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Loss of Heterozygosity (LOH) Analysis Getting Started Guide

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Contents

Preface

How to Use This Guide

Purpose of This Guide

The *GeneMapper® Software Version 4.1 LOH Analysis Getting Started Guide* provides brief, step-by-step instructions for sizing, genotyping, and evaluating LOH microsatellite data generated using any of the compatible Applied Biosystems electrophoresis instruments and Data Collection Software. It describes how to troubleshoot, print and export data, and create reports. It is designed to help you quickly learn to use basic functions of the GeneMapper Software.

Audience

This guide is intended for novice GeneMapper Software users.

Assumptions

This guide assumes that:

- You have installed GeneMapper Software version 4.1 as described in the *GeneMapper® Software Version 4.1 Installation and Administration Guide* (PN 4403614).
- You have a working knowledge of the Microsoft® Windows® 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 4.1 Installation and Administration Guide* (PN 4403614).

How to Obtain More Information

Safety Information

For safety information, see the *GeneMapper® Software Version 4.1 Installation and Administration Guide* (PN 4403614).

Software Warranty and License

For all warranty and licensing information, see the *GeneMapper® Software Version 4.1 Installation and Administration Guide* (PN 4403614).

Related Documentation

The following related documents are shipped with the software:

- ***GeneMapper® Software Version 4.1 Installation and Administration Guide*** (PN 4403614) – Provides procedures for installing, securing, and maintaining version 4.1 of the GeneMapper Software.
- ***GeneMapper® Software Version 4.1 Getting Started Guides*** for microsatellite analysis (PN 4403672), loss of hetereozygosity (LOH) analysis (PN 4403621), AFLP® system analysis (PN 4403620), SNaPshot® kit analysis (PN 4403618), and SNPlex™ system analysis (PN 4403617) – 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 4.1 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 4.1 Quick Reference Guide*** (PN 4403615) – Provides workflows for specific analysis types and lists instruments, software, and analysis applications compatible with the GeneMapper Software.
- ***GeneMapper® Software Version 4.1 Reference and Troubleshooting Guide*** (PN 4403673) – 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 4.1 Documentation CD*.

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

Obtaining Information from Online Help

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

Send Us Your Comments

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

techpubs@appliedbiosystems.com

How to Obtain Support

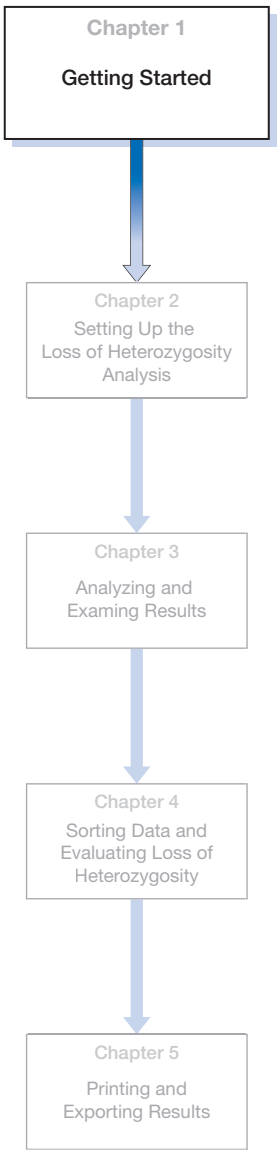
For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

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

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

1 Getting Started



This chapter includes:

About LOH Microsatellite Analyses

Microsatellite Markers

Microsatellite markers, also known as short tandem repeats (STRs), are polymorphic DNA loci consisting of a repeated nucleotide sequence. The repeat sequence can be from 2 to 7 base pairs long. The number of repeat units varies in a population, thereby creating multiple alleles for a microsatellite locus.

Microsatellite Analysis

In a typical microsatellite analysis, microsatellite loci are amplified by PCR using fluorescently labeled forward and unlabeled reverse primers. The PCR amplicons are separated by size using electrophoresis; then the dye labeled products are identified by fluorescence detection. You can then use the GeneMapper Software to size and genotype the alleles.

What is LOH?

In the two-hit model used to describe inactivation of tumor suppressor genes (TSGs), the first mutation or "hit" results in a heterozygous state for the TSG with one wild-type and one mutant allele. If this is followed by a second "hit," in which all or part of the chromosome that contains the wild-type allele of the TSG is deleted, the chances of tumorigenesis increase. This phenomenon is known as Loss of Heterozygosity or LOH. While this is a rare event, it occurs more frequently in familial forms of cancer, in which a mutation of one of the TSG alleles is inherited.

LOH Microsatellite Analysis

An LOH microsatellite analysis is the screening of tumor samples for LOH using microsatellite markers. Because LOH can be caused by deletion of genomic DNA regions containing the wild-type copy of a TSG, researchers can use microsatellite markers to screen samples for LOH.

A typical LOH assay compares amplified microsatellite markers from the suspected cancerous tissue to the same markers from the healthy tissue, both from the same individual. The healthy tissue should show two alleles for a given heterozygous microsatellite marker. If a given tumor sample has undergone LOH, it will be evident by a decrease in peak height of one of the two alleles (relative to the healthy allele peak heights) (Figure 1-1). The reason the tumor sample shows a decrease in peak height (instead of an absence of the peak) is that during isolation, the tumor sample is contaminated with healthy cells, thus introducing some wild-type DNA into the tumor specimen.

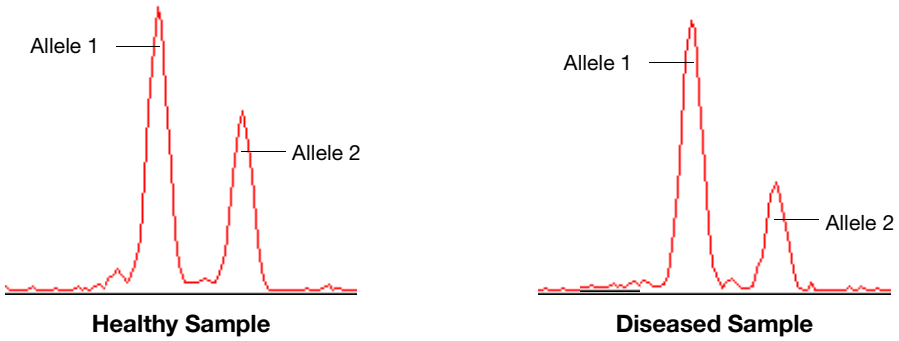


Figure 1-1 Diseased sample shows loss in peak height of Allele 2

After performing a microsatellite analysis, you can use the Report Manager in the GeneMapper® Software to calculate and compare peak height ratios of microsatellite alleles between healthy and diseased tissues, as well as identify and flag LOH candidates based on set peak-height ratio thresholds. You can alter these thresholds based on the observed level of wild-type DNA contamination in the tumor samples.

Custom Primers

Applied Biosystems provides custom primers for PCR amplification of LOH microsatellite 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 LOH microsatellite analyses, see the *GeneMapper® Software Version 4.1 Quick Reference Guide* (PN 4403615).

About the Example Data

Sample File Naming Conventions

The naming convention for the sample files used in this guide allows you to take advantage of the LOH Default report setting provided with the GeneMapper® Software. Specifically, healthy and tumor samples from the same individual start with the same letter or number, so they list consecutively when sorted by sample file and marker.

> **Example:** 1_Healthy.fsa, 1_Tumor.fsa, 2_Healthy.fsa, 2_Tumor.fsa, and so on

When analyzing your own LOH data, if you want to use the LOH Default report setting, your sample files must be named as described above.

If your LOH data sample files are not named as described above, you can still use the sorting and LOH reporting features in the GeneMapper® Software, but you will have to edit the report setting as described in "Editing or Creating a Custom LOH Report Setting" on page 59. Below is an example naming convention in which healthy and tumor samples from the same individual do *not* list consecutively after being sorted by sample file and marker.

> **Example:** Healthy_1.fsa, Healthy_2.fsa, …,Tumor_2.fsa, Tumor_1.fsa, and so on

Sample File Location

To perform the exercise described in this getting started guide, use the four sample files (.fsa) located on your computer hard drive at:

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

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 PCR-amplified and fluorescently tagged LOH samples on an ABI PRISM® 3100 Genetic Analyzer using the GeneScan™ 500 LIZ® Size Standard.

Marker Information

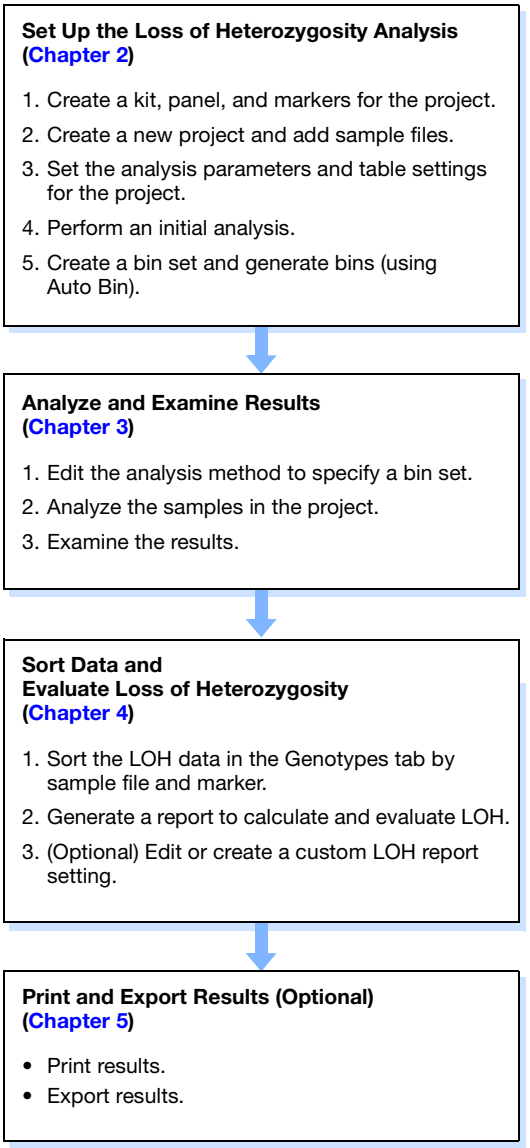
The sample files contain the following six markers.

Marker	Dye Label	Allele Size Range (bp)
R5	Blue	120 – 186
R26	Blue	193 – 295
R14	Blue	300 – 470
R21	Yellow	100 – 160
R24	Yellow	170 – 250
R2	Green	157 – 204

You will use this marker information when creating a panel and markers in Chapter 2, “Setting Up the Loss of Heterozygosity Analysis.”

LOH Microsatellite Analysis Workflow

The following flowchart summarizes the steps for performing an LOH analysis using the GeneMapper® Software:



GeneMapper® Software Terms

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

Starting the Software and Logging In

To start the GeneMapper® Software and log in:

1. Select **Start ▸ All Programs ▸ Applied Biosystems ▸ GeneMapper ▸ GeneMapper 4.1**.
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 LOH microsatellite analysis workflow when analyzing your own sample files. For information on advanced software features, see the *GeneMapper® Software Online Help*.

Alternatives to the Procedures in This Guide

Overview

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

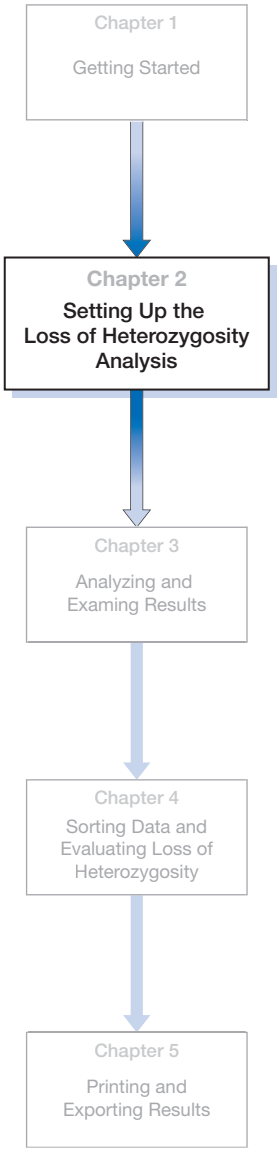
Using Autoanalysis to Set Up Projects

The GeneMapper Software includes an Autoanalysis feature that can eliminate most of the tasks leading up to the analysis of a microsatellite LOH project. Much of Chapter 2, "Setting Up the Loss of Heterozygosity Analysis," explains how to manually create, add samples to, and analyze projects for use in microsatellite LOH projects. When configured for Autoanalysis, the GeneMapper Software can automatically accomplish these tasks by coordinating with the Data Collection Software. For a more detailed explanation of how to use the Autoanalysis feature to set up microsatellite LOH projects, see the *GeneMapper® Software Version 4.1 Installation and Administration Guide* (PN 4403614).

Using the Command Line Interface to Set Up Projects

The GeneMapper Software features a command line interface that can perform most of the major functions of the software. The command line interface can be a useful tool when analyzing microsatellite LOH projects because it automate many of the tasks explained in Chapter 2, "Setting Up the Loss of Heterozygosity Analysis." For a complete description of the command line interface and how it can be used to automate the functions of the GeneMapper Software, see the *GeneMapper® Software Version 4.1 Installation and Administration Guide* (PN 4403614).

2 Setting Up the Loss of Heterozygosity Analysis



This chapter includes:

Overview

In This Chapter

In this chapter you will learn how to:

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

For More Information

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

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

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

Creating a Kit, Panel, and Markers

Overview

You create the following hierarchical objects in the Panel Manager:

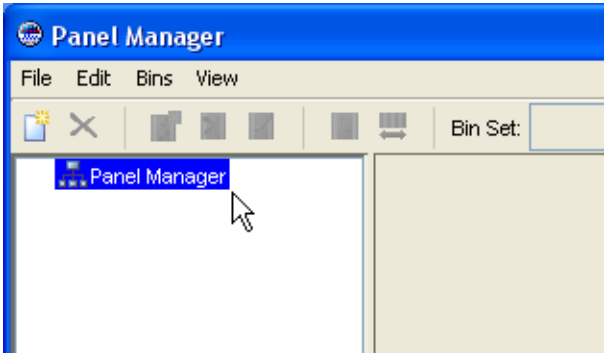
- **Kit** – A group of panels
- **Panel** – A group of markers
- **Marker** – A fragment size range (bp), dye color, and repeat length

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

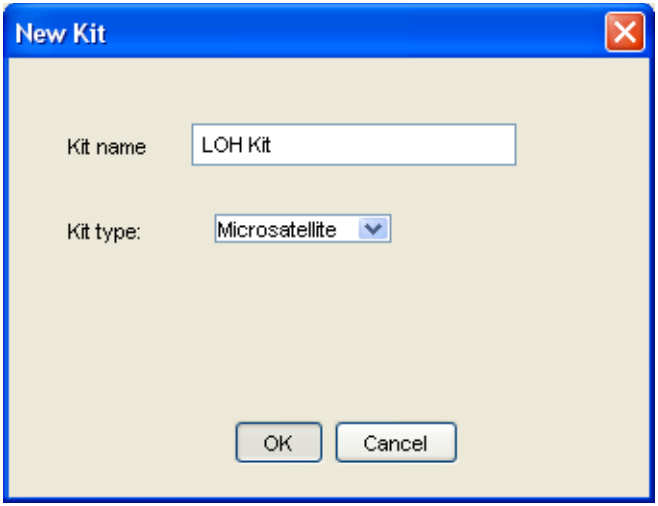
Creating a Kit, Panel, and Markers

To create a kit, panel, and markers:

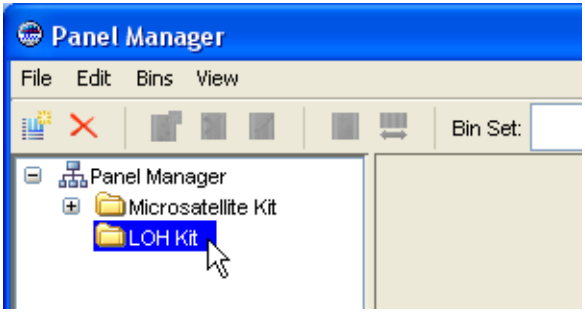
1. Open the Panel Manager by clicking  (**Tools ▸ Panel Manager**).
2. Select **Panel Manager** at the top of the Navigation Pane (left side), then click  (**File ▸ New Kit**).



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



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

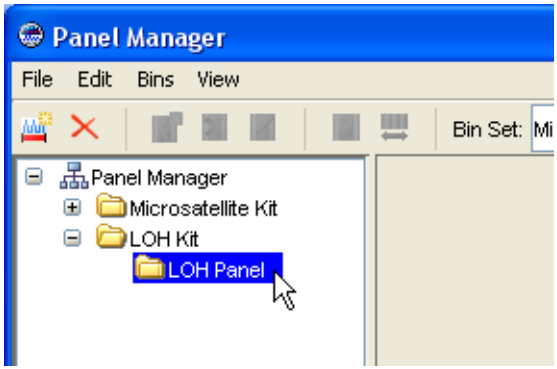


4. Select the **LOH Kit** in the Navigation Pane, then click  (**File ▸ New Panel**).

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

	Panel Name	Comment
1	LOH Panel	none

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



6. Select the **LOH Panel** in the Navigation Pane, then click  (**File ▸ New Marker**).

7. In the right pane of the Panel Manager, type the following marker information:

	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker Repeat
1	R5	blue	120.0	186.0		2

8. Repeat steps 6 through 7 to create the following markers:

Marker	Dye Color	Minimum Size (bp)	Maximum Size (bp)	Marker Repeat
R26	Blue	193	295	2
R14	Blue	300	470	2
R21	Yellow	100	160	2
R24	Yellow	170	250	2
R2	Green	157	204	2

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

Next Steps

Create a new project and add sample files (.fsa) to it as described on page 15.

Creating a New Project and Adding Sample Files

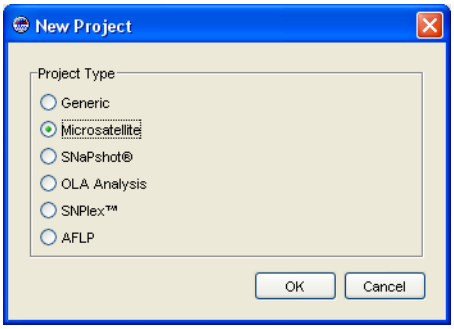
Overview

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

Creating a New Project and Adding Sample Files

To create a new project and add sample files:

1. Click (**File ▸ New Project**).



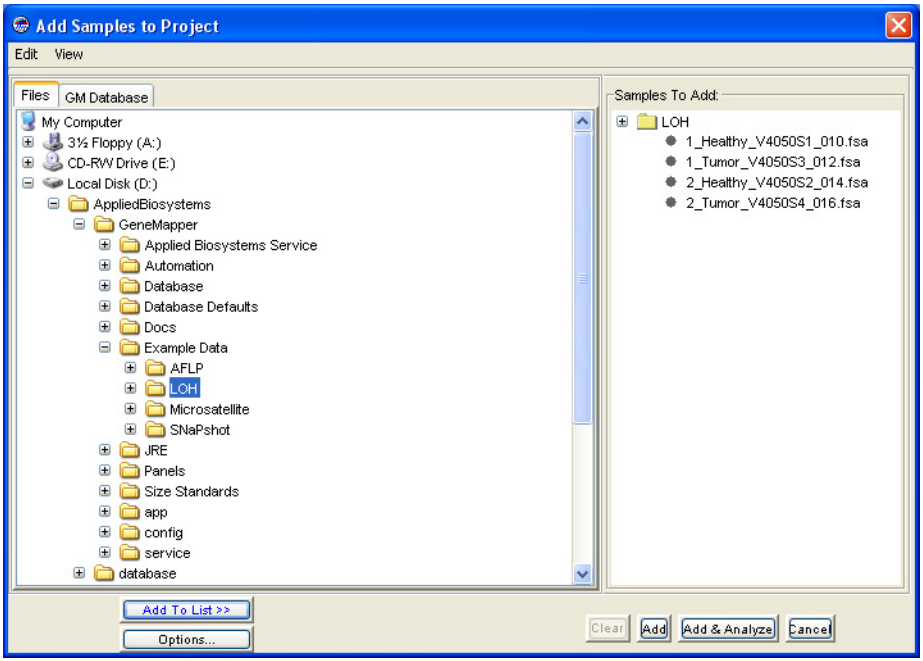
2. In the New Project dialog box, select **Microsatellite**, then click **OK**.
3. From the GeneMapper window, click (**File ▸ Add Samples to Project**).
4. In the Add Samples to Project dialog box, in the Files tab, navigate to:

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

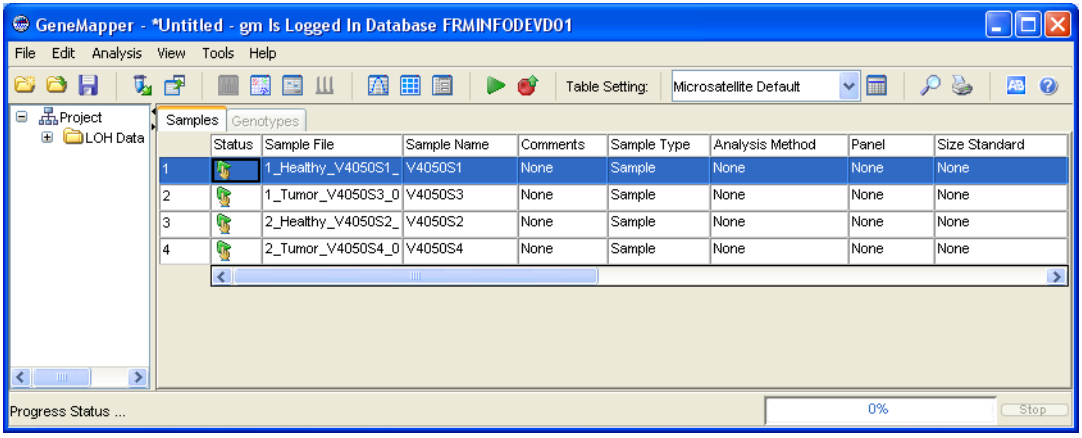
   Note: The above location will vary depending on the installation of the GeneMapper® Software. The default installation is the D drive.

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

   Note: For this guide you added all sample files in the LOH 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 four sample files from the LOH 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 17.

Setting Analysis Parameters and Table Settings for the Project

Overview

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

Analysis parameters include:

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

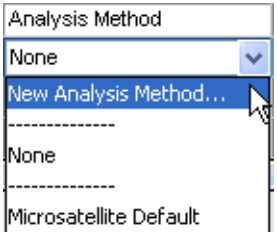
You set analysis parameters that determine the peak detection, sizing, and genotyping algorithms used by the GeneMapper® Software to analyze all sample files in a project.

Display settings include Table Settings and Plot Settings.

Setting Analysis Parameters

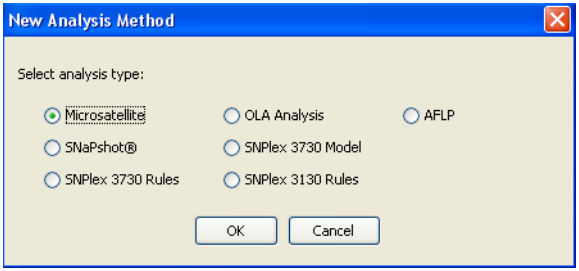
To set analysis parameters for the project:

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

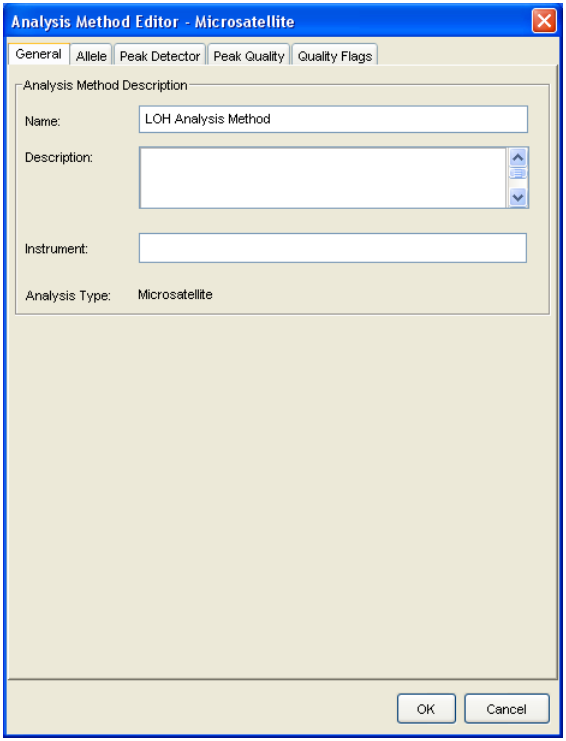


Note: You can also create a new analysis method from the Analysis Method tab in the GeneMapper Manager.

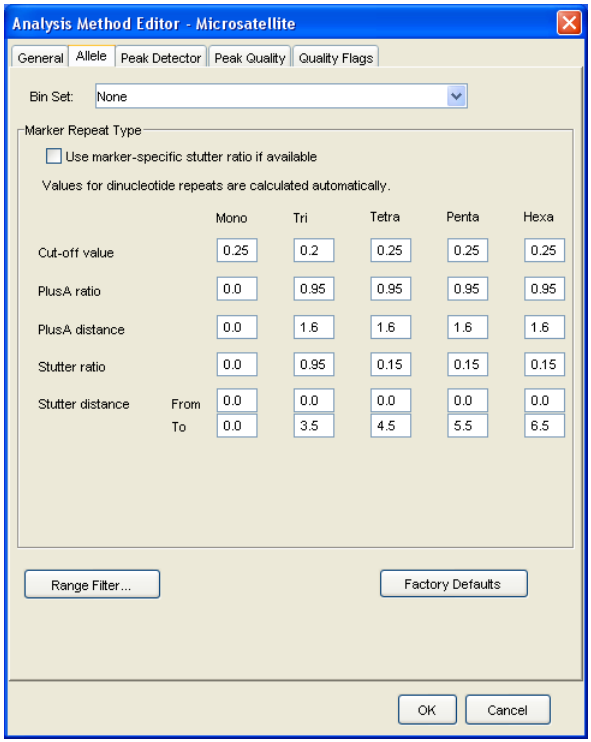
3. In the New Analysis Method dialog box, select **Microsatellite** for Analysis Type, then click **OK**.



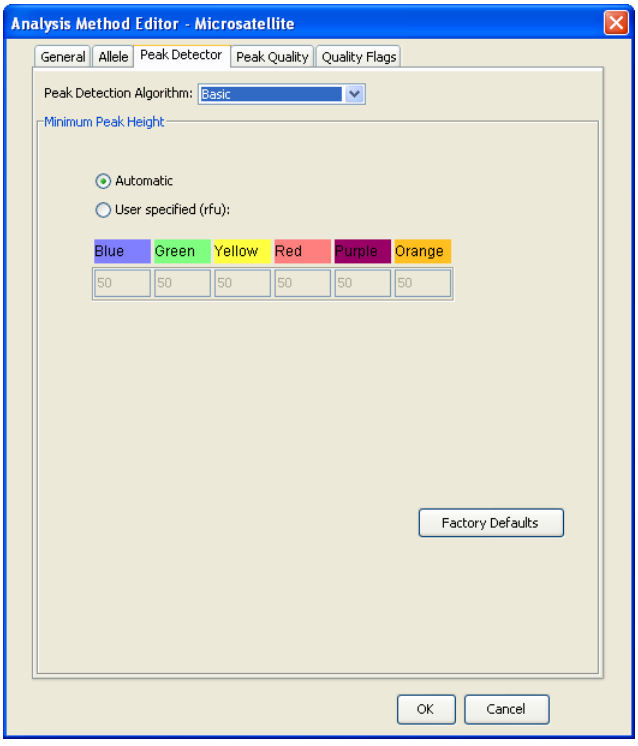
4. In the Analysis Method Editor dialog box, select and edit the five tabs:
   - **General** – This tab includes reference information about the method. Type **LOH Analysis Method** for the Name. Optionally, type a description and the instrument on which the data was generated.



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

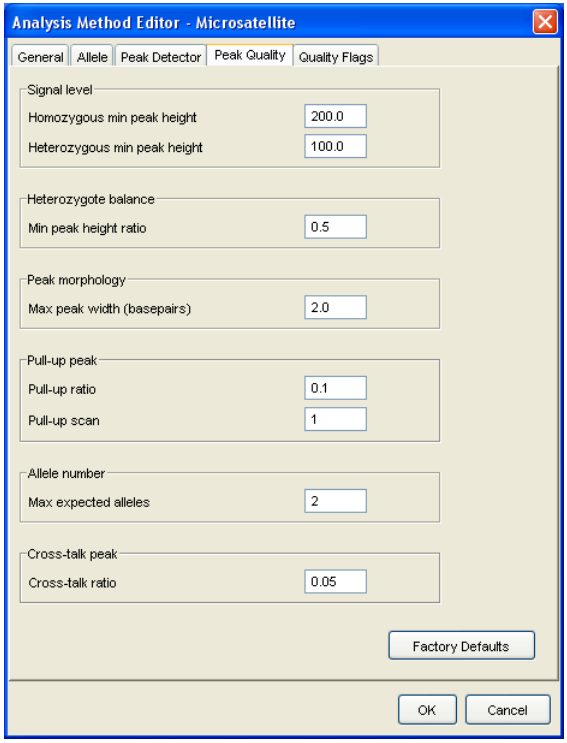


- **Peak Detector** – This tab includes settings that determine peak detection and sizing of peaks. Select **Basic** for the Peak Detection Algorithm. Leave the default values for all other settings.



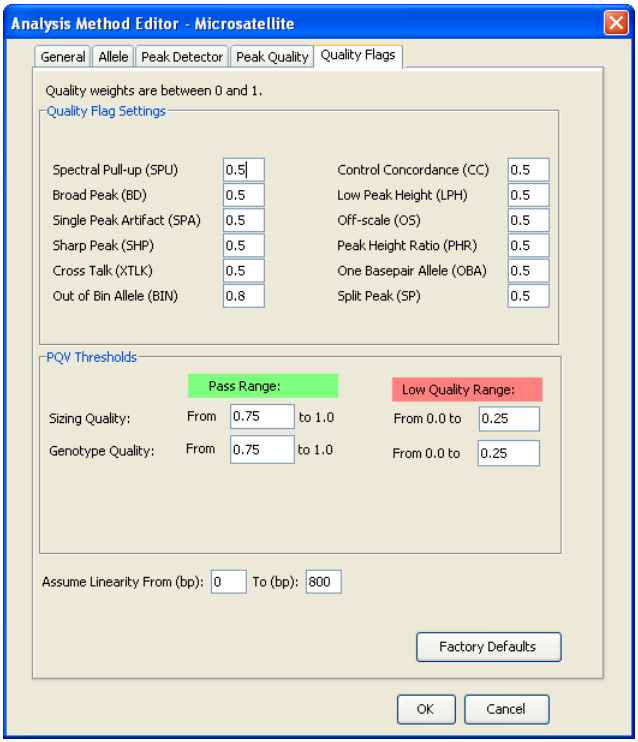
- **Peak Quality** – This tab includes settings that determine when specific PQVs are left green (Pass) or flagged yellow (Check).

  Type **2.0**. for the Max peak width (basepairs).
  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.
  - Assume Linearity Range, where the size calling algorithm assumes the fragment migration is linear for a given size range when calculating the Sizing Quality (SQ).

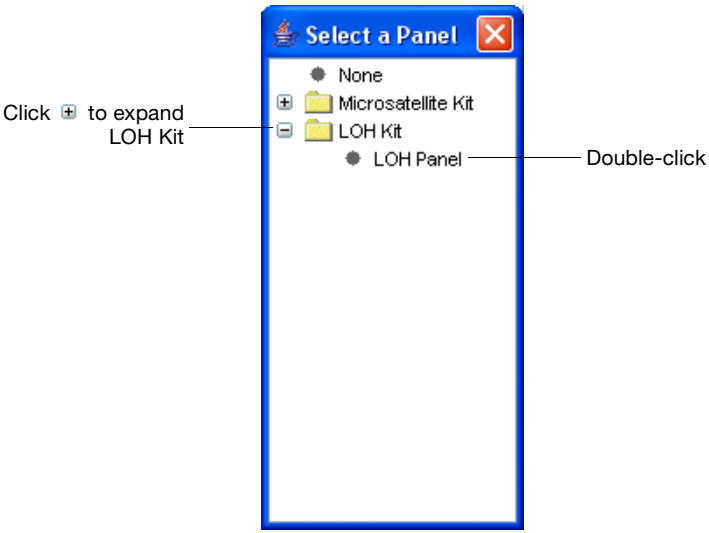
  Leave the default values for all settings.



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, expand the **LOH Kit**, then double-click **LOH Panel**. (This is the Panel you created on page 11.)



7. Select the first row in the **Size Standard** column, then select **GS500(-35, -250, -340)LIZ** from the drop-down list.

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

- GS500(-250)LIZ – excludes the 250-bp peak
- GS500(-35,-250,-340)LIZ – excludes the 35-, 250-, and 340-bp peaks
- GS500LIZ – includes all peaks present in the actual GeneScan™ 500 LIZ® Size Standard

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

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
LOH Analysis Method	LOH Panel	GS500(-35,-250,-340)LIZ
None	None	None
None	None	None
None	None	None

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

Selecting Table Setting

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

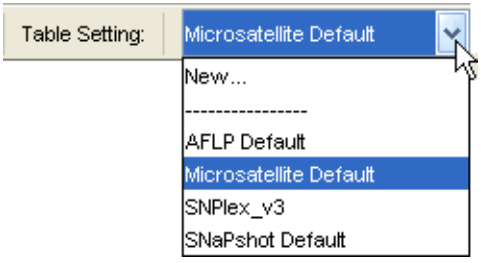


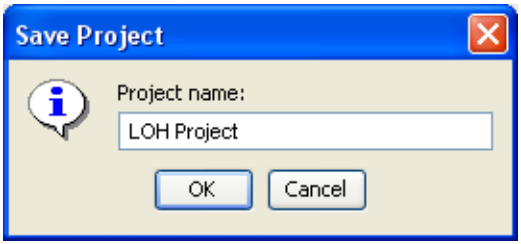
Table Settings control the information displayed in the Samples tab and Genotypes tab after analysis. Microsatellite Default is one of the default Table Settings provided with the GeneMapper Software.

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

Saving the Project

To save the project:

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



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

Next Steps

Perform the initial analysis on the project as described on page 26.

Performing the Initial Analysis on the Project

Overview

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

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

To perform the initial analysis:

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

Analyzing the Project

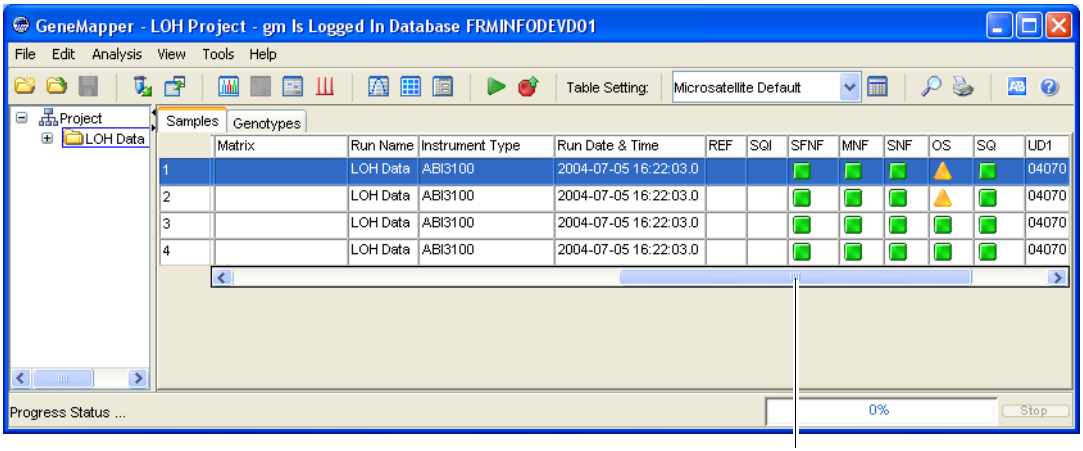
Click  (**Analysis** ▸ **Analyze**).

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

Reviewing the SQ and PQVs

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

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



Click-drag scroll bar to right to view SQ column

If you followed the procedures and used the example data indicated in this guide, the SQ for each sample is (Pass). 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 28.

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**).

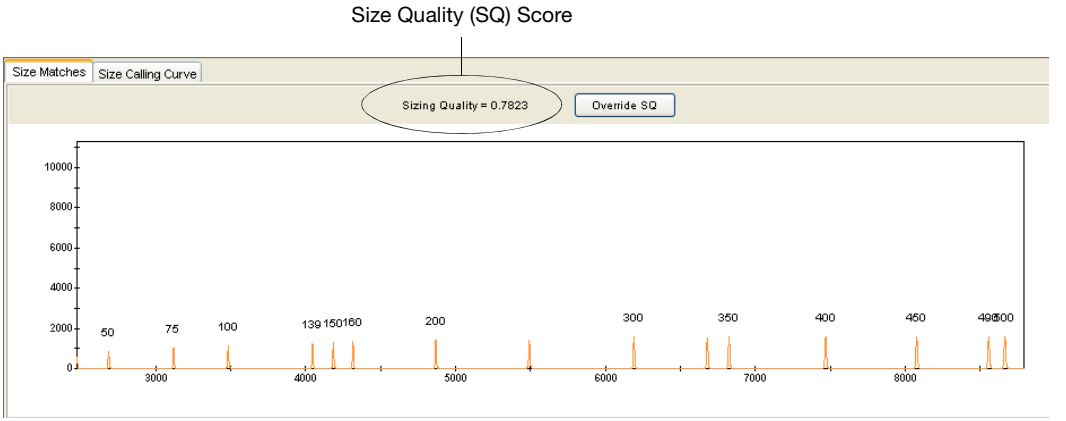


Figure 2-1 Size Match Editor – Size Matches tab

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).

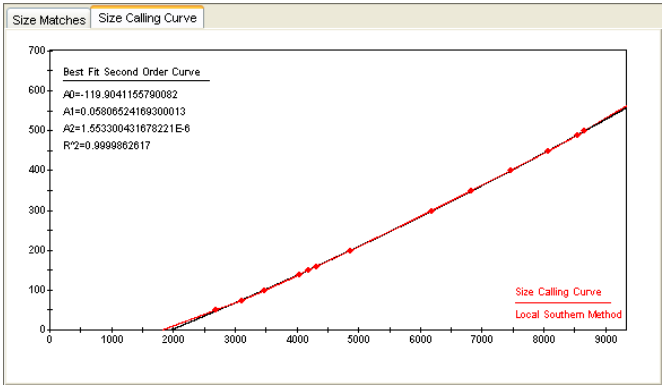
   However, when analyzing you own data you may notice the Sizing Quality is less and the SQ displays (Check) or (Low Quality). For troubleshooting help, see Table 2-1 on page 30.

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 30.

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

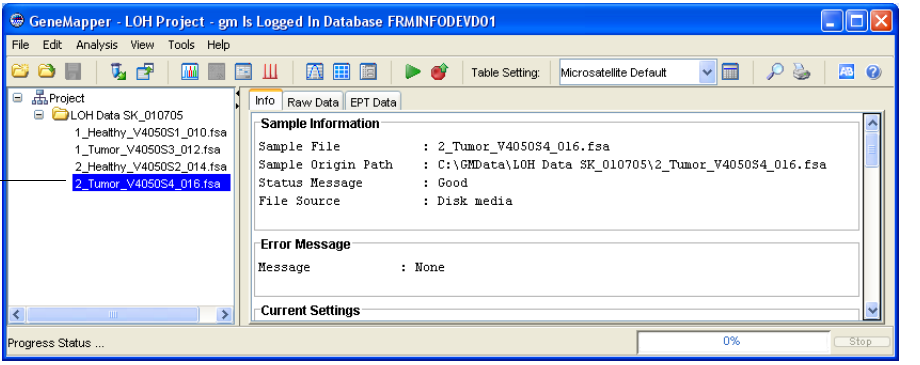
Problem	Action
Sizing Quality score is low and the SQ displays (Check) or (Low Quality), but all size standard peaks are present and labeled correctly.	Override the Sizing Quality by clicking **Override SQ** at the top of the Size Matches tab (Figure 2-1). Overriding changes the Sizing Quality score 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.

Select sample file to view sample information and raw data

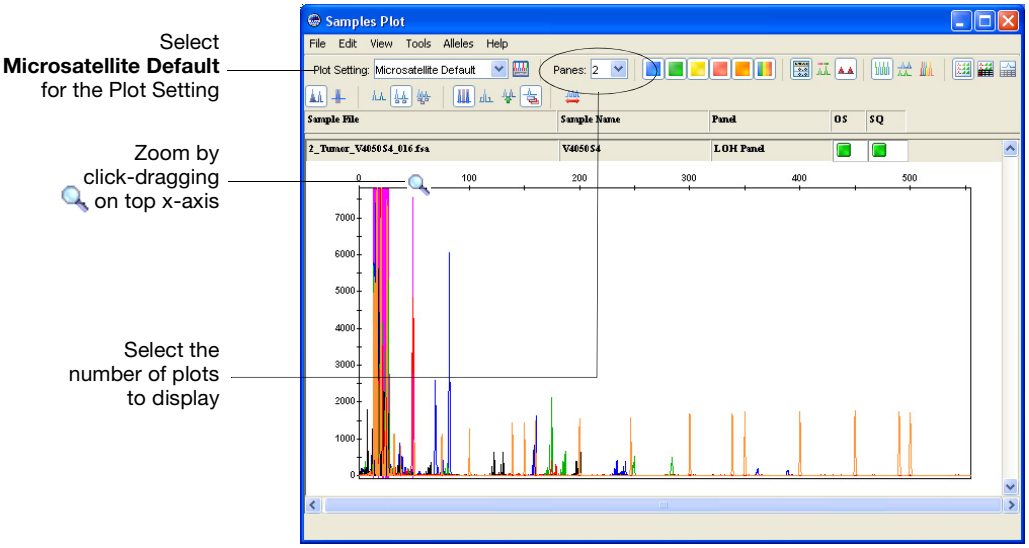


Viewing Sample Plots

To view the plots of the samples:

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

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



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

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

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

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

Examining Data in the Samples Plot Window

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

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

See Figure 2-2 on page 34 for an illustration of some of the above features.

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

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

Next Steps

Create a bin set and generate bins using the Auto Bin feature and reference data as described on page 35.

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

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

Overview

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

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

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

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

Creating a Bin Set

To create a bin set:

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

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

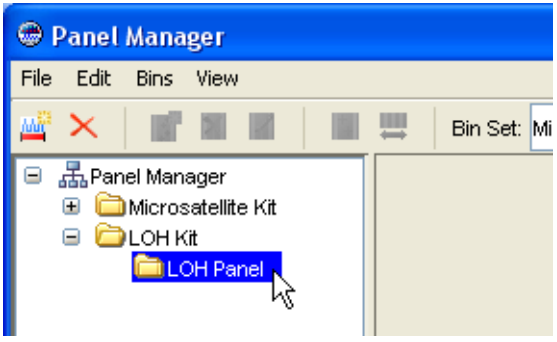
Adding Reference Data to a Panel and Bin Set

Note: You can add all or only a subset of the sample files in a project as reference data. Because the tumor sample files would contain the same alleles as their healthy counterparts, it is not necessary to add all the samples files. In this guide, you will use only the healthy sample files as the reference data for use in creating a panel and bin set.

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

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

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



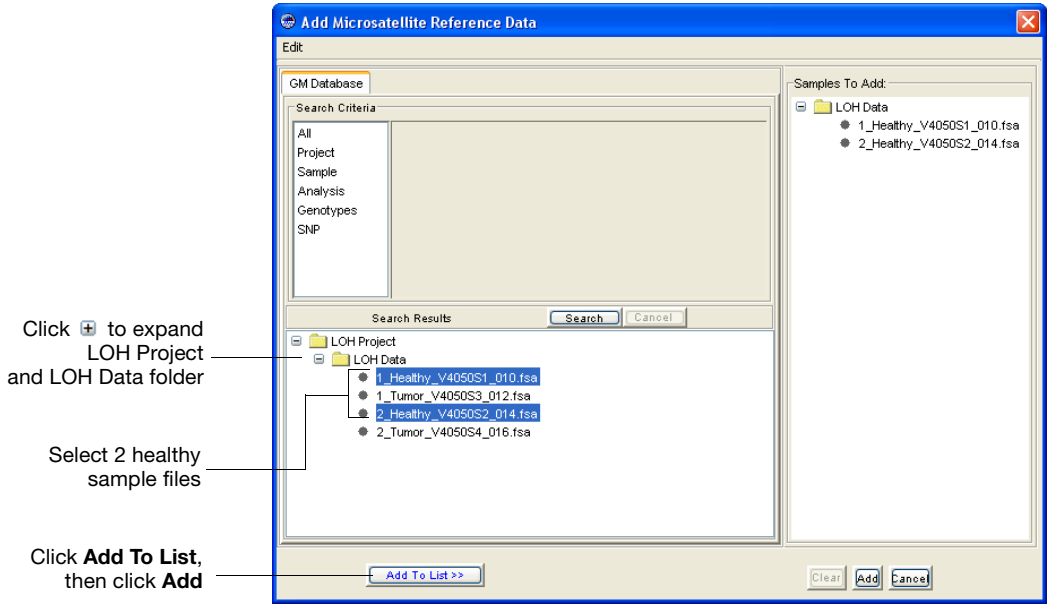
All the markers you created display on the right pane of the Panel Manager (Figure 2-3 on page 38).

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

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

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

4. In the Add Microsatellite Reference Data dialog box:
   a. Expand **LOH Project**.
   b. Expand the **LOH** folder.
   c. Press and hold **Ctrl** to select both healthy sample files.
   d. Click **Add to List**.
   e. Click **Add**.



The selected sample files are added as reference samples to the LOH Panel and appear in the lower half of the Navigation Pane in the Panel Manager (Figure 2-3 on page 38).

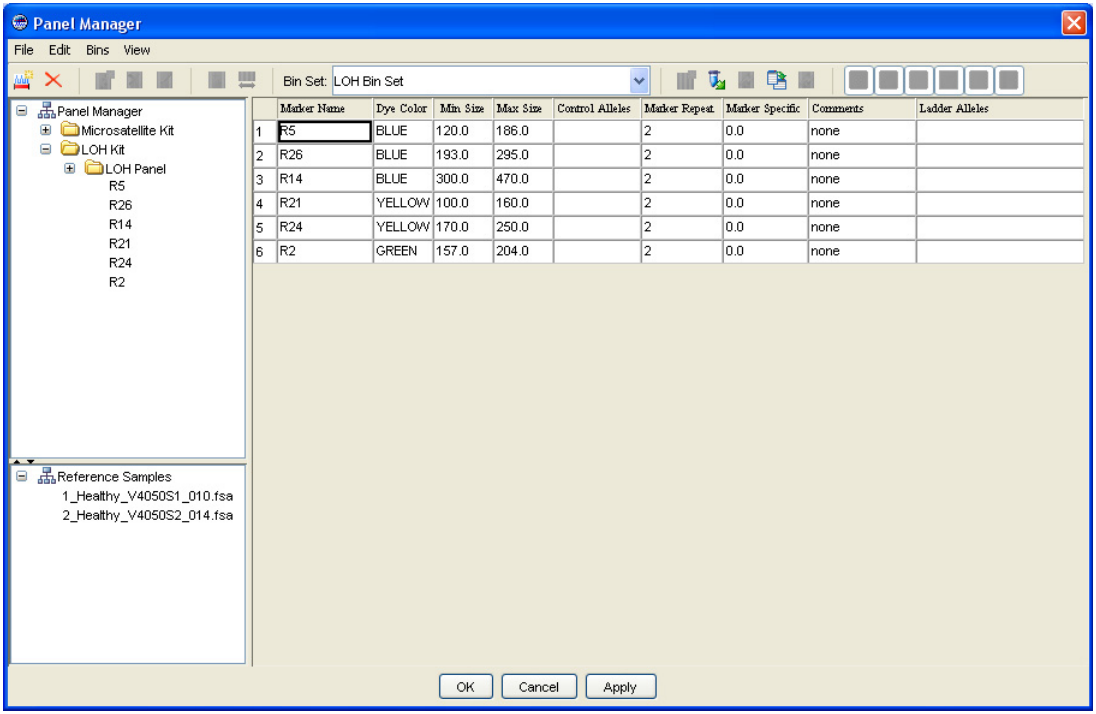
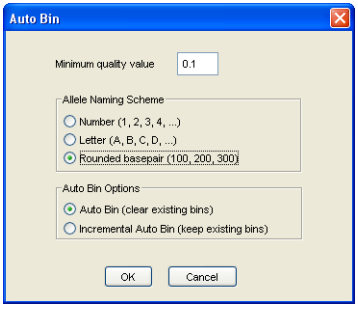


Figure 2-3 Panel Manager displaying markers and reference sample files for selected panel

Generating Bins using Auto Bin

To generate bins using the Auto Bin feature:

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



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

Reviewing the Markers and Bins

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

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

   A plot (Figure 2-4) displays:
   - Marker (pink line)
   - Bins (grey columns) for that marker
   - Reference alleles (red cross hatches) for each bin
2. With a marker selected in the upper half of the Navigation Pane, select a reference sample in the lower half of the Navigation Pane.

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

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

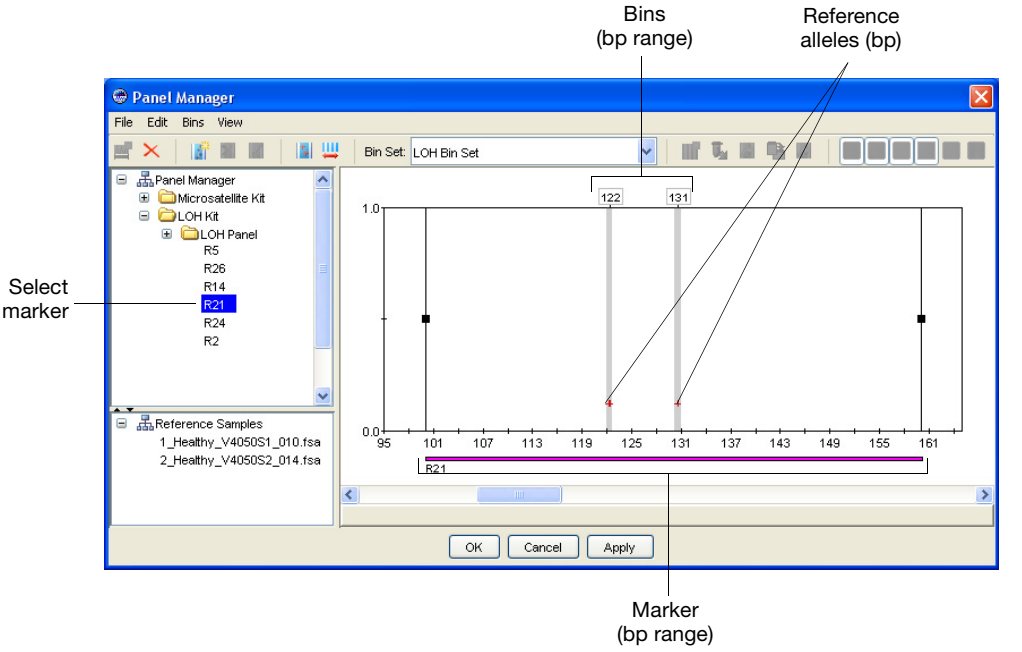


Figure 2-4 Selecting a marker

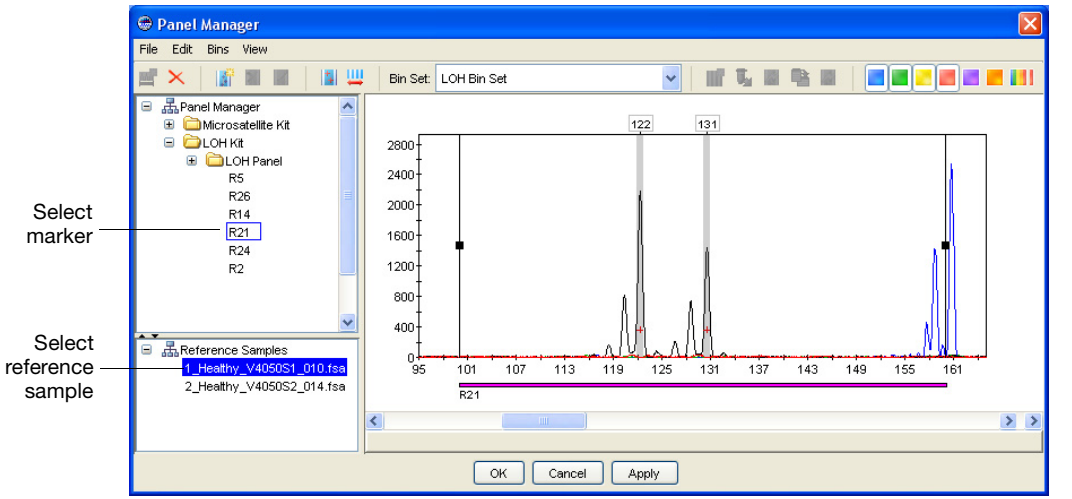


Figure 2-5 Selecting a marker and reference sample

Accepting the Bin Set

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

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

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

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

Adding a Bin to a Marker

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

Editing a Bin

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

Editing a Bin Graphically

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

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

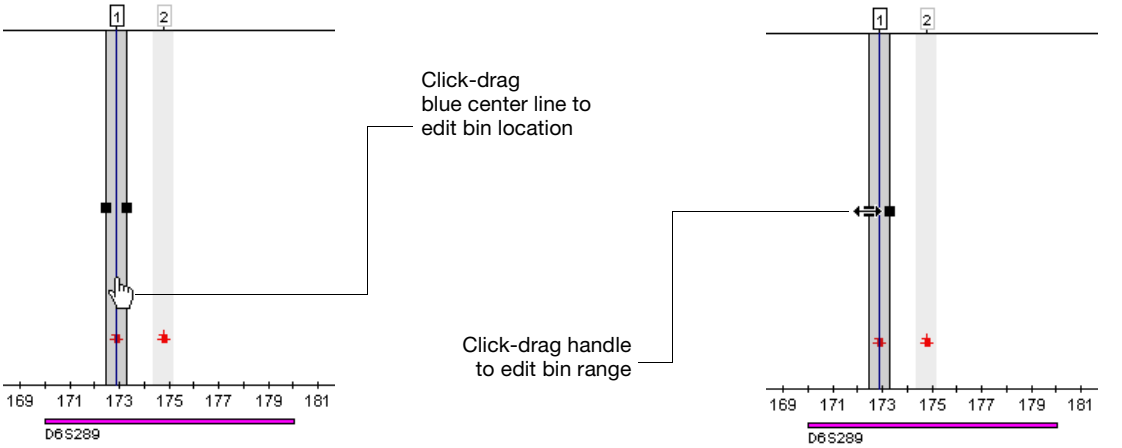


Figure 2-6 Editing a bin graphically

Deleting a Bin

To delete a bin from a marker:

- Select the bin, then click  (**Bins ▸ Delete Bin**)

  or
- Select the bin, right-click the bin, then select **Delete Bin**.

Editing a Marker

1. Select the marker in the upper Navigation Pane (Figure 2-7 on page 43).
2. Click-drag the left or right handles that define the marker range.

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

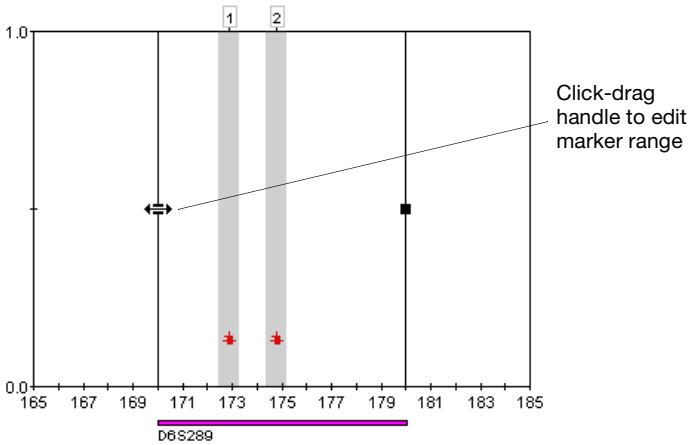


Figure 2-7 Editing a marker

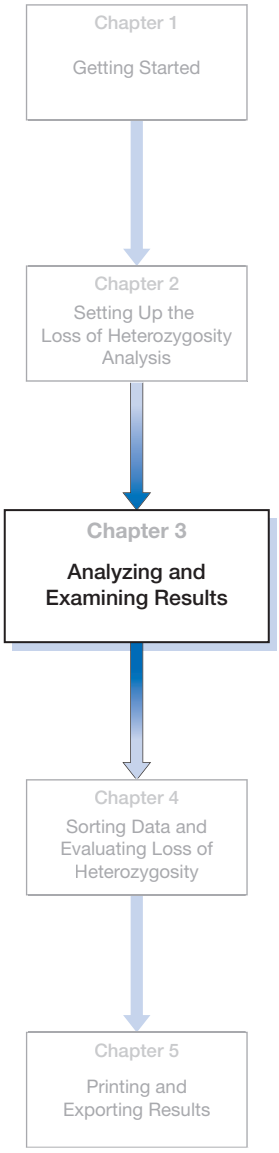
Deleting a Marker From a Panel

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

Next Steps

Analyze and examine the data in the LOH project as described in Chapter 3.

3 Analyzing and Examining Results



This chapter includes:

Editing the Analysis Method

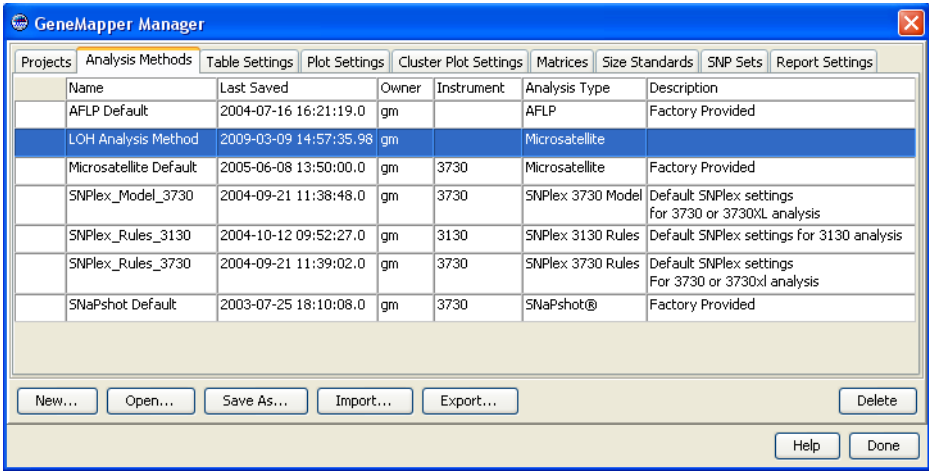
Overview

When you created the LOH Analysis Method on page 17, for the initial analysis, you did not select a bin set in the method. Now that you have created a bin set, select that bin set in the analysis method before analyzing the data. The bin set will allow the GeneMapper® Software to make allele calls when you analyze the sample files in the project.

Editing the Analysis Method

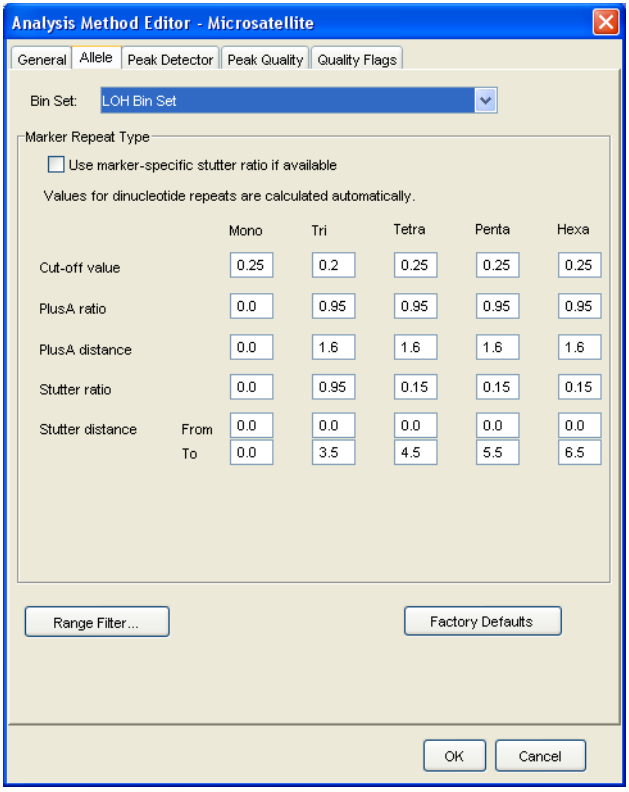
To edit the LOH Analysis Method:

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



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

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



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

Note: You can also access the Analysis Method Editor by double-clicking any row in the Analysis Method column in the Samples tab of the GeneMapper window (see page 17).

Next Steps

Analyze the project as described on page 48.

Analyzing the Project

Overview

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

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

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

Analyzing

Click (**Analysis ▸ Analyze**).

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

Next Steps

Examine the results as described below.

Examining the Results

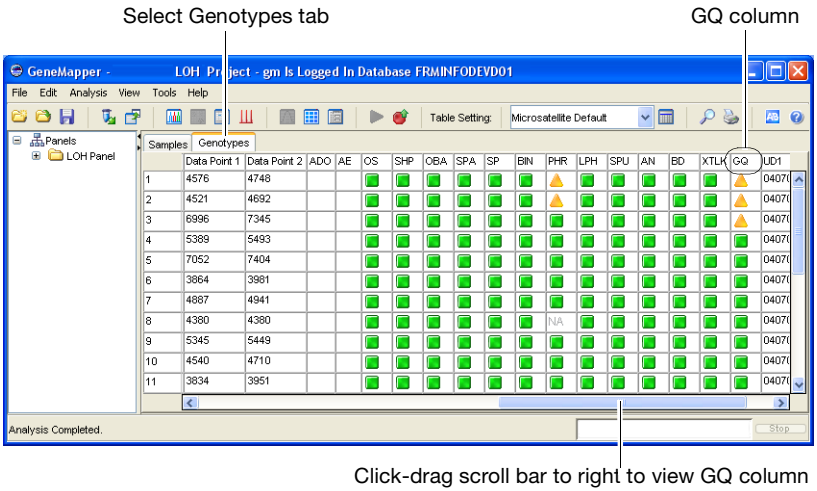
Overview

To examine the sizing and genotyping results:

- Review the SQ, associated PQVs, size standard, sample information, and samples plots (described on pages 27 through 33)
- Review the GQ and contributing PQVs (page 49)
- Review the allele calls for each sample (page 50)
- View genotype plots (page 50)
- Examine data in Genotypes Plot window (page 52)
- View project alleles (page 52)

Reviewing the GQ and PQVs

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



If you followed the procedures and used the example data indicated in this guide, the GQ for most samples should be (Pass). The Process Quality Values (PQVs) that contribute the GQ (AN, BD, BIN, LPH, OBA, OS, PHR, SHP, SP, SPA, SPU, and XTLK) should also be , except for a few samples with PHR (Peak Height Ratio). However, yellow PHR is expected for LOH data.

Hint: When analyzing LOH data, to reduce the likelihood of PHR being flagged, you may want to edit the Analysis Method to reduce the Minimum Peak Height Ratio in the Heterozygote Balance section of the Peak Quality tab (see page 21).

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, LPH, OBA, OS, PHR, SHP, SP, SPA, SPU, and XTLK) to be , indicating issues with the data, marker or bin definitions, or analysis parameters. To investigate and correct these issues, see the *GeneMapper® Software Reference and Troubleshooting Guide*.

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

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	Dye	Allele 1	Allele 2	Size 1	Size 2	Height 1
15	Tumor1_V4050S3_012.fsa	V4050S3	LOH Panel	1Green	G	175	187	174.54	186.95	3014
16	Tumor2_V4050S4_016.fsa	V4050S4	LOH Panel	3Yellow	Y	197	201	196.97	200.96	399
17	Tumor2_V4050S4_016.fsa	V4050S4	LOH Panel	3Blue	B	236	245	236.61	244.89	409
18	Tumor2_V4050S4_016.fsa	V4050S4	LOH Panel	2Yellow	Y	122	131	122.44	130.62	649
19	Tumor2_V4050S4_016.fsa	V4050S4	LOH Panel	2Blue	B	161	161	160.64	160.64	1620
20	Tumor2_V4050S4_016.fsa	V4050S4	LOH Panel	1Yellow	Y	65	65	64.79	64.79	354
21	Healthy2_V4050S2_014.fsa	V4050S2	LOH Panel	3Blue	B	236	245	236.52	244.86	880
22	Healthy2_V4050S2_014.fsa	V4050S2	LOH Panel	4Blue	B	362	389	361.5	388.71	961
23	Healthy2_V4050S2_014.fsa	V4050S2	LOH Panel	1Green	G	175	187	174.69	187.01	2385
24	Healthy2_V4050S2_014.fsa	V4050S2	LOH Panel	2Yellow	Y	122	131	122.36	130.61	1770

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 **Microsatellite Default** for the Plot Setting

Select the number of plots to display

Zoom by click-dragging on top x-axis

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

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

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

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

Examining Data in the Genotypes Plot Window

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

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

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

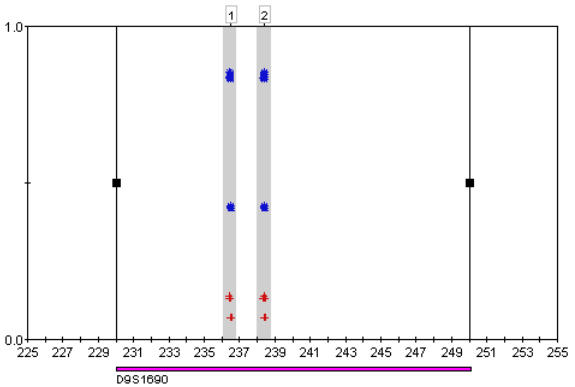
When done viewing the Genotypes Plot, click to close the window.

Viewing All Project Alleles

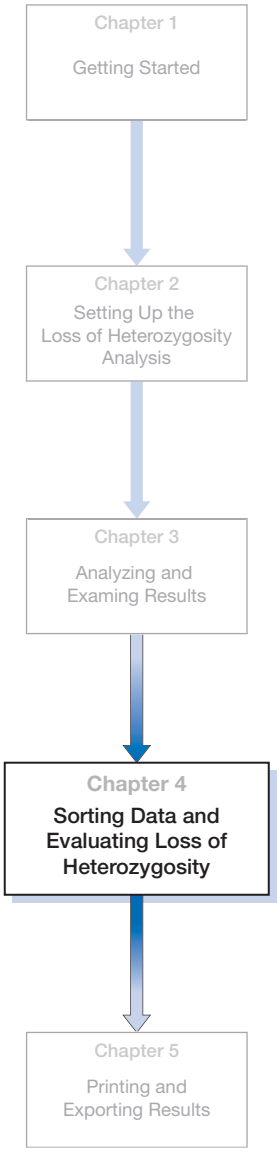
To view all the alleles detected in the sample data for each marker:

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

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



4 Sorting Data and Evaluating Loss of Heterozygosity



This chapter includes:

Overview

An LOH analysis investigates and compares healthy tissue and suspected diseased tissue from the same individual. Both the healthy and diseased samples should show two alleles for a given heterozygous microsatellite marker. If LOH is occurring in the diseased tissue, it will be evident by a decrease in peak height of one of the two alleles (relative to the healthy allele peak heights). The reason the tumor sample shows a decrease in peak height (instead of an absence of the peak) is that during isolation, the tumor sample is contaminated with healthy cells, thus introducing some wild-type DNA into the tumor specimen.

The Report Manager in the GeneMapper Software includes an LOH Default report setting that calculates and compares peak height ratios of microsatellite alleles between healthy and diseased tissues (Figure 4-1). It then evaluates and flags any samples that show potential LOH based on set peak-height ratio thresholds, so these samples can be further investigated. You can alter these thresholds based on the observed level of wild-type DNA contamination in the tumor samples.

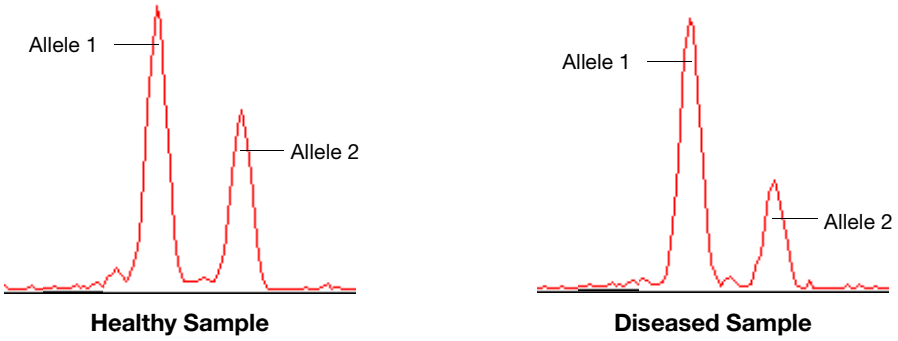


Figure 4-1 Diseased sample shows loss in peak height of Allele 2

The calculations and LOH criteria used by the LOH Default report setting are summarized below:

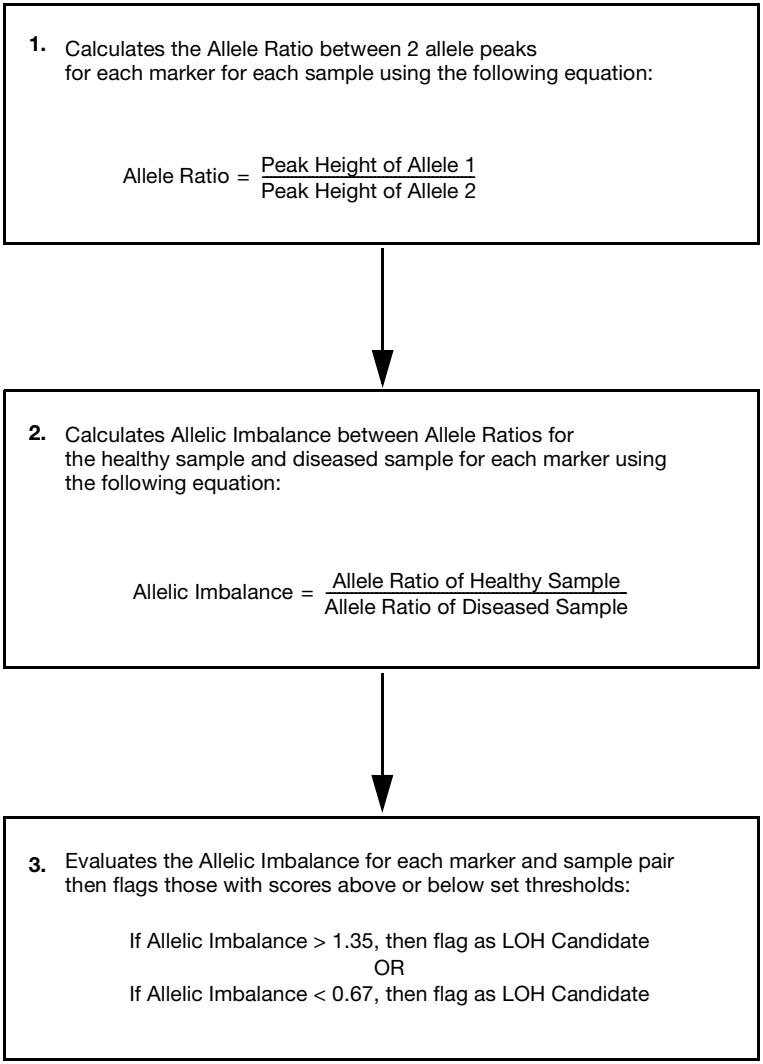


Figure 4-2 Calculations and thresholds used by the LOH Default report setting

IMPORTANT! You can edit the LOH Default report setting or create your own report setting as appropriate for your data (see page 59).

Sorting the LOH Data

Overview

Before you can apply the LOH Default report setting to calculate and evaluate potential LOH, it is critical to sort the data so that healthy samples and diseased samples for each marker are listed consecutively.

Sorting

To sort the LOH data:

1. Select the **Genotypes tab** in the GeneMapper window.
2. Sort by sample files by pressing and holding the **Shift** key, then clicking the **Sample File** column header.

   All markers from the 1_Healthy sample file are listed first, followed by all samples from the 1_Tumor sample file, and so on.

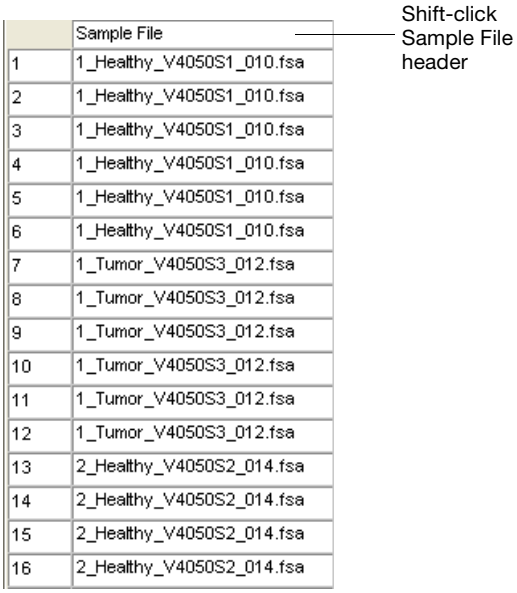


Figure 4-3 Sorting by sample file

3. Sort by Marker by pressing and holding the **Shift** key, then clicking the **Marker** column header.

   This sorts the results so that healthy samples and diseased samples for the same marker are listed consecutively.

Shift-click Marker header

	Sample File	Sample Name	Panel	Marker
1	1_Healthy_V4050S1_010.fsa	V4050S1	M2 LOH Panel	R14
2	1_Tumor_V4050S3_012.fsa	V4050S3	M2 LOH Panel	R14
3	2_Healthy_V4050S2_014.fsa	V4050S2	M2 LOH Panel	R14
4	2_Tumor_V4050S4_016.fsa	V4050S4	M2 LOH Panel	R14
5	1_Healthy_V4050S1_010.fsa	V4050S1	M2 LOH Panel	R2
6	1_Tumor_V4050S3_012.fsa	V4050S3	M2 LOH Panel	R2
7	2_Healthy_V4050S2_014.fsa	V4050S2	M2 LOH Panel	R2
8	2_Tumor_V4050S4_016.fsa	V4050S4	M2 LOH Panel	R2
9	1_Healthy_V4050S1_010.fsa	V4050S1	M2 LOH Panel	R21
10	1_Tumor_V4050S3_012.fsa	V4050S3	M2 LOH Panel	R21
11	2_Healthy_V4050S2_014.fsa	V4050S2	M2 LOH Panel	R21
12	2_Tumor_V4050S4_016.fsa	V4050S4	M2 LOH Panel	R21
13	1_Healthy_V4050S1_010.fsa	V4050S1	M2 LOH Panel	R24
14	1_Tumor_V4050S3_012.fsa	V4050S3	M2 LOH Panel	R24
15	2_Healthy_V4050S2_014.fsa	V4050S2	M2 LOH Panel	R24
16	2_Tumor_V4050S4_016.fsa	V4050S4	M2 LOH Panel	R24

Figure 4-4 Sorting by sample file and marker

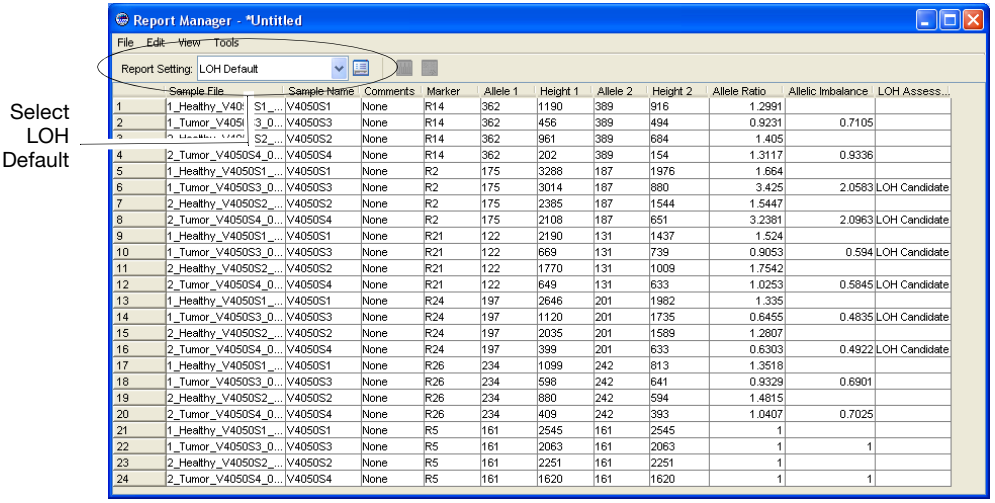
Next Steps

Generate a report to calculate and evaluate the LOH as described on page 58.

Generating a Report to Calculate and Evaluate LOH

To generate a report that calculates and evaluates loss of heterozygosity:

1. Make sure you sorted the data as described on page 56.
2. Select all the samples in the Genotypes tab by selecting **Edit ▸ Select All**.
3. Select **Analysis ▸ Report Manager**.
4. In the Report Manager dialog box, select **LOH Default** from the Report Setting drop-down list.



	Sample File		Sample Name	Comments	Marker	Allele 1	Height 1	Allele 2	Height 2	Allele Ratio	Allelic Imbalance	LOH Assess...
1	1_Healthy_V40:	S1_...	V4050S1	None	R14	362	1190	389	916	1.2991		
2	1_Tumor_V405(	3_0...	V4050S3	None	R14	362	456	389	494	0.9231	0.7105	
3	[illegible]	S2_...	V4050S2	None	R14	362	961	389	684	1.405		
4	2_Tumor_V4050S4_0...		V4050S4	None	R14	362	202	389	154	1.3117	0.9336	
5	1_Healthy_V4050S1_...		V4050S1	None	R2	175	3288	187	1976	1.664		
6	1_Tumor_V4050S3_0...		V4050S3	None	R2	175	3014	187	880	3.425	2.0583	LOH Candidate
7	2_Healthy_V4050S2_...		V4050S2	None	R2	175	2385	187	1544	1.5447		
8	2_Tumor_V4050S4_0...		V4050S4	None	R2	175	2108	187	651	3.2381	2.0963	LOH Candidate
9	1_Healthy_V4050S1_...		V4050S1	None	R21	122	2190	131	1437	1.524		
10	1_Tumor_V4050S3_0...		V4050S3	None	R21	122	669	131	739	0.9053	0.594	LOH Candidate
11	2_Healthy_V4050S2_...		V4050S2	None	R21	122	1770	131	1009	1.7542		
12	2_Tumor_V4050S4_0...		V4050S4	None	R21	122	649	131	633	1.0253	0.5845	LOH Candidate
13	1_Healthy_V4050S1_...		V4050S1	None	R24	197	2646	201	1982	1.335		
14	1_Tumor_V4050S3_0...		V4050S3	None	R24	197	1120	201	1735	0.6455	0.4835	LOH Candidate
15	2_Healthy_V4050S2_...		V4050S2	None	R24	197	2035	201	1589	1.2807		
16	2_Tumor_V4050S4_0...		V4050S4	None	R24	197	399	201	633	0.6303	0.4922	LOH Candidate
17	1_Healthy_V4050S1_...		V4050S1	None	R26	234	1099	242	813	1.3518		
18	1_Tumor_V4050S3_0...		V4050S3	None	R26	234	598	242	641	0.9329	0.6901	
19	2_Healthy_V4050S2_...		V4050S2	None	R26	234	880	242	594	1.4815		
20	2_Tumor_V4050S4_0...		V4050S4	None	R26	234	409	242	393	1.0407	0.7025	
21	1_Healthy_V4050S1_...		V4050S1	None	R5	161	2545	161	2545	1		
22	1_Tumor_V4050S3_0...		V4050S3	None	R5	161	2063	161	2063	1	1	
23	2_Healthy_V4050S2_...		V4050S2	None	R5	161	2251	161	2251	1		
24	2_Tumor_V4050S4_0...		V4050S4	None	R5	161	1620	161	1620	1	1	

The LOH Default report setting is applied to the LOH Project data. The resulting report shows the Allele Ratio, Allelic Imbalance, and LOH Assessment for each sample or pair of samples.

5. Save, print, or export the generated report by selecting the appropriate command from the File menu.

IMPORTANT! When generating a report for your own data, make sure samples do not have a Low Quality SQ score in the Samples tab. If they do, the samples and associated markers appear in the Genotypes tab, but with no data to be used by the report setting.

Editing or Creating a Custom LOH Report Setting

Overview

The LOH Default report setting provided with the GeneMapper Software assumes the following:

- **Allelic Imbalance Row Calculation** – When calculating the Allelic Imbalance (ratio of peak-height ratios) between two samples, the report setting compares every two rows in the Genotypes tab. This calculation assumes your sample files are named so that healthy samples and diseased samples for each individual and marker are listed consecutively (in pairs) after being sorted by sample file and marker.
- **LOH Assessment Thresholds** – The threshold values used to flag samples as LOH candidates are > 1.35 and < 0.67.

You can edit the above settings in the LOH Default report setting as appropriate for your samples and experiment.

Editing the Allelic Imbalance Row Calculation

Depending on how your sample files are named, healthy and tumor files from the same individual may or may not list consecutively after being sorted by sample file and marker (Figure 4-5).

1_Healthy.fsa	Healthy_1.fsa
1_Tumor.fsa	Healthy_2.fsa
2_Healthy.fsa	Healthy_3.fsa
2_Tumor.fsa	.
3_Healthy.fsa	.
3_Tumor.fsa	.
.	Healthy_n.fsa
.	Tumor_1.fsa
.	Tumor_2.fsa
n_Healthy.fsa	Tumor_3.fsa
n_Tumor.fsa	.
	.
	.
	Tumor_n.fsa
Healthy and tumor files from same individual list consecutively	**All healthy files list first, followed by all tumor files**

Figure 4-5 How sample file naming convention affects the order files list after being sorted by sample and marker

To change the row calculation for the Allelic Imbalance in the LOH Default report setting:

1. Open the GeneMapper Manager by clicking (**Tools ▸ GeneMapper Manager**).
2. Select the **Report Settings** tab (Figure 4-5).
3. Select the **LOH Default** report setting, then click **Open**.

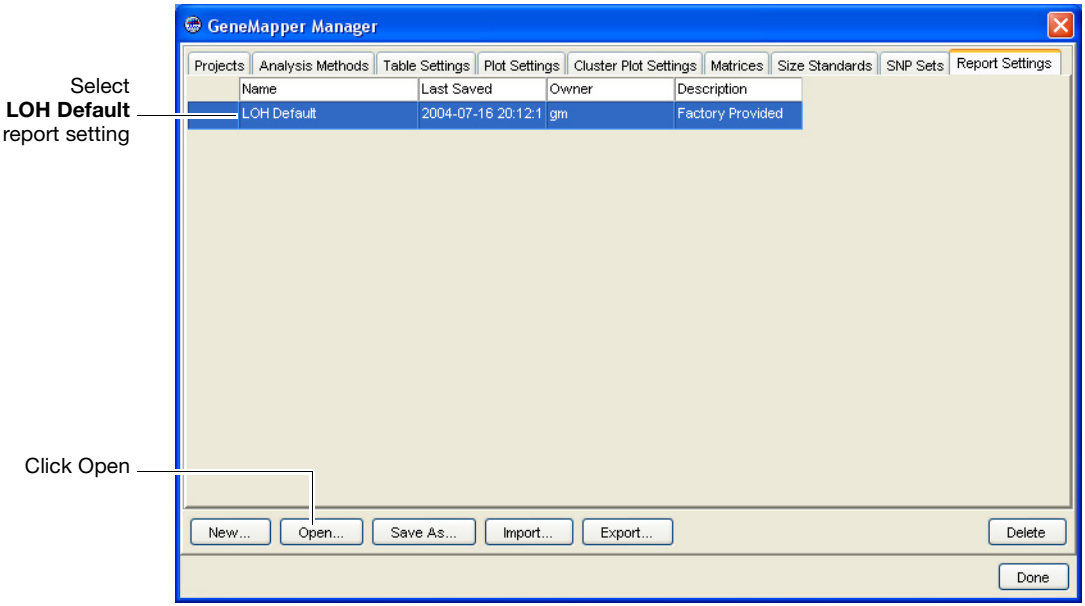


Figure 4-6 GeneMapper Manager Report Settings tab

4. In the Report Settings Editor (Figure 4-5):
   a. Select the **Calculation** tab.
   b. Select **Allelic Imbalance** from the Selected Columns list (right pane).
   c. Click to move Allelic Imbalance to the Calculation tab.

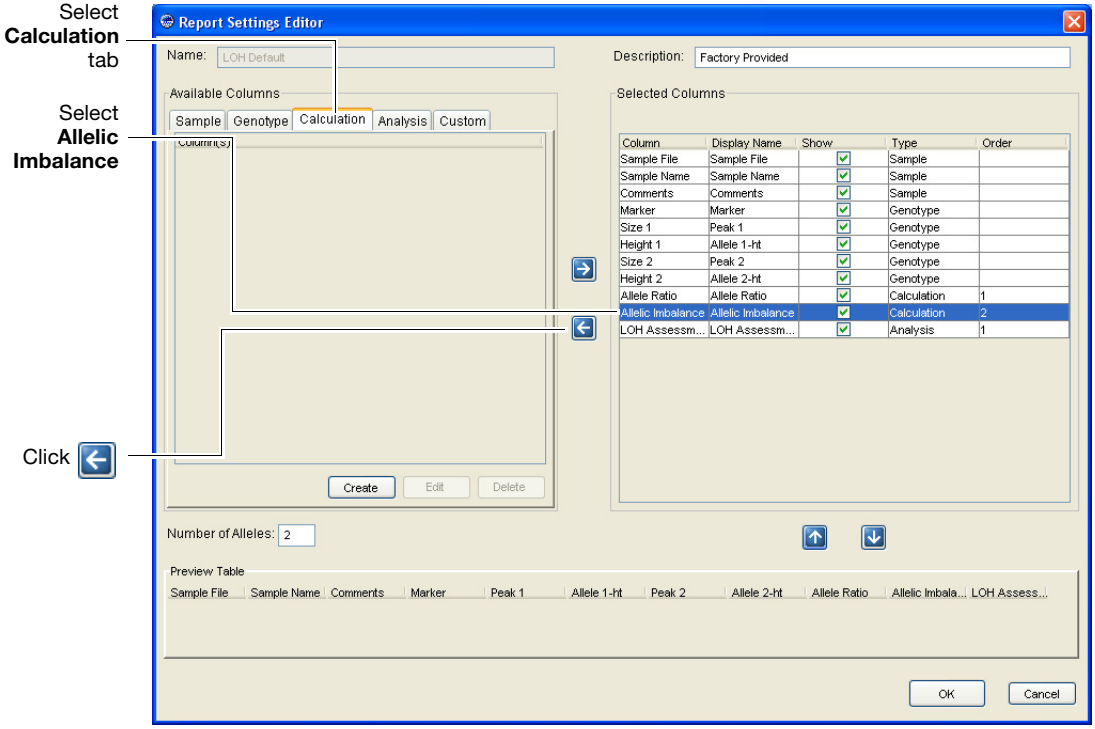


Figure 4-7 Report Settings Editor

5. Select **Allelic Imbalance** in the Calculation tab, then click **Edit**.

6. In the Edit Calculation dialog box, replace 2 with *n + 1* in the two fields shown in Figure 4-5.

Note: The value *n* is the sample set number, that is the number of healthy samples or the number of tumor samples (see Figure 4-5 on page 59). For example, if your sample set contains 5 healthy and 5 tumor samples, you would type 6.

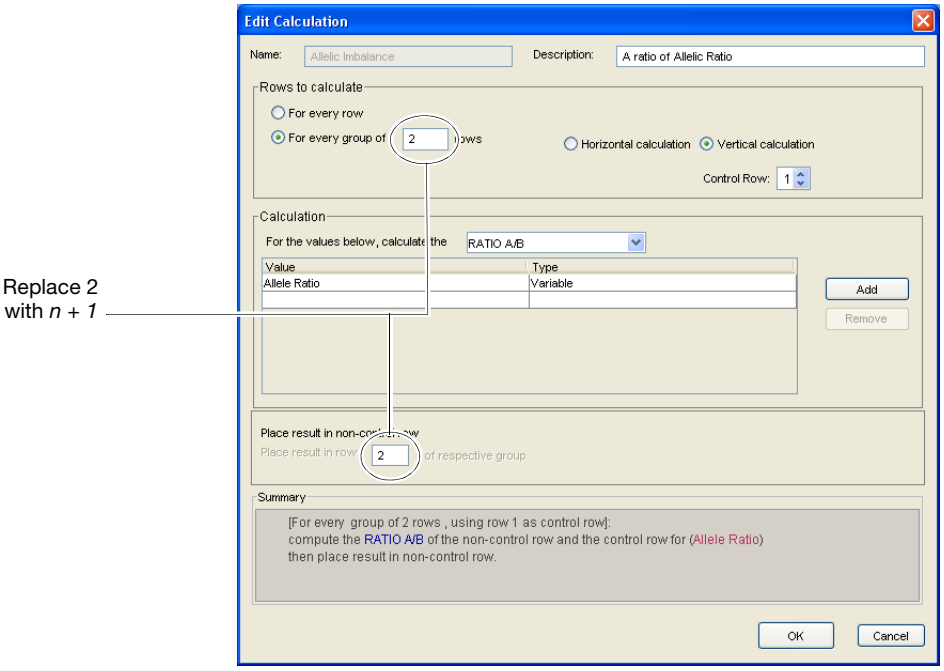


Figure 4-8 Edit Calculation dialog box

7. Click **OK** to close the Edit Calculation dialog box.
8. In the Report Settings Editor (Figure 4-5), click  to move Allelic Imbalance from the Calculation tab to the Selected Columns list.
9. In the Report Settings Editor (Figure 4-5):
   a. Select the **Analysis** tab.
   b. Select **LOH Assessment** from the Selected Columns list (right pane).
   c. Click  to move LOH Assessment to the Analysis tab.
10. Select **LOH Assessment** in the Analysis tab, then click **Edit**.

11. In the Analysis section of the Edit Analysis dialog box, select **For every group of**, then type *n + 1* in the two fields shown in Figure 4-5.

Note: The value *n* is the sample set number, that is the number of healthy samples or the number of tumor samples (see Figure 4-5 on page 59). For example, if your sample set contains 5 healthy and 5 tumor samples, you would type 6.

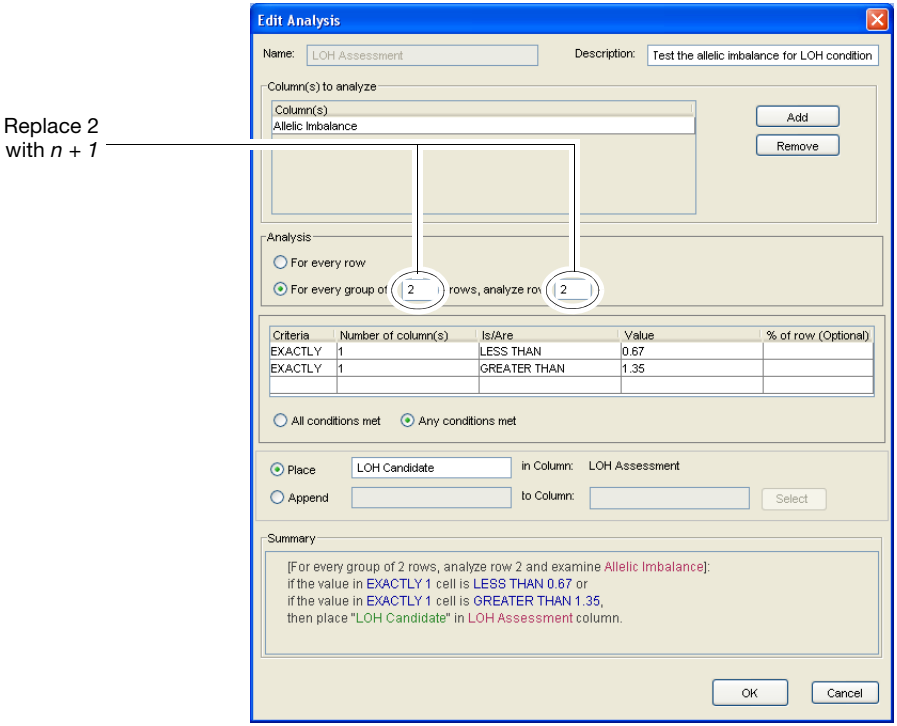


Figure 4-9 Edit Analysis dialog box

12. Click **OK** to close the Edit Analysis dialog box.

13. In the Report Settings Editor (Figure 4-5 on page 61), click  to move LOH Assessment from the Analysis tab to the Selected Columns list.

14. Click **OK** to close the Report Settings Editor. Click **Done** to close the GeneMapper Manager.

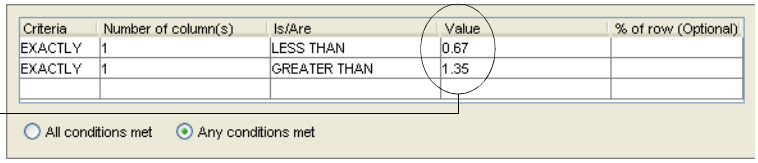
Editing the LOH Assessment Thresholds

Select LOH Assessment thresholds appropriate for the observed level of wild-type DNA contamination in tumor samples. For more information, see the discussion on page 54.

To change the thresholds for the LOH Assessment in the LOH Default report setting:

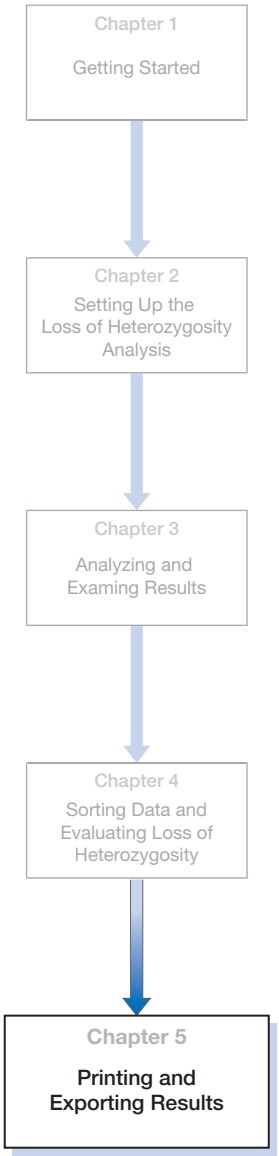
1. Open the GeneMapper Manager by clicking  (**Tools ▸ GeneMapper Manager**).
2. Select the **Report Settings** tab (Figure 4-5 on page 60).
3. Select the **LOH Default** report setting, then click **Open**.
4. In the Report Settings Editor (Figure 4-5 on page 61):
   a. Select the **Analysis** tab.
   b. Select **LOH Assessment** from the Selected Columns list (right pane).
   c. Click  to move LOH Assessment to the Analysis tab.
5. Select LOH Assessment in the Analysis tab, then click **Edit**.
6. In the Edit Analysis dialog box, in the Value column, replace the lower and upper thresholds of 0.67 and 1.35 with thresholds appropriate for your data.

Change these values as appropriate for your data



7. Click **OK** to close the Edit Analysis dialog box.
8. In the Report Settings Editor (Figure 4-5), click  to move LOH Assessment from the Analysis tab to the Selected Columns list.
9. Click **OK** to close the Report Settings Editor. Click **Done** to close the GeneMapper Manager.

5 Printing and Exporting Results



This chapter includes:

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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