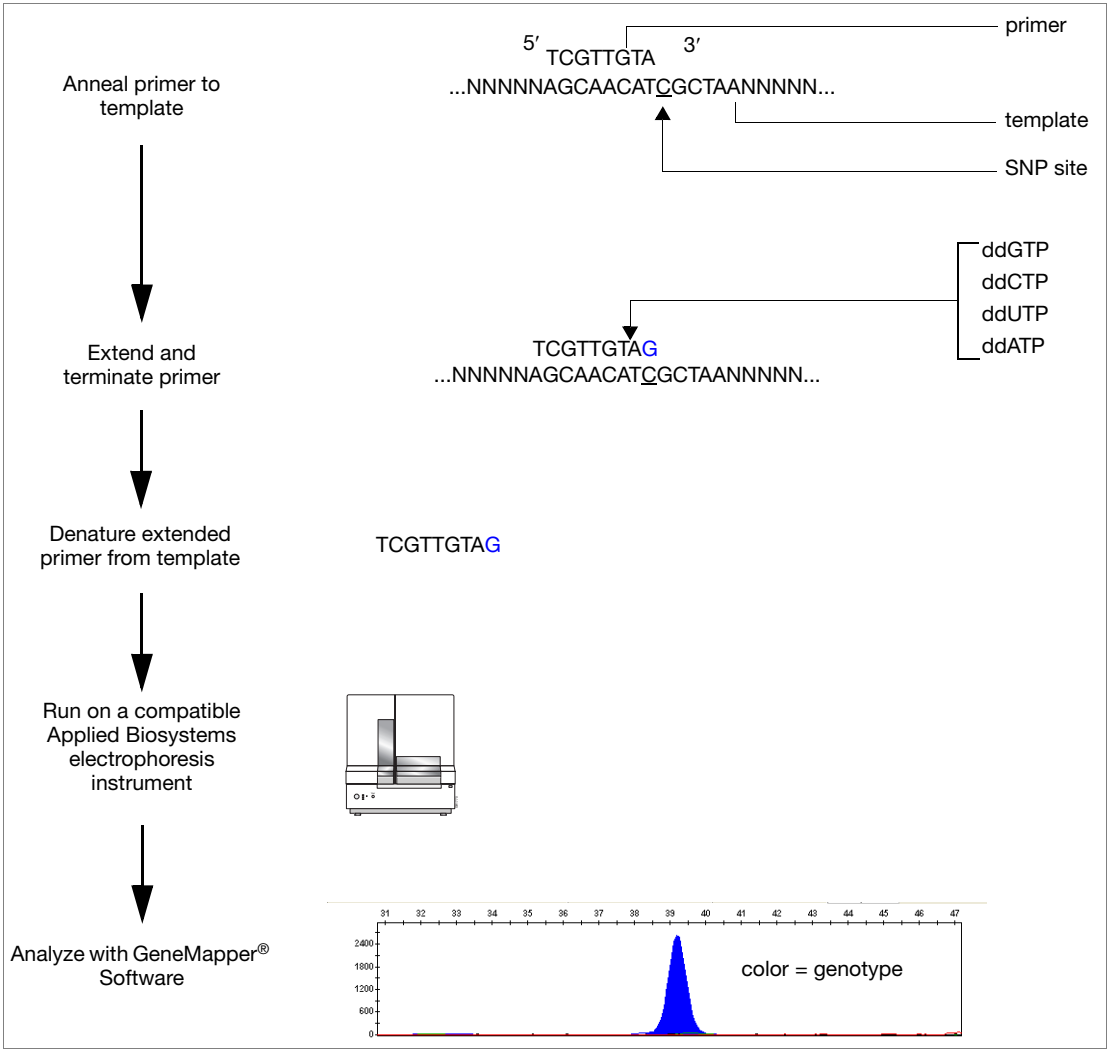



**Figure 1-1 Overview of SNaPshot® Multiplex Kit workflow**

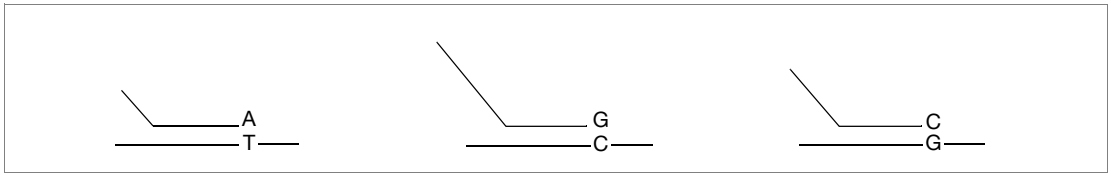


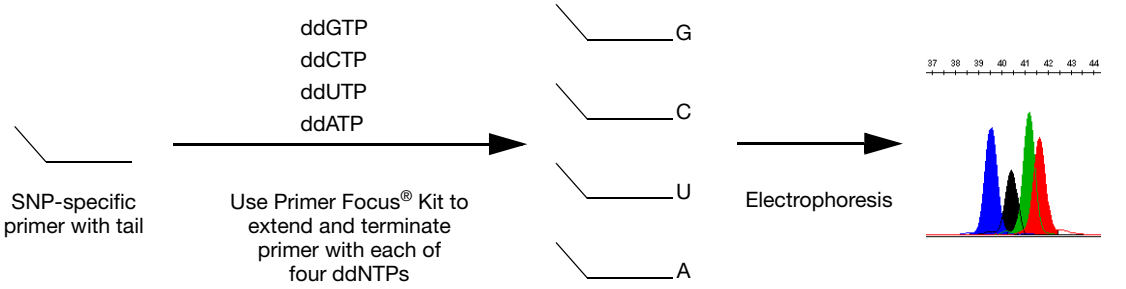

**Figure 1-2 Tailed primers annealed to templates and extended**

### Primer Focus® Kit

Optionally, you can use a Primer Focus kit to create all four possible alleles of your SNP markers of interest. This kit contains reagents that allow you to add all four possible ddNTPs to the 3′ end of your unlabeled SNaPshot primers without using a template.

The GeneMapper Software can then use the sample file from the Primer Focus kit sample to:

- Evaluate the mobilities of any tailed extension products to verify the SNP markers are not overlapping.
- Automatically generate bins for each SNP marker allele using the Auto Panel feature.




## SNaPshot® Kits

The following kits are available from Applied Biosystems:

- SNaPshot Multiplex Kit (part numbers 4323159, 4323161, 4323163)
- Primer Focus Kit (part number 4329538)

## Custom Primers

Applied Biosystems provides custom primers for PCR amplification of SNP markers. For more information, visit the Applied Biosystems Web site at **www.appliedbiosystems.com**.

## Compatible Instruments

For information about Applied Biosystems electrophoresis instruments that are compatible with SNaPshot analyses, see the *GeneMapper® Software Version 4.0 Quick Reference Guide* (PN 4362816).

# About the Example Data

## Sample File Location

To perform the exercise described in this getting started guide, use the three sample files (.fsa) and, optionally, the six Primer Focus kit sample files located on your computer hard drive at:

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

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

## Instrument and Size Standard

Sample files were generated by running samples from a SNaPshot Multiplex Kit on an ABI PRISM® 3100 Genetic Analyzer using the GeneScan™ 120 LIZ® size standard. Additionally, sample files containing all possible marker alleles were generated by running samples from a Primer Focus Kit on an ABI PRISM® 310 Genetic Analyzer, using the GeneScan™ 120 LIZ® size standard.

## Marker Information

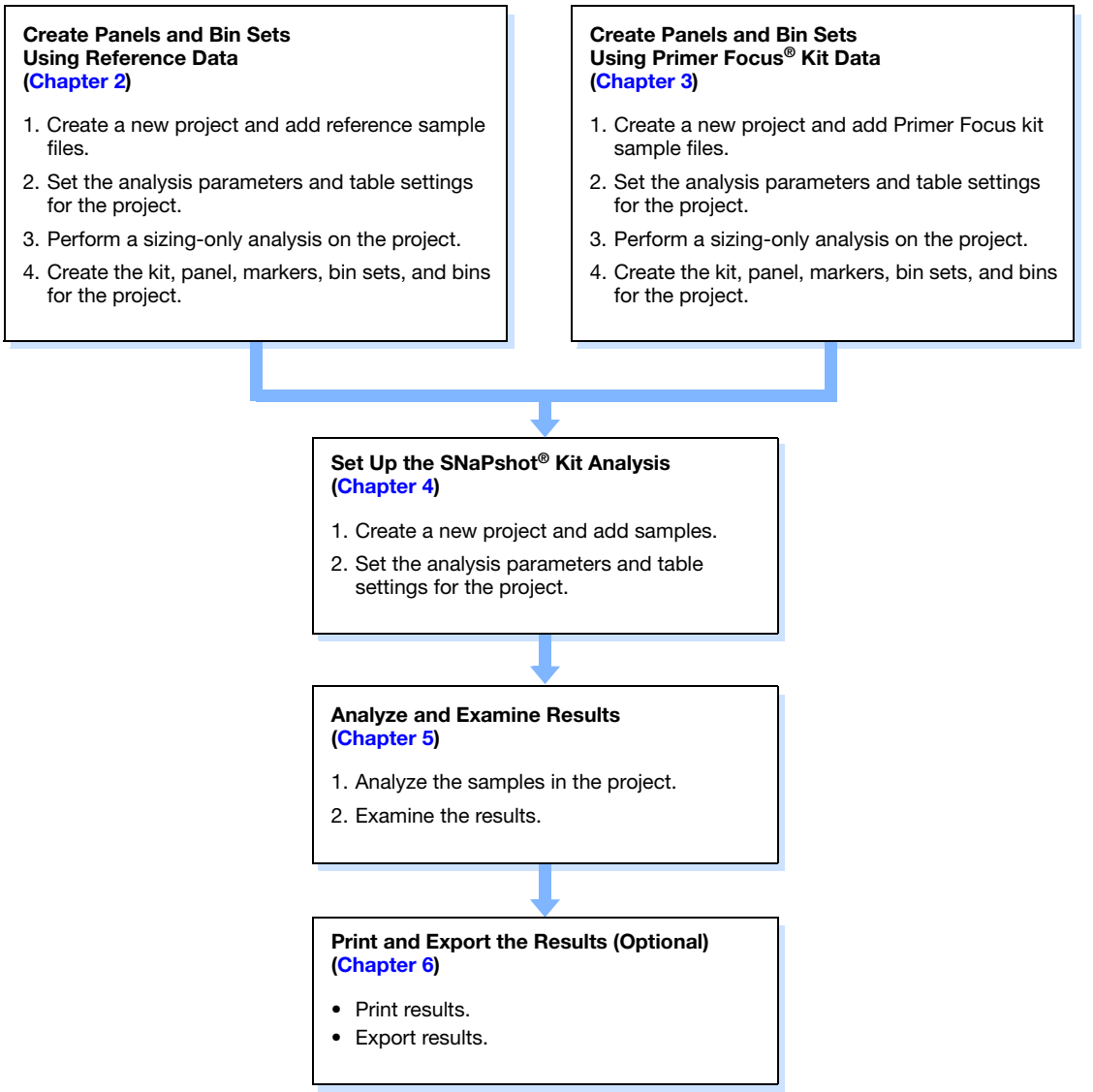
The example SNaPshot kit data includes six markers.

In Chapter 2 you will learn how to *manually* create markers and bins (allele definitions) for your project using reference data. The marker and allele information for the sample data is provided there.

In Chapter 3 you will learn how to *automatically* create markers and bins (allele definitions) for your project by using Primer Focus kit data and the Auto Panel feature in the GeneMapper Software.

# SNaPshot® Kit Analysis Workflow

The following flowchart summarizes the steps for performing a SNaPshot 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 SNP marker is defined by a name and fragment size range (bp). |
| 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 SNaPshot kit 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 SNaPshot kit 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 SNaPshot kit 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.

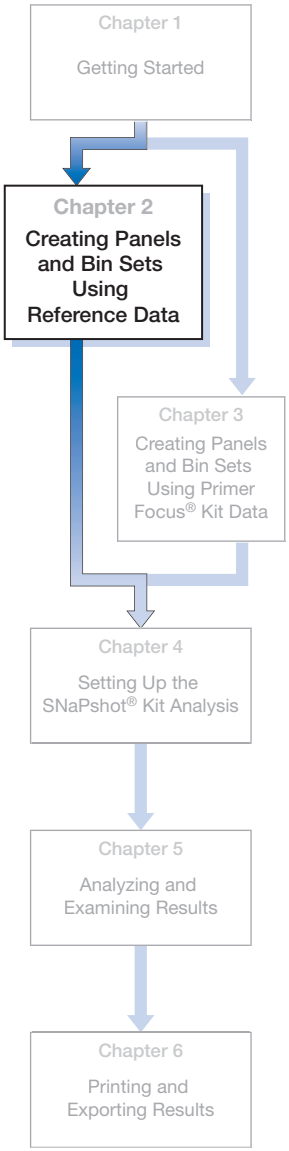
## Using Autoanalysis to Set Up Projects

The GeneMapper Software includes an Autoanalysis feature that can eliminate most of the tasks leading up to the analysis of a SNaPshot kit project. Much of Chapter 2, Chapter 3, and Chapter 4 explain how to manually create, add samples to, and analyze projects for use in SNaPshot kit 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 SNaPshot kit 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 SNaPshot kit projects because it automate many of the tasks explained in Chapter 2, Chapter 3, and Chapter 4. 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).

# 2 Creating Panels and Bin Sets Using Reference Data




This chapter includes:


- Creating a New Project and Adding Reference Sample Files 12
- Setting Analysis Parameters and Table Settings . . . . . 14
- Performing a Sizing-Only Analysis on the Project . . . . . 17
- Creating a Kit, Panel, Markers, Bin Set, and Bins . . . . . 26


**IMPORTANT!** Follow the instructions in this chapter only if you want to learn how to *manually* create markers and bins (allele definitions) for your project using reference data.

If you want to learn how to learn how to *automatically* create markers and bins (allele definitions) for your project by using Primer Focus kit data and the Auto Panel feature, follow the instructions in Chapter 3 instead.

# Creating a New Project and Adding Reference Sample Files

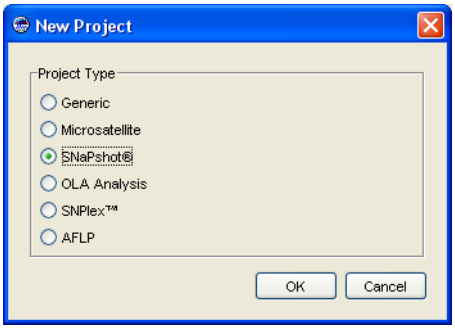
## Overview

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**).



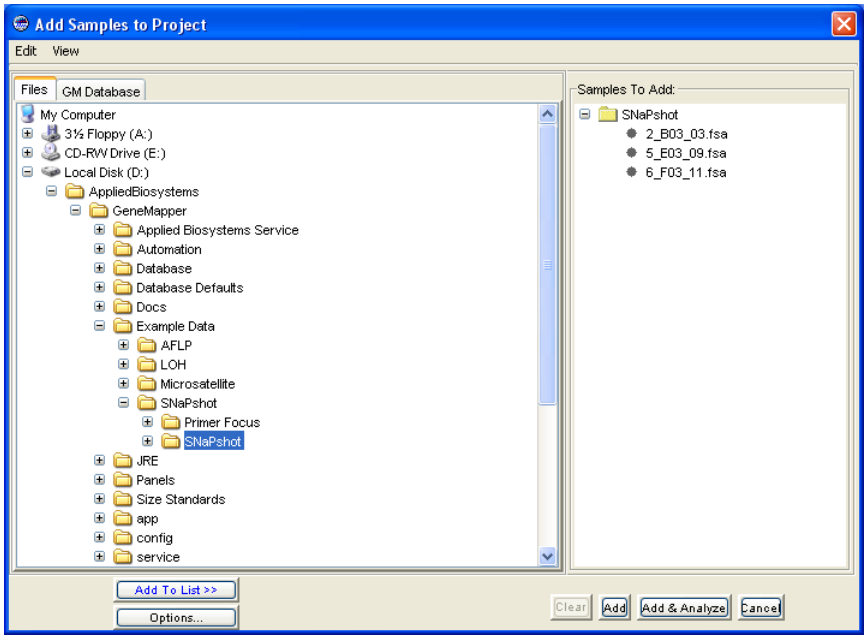

2. In the New Project dialog box, select **SNaPshot**, then click **OK**.
3. From the GeneMapper window, click (**File ▸ Add Samples to Project**).
4. In the Add Samples to Project dialog box, in the Files tab, navigate to

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

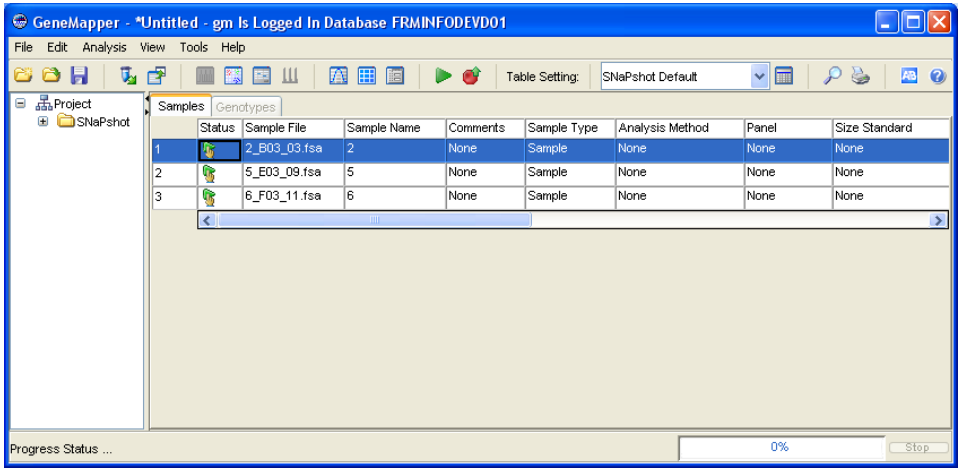
   **Note:** The above location will vary depending on the installation of the GeneMapper® Software. The default installation is the D drive.

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

   **Note:** For this guide you added all three sample files in the SNaPshot folder. However, you can add a subset of files 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 SNaPshot 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 14.

# Setting Analysis Parameters and Table Settings

## Overview

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

Analysis parameters include:

- Analysis Method (including bin set)
- Size Standard
- Panel (set of markers)

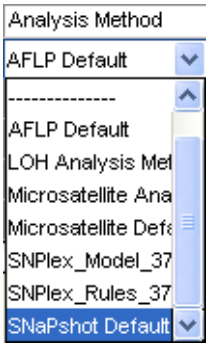
You set analysis parameters that determine the peak detection, sizing, and genotyping algorithms used by the GeneMapper® Software to analyze all sample files in a project.

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 S**NaPshot Default** from the drop-down list.



3. Leave the Panel column set to None.

   **Note:** Selecting a panel is optional. If you do not select a panel, the GeneMapper Software can size the data, but not genotype it. Because you are creating this project to do a sizing-only analysis of reference data for use in creating panels and bin sets, you will not select a panel as part of your analysis parameters.

4. Select the first row in the **Size Standard** column, then select **GS120LIZ** from the drop-down list. (This was the size standard run with the samples.)

5. 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 |
|---|---|---|
| SNaPshot Default | None | GS120LIZ |
| 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 **SNaPshot Default** from the Table Settings drop-down list.

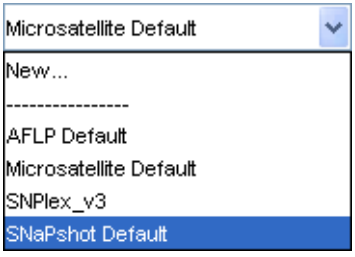


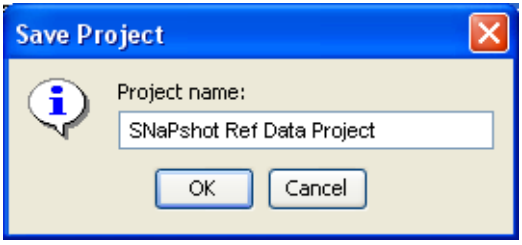

Table Settings control the information displayed in the Samples tab and Genotypes tab after analysis. SNaPshot Default is one of the default Table Settings provided with the GeneMapper Software.

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 **SNaPshot Ref Data Project**, then click **OK**.




SNaPshot Ref Data Project appears in the title bar of the GeneMapper window.

## Next Steps

Perform a sizing-only analysis on the project as described on page 17.

# Performing a Sizing-Only Analysis on the Project

## Overview

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

To perform the sizing-only 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

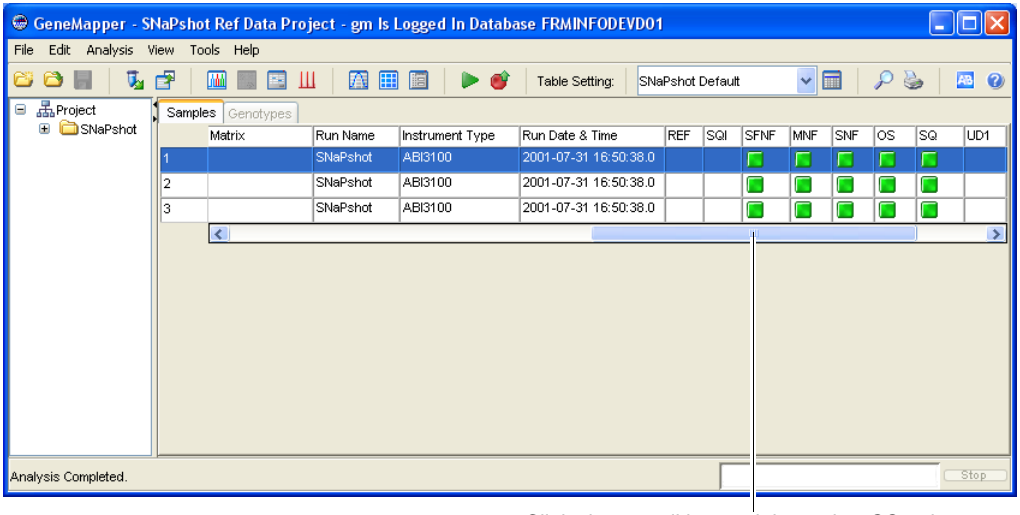
Click  (**Analysis** ▸ **Analyze**).

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 19.

**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**).

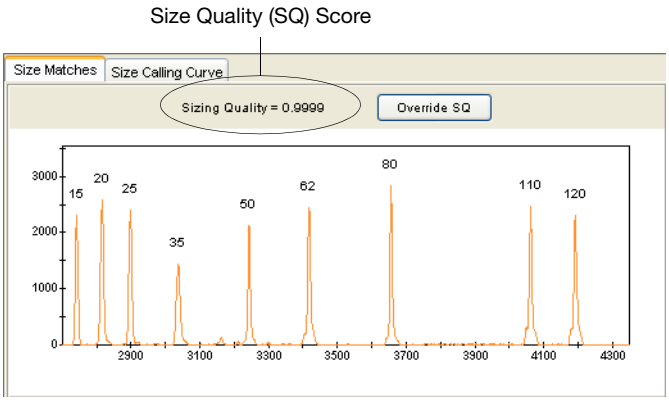



**Figure 2-1 Size Match Editor – Size Matches tab**

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).

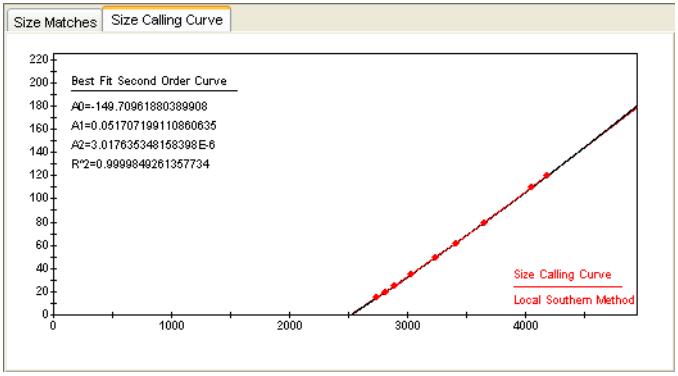
   However, when analyzing you own data you may notice the Sizing Quality is less and the SQ displays (Check) or (Low Quality). For troubleshooting help, see Table 2-1 on page 21.

5. 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 you own data you may find some size standards peaks to be incorrectly labeled or missing. For troubleshooting help, see Table 2-1 on page 21.

6. 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.




7. Select another sample from the left pane of the Size Match Editor, then repeat steps 3 through 6.

8. Click **OK** to close the Size Match Editor.

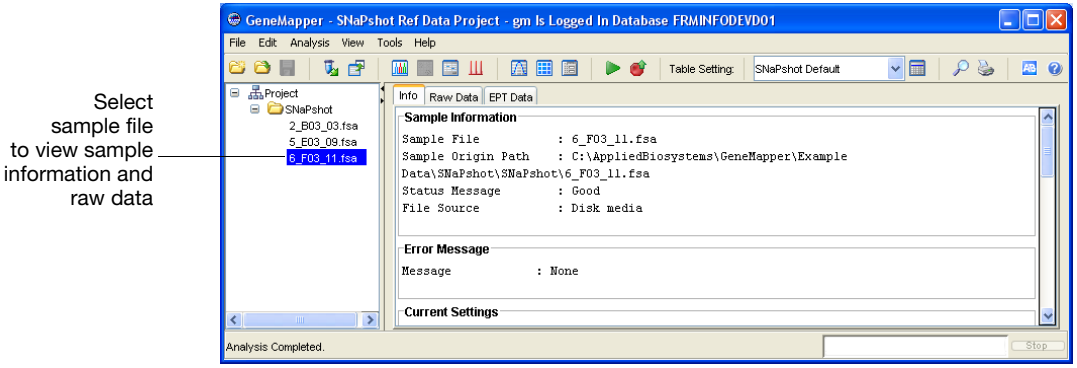
**Table 2-1 Troubleshooting the size standard**

| 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 *GeneMapper® Software Online Help*. |
| Some size standard peaks are not present. | Create a custom size standard in the software. For more information, see the *GeneMapper® Software Online Help*. |

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.

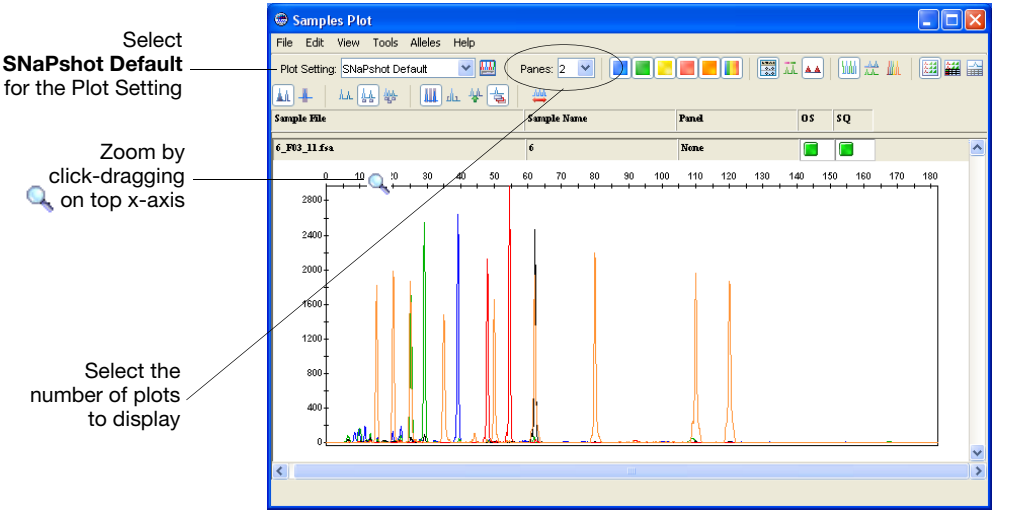



## Viewing Sample Plots

**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.



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

**Note:** Plot Settings control the information displayed in the Samples Plot window after analysis. SNaPshot 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.<br>or<br>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.<br>or<br>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.<br>or<br>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 25 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*.

---

**Hint:** Using the Samples Plot window to compare allele peaks in different sample files is useful to determine which sample files to use as reference data and to determine the minimum and maximum fragment lengths for bins. This information is useful when adding reference data to a kit (page 29), and when creating markers and bins (page 33).

---

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

## Next Steps

Create a kit, panel, markers, bin set, and bins as described on page 26.

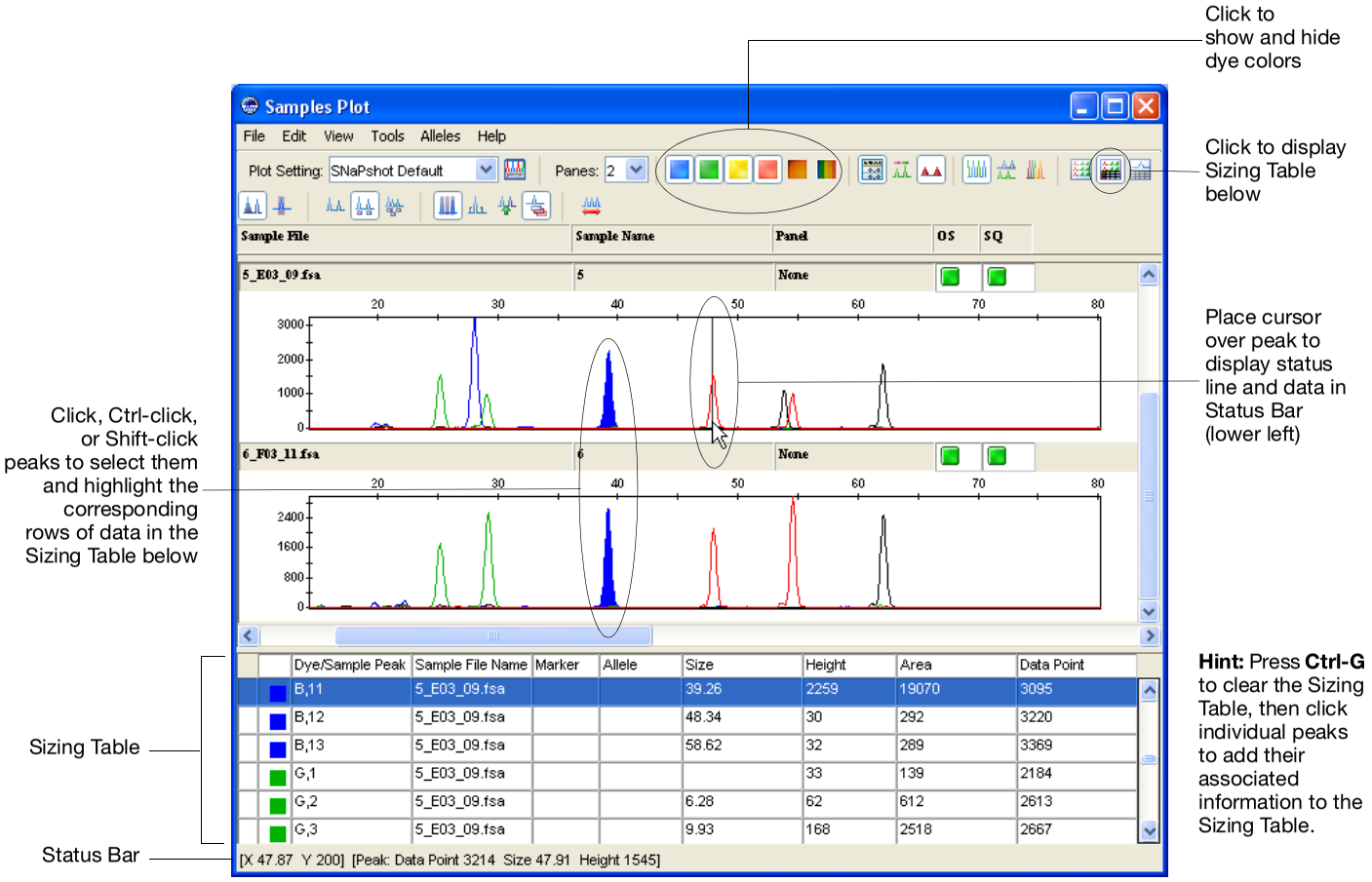



Figure 2-2 Examining and comparing data from different sample files in the Samples Plot

# Creating a Kit, Panel, Markers, Bin Set, and Bins

## 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)

**Note:** In this guide, you will learn how to create or generate panels and markers. However, you can also import panels (text files) that contain marker information. For more information on importing panels, see the *GeneMapper® Software Online Help*.

You also use the Panel Manager to create:

- **Bin Set** – A collection of bins
- **Bin** – An allele definition; a fragment size (bp) and dye color

Before you create a bin set, you must select a kit. You can create only one bin set in a SNP kit. The bin set can then be associated with any panels in that SNP kit.

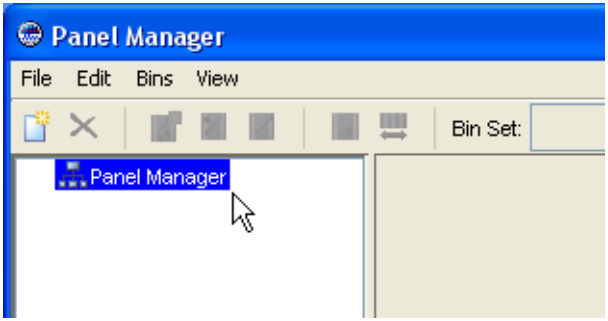
Before you create or generate bins, you must select a panel and a bin set. The bins will be associated with markers in the selected panel and will be stored in the selected bin set.

**Note:** In this chapter, you will learn how to manually create bins using reference data. However, you can also automatically create bins, using Primer Focus kit data and the Auto Panel feature, or import bin sets (text files) that contain bin information. For more information in automatically creating bins, see Chapter 3, "Creating Panels and Bin Sets Using Primer Focus® Kit Data." For more information on importing bin sets, see the *GeneMapper® Software Online Help*.

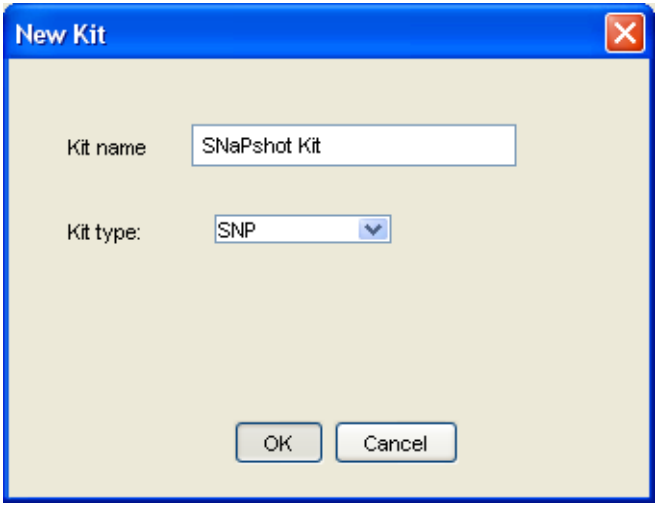
## Creating a Kit and Panel

**To create a kit and panel:**

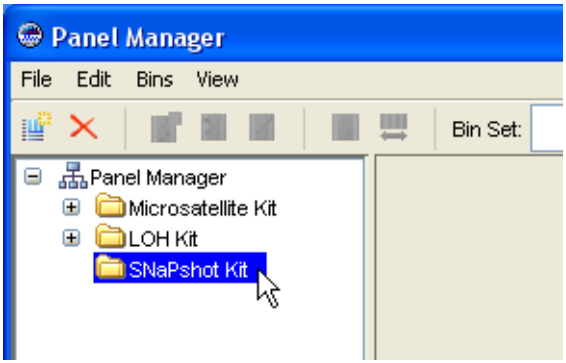
1. Open the Panel Manager by clicking (**Tools ▸ Panel Manager**).
2. Select **Panel Manager** at the top of the Navigation Pane (left side), then click (**File ▸ New Kit**).




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

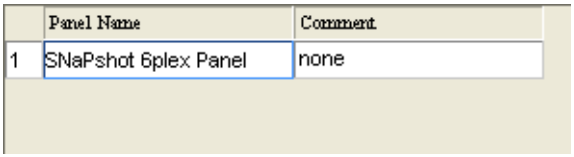


SNaPshot Kit appears in the Navigation Pane (left side).

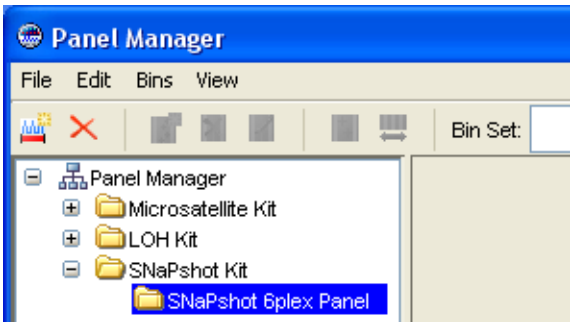



4. Select the **SNaPshot Kit** in the Navigation Pane, then click  (**File ▸ New Panel**).

5. In the right pane of the Panel Manager, select **New Panel**, type **SNaPshot 6plex Panel** for the Panel Name, then press **Enter**.



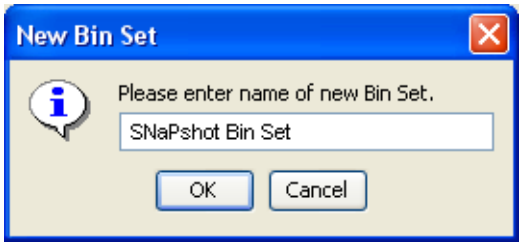

SNaPshot 6plex Panel appears under SNaPshot Kit in the Navigation Pane (left side).



## Creating a Bin Set

**To create a bin set:**

1. In the Navigation Pane (left), select the **SNaPshot Kit** you created on page 27.
2. Click (**Bins ▸ New Bin Set**).
3. In the New Bin Set dialog box, type **SNaPshot Bin Set** for the Bin Set Name, then click **OK**.



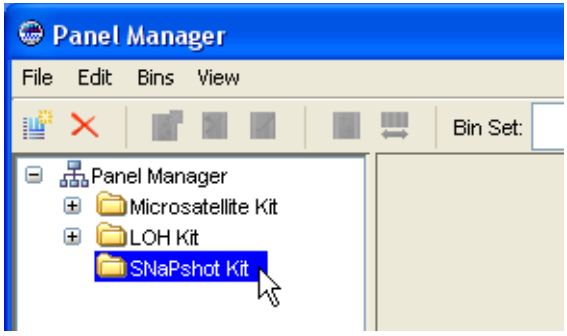

The SNaPshot Bin Set is added to the Bin Set drop-down list at the top of the Panel Manager. The SNaPshot Bin Set can now be associated with the SNaPshot Panel (or any other panels added to the SNaPshot Kit).

## Adding Reference Data to a Kit

**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.

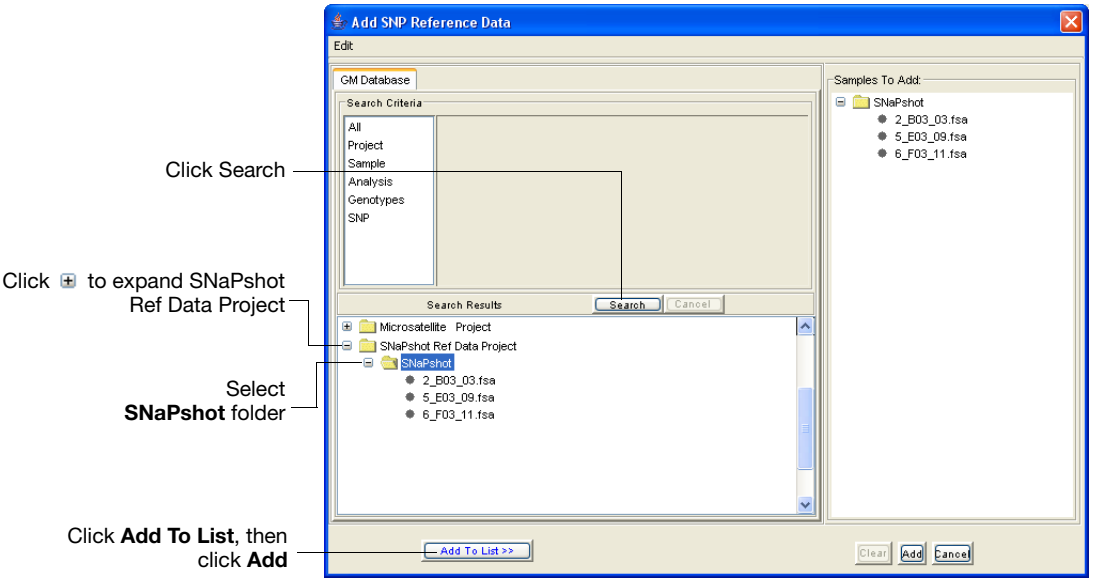
**To add reference data to the SNaPshot Kit:**

1. In the Navigation Pane (left), select the **SNaPshot Kit** you created on page 27.



2. Click (**Bins ▸ Add Reference Data**).

   The Add SNP Reference Data dialog box opens.

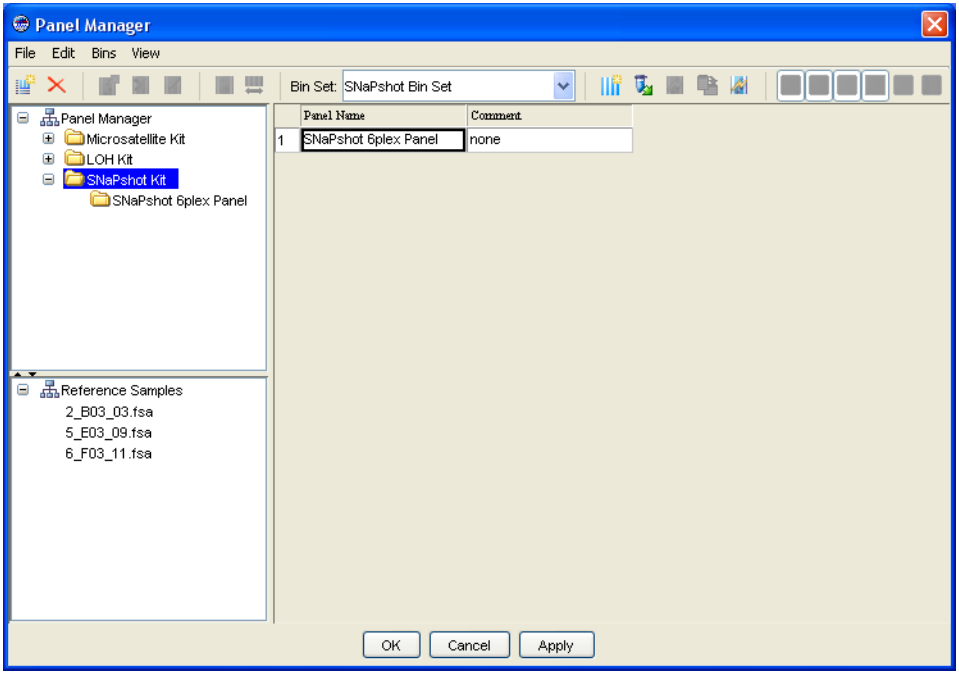



3. Click **Search**.

   The lower left pane displays all projects in the GeneMapper database that have been sized.

4. Expand the **SNaPshot Ref Data Project**, select the **SNaPshot** folder, click **Add to List**, then click **Add**.

All three sample files in the SNaPshot Data folder are added as reference samples to the SNaPshot Kit and appear in the lower half of the Navigation Pane in the Panel Manager.

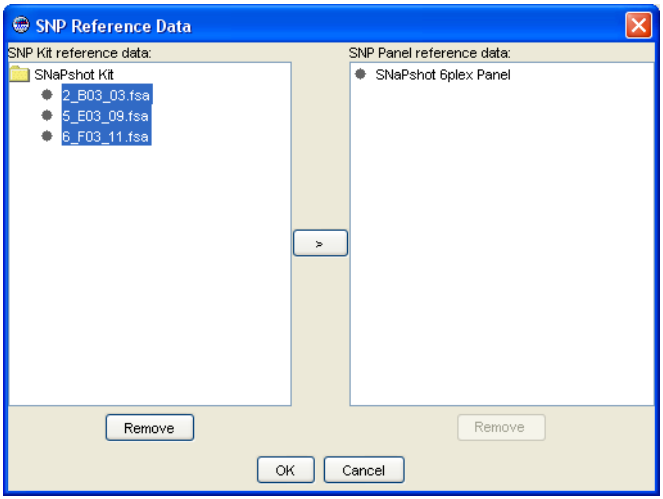


## Adding Reference Data to a Panel

After you add reference data to a kit, you can add the sample files to individual panels in the kit. This is also called panelizing the reference data.

**To add reference data to the SNaPshot 6plex Panel:**

1. In the upper Navigation Pane, select the **SNaPshot 6plex Panel** you created on page 27.
2. Select **Bins ▸ Panel Reference Data**.



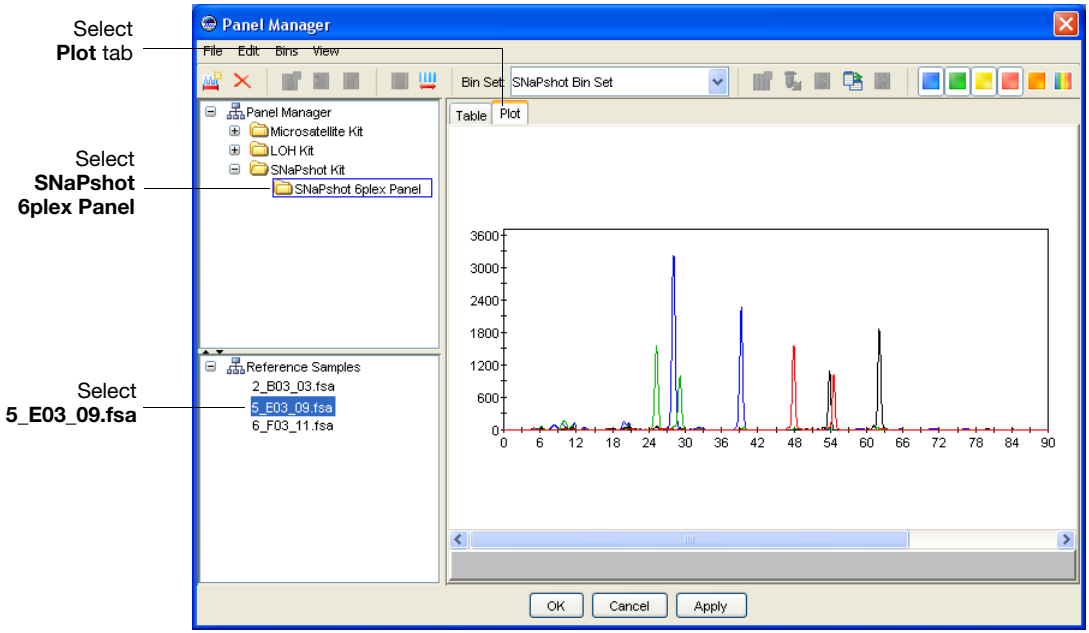

3. In the SNP Reference Data dialog box, select the **SNaPshot Kit** folder, then press and hold **Shift** while selecting all three sample files.
4. Click [ > ] to add the three sample files from the SNaPshot Kit folder to the SNaPshot 6plex Panel.
5. Click **OK**.

## Manually Creating Markers and Bins From Reference Data

**To manually create markers and bins from the three reference sample files:**

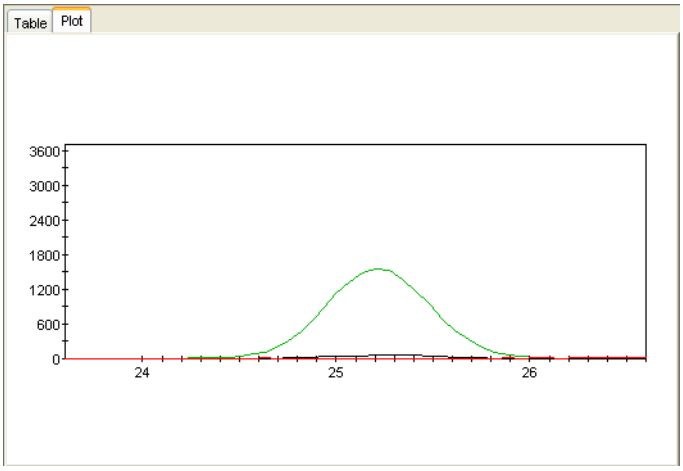
1. In the Panel Manager window, select the Plot tab.
2. In the Navigation Pane:
   a. Select the **SNaPshot 6plex Panel** in the upper half.
   b. Select the **5_E03_09.fsa** sample file in the lower half.

   An electropherogram of the 5_E03_09.fsa sample file appears in the Plot tab.

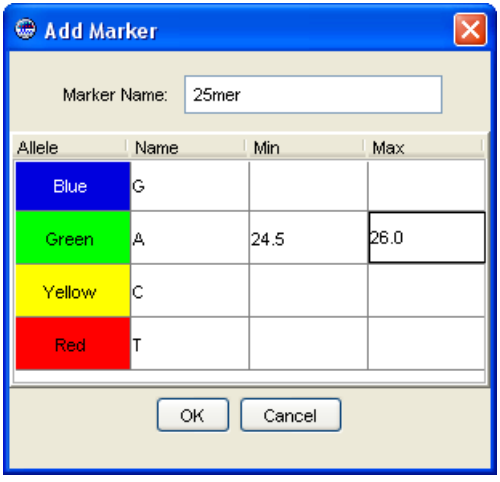



3. Practice viewing the data in different ways:
   - Change the scales for the X and Y axes by selecting commands from the View menu or by right-clicking the labels of the X and Y axes.
   - Show and hide specific dye color peaks by clicking the color buttons on the toolbar.

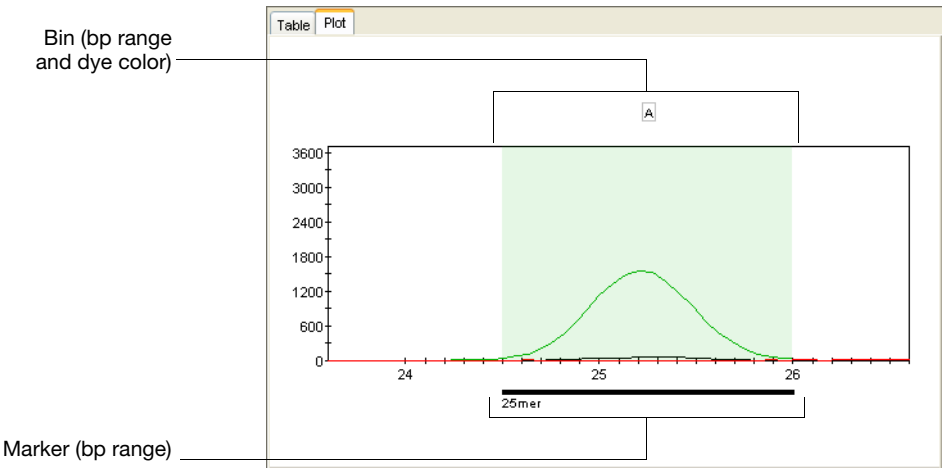
4. In the electropherogram, zoom in on the first green peak (a homozygote allele located at approximately 25 basepairs) by placing the cursor on the x-axis, then click-dragging the right or left.




5. Select **File ▸ New SNP Marker**.

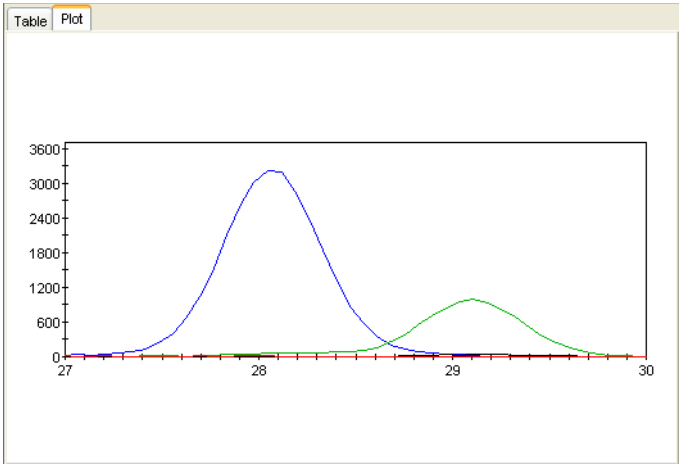


6. In the Add Marker dialog box:
   a. Type **25mer** for the Marker Name.
   b. Type **24.5** for the Min and **26.0** for the Max for the Green Allele.
   c. For the Name, leave the default settings, which are the ddNTP base names (G, A, C, T). These allele names will be used to name the bins generated from the Primer Focus kit data.
   d. Click **OK** to save the marker and bin information to the SNaPshot 6plex Panel and display the marker and bin in the eletropherogram.

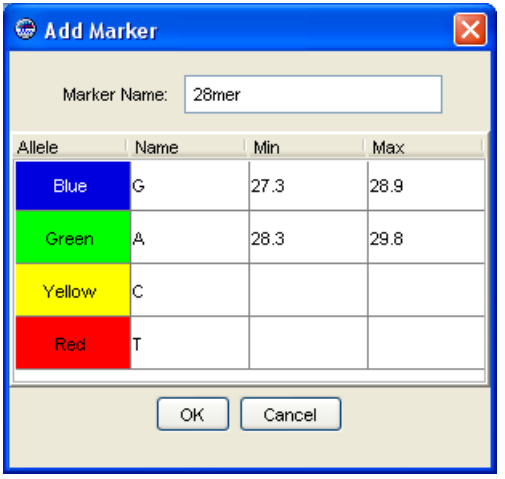



7. Zoom out to view other allele peaks by clicking  (**View ▸ Full View**).

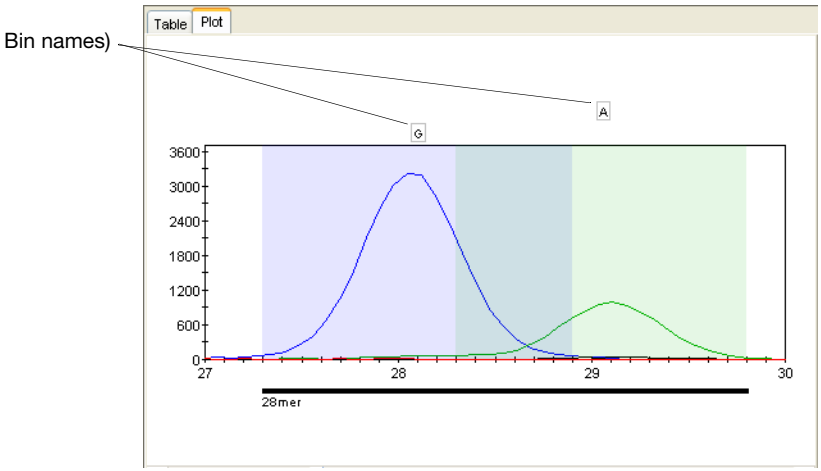
8. Zoom in on the blue and green peaks (a heterozygote allele located at approximately 28 basepairs).




9. Repeat steps 5 through 6 to create another marker with the following information:



The resulting marker and bins are saved to the SNaPshot 6plex Panel and they should appear as below:



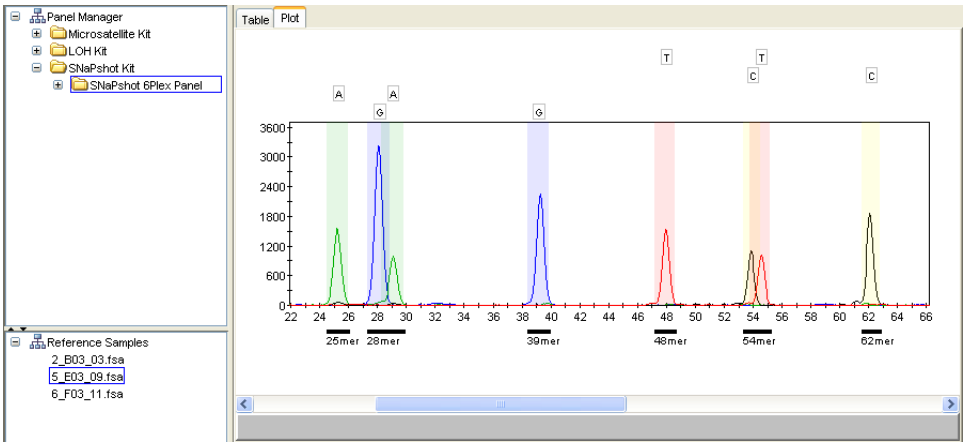

**Note:** The GeneMapper Software automatically calculates the marker size range based on the size ranges you provide for the bins.

**10.** Create markers and bins for the four additional markers (39mer, 48mer, 54mer, and 62mer) by repeating the above steps and using the bin colors, names, and ranges shown below.

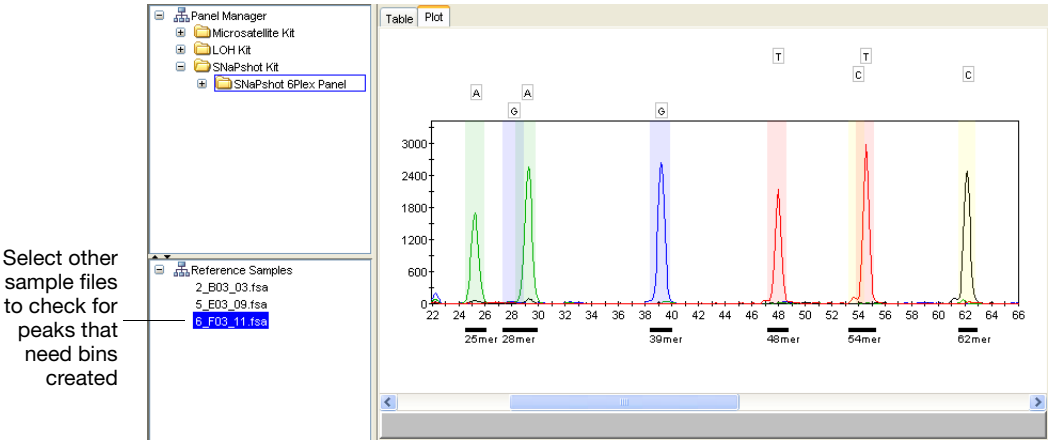
| Marker Name | Allele Color | Allele Name | Min | Max |
|---|---|---|---|---|
| 39mer | Blue | G | 38.4 | 39.9 |
| 48mer | Red | T | 47.2 | 48.6 |
| 54mer | Yellow | C | 53.3 | 54.5 |
| | Red | T | 53.8 | 55.2 |
| 62mer | Yellow | C | 61.5 | 62.8 |

**Note:** As a general rule, bin ranges should be approximately 1.5 bp.

Your finished plot should look similar to the plot below:




**11.** In the lower Navigation Pane, select the other two reference sample files you added to the SNaPshot 6plex Panel (2_B03_03.fsa and 6_F03_11.fsa). In the Plot tab, notice how the peaks in each sample file fit into the bins you created from the 5_E03_09.fsa reference sample file.




Select other sample files to check for peaks that need bins created

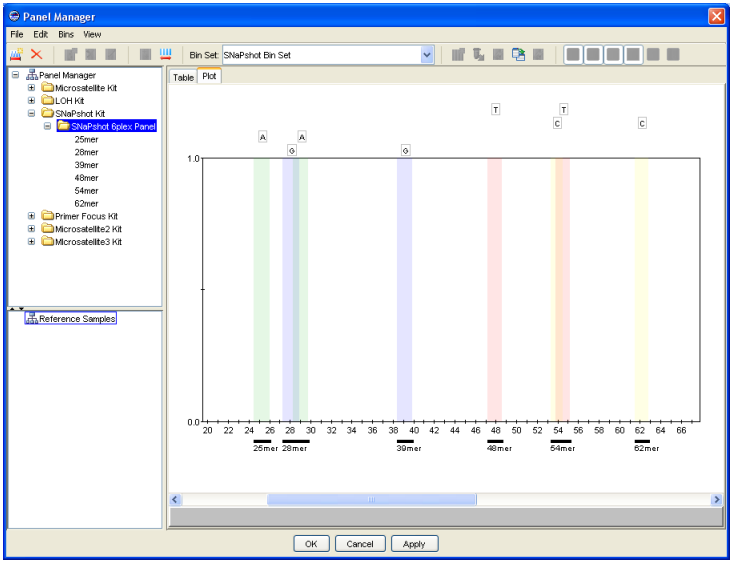
**IMPORTANT!** Typically, each reference sample file would contain peaks that do *not* have bins created from the previous sample file. You would need to repeat steps 4 through 7 to create bins for these peaks.

## Reviewing the Markers and Bins

**To review marker and bin information:**

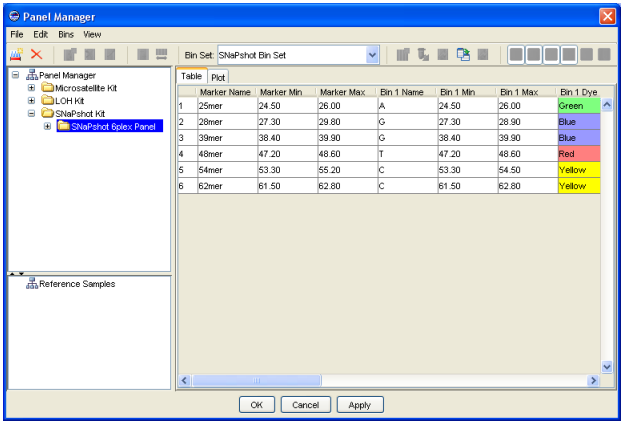
1. In the Panel Manager, select the **SNaPshot 6plex Panel** in the upper half of the Navigation Pane, select **Reference Samples** in the lower half of the Navigation Pane, then select the **Plot** tab.

   The panel displays with markers denoted on the lower x-axis and bins denoted on the upper x-axis.




2. In the Panel Manager, select the **Table** tab.

   The marker and bin information display in a table format.



## Accepting the Bin Set

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

## Editing Bins and Markers (Optional)

To complete the experiment in this guide, you do *not* need to add, edit, or delete any markers or bins. However, you may wish to test these functions by opening the Panel Manager, then selecting the SNaPshot Kit and SNaPshot 6plex Panel.

**IMPORTANT!** If you edit or delete any markers or bins, 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 Plot tab (lower x-axis) or Table tab (row).
2. Click  (**Bins** ▸ **Add Bin**).
3. In the Edit SNP Marker dialog box, type a **Name**, **Min**, and **Max** for the bin, then click **OK**.

### Editing a Bin

1. Select the bin in the Plot tab (upper x-axis).
2. Click  (**Bins** ▸ **Edit Bin**) or right-click the bin, then select **Edit SNP Marker**.
3. In the Edit SNP Marker dialog box, edit the **Name**, **Min**, and **Max** for the bin, then click **OK**.

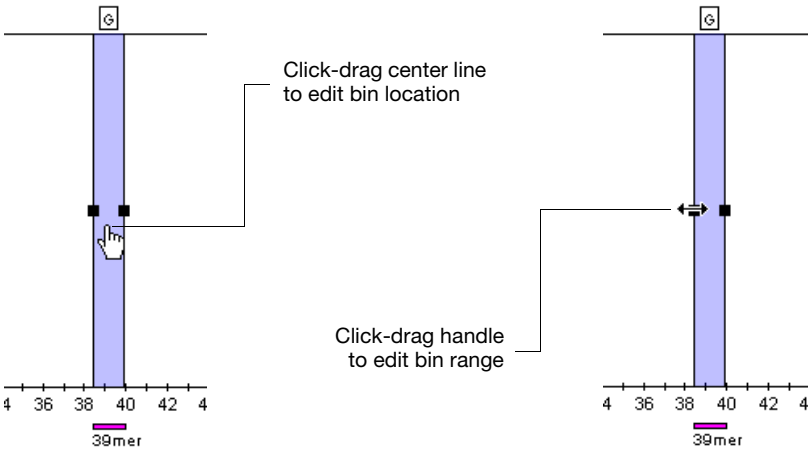
or

1. Select the **Table** tab.
2. Edit any of the following information:
   - Bin Name
   - Bin Min
   - Bin Max

### Editing a Bin Graphically

1. Select the bin in the Plot tab (upper x-axis).
2. Click-drag the blue center line that defines the bin location.
3. Click-drag the left of right handles that define the bin offsets (range).

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




### Deleting a Bin

To delete a bin from a marker:

- In the Plot tab or Table tab, select the bin, then click  (**Bins ▸ Delete Bin**)

  or

- In the Plot tab, select the bin (upper x-axis), right-click the bin, then select **Delete Bin**.

### Editing a Marker

1. Select the marker in the Plot tab (lower x-axis).
2. Click  (**Bins ▸ Edit SNP Marker**) or right-click the marker, then select **Edit SNP Marker**.
3. In the Edit SNP Marker dialog box, edit the **Marker Name**, then click **OK**.

or

In the Table tab, edit the Marker Name.

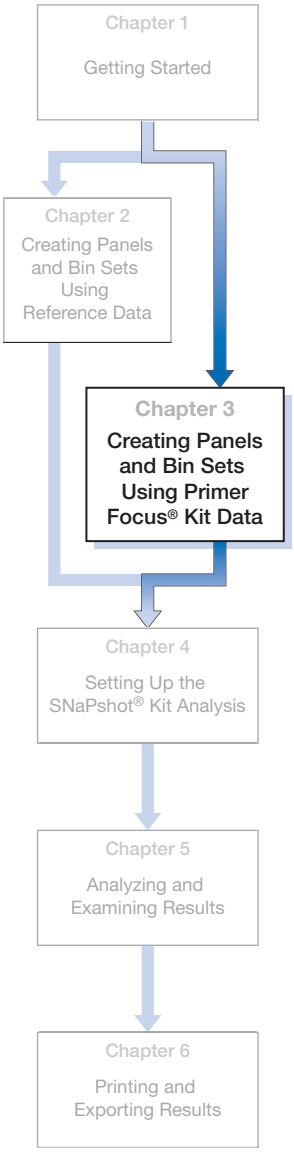
### Deleting a Marker from a Panel

To delete a marker from a panel, in the Plot tab select the marker (lower x-axis), right-click the marker, then select **Delete Marker**.

## Next Steps

Set up the SNaPshot analysis as described in Chapter 4.

# 3 Creating Panels and Bin Sets Using Primer Focus® Kit Data




This chapter includes:


- Creating a New Project and Adding Primer Focus® Kit Sample Files . . . . . 44
- Setting Analysis Parameters and Table Settings . . . . . 46
- Performing a Sizing-Only Analysis on the Project . . . . . 49
- Creating a Kit, Panel, Markers, Bin Set, and Bins . . . . . 58


**IMPORTANT!** Follow the instructions in this chapter only if you want to learn how to *automatically* create markers and bins (allele definitions) for your project by using Primer Focus kit data and the Auto Panel feature.

If you want to learn how to learn how to *manually* create markers and bins (allele definitions) for your project using reference data, follow the instructions in Chapter 2 instead.

# Creating a New Project and Adding Primer Focus® Kit Sample Files

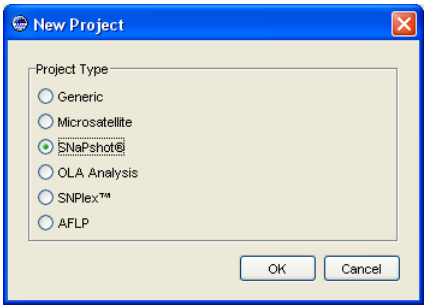
## Overview

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**).



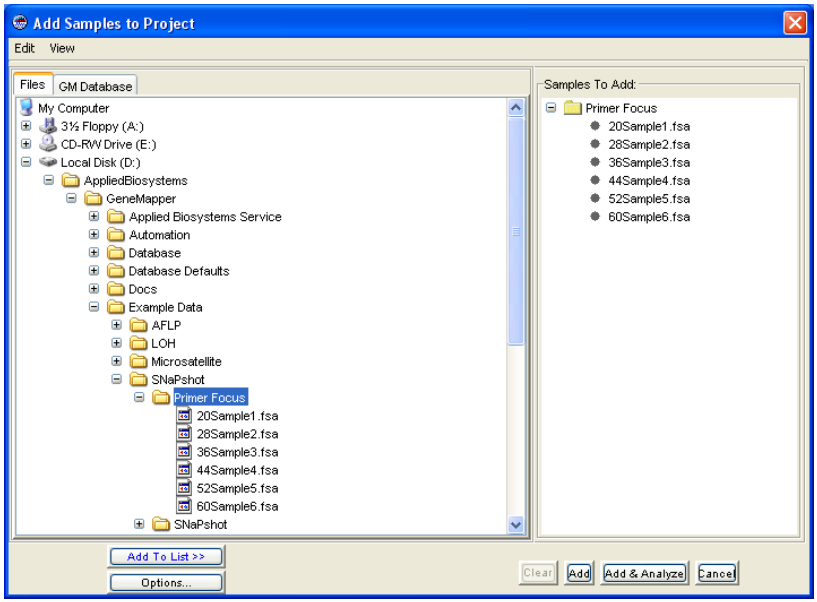

2. In the New Project dialog box, select **SNaPshot**, then click **OK**.
3. From the GeneMapper window, click (**File ▸ Add Samples to Project**).
4. In the Add Samples to Project dialog box, in the Files tab, navigate to:

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

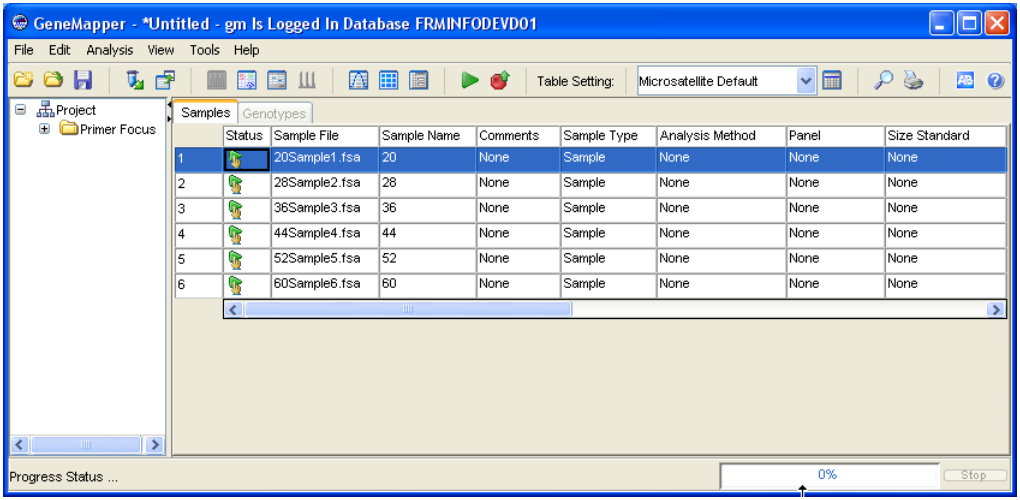
   **Note:** The above location will vary depending on the installation of the GeneMapper® Software. The default installation is the D drive.

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

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




The six sample files from the Primer Focus 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 46.

# Setting Analysis Parameters and Table Settings

## Overview

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

Analysis parameters include:

- Sample Type
- Analysis Method (including bin set)
- Size Standard
- Panel (set of markers)

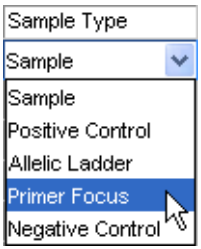
You set analysis parameters that determine the peak detection, sizing, and genotyping algorithms used by the GeneMapper® Software to analyze all sample files in a project.

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 **Sample Type** column, then select **Primer Focus** from the drop-down list.




3. Click the first row in the **Analysis Method** column, then select S**NaPshot Default** from the drop-down list.

4. Leave the Panel column set to None.

   **Note:** Selecting a panel is optional. If you do not select a panel, the GeneMapper Software can size the data, but not genotype it. Because you are creating this project to do a sizing-only analysis of reference data for use in creating panels and bin sets, you will not select a panel as part of your analysis parameters.

5. Select the first row in the **Size Standard** column, then select **GS120LIZ** from the drop-down list. (This was the size standard run with the samples.)

6. 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.

| Sample Type | Analysis Method | Panel | Size Standard |
|---|---|---|---|
| Primer Focus | SNaPshot Default | None | GS120LIZ |
| Sample | None | None | None |
| Sample | None | None | None |
| Sample | None | None | None |

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

## Selecting Table Setting

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

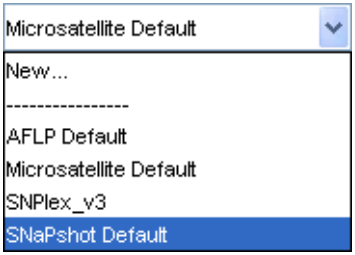


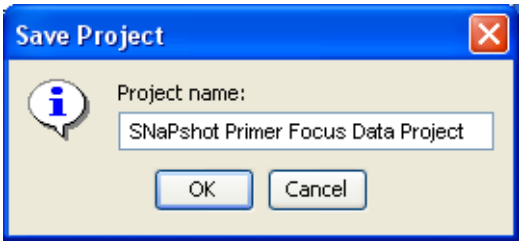

Table Settings control the information displayed in the Samples tab and Genotypes tab after analysis. SNaPshot Default is one of the default Table Settings provided with the GeneMapper Software.

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 **SNaPshot Primer Focus Data Project**, then click **OK**.




SNaPshot Primer Focus Data Project appears in the title bar of the GeneMapper window.

## Next Steps

Perform a sizing-only analysis on the project as described on page 49.

# Performing a Sizing-Only Analysis on the Project

## Overview

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

To perform the sizing-only 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

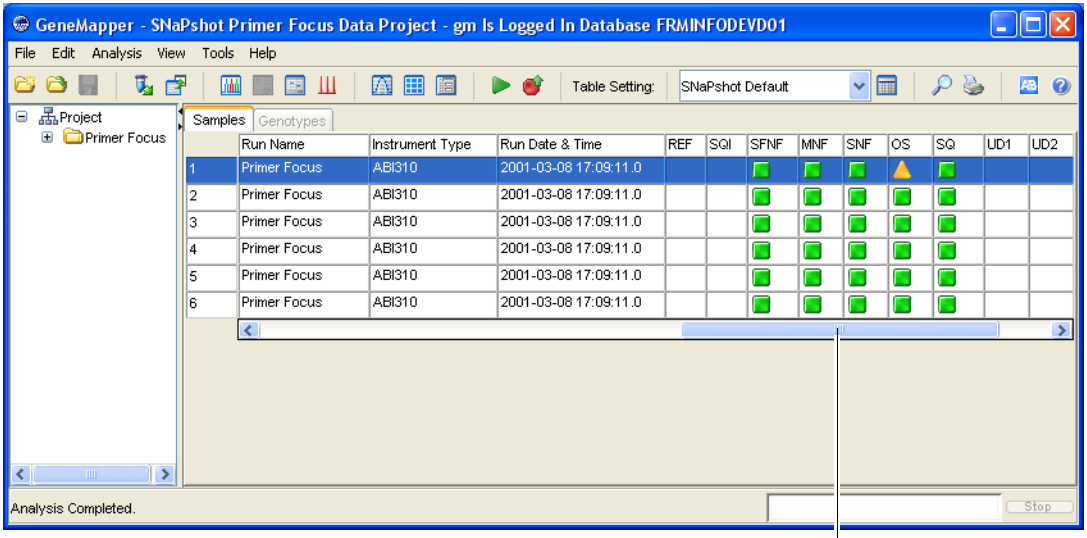
Click  (**Analysis** ▸ **Analyze**).

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). Most of 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 51.

**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**).

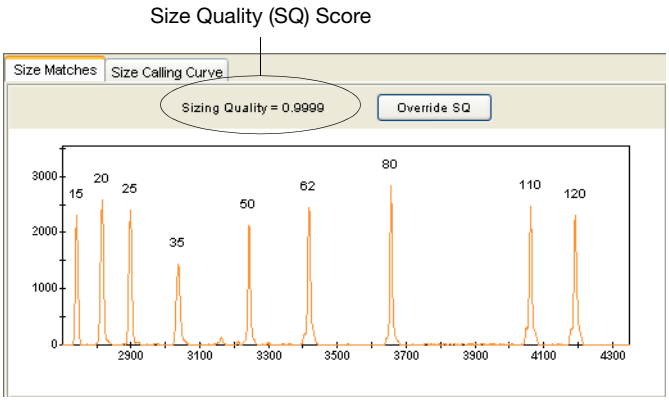



**Figure 3-1 Size Match Editor – Size Matches tab**

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 3-1) 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 you own data you may notice the Sizing Quality is less and the SQ displays (Check) or (Low Quality). For troubleshooting help, see Table 3-1 on page 53.

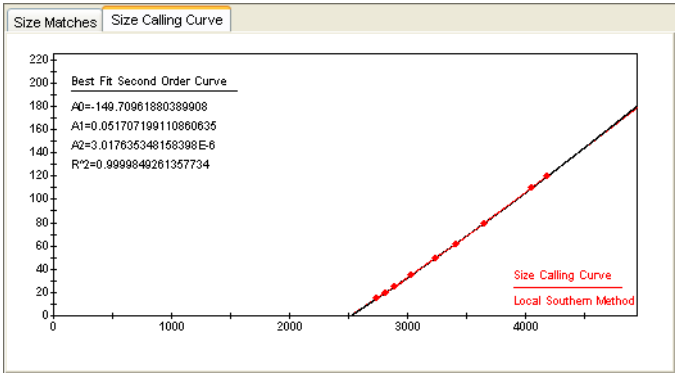
5. Determine if all peaks in the size standard are present and labeled correctly.

   If you followed instructions in this guide, all peaks are present and labeled correctly as shown in Figure 3-1.

   However, when analyzing you own data you may find some size standards peaks to be incorrectly labeled or missing. For troubleshooting help, see Table 3-1 on page 53.

   *GeneMapper® Software Reference and Troubleshooting Guide*.

6. 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.




7. Select another sample from the left pane of the Size Match Editor, then repeat steps 3 through 6.

8. Click **OK** to close the Size Match Editor.

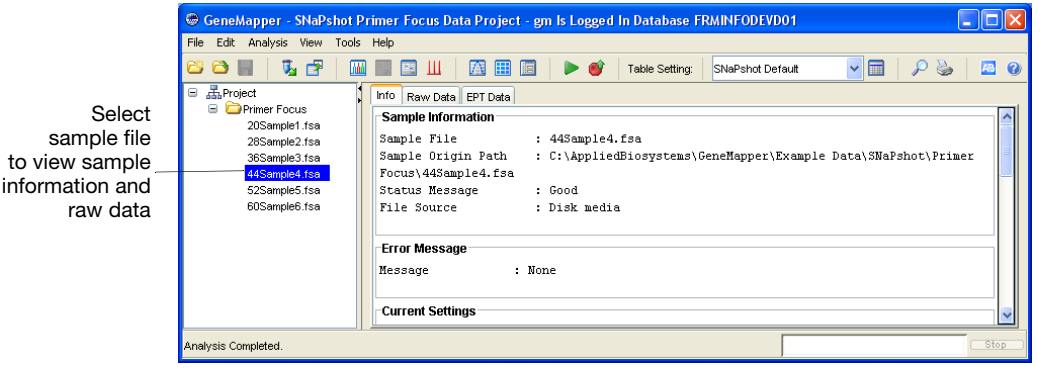
**Table 3-1 Troubleshooting the size standard**

| 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 3-1). 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 *GeneMapper® Software Online Help*. |
| Some size standard peaks are not present. | Create a custom size standard in the software. For more information, see the *GeneMapper® Software Online Help*. |

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.

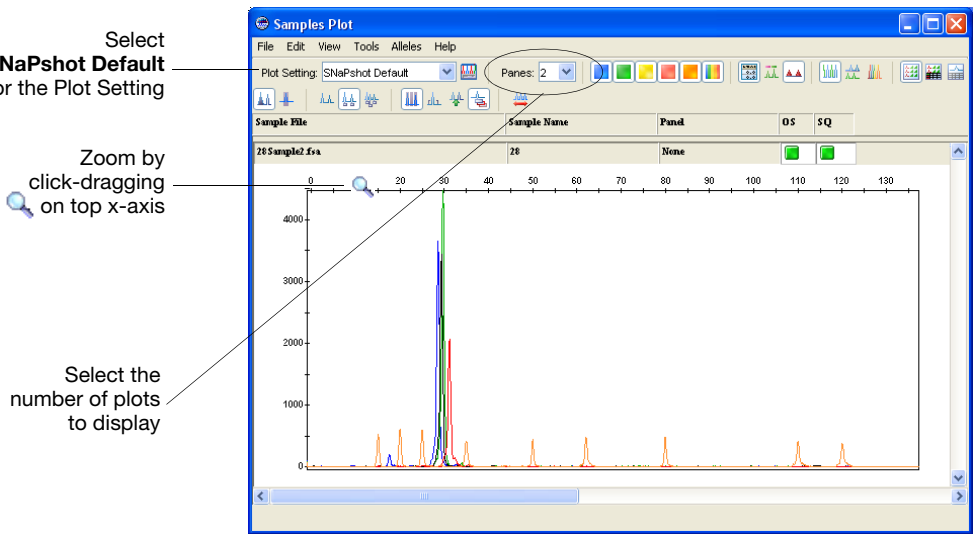



## Viewing Sample Plots

**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.



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

**Note:** Plot Settings control the information displayed in the Samples Plot window after analysis. SNaPshot 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.<br>or<br>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.<br>or<br>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.<br>or<br>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 3-2 on page 57 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 kit, panel, markers, bin set, and bins as described on page 58.

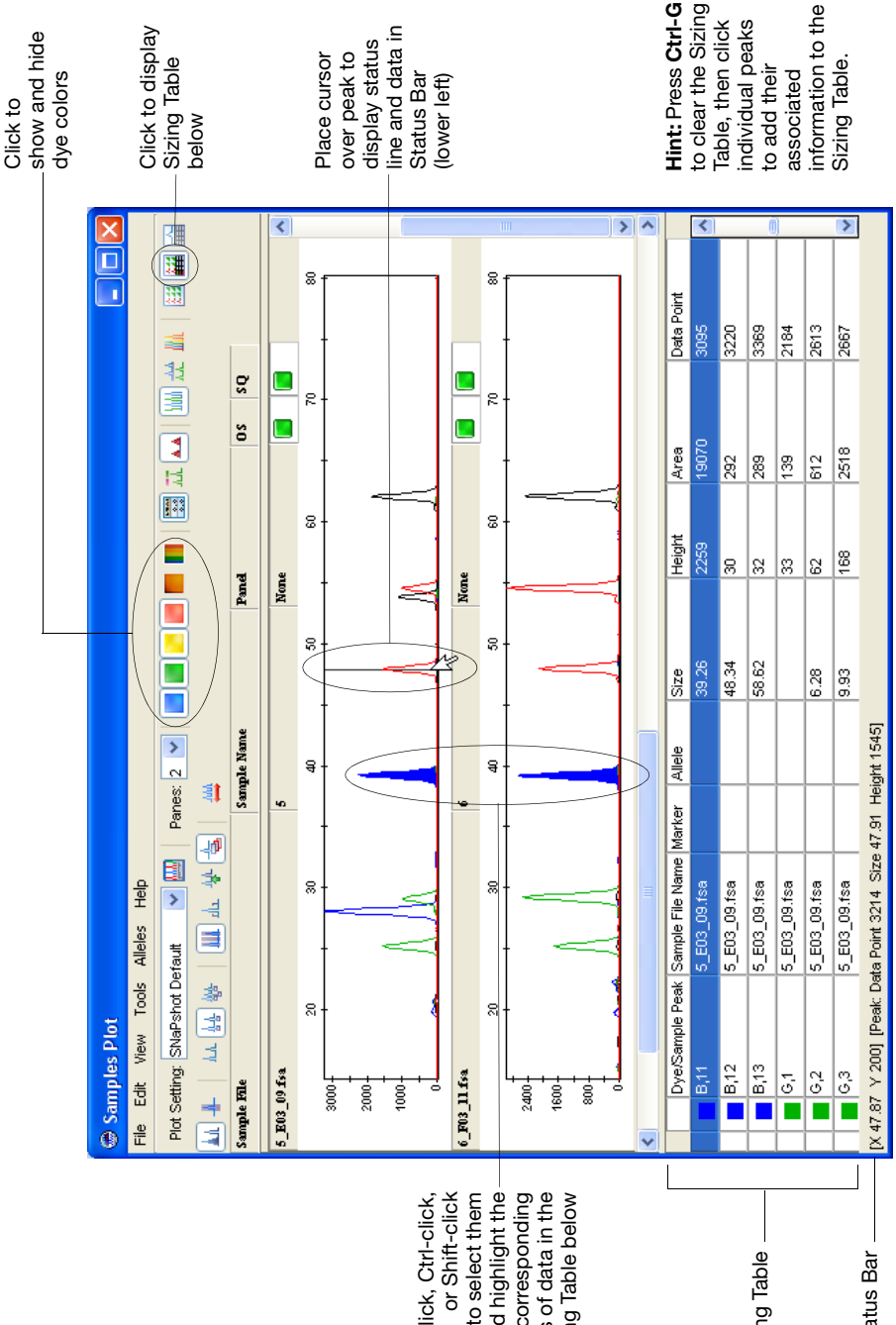



**Figure 3-2 Examining and comparing data from different sample files in the Samples Plot**

# Creating a Kit, Panel, Markers, Bin Set, and Bins

## 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)

**Note:** In this guide, you will learn how to create or generate panels and markers. However, you can also import panels (text files) that contain marker information. For more information on importing panels, see the *GeneMapper® Software Online Help*.

You also use the Panel Manager to create:

- **Bin Set** – A collection of bins
- **Bin** – An allele definition; a fragment size (bp) and dye color

Before you create a bin set, you must select a kit. You can create only one bin set in a SNP kit. The bin set can then be associated with any panels in that SNP kit.

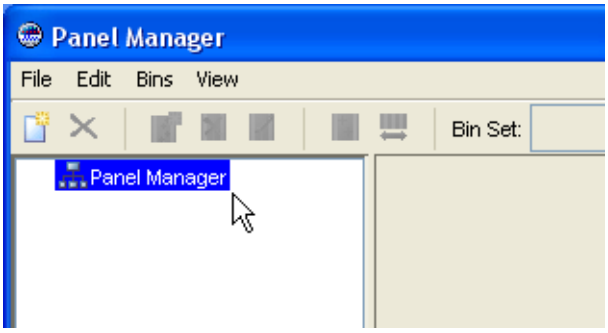
Before you create or generate bins, you must select a panel and a bin set. The bins will be associated with markers in the selected panel and will be stored in the selected bin set.

**Note:** In this chapter, you will learn how to automatically create bins using Primer Focus kit data and the Auto Panel feature. However, you can also manually create bins, or import bin sets (text files) that contain bin information. For more information in manually creating bins, see Chapter 2, "Creating Panels and Bin Sets Using Reference Data." For more information on importing bin sets or creating bins manually, see the *GeneMapper® Software Online Help*.

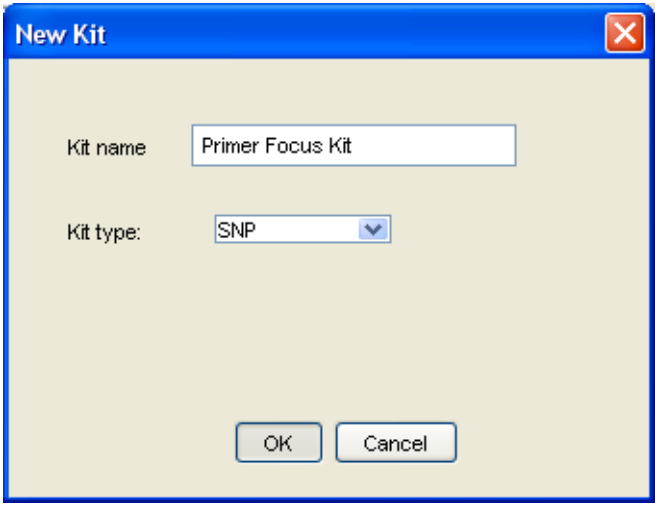
## Creating a Kit

**To create a kit:**

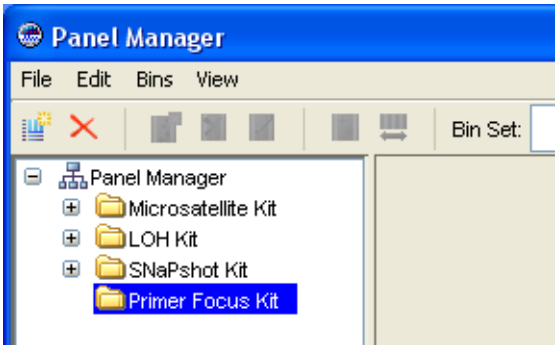
1. Open the Panel Manager by clicking (**Tools ▸ Panel Manager**).
2. Select **Panel Manager** at the top of the Navigation Pane (left side), then click (**File ▸ New Kit**).




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



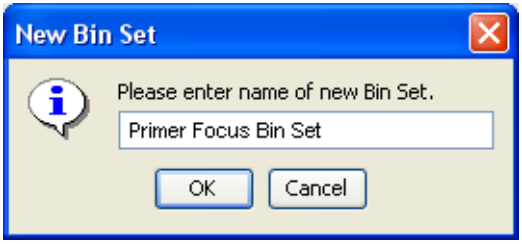
Primer Focus Kit appears in the Navigation Pane (left side).




## Creating a Bin Set

**To create a bin set:**

1. In the Navigation Pane (left), select the **Primer Focus Kit** you created on page 59.
2. Click  (**Bins ▸ New Bin Set**).
3. In the New Bin Set dialog box, type **Primer Focus Bin Set** for the Bin Set Name, then click **OK**.

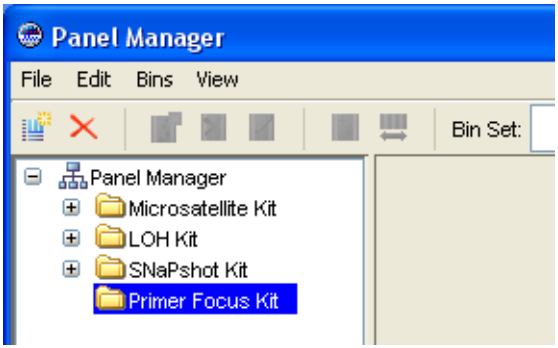



The Primer Focus Bin Set is added to the Bin Set drop-down list at the top of the Panel Manager. The Primer Focus Bin Set can now be associated with any panels added to the Primer Focus Kit.

## Adding Primer Focus Kit Data to the Kit

**To add Primer Focus kit reference data to the Primer Focus Kit:**

1. In the Navigation Pane (left), select the **Primer Focus Kit** you created on page 59.



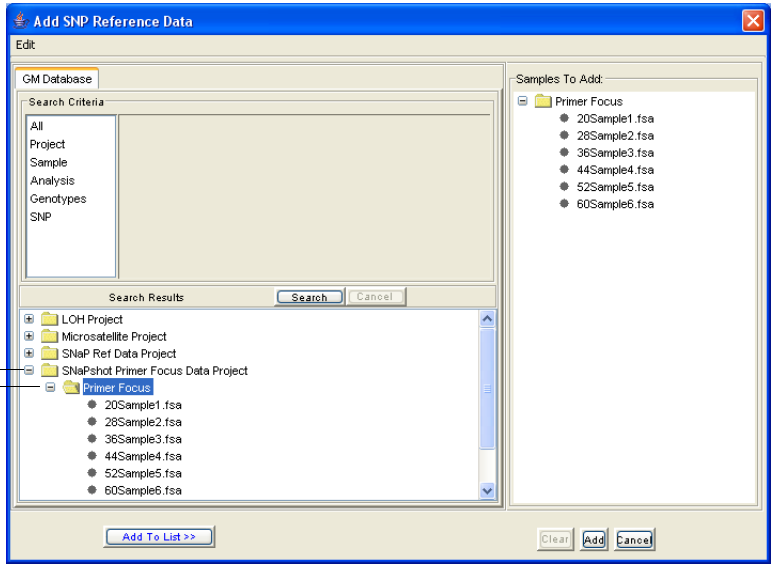

2. Click  (**Bins ▸ Add Reference Data**).

   The Add SNP Reference Data dialog box opens displaying all projects containing Primer Focus kit samples in the lower left pane.

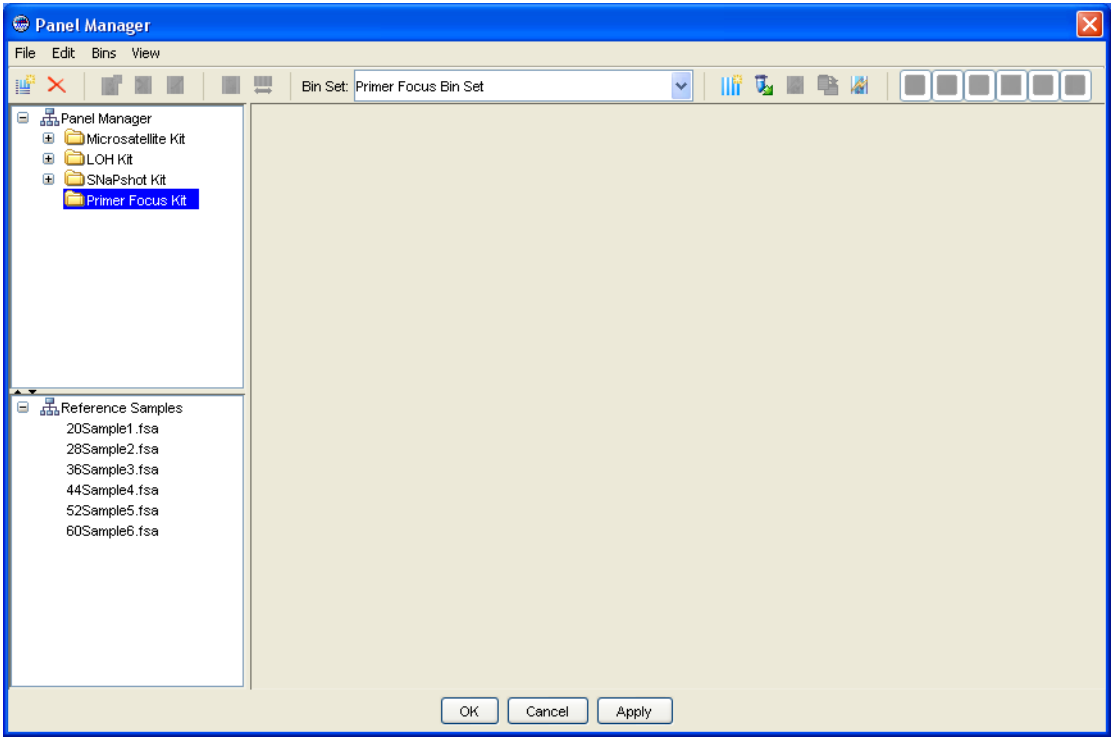
3. Expand the **SNaPshot Primer Focus Data Project**, select the **Primer Focus** folder, click **Add to List**, then click **Add**.

Click ⊞ to expand SNaPshot Primer Focus Data Project created on page 44

Select Primer Focus folder



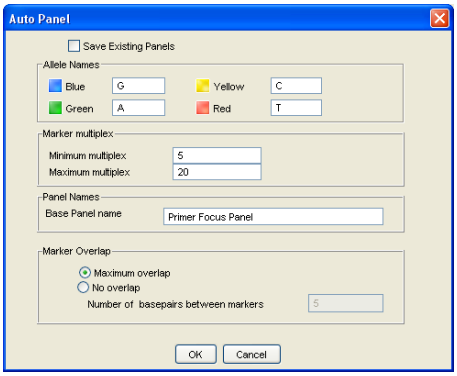

All six Primer Focus kit sample files in the SNaPshot Primer Focus Data Project are added as reference samples to the Primer Focus Kit, and they appear in the lower half of the Navigation Pane in the Panel Manager.


Panel Manager
File
Edit
Bins
View
Bin Set:
Primer Focus Bin Set
Panel Manager
Microsatellite Kit
LOH Kit
SNaPshot Kit
Primer Focus Kit
Reference Samples
20Sample1.fsa
28Sample2.fsa
36Sample3.fsa
44Sample4.fsa
52Sample5.fsa
60Sample6.fsa
OK
Cancel
Apply

## Generating a Panel, Markers, and Bins Using Auto Panel

**To create a panel, markers, and bins using the Auto Panel feature:**

1. In the Navigation Pane (left), select the **Primer Focus Kit** you created on page 59.
2. Click  (**Bins ▸ Auto Panel**).




3. In the Auto Panel dialog box, edit the following sections:
   - **Allele Names** – Leave the default settings, which are the ddNTP base names (G, A, C, T). These allele names will be used to name the bins generated from the Primer Focus data.

     **Note:** The marker names will be based on the Primer Focus kit sample file name.

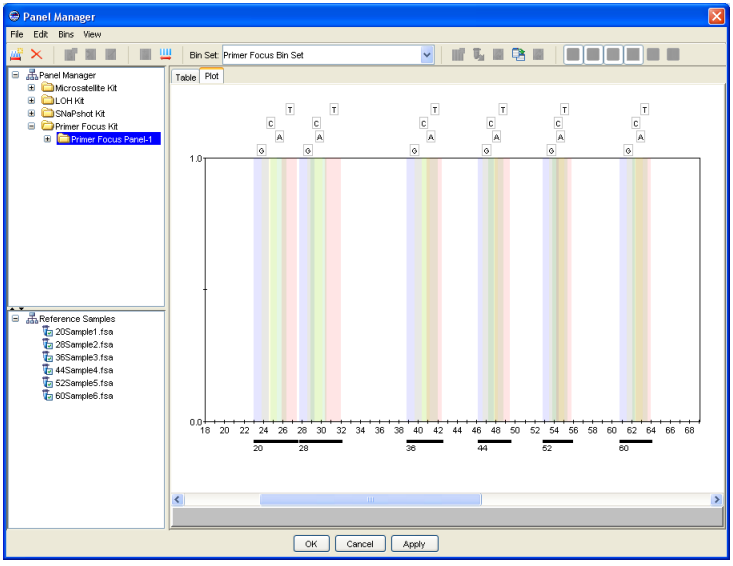
   - **Marker Multiplex** – Leave the default settings of 5 and 20. These are the minimum and maximum number of markers the Auto Panel feature will create per panel.
   - **Panel Names** – Type **Primer Focus Panel**. This is the base name for the panels. If the software needs to create multiple panels (based on the Marker Multiplex settings), the panel names will be Primer Focus Panel_1, Primer Focus Panel_2, and so on.
   - **Marker Overlap** – Select **Maximum Overlap**. Maximum overlap allows markers in the same panel to overlap as long as bins of the same color do not overlap.
   - **Save Existing Panels** – Do *not* select this check box. Selecting this option allows you to add markers to pre-existing panels.
4. Click **OK** to Auto Panel the Primer Focus kit data.

## Reviewing the Markers and Bins

**To review the panels created by the Auto Panel feature:**

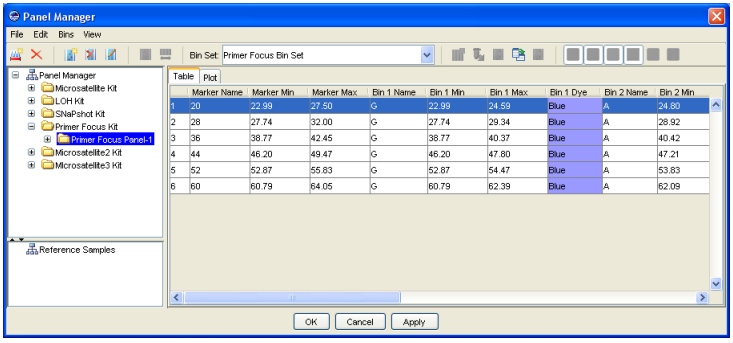
1. In the Navigation Pane, expand the **Primer Focus Kit**.
2. Select the **Primer Focus Panel-1**.
3. Select the **Plot** tab.

   The panel displays with markers denoted on the lower x-axis and bins denoted on the upper x-axis. In the lower Navigation Pane, a green check appears next to Primer Focus kit reference samples used to create the panel.




4. Select the **Table** tab.

   The marker and bin information display in a table format.



## Accepting the Panel and Bin Set

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

## Editing Bins and Markers (Optional)

To complete the experiment in this guide, you do *not* need to add, edit, or delete any markers or bins. However, you may wish to test these functions by opening the Panel Manager, then selecting the Primer Focus Kit and Primer Focus Panel-1.

**IMPORTANT!** If you edit or delete any markers or bins, 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 Plot tab (lower x-axis) or Table tab (row).
2. Click  (**Bins** ▸ **Add Bin**).
3. In the Edit SNP Marker dialog box, type a **Name**, **Min**, and **Max** for the bin, then click **OK**.

### Editing a Bin

1. Select the bin in the Plot tab (upper x-axis).
2. Click  (**Bins** ▸ **Edit Bin**) or right-click the bin, then select **Edit SNP Marker**.
3. In the Edit SNP Marker dialog box, edit the **Name**, **Min**, and **Max** for the bin, then click **OK**.

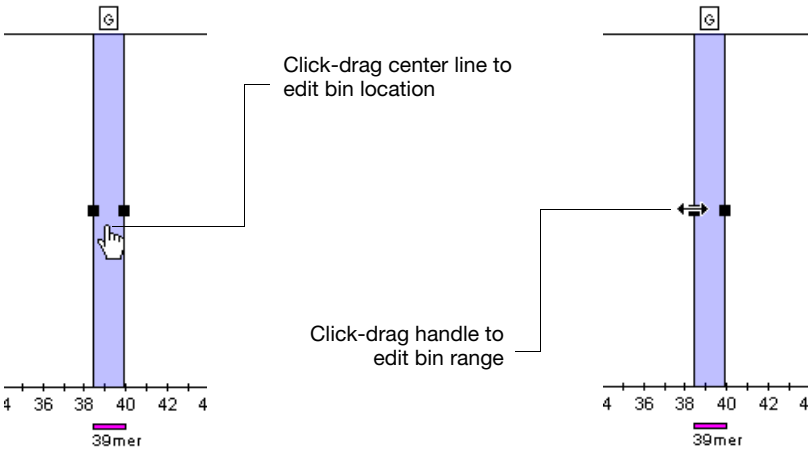
or

1. Select the **Table** tab.
2. Edit any of the following information:
   - Bin Name
   - Bin Min
   - Bin Max

### Editing a Bin Graphically

1. Select the bin in the Plot tab (upper x-axis).
2. Click-drag the blue center line that defines the bin location.
3. Click-drag the left of right handles that define the bin offsets (range).

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




### Deleting a Bin

To delete a bin from a marker:

- In the Plot tab or Table tab, select the bin, then click  (**Bins ▸ Delete Bin**)

  or

- In the Plot tab, select the bin (upper x-axis), right-click the bin, then select **Delete Bin**.

### Editing a Marker

1. Select the marker in the Plot tab (lower x-axis).
2. Click  (**Bins ▸ Edit SNP Marker**) or right-click the marker, then select **Edit SNP Marker**.
3. In the Edit SNP Marker dialog box, edit the **Marker Name**, then click **OK**.

or

In the Table tab, edit the Marker Name.

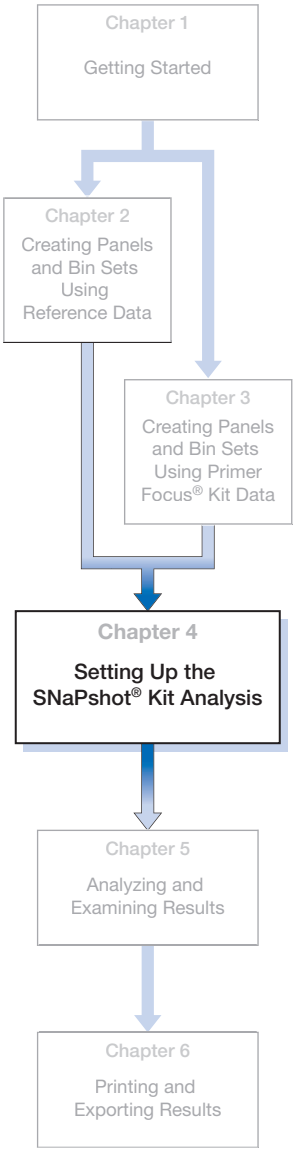
### Deleting a Marker from a Panel

To delete a marker from a panel, in the Plot tab select the marker (lower x-axis), right-click the marker, then select **Delete Marker**.

## Next Steps

Set up the SNaPshot kit analysis as described in Chapter 4.

# 4 Setting Up the SNaPshot® Kit Analysis




This chapter includes:

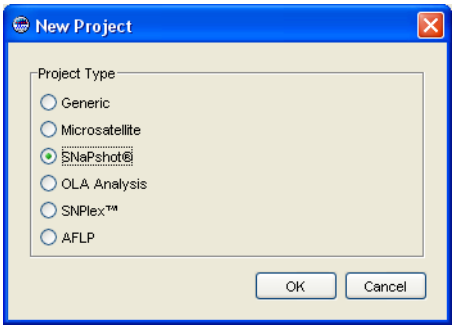

- Creating a New Project and Adding Sample Files . . . . . 70
- Setting Analysis Parameters and Table Settings for the Project . . . . . 72

# Creating a New Project and Adding Sample Files

## Creating a New Project and Adding Sample Files

**To create a new project and add sample files:**

1. Click (**File ▸ New Project**).



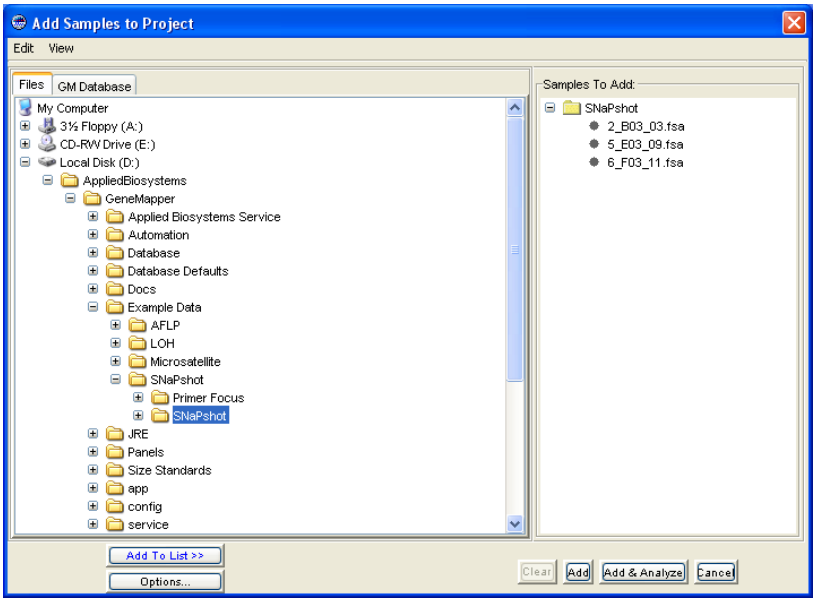

2. In the New Project dialog box, select **SNaPshot**, then click **OK**.
3. From the GeneMapper window, click (**File ▸ Add Samples to Project**).
4. In the Add Samples to Project dialog box, in the Files tab, navigate to:

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

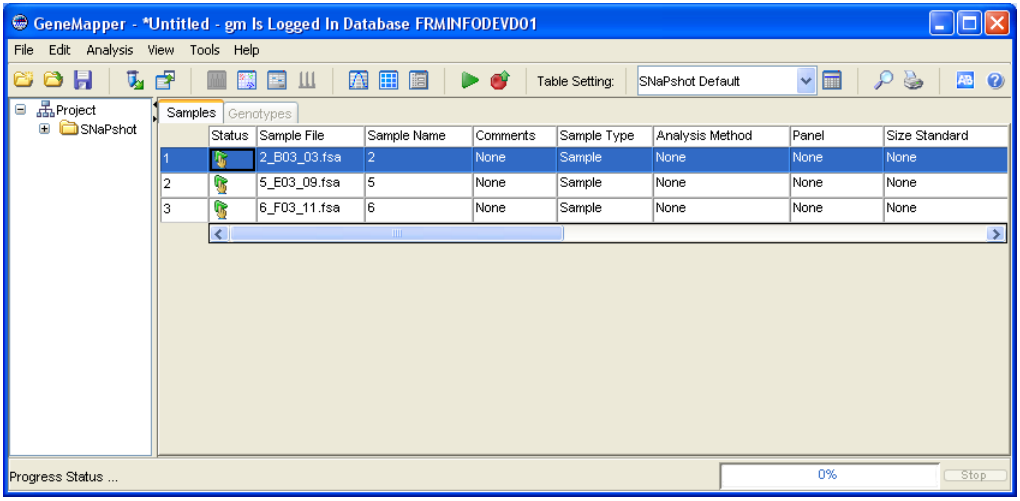
   **Note:** The above location will vary depending on the installation of the GeneMapper® Software. The default installation is the D drive.

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

   **Note:** For this guide you added all three sample files in the SNaPshot 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 SNaPshot Data 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 72.

# 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

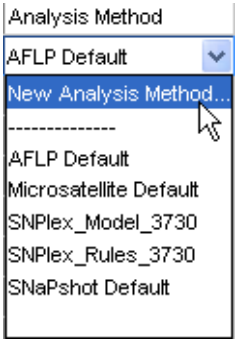
You set analysis parameters that determine the peak detection, sizing, and genotyping algorithms used by the GeneMapper® Software to analyze all sample files in a project.

Display settings include Table Settings and Plot Settings.

## Setting Analysis Parameters

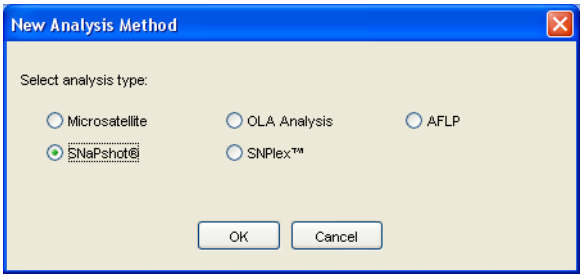
**To set analysis parameters for the project:**

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.

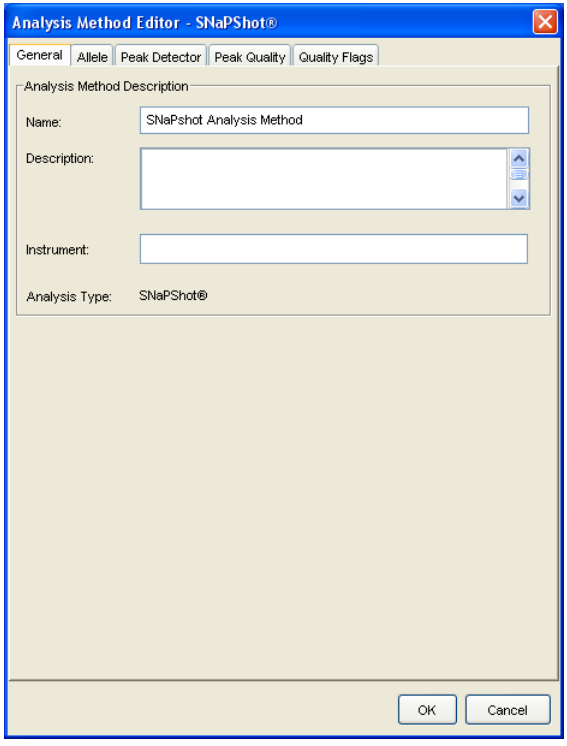



**Note:** You can also create a new analysis method from the Analysis Method tab in the GeneMapper Manager.

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

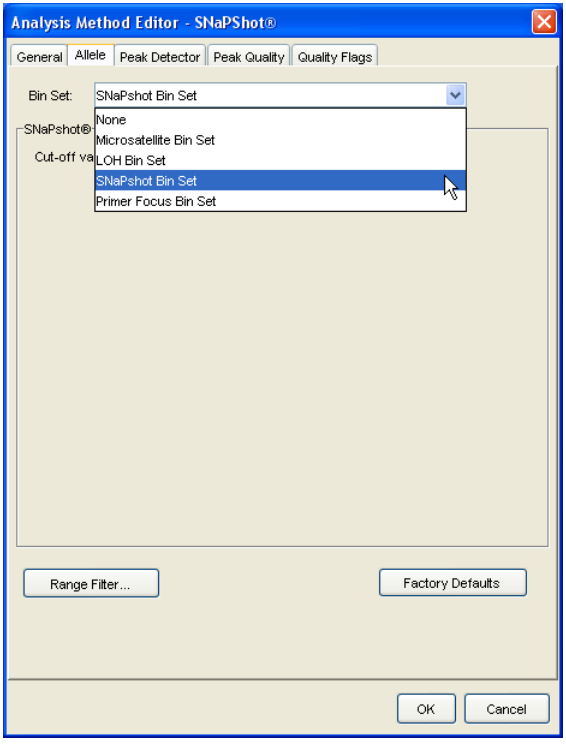



4. In the Analysis Method Editor dialog box, select and edit the five tabs.
   - **General** – This tab includes reference information about the method. Type **SNaPshot Analysis Method** for the Name. Optionally, type a description and the instrument on which the data was generated.

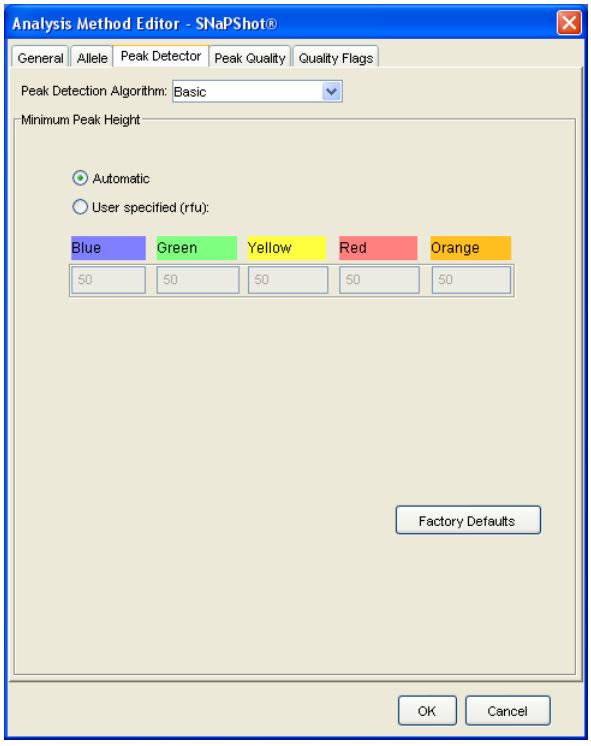


- **Allele** – This tab includes settings that determine allele calling. Select one of the following for the Bin Set:
  - **SNaPshot Bin Set** – Select if you created this bin set using SNaPshot reference data as described in Chapter 2.
  - **Primer Focus Bin Set** – Select if you created this bin set using Primer Focus kit reference data as described in Chapter 3.

  Leave the default values for all other settings.

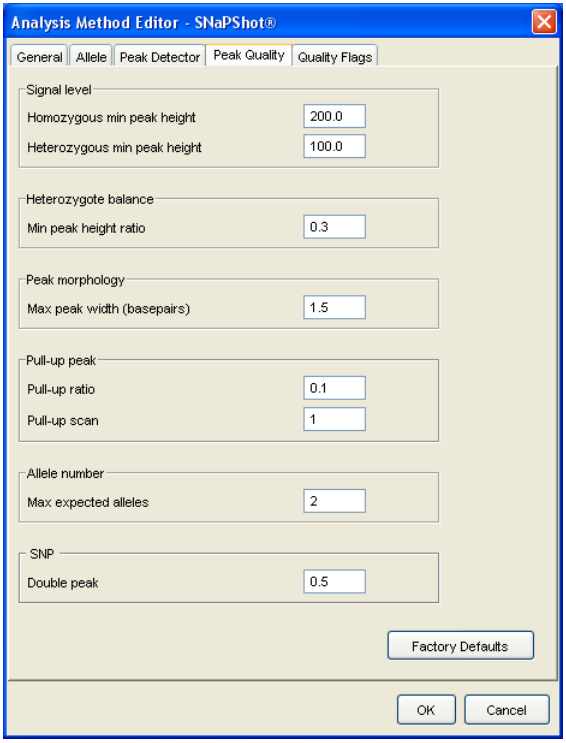


- **Peak Detector** – This tab includes settings that determine peak detection and sizing of peaks. Select **Basic** for the Peak Detection Algorithm. Leave the default values for all other settings.



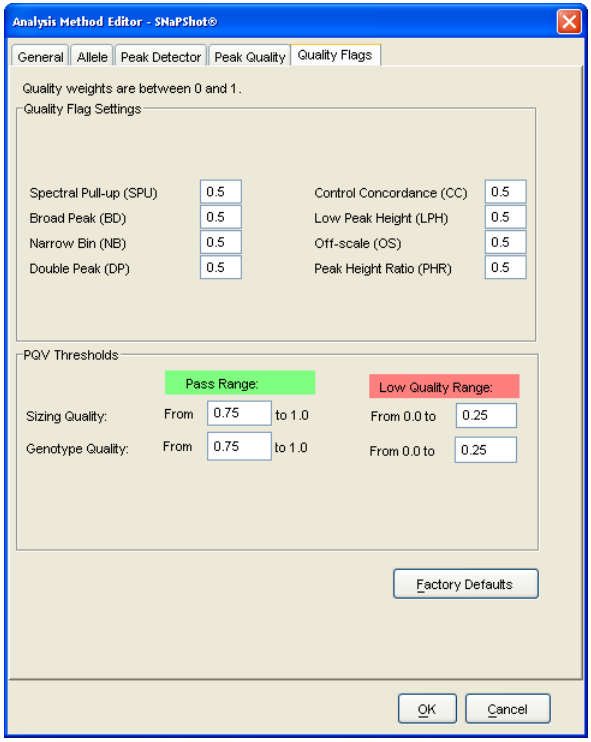
- **Peak Quality** – This tab includes settings that determine when specific PQVs are left green (Pass) or flagged yellow (Check).

  Type **0.3** for the Min peak height ratio. Leave the default values for all other settings.



- **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.

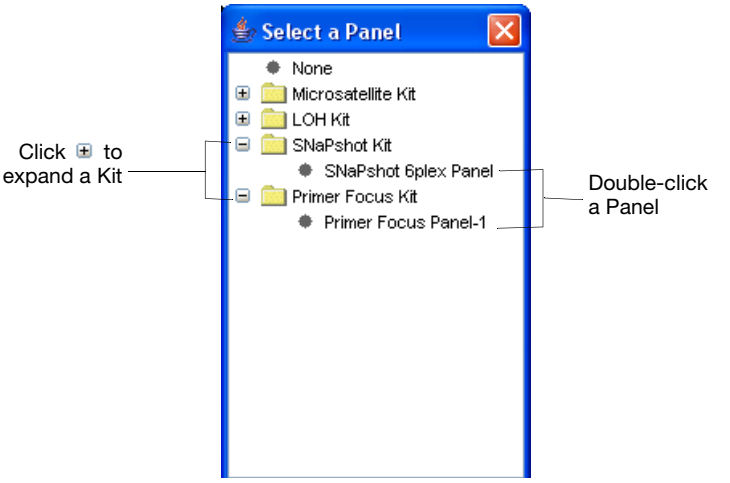
  Leave the default values for all settings.




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

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

6. Select the first row in the **Panel** column. From the Select a Panel dialog box, do one of the following:
   - Expand the **SNaPshot Kit**, then double-click **SNaPshot 6plex Panel**. (If you created this panel using SNaPshot reference data as described in Chapter 2.)
   - Expand the **Primer Focus Kit**, then double-click **Primer Focus Panel-1**. (If you created this panel using Primer Focus kit reference data as described in Chapter 3.)




7. Select the first row in the **Size Standard** column, then select **GS120LIZ** from the drop-down list. (This was the size standard run with the samples.)

8. 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 |
|---|---|---|
| SNaPshot Analysis Method | SNaPshot 6plex Panel | GS120LIZ |
| 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 **SNaPshot Default** from the Table Settings drop-down list.

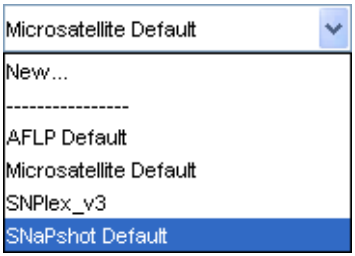


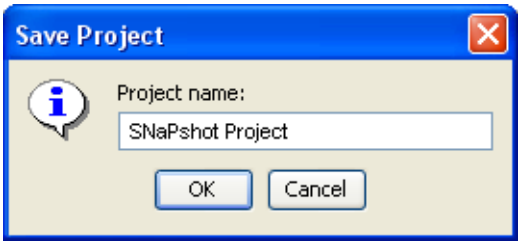

Table Settings control the information displayed in the Samples tab and Genotypes tab after analysis. SNaPshot Default is one of the default Table Settings provided with the GeneMapper Software.

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 **SNaPshot Project**, then click **OK**.

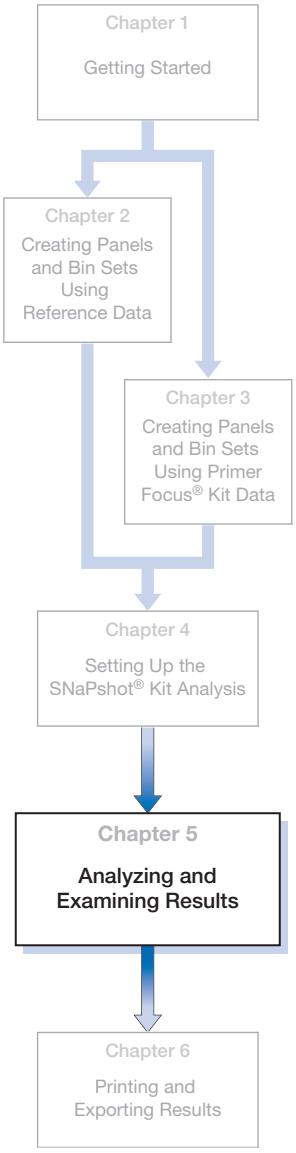



SNaPshot Project appears in the title bar of the GeneMapper window.

## Next Steps

Analyze and examine the data in the SNaPshot project as described in Chapter 5.

# 5 Analyzing and Examining Results




This chapter includes:


- Analyzing the Project . . . . . 82
- Examining the Results . . . . . 82

# Analyzing the Project

**Overview**

Now that you created a project with analysis parameters that include both a panel and an analysis method that 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.

**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

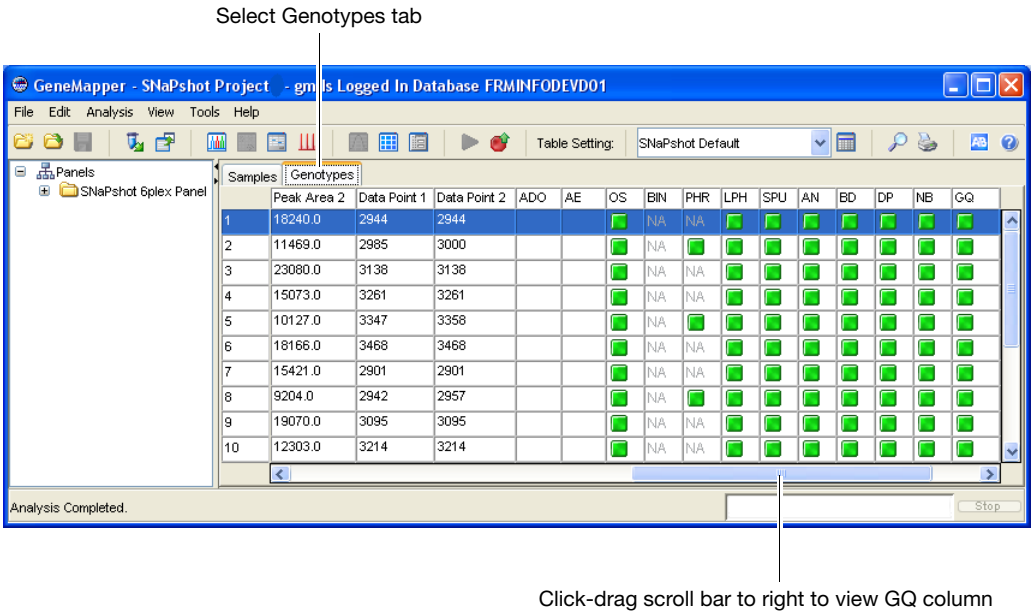
**Overview**

To examine the sizing and genotyping results:

- Review the SQ, associated PQVs, size standard, sample information, and samples plots (described on pages 18 through 24 or pages 50 through 56.)
- Review the GQ and contributing PQVs (page 83)
- Review the allele calls for each sample (page 84)
- View genotypes plots (page 84)
- Examine the data in the Genotypes Plot window (page 86)

## Reviewing the GQ and PQVs

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




If you followed the procedures and used the example data indicated in this guide, the GQ for each sample should be (Pass). The Process Quality Values (PQVs) that contribute the GQ (AN, BD, BIN, DP, LPH, NB, OS, PHR, and SPU) 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, DP, LPH, NB, OS, PHR, and SPU) 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.

## Reviewing the Allele Calls

To review the allele calls for each marker in each sample, select the Genotypes tab, then view the Allele 1 and Allele 2 columns.

View allele calls for each marker

Samples | Genotypes

| | Sample File | Sample Name | Panel | Marker | Allele 1 | Allele 2 | Size 1 | Size 2 | Height 1 |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 2_B03_03.fsa | 2 | SNaPshot 6plex Panel | 54mer | C | T | 53.89 | 54.63 | 1343 |
| 6 | 2_B03_03.fsa | 2 | SNaPshot 6plex Panel | 62mer | C | C | 62.07 | 62.07 | 2202 |
| 7 | 5_E03_09.fsa | 5 | SNaPshot 6plex Panel | 25mer | A | A | 25.2 | 25.2 | 1554 |
| 8 | 5_E03_09.fsa | 5 | SNaPshot 6plex Panel | 28mer | G | A | 28.05 | 29.11 | 3220 |
| 9 | 5_E03_09.fsa | 5 | SNaPshot 6plex Panel | 39mer | G | G | 39.26 | 39.26 | 2259 |
| 10 | 5_E03_09.fsa | 5 | SNaPshot 6plex Panel | 48mer | T | T | 47.91 | 47.91 | 1545 |
| 11 | 5_E03_09.fsa | 5 | SNaPshot 6plex Panel | 54mer | C | T | 53.82 | 54.58 | 1096 |
| 12 | 5_E03_09.fsa | 5 | SNaPshot 6plex Panel | 62mer | C | C | 62.07 | 62.07 | 1854 |
| 13 | 6_F03_11.fsa | 6 | SNaPshot 6plex Panel | 25mer | A | A | 25.21 | 25.21 | 1705 |
| 14 | 6_F03_11.fsa | 6 | SNaPshot 6plex Panel | 28mer | A | A | 29.23 | 29.23 | 2548 |

## Viewing Genotype Plots

**To view the genotypes plots of the samples:**

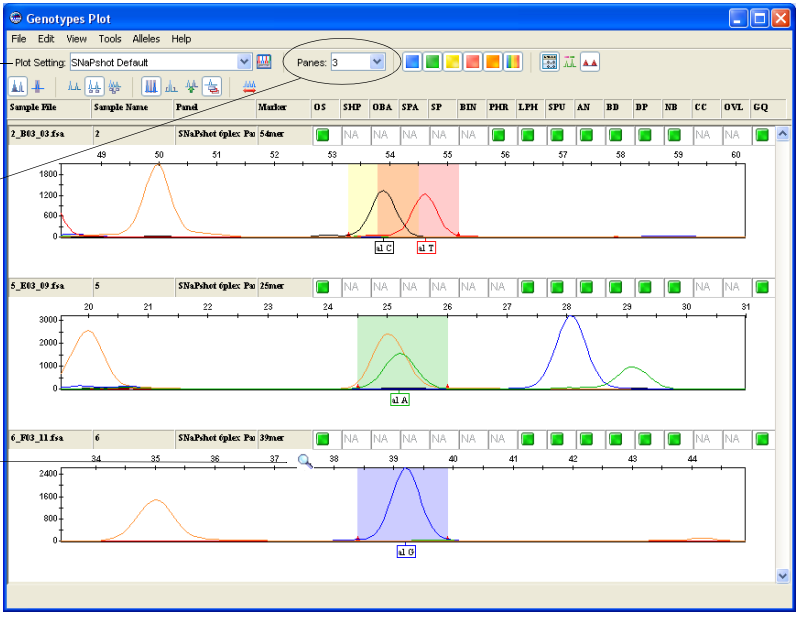
1. Select a sample and marker (row) in the Genotypes tab. To select multiple markers, press and hold **Shift** or **Ctrl**. To select all markers, select **Edit ▸ Select All**.
2. Click (**Analysis ▸ Display Plots**).

   The Genotypes Plot window displays an electropherogram for each selected marker.

Select **SNaPshot Default** for the Plot Setting

Select the number of plots to display

Zoom by click-dragging on top x-axis



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

**Note:** Plot Settings control the information displayed in the Genotypes Plot window after analysis. SNaPshot 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.<br>or<br>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.<br>or<br>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.<br>or<br>Right-click the x-axis or y-axis, then select **Full View**. |

## Examining Data in the Genotypes Plot Window

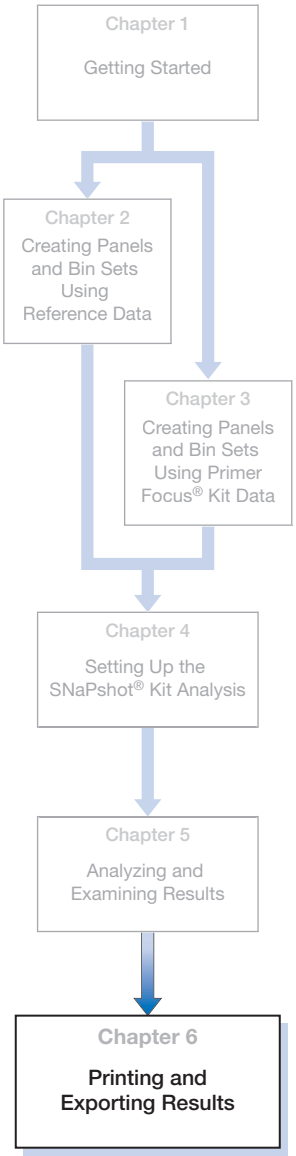
Other tasks you can perform in the Genotypes Plot window include:

- 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.

# 6 Printing and Exporting Results




This chapter includes:


- Printing Results . . . . . 88
- Exporting Results . . . . . 89

# 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*.

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