ABI PRISM® Genotyper® 2.5 Software

User's Manual

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

Notice to Purchaser:

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Introduction to Genotyper Software Version 2.5

New Features

The latest release of the ABI PRISM® Genotyper® Software, version 2.5, has two new features:

- The ability to import ABI PRISM® GeneScan® Analysis data from a BioLIMS® 2.0 database.

  See Chapter 13 for more information.

- The ability to import and process GeneScan sample files containing a 5th dye.

  See Chapter 14 for more information.

IMPORTANT Genotyper v. 2.5 runs only on a Power Macintosh® computer.

Note The user interface for importing dye colors and raw data has been changed in this version (v. 2.5) of Genotyper Software. This change is described in Chapter 14.

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4. This Agreement and the Applied Biosystems Sales Quotation constitute the entire agreement between Applied Biosystems and you concerning the SOFTWARE.

Introducing Genotyper 1

Chapter Overview

Introduction

This chapter describes ABI PRISM® Genotyper® 2.5, the components of the ABI PRISM Genotyping Software System, and requirements for installing and starting Genotyper.

In This Chapter

This chapter contains the following topics:

Overview of ABI PRISM Genotyper 2.5

Definition Genotyper is a software application that enables you to analyze and interpret nucleic acid fragment size and quantitation data by converting it into user defined results.

You can transfer these results to:

- GenBase for storage
- GenoPedigree for display on pedigrees
- Other databases for storage, spreadsheets for statistical analysis or linkage analysis.

How You Can Use Genotyper

You can use Genotyper, as well as the other components of the ABI PRISM Genotyping Software System, to automate and assist you with many different genetic research projects including:

Microsatellite Analysis

- Fluorescent genotyping for genetic linkage studies
- Paternity identification
- Forensic identification of samples
- Determination of loss of heterozygosity
- Microsatellite instability
- Trisomy analysis

AFLP Analysis

- Gene Mapping using amplified fragment polymorphisms (AFLP)
- Quantitative expression of gene products

Gene Expression Profiling

- Differential display
- Quantitative expression of gene products

Mutation Detection

- Single strand conformation polymorphisms (SSCP)
- Heteroduplex mobility assays (HMA)
- Mismatch cleavage

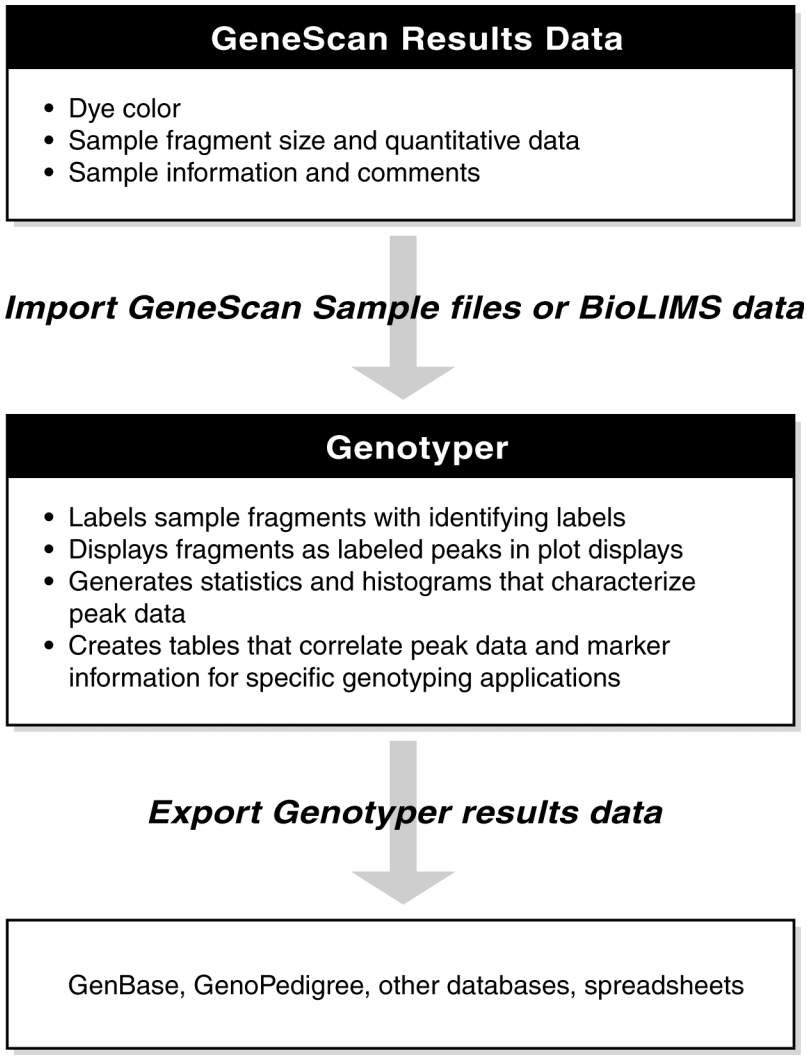
Mutation Screening

- Oligonucleotide ligation assays (OLA)
- Allele-specific PCR
- Gene dosage PCR
- RNAse protection assays

For more information on ABI PRISM Genotyping Software System components, see “Overview of the ABI PRISM Genotyping Software System” on page 1-5.

How Genotyper Works

The following figure shows how Genotyper analyzes data imported from ABI PRISM GeneScan® and BioLIMS®:



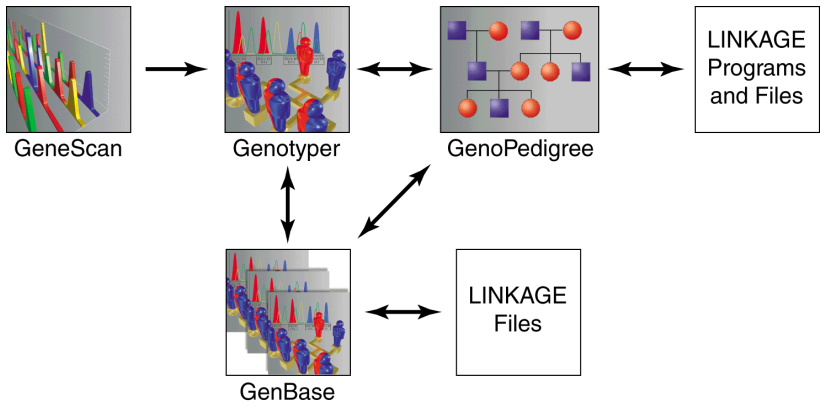
Overview of the ABI PRISM Genotyping Software System

Definition

Genotyper®, GeneScan, GenBase™, and GenoPedigree™ are four stand-alone software applications that when used together make up the ABI PRISM Genotyping Software System.

Relationship of System Components

The diagram below illustrates how the ABI PRISM Genotyping Software System integrates Genotyper with GenoPedigree and linkage analysis programs through GenBase.



Component Descriptions

The following table describes the components of the ABI PRISM Genotyping Software System.

Components of the ABI PRISM Genotyping Software System:

Component	Description
ABI PRISM GeneScan	Analyzes nucleic acid fragment data collected by an ABI PRISM instrument and sizes and quantifies detected fragments, putting the results in GeneScan files.
ABI PRISM Genotyper	Analyzes data from GeneScan files or BioLIMS, labeling fragment data and creating tables specific to your genotyping studies.
ABI PRISM GenoPedigree	An interactive pedigree diagram editor that reads and writes its own documents containing pedigree, layout, and style information.
ABI PRISM GenBase	A database application that stores data for genotypes, pedigrees, markers, traits (diseases), and other relevant information. You can import data from or export data to GenBase from Genotyper, and GenoPedigree.

Related Publications

The following table describes publications that you can refer to for detailed information about ABI PRISM Genotyping Software System component applications.

Publication List:

Publication	Description
Hard-copy Publications	
ABI PRISM Genotyper User's Manual	Explains how to use features of Genotyper to analyze data from GeneScan files and BioLIMS, and produce results data specific to your Genotyping application.
ABI PRISM Genotyper Applications Tutorials	Contains tutorial information for using Genotyper and Genotyping Software System components to perform typical genotyping applications.
ABI PRISM GenoPedigree User's Manual	Explains how to use GenoPedigree to display, generate, and analyze, import and export pedigree diagrams and data.
ABI PRISM GenBase User's Manual	Explains how to use a database application that stores data for genotypes, pedigrees, markers, traits (diseases), and other relevant information. You can import data from or export data to GenBase from Genotyper and GenoPedigree.
ABI PRISM GeneScan User's Manual	Explains how to use GeneScan Analysis software to size and quantify nucleic acid fragments detected on an ABI PRISM instrument.
CD-ROM Publications	
ABI PRISM Genotyper User's Manual	Allows you to use online navigation tools to search for information contained in the Genotyper User's Manual, and access user documentation for other Genotyping System Software components.
ABI PRISM Genotyper Applications Tutorials	Allows you to use online navigation tools to search for information contained in the Genotyper Applications Tutorials, and access user documentation for other Genotyping System Software components.
ABI PRISM GenBase User's Manual	Allows you to use online navigation tools to search for information contained in the GenBase User's Manual, and access user documentation for other Genotyping System Software components.

Publication List: *(continued)*

Publication	Description
ABI PRISM GenoPedigree User's Manual	Allows you to use online navigation tools to search for information contained in the GenoPedigree User's Manual, and access user documentation for other Genotyping System Software components.

Web Site Information

Information on the software and user manuals of the ABI PRISM® Genotyping Software System can be accessed from the Applied Biosystems:

www.appliedbiosystems.com/techsupport

Installing and Starting Genotyper

Registering Your Copy of Genotyper

When you register your copy of Genotyper you become eligible for telephone and field service support from Applied Biosystems for 100 days from the date of shipment. To register, fill out the registration card included in this package and return it to Applied Biosystems.

For Applied Biosystems technical support telephone and address information, see “Technical Support” on page 1-16.

Registration also allows you to purchase upgrades to the software at a lower price than it would cost you to purchase a new upgraded package.

IMPORTANT These privileges are available only if you have returned your registration card.

Compatibility With Previous Versions

Genotyper 2.5 can read files created by Genotyper 1.0, 1.1, 1.1.1, and 2.0.

Previous versions of Genotyper cannot read files created by Genotyper 2.5.

The commands Add Rows to Tables and Add Rows to Link are supported in Genotyper 2.5 for compatibility with Genotyper 1.X macros. However, there are many new table features in Genotyper 2.0 that require the new versions of these commands. In particular, tables written with Add Rows to Table will need to be rewritten with Setup Table and Append to Table. The older commands may be removed in future versions.

For more information on using the new table features, see “Working with Tables” on page 8-1.

Hardware and Software Requirements

This table describes the components your computer system requires to run Genotyper 2.5.

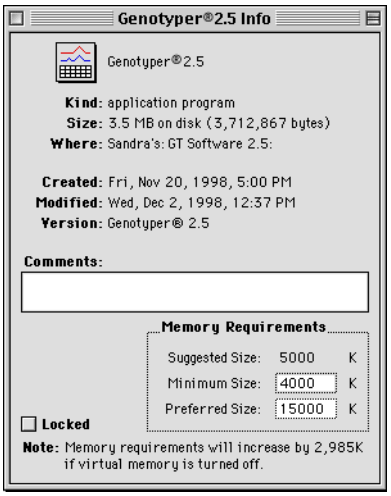
Genotyper hardware and software requirements:

System Component	Description	
	Recommended	**Minimum Requirement**
Computer	A Power Macintosh G3 computer with a 233 MHz or faster processor	A PowerPC Macintosh
Monitor	A seventeen inch monitor	A 640 x 480 pixels size monitor
	Note Color monitors are useful, but not required.	
Operating System	Macintosh Operating System 8.0 or later	Macintosh Operating System 7.5.3 with 32 MB of RAM.
Memory Allocated to Genotyper	15 MB of RAM (without virtual memory 16.5). This allows you to import GeneScan files from approximately one Gel file.	5 MB of RAM (without virtual memory 6.5). This allows you to import only a limited number of GeneScan files at one time.
	Note Genotyper performance increases when you allocate more memory to the program. Performance decreases, as you import more GeneScan files. Actual results will vary depending on your type of computer system and the kinds of Genotyper tasks you are performing.	
Disk Drive	If you intend to use Genotyper with GenBase, note that the GenBase data file is a single, large disk file, and performance depends on having sufficient unfragmented space on your hard drive. GenBase requires at least 5 MB of free space to launch successfully.	

Setting Memory Allocation

Because the sizes of the GeneScan data you import can vary, you may need to allocate more memory to Genotyper. You can estimate that each additional megabyte of memory allocated to Genotyper allows you to import approximately 12 GeneScan files when raw data is imported or 20 GeneScan files if raw data is not imported (actual sizes may vary).

To change the memory allocation to Genotyper:

Step	Action
1	Quit Genotyper.
2	Click the Genotyper application icon to select it.
3	Choose the Get Info command from the Finder's File menu. The Get Info dialog box appears. 
4	Enter a new value for the Preferred size **Note** In some versions of the Macintosh System, the Preferred size value is referred to as the Current size.
5	Click the close box in the upper left corner.

How to Install Genotyper

Genotyper comes on a single CD-ROM disc containing the:

- Genotyper Program file
- Tutorials & Examples files
- Genotyper User Manual in Portable Document Format (pdf)

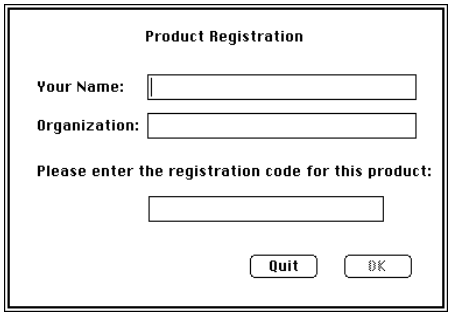
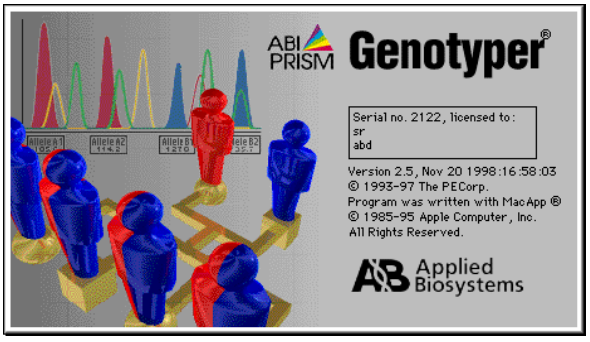
IMPORTANT Do not work off of the CD-ROM disc.

To install Genotyper for the first time:

Step	Action
1	Insert the Genotyper CD into your computer CD-ROM drive.
2	Double-click the Genotyper Installer icon.
3	Choose the hard-drive on which you want the program to be installed.
4	Follow the prompts on the installer to install the program.
5	Eject the Genotyper CD.

How to Start Genotyper

To start Genotyper:

Step	Action
1	Double click the Genotyper icon in the Finder. The first time you start Genotyper, the Registration dialog box appears. 
2	Enter your name, your organization, and your registration code. **Note** The first time you use the application, you are asked to enter the registration code on your registration card. Genotyper then verifies the code. **IMPORTANT** Keep your registration code in a place where you can easily retrieve it. If you need to re-install the software at any time, you will be prompted for the registration code once again.
3	Click OK. The Genotyper start-up screen appears briefly.  You are now ready to use Genotyper.

Using the Macintosh

Knowledge Assumptions

For the purposes of this manual, it is assumed that you have used a Macintosh computer. If you are not familiar with the terms or procedures in the following table, refer to the *Macintosh System Software User's Guide* for more information.

The table below describes Macintosh procedures that you should be familiar with to use Genotyper.

Macintosh procedures:

Procedure	Description
Using the mouse	Clicking and double-clicking, selecting, and dragging.
Choosing commands	Using pull-down and pop-up menus, dialog boxes, radio buttons and checkboxes.
Working with windows	Opening and closing, re-sizing and repositioning, scrolling, understanding the active window.
Using the Macintosh hierarchical file system	Finding files and creating folders.

Performance and Maintenance Guidelines

When using Genotyper, you will often be working with many files, and accessing the hard disk often, so it is essential that you follow guidelines to minimize the occurrence of errors during operation of the computer.

Follow these general guidelines for optimal performance:

- Install only one Macintosh System per hard disk.
- Back up all programs and files regularly.
- Use discretion when adding software programs, especially control panel and System extension (INIT) files.

Macintosh Terms Used in This Manual

Definitions The following terms are used in this manual:

Term	Definition
Dialog Boxes	Appear when you need to make a decision or enter information. All other action on the monitor screen is suspended until you close the dialog box by clicking a button such as Cancel, OK, or Done.
Menus	Provide access to various functions you can perform with the software. A triangle after a menu item indicates that a submenu appears. When you click that choice and hold the mouse button down, a submenu appears. These menus allow you to choose dialog box entries from specific lists of items.
Pop-up menus	Display a triangle and are found in dialog boxes. When you click a pop-up menu and hold the mouse button down, a submenu appears. These menus allow you to choose dialog box entries from specific lists of items.
Windows	Display information, and in some cases allow you to edit or enter additional information. The top border of an active window always has six horizontal lines and usually has a close box in the upper left corner. If many windows are open, click one window to make it active. When a window is active, you can click the top border, hold the mouse button down, and drag the window to another location on the screen. When you are finished working with a window, click the close box to remove the window from the screen, or click another window.
Entry fields	Rectangular areas in which you can enter information. Click in an entry field to display a cursor, and use the keyboard to enter the information.
Check boxes	Boxes that you click to select certain options in a dialog box. When you click an empty checkbox, an x appears in it, indicating that you have selected the option. You can usually select multiple checkboxes.
Radio Buttons	Small circles that appear in front of choices. When you click a radio button with the cursor, a black dot appears in the center of the circle to indicate your choice. You can select only one at a time.
Buttons	Rectangles with rounded corners that allow you to accept or cancel the contents of a dialog box or perform functions (such as printing) within the dialog box. A button with a heavy outline is the default button that applies if you press the Return key.

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section “To Obtain Documents on Demand” following the telephone information below).

To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers	tsupport@appliedbiosystems.com
Applied Biosystems/MDS Sciex	api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

Hours for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

Product	Hours
Chemiluminescence	8:30 a.m. to 5:30 p.m. Eastern Time
Framingham support	8:00 a.m. to 6:00 p.m. Eastern Time
All Other Products	5:30 a.m. to 5:00 p.m. Pacific Time

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Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM® 3700 DNA Analyzer	**1-800-831-6844,** then press **8**	**1-650-638-5981**
DNA Synthesis	**1-800-831-6844**, then press **21**	**1-650-638-5981**
Fluorescent DNA Sequencing	**1-800-831-6844**, then press **22**	**1-650-638-5981**
Fluorescent Fragment Analysis (includes GeneScan® applications)	**1-800-831-6844**, then press **23**	**1-650-638-5981**
Integrated Thermal Cyclers (ABI PRISM®877 and Catalyst 800 instruments)	**1-800-831-6844**, then press **24**	**1-650-638-5981**
ABI PRISM® 3100 Genetic Analyzer	**1-800-831-6844**, then press **26**	**1-650-638-5981**
BioInformatics (includes BioLIMS™, BioMerge™, and SQL GT™ applications)	**1-800-831-6844**, then press **25**	**1-505-982-7690**
Peptide Synthesis (433 and 43X Systems)	**1-800-831-6844**, then press **31**	**1-650-638-5981**
Protein Sequencing (Procise® Protein Sequencing Systems)	**1-800-831-6844**, then press **32**	**1-650-638-5981**
PCR and Sequence Detection	**1-800-762-4001**, then press **1** for PCR, **2** for the 7700 or 5700, **6** for the 6700 or dial **1-800-831-6844**, then press **5**	**1-240-453-4613**

Product or Product Area	Telephone Dial...	Fax Dial...
Voyager™ MALDI-TOF Biospectrometry and Mariner™ ESI-TOF Mass Spectrometry Workstations	**1-800-899-5858**, then press **13**	**1-508-383-7855**
Biochromatography (BioCAD® Workstations and Poros® Perfusion Chromatography Products)	**1-800-899-5858**, then press **14**	**1-508-383-7855**
Expedite™ Nucleic acid Synthesis Systems	**1-800-899-5858**, then press **15**	**1-508-383-7855**
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	**1-800-899-5858**, then press **15**	**1-508-383-7855**
PNA Custom and Synthesis	**1-800-899-5858,** then press **15**	**1-508-383-7855**
FMAT™ 8100 HTS System and Cytofluor® 4000 Fluorescence Plate Reader	**1-800-899-5858**, then press **16**	**1-508-383-7855**
Chemiluminescence (Tropix)	**1-800-542-2369** (U.S. only), or **1-781-271-0045**	**1-781-275-8581**
Applied Biosystems/MDS Sciex	**1-800-952-4716**	**1-650-638-6223**

Outside North America

Region	Telephone Dial...	Fax Dial...
Africa and the Middle East		
Africa (English Speaking) and West Asia (Fairlands, South Africa)	**27 11 478 0411**	**27 11 478 0349**
South Africa (Johannesburg)	**27 11 478 0411**	**27 11 478 0349**
Middle Eastern Countries and North Africa (Monza, Italia)	**39 (0)39 8389 481**	**39 (0)39 8389 493**

Region	Telephone Dial...	Fax Dial...
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 758 8268**	**60 3 754 9043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 2358 2838**	**886 2 2358 2839**
Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 712 5555**	**32 (0)2 712 5516**
Czech Republic and Slovakia (Praha)	**420 2 61 222 164**	**420 2 61 222 168**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0) 6150 101 0**	**49 (0) 6150 101 101**
Hungary (Budapest)	**36 (0)1 270 8398**	**36 (0)1 270 8288**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**
Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 (22) 866 40 10**	**48 (22) 866 40 20**
Portugal (Lisboa)	**351 (0)22 605 33 14**	**351 (0)22 605 33 15**
Russia (Moskva)	**7 095 935 8888**	**7 095 564 8787**
South East Europe (Zagreb, Croatia)	**385 1 34 91 927**	**385 1 34 91 840**
Spain (Tres Cantos)	**34 (0)91 806 1210**	**34 (0)91 806 1206**
Sweden (Stockholm)	**46 (0)8 619 4400**	**46 (0)8 619 4401**
Switzerland (Rotkreuz)	**41 (0)41 799 7777**	**41 (0)41 790 0676**
The Netherlands (Nieuwerkerk a/d IJssel)	**31 (0)180 331400**	**31 (0)180 331409**

Region	Telephone Dial...	Fax Dial...
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**
All other countries not listed (Warrington, UK)	**44 (0)1925 282481**	**44 (0)1925 282509**
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6230**	**81 3 5566 6507**
Latin America		
Del.A. Obregon, Mexico	**305-670-4350**	**305-670-4349**

To Reach Technical Support Through the Internet

We strongly encourage you to visit our Web site for answers to frequently asked questions and for more information about our products. You can also order technical documents or an index of available documents and have them faxed or e-mailed to you through our site. The Applied Biosystems Web site address is

http://www.appliedbiosystems.com/techsupp

To submit technical questions from North America or Europe:

Step	Action
1	Access the Applied Biosystems Technical Support Web site.
2	Under the **Troubleshooting** heading, click **Support Request Forms**, then select the relevant support region for the product area of interest.
3	Enter the requested information and your question in the displayed form, then click **Ask Us RIGHT NOW** (blue button with yellow text).
4	Enter the required information in the next form (if you have not already done so), then click **Ask Us RIGHT NOW**. You will receive an e-mail reply to your question from one of our technical experts within 24 to 48 hours.

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Planning Genotyper Applications 2

Chapter Overview

Introduction

This chapter discusses techniques for collecting and preparing sample data that will help you improve the overall quality of the GeneScan data you import into Genotyper. It also discusses how to plan for use of Genotyper features in your genotyping application.

In This Chapter

This chapter contains the following topics:

Preparing Sample Data for Genotyper

Introduction

Genotyper analyzes the results of fragment analysis data collected by an ABI PRISM instrument, and generated by GeneScan.

Perfecting techniques for preparing fragment samples, collecting sample data, and using GeneScan to size and quantify fragments will simplify automation of many Genotyper tasks, and minimize editing tasks required to achieve quality genotyping results.

Preparing Fragment Samples

The source of your fragment samples often affects peak resolution in Genotyper plot displays.

When preparing fragment samples for eventual sizing and quantitation by GeneScan, adjust pooling conditions to dilute the amplified products that consistently yield off-scale data. You can obtain optimal results with peak heights of ~1000 fluorescent units.

For more information on optimizing sample preparations for GeneScan analysis, see the *GeneScan Chemistry Guide*.

Optimizing Data Collection

Optimizing run conditions on your ABI PRISM instrument will ensure a higher quality of fragment data.

- Make sure gel run parameters are consistent from run-to-run.
- Choose the right Matrix for the right type of gel and run parameters.

For more information on optimizing run conditions, see the user's manual for the ABI PRISM instrument that you are using.

Completing a GeneScan Sample Sheet

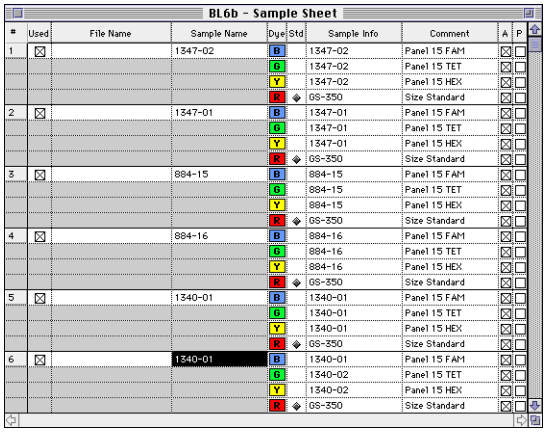
Introduction

You must completely fill out a GeneScan Sample Sheet before running samples on your ABI PRISM instrument (Figure 2-1).

For detailed information on how to correctly fill out a GeneScan Sample Sheet, see the instrument user's manual for the ABI PRISM instrument that you are using or the *GeneScan Analysis User's Manual*.

Example of a GeneScan Sample Sheet

Sample Sheets identify the lane number and contents of each sample that you run when electrophoresing samples on an ABI PRISM instrument.



BL6b - Sample Sheet

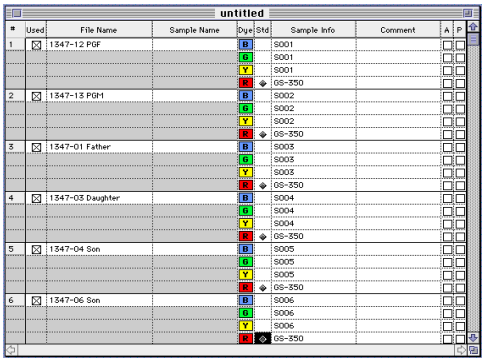
#	Used	File Name	Sample Name	Dye	Std	Sample Info	Comment	A	P
1	☒		1347-02	B		1347-02	Panel 15 FAM	☒	☐
				G		1347-02	Panel 15 TET	☒	☐
				Y		1347-02	Panel 15 HEX	☒	☐
				R	◆	GS-350	Size Standard	☒	☐
2	☒		1347-01	B		1347-01	Panel 15 FAM	☒	☐
				G		1347-01	Panel 15 TET	☒	☐
				Y		1347-01	Panel 15 HEX	☒	☐
				R	◆	GS-350	Size Standard	☒	☐
3	☒		884-15	B		884-15	Panel 15 FAM	☒	☐
				G		884-15	Panel 15 TET	☒	☐
				Y		884-15	Panel 15 HEX	☒	☐
				R	◆	GS-350	Size Standard	☒	☐
4	☒		884-16	B		884-16	Panel 15 FAM	☒	☐
				G		884-16	Panel 15 TET	☒	☐
				Y		884-16	Panel 15 HEX	☒	☐
				R	◆	GS-350	Size Standard	☒	☐
5	☒		1340-01	B		1340-01	Panel 15 FAM	☒	☐
				G		1340-01	Panel 15 TET	☒	☐
				Y		1340-01	Panel 15 HEX	☒	☐
				R	◆	GS-350	Size Standard	☒	☐
6	☒		1340-01	B		1340-01	Panel 15 FAM	☒	☐
				G		1340-02	Panel 15 TET	☒	☐
				Y		1340-02	Panel 15 HEX	☒	☐
				R	◆	GS-350	Size Standard	☒	☐

Figure 2-1 Example of a GeneScan Sample Sheet

How Genotyper Uses Sample Sheet Information

Sample Sheet information from GeneScan is essential for associating the nature of sample fragments with individual dye/lanes and tables in Genotyper. For example, Figure 2-2 shows how Genotyper incorporates information entered in the Sample Info and File Name fields of a GeneScan Sample Sheet into Genotyper tables.

GeneScan Sample Sheet



Genotyper table

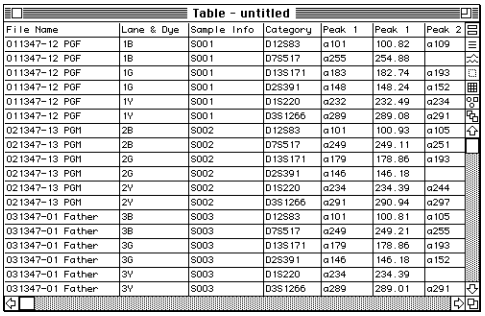
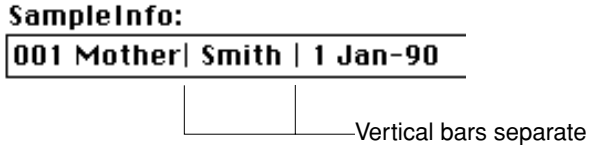


Figure 2-2 GeneScan Sample Sheet data used in Genotyper tables

Sample Subfield Example

When you fill in the Sample Info field of the GeneScan Sample Sheet, you can edit the Sample Info field and create Sample subfields. You can use Sample subfields for Genotyper table entries when you create tables.

Example



Plan for the Find Command

You can increase the utility of the Find command in Genotyper by carefully planning the format of the information you put into the Sample Info field of the GeneScan Sample Sheet.

Example

If you have 12 samples, numbered 1, 2, 3, ..., 12, and you enter these numbers into the Sample Info field, then, when you search for all dye/lanes containing a “1” in the Sample Info field, not only will you select sample 1, you will also get samples 10, 11, and 12.

A better plan would be to number the samples 01, 02, 03, and so on, so that a search for the text “01” would select only the desired dye/lanes.

In addition, you can place key words in the Sample Comments field that distinguish samples from each other. For example, enter “ladder” for those lanes or capillaries containing allelic ladders.

Planning for Automation

Introduction

In Genotyper, you can automate many of the repetitive genotyping tasks, simplifying such analysis procedures as importing GeneScan data, labeling peaks, filtering peak labels, and working with plot and table information.

Planning for automation involves choosing the appropriate Genotyper automation feature and the appropriate genotyping tasks to automate for each project.

Ways to Automate

To automate different genotyping tasks:

Genotyping Task	Automation Method	See
Importing Sample files	Running the Set Import Macro	"Importing GeneScan Files" on page 3-13
	Creating a Macro	"Creating Macros from the Step List" on page 4-12
	Using a Template	"Using and Creating Templates" on page 4-16
Defining categories	Defining Categories	"Defining Categories for Labeling" on page 6-4
	Creating a Macro	"Creating Macros from the Step List" on page 4-12
	Using a Template	"Using and Creating Templates" on page 4-16
Selecting Dye/lanes	Using the Find Command	"Searching and Sorting Through Lists" on page 5-9
	Creating a Macro	"Creating Macros from the Step List" on page 4-12
	Using a Template	"Using and Creating Templates" on page 4-16

To automate different genotyping tasks: *(continued)*

Genotyping Task	Automation Method	See
Labeling peaks	Automatic Peak Labeling	"Automatic Peak Labeling" on page 6-26
	Defining Categories	"Defining Categories for Labeling" on page 6-4
	Creating a Macro	"Creating Macros from the Step List" on page 4-12
	Using a Template	"Using and Creating Templates" on page 4-16
Filtering labels	Automatic Label filtering	"Filtering Labels" on page 6-29
	Defining Categories	"Defining Categories for Labeling" on page 6-4
	Creating a Macro	"Creating Macros from the Step List" on page 4-12
	Using a Template	"Using and Creating Templates" on page 4-16
Generating plots	Defining Categories	"Defining Categories for Labeling" on page 6-4
	Creating a Macro	"Creating Macros from the Step List" on page 4-12
	Using a Template	"Using and Creating Templates" on page 4-16

To automate different genotyping tasks: *(continued)*

Genotyping Task	Automation Method	See
Generating tables	Defining Categories	"Defining Categories for Labeling" on page 6-4
	Creating a Macro	"Creating Macros from the Step List" on page 4-12
	Using a Template	"Using and Creating Templates" on page 4-16
Exporting genotyping data	Using a Template	"Using and Creating Templates" on page 4-16
	Creating a Macro	"Creating Macros from the Step List" on page 4-12
	Setting Linked Program Preferences	"Planning for Linking to GenBase" on page 2-9

Planning for Linking to GenBase

Introduction

One of the components of the ABI PRISM Genotyping Software System, GenBase, is a genotype and phenotype database. It can store relevant sample, or disease information, phenotype information as well as Genotyper results data.

If you are planning to link to the GenBase database application after performing genotyping tasks, you must first install GenBase, and prepare the database for data exchanges.

Preparing GenBase for Linking

The following table shows which topics to refer to in the *ABI PRISM GenBase User's Manual* for instructions.

For instructions on...	See
Installing GenBase	"Chapter 1, Introduction"
Planning for the kinds of Tables you are going to use when Linking to GenBase	"Chapter 2, Preparing the Database"
Preparing the database for data exchange with Genotyper	"Chapter 2, Preparing the Database"

3 Getting Started

Chapter Overview

Introduction

This chapter discusses how to work with Genotyper Documents to produce results meaningful to your particular research activities. It covers some of the basic Genotyper procedures required for all genotyping applications.

In This Chapter

This chapter contains the following topics:

Choosing a Genotyping Application

Introduction

The first step in using Genotyper is to decide what kind of genotyping application you want to run. Choosing a genotyping application is the first step in planning your Genotyper project. The features of Genotyper that you use, and how you use them depend on what kind of genotyping application you choose to run.

Kinds of Genotyping Applications

Genotyper enables you to organize your analyzed fragment data into tables. Tables can provide an interpretation of peak data meaningful to your particular genotyping studies. You can print Genotyper tables or export them to databases or other software applications for further analysis.

Kinds of tables Genotyper can produce for some genotyping applications:

For this application...	Genotyper can produce...	For details see...
Linkage Mapping	A table of alleles that you can export to a mapping application or a database.	"Linkage Mapping" on page 3-4
Gene Expression Profiling	A comparative analysis table containing normalized data.	"Gene Expression Profiling" on page 3-6
AFLP	A comparative analysis table of polymorphic peaks that shows the presence or absence of peaks.	"Web Site Information" on page 1-8
Paternity Testing	A table of alleles that have been checked for Mendelian inheritance.	"Checking for Mendelian Inheritance" on page 8-37
Forensics/Human Identification	A genotype table to discriminate between individuals.	The *ABI PRISM Genotyper 2.0 Applications Tutorials*
SSCP	A table of alleles identifying mutants and wild types.	The *ABI PRISM Genotyper 2.0 Applications Tutorials*

Kinds of tables Genotyper can produce for some genotyping applications: *(continued)*

For this application...	Genotyper can produce...	For details see...
Loss of Heterozygosity	A table of peak height ratios that identifies loss of heterozygosity in DNA samples from tumor cells and normal cells.	"Using Analyze and Calculate in Table Commands–An LOH Example" on page 8-26

Linkage Mapping

Linkage mapping applications identify polymorphic fragments by size.

Steps for using Genotyper for linkage mapping applications and where to find related task information for performing each step follow.

To import and analyze data:

Step	Action	See
1	Allocate enough memory for the number of GeneScan files that you are going to import.	"Setting Memory Allocation" on page 1-11
2	Import GeneScan data.	"Importing GeneScan Files" on page 3-13
3	Are you using a Template? Yes, go to step 5. No, go to next step.	"Using and Creating Templates" on page 4-16
4	Define categories.	"Defining Categories for Labeling" on page 6-4
5	Select dye lanes.	"Working with Dye/lane Lists" on page 5-1
6	Label peaks.	"Approaches to Labeling" on page 6-2
7	Filter labels.	"Filtering Labels" on page 6-29
8	Make a table.	"Setting Up a Table" on page 8-2

To review analyzed data:

Step	Action	See
1	View plot data.	"Viewing Plots of Imported Dye/Lanes" on page 7-2
2	Check for overflows. Are there more than two alleles? Yes, manually remove unwanted labels. No, go to next step.	"Removing Labels" on page 6-42
3	Update table.	"Updating Tables" on page 8-35

To export data:

Step	Action	See
1	Export data:	
	♦ To GenBase	♦ "Importing and Exporting Results Data" on page 10-4
	♦ To a spreadsheet or linked programs	♦ "Linking to Programs and Files" on page 11-1
	♦ To a file	♦ "Exporting Tables" on page 8-46
2	Print results.	"Printing Genotyper Document Windows" on page 3-20

Gene Expression Profiling

Gene expression applications analyze the quantities of nucleic acid fragments in analyzed samples in terms of peak heights and peak areas.

Steps for comparing peak quantities and where to find related task information for performing each step.

To quantify sample fragments:

Step	Action	See
1	Allocate enough memory for number of GeneScan files you are going to import.	"Setting Memory Allocation" on page 1-11
2	Import GeneScan data.	"Importing GeneScan Files" on page 3-13.
3	Are you using a Template? Yes, skip Defining categories. No, go to next step.	"Using and Creating Templates" on page 4-16
4	Define categories.	"Defining Categories for Labeling" on page 6-4
5	Select dye/lanes in the Dye/lane list.	"Working with Dye/lane Lists" on page 5-1
6	Label peaks.	"Approaches to Labeling" on page 6-2
7	Filter labels.	"Filtering Labels" on page 6-29

To compare sample quantities:

Step	Action	See
1	Normalize labels by control peaks	"Labeling Normalized Peaks—an Example" on page 6-45
2	View plot data	"Viewing Plots of Imported Dye/Lanes" on page 7-2
3	Generate a table	"Setting Up a Table" on page 8-2

To compare sample quantities: *(continued)*

Step	Action	See
4	Normalize Labels	
	♦ Using scale factors	♦ "Using Scale Factors for Quantitative Applications" on page 5-15
	♦ Using Normalize Peaks command	♦ "Labeling Normalized Peaks—an Example" on page 6-45
	♦ Using Calculate in Table command	♦ "Calculating Results from Table Data" on page 8-19

To export results data:

Step	Action	See
1	Export data	
	♦ To a third-party application	♦ "Linking to Programs and Files" on page 11-1
	♦ To a file	♦ "Exporting Tables" on page 8-46
2	Print results	"Printing Genotyper Document Windows" on page 3-20

Opening Genotyper Documents

Definition

In Genotyper, you perform all tasks in a Genotyper Document. All Genotyper Documents show different representations of size and quantity data for associated GeneScan files and results of all analysis tasks performed in Genotyper.

You can view the different parts of a Genotyper Document by opening document windows from the Main window.

How to Open Genotyper Documents

The steps for opening Genotyper Documents differ depending on whether you are opening an existing Genotyper Document, or creating a new one.

To open a Genotyper document:

If you...	Then...
Are working with an existing Genotyper Document or a template file	a. Choose Open...from the File menu. b. Locate and select the document you want to open. c. Click Open.
Do not have an existing Genotyper Document	Choose New from the File menu.

Starting from the Main Window

Definition

The Main window displays all parts of a Genotyper Document. You perform all Genotyper tasks from the Main window, or from Document windows you can open within the Main window that let you view GeneScan file data, and results data in different formats.

The Main Window

When you open a new Genotyper Document, a blank Main window appears. Once you import GeneScan data and create all the parts of a Genotyper Document, the Main window will look like the figure below.

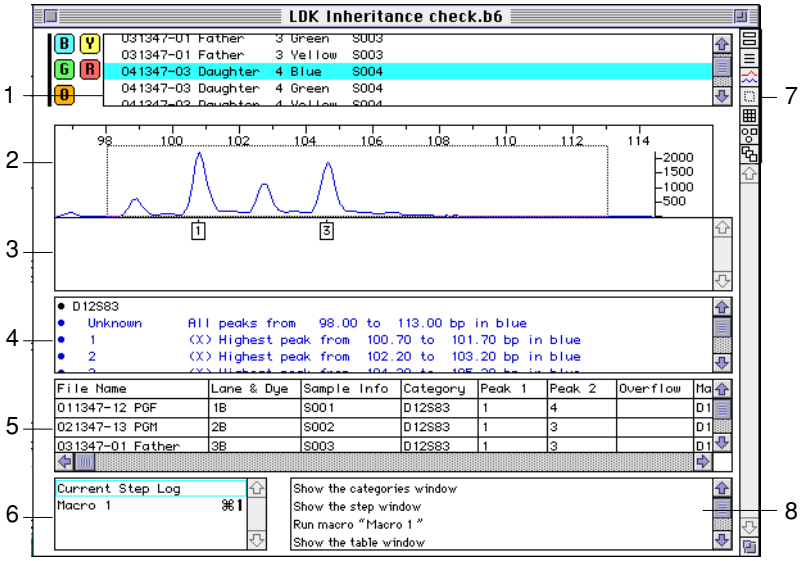


Figure 3-1 The Main Window

Parts of the Main Window

The following table describes the parts of the Main Window in Figure 3-1 on page 3-9.

Item	Name	Description
1	Dye/lane list	Shows specific dye/lanes available for analysis.
2	Upper Graphical Area	Shows electropherogram plots.
3	Lower Graphical Area	Shows peak labels.
4	Category list	Shows criteria for a group of peaks selected on the basis of parameters you define using the category features. For example, dye color, size, or height.
5	Table Area	Shows tabular data for created tables.
6	Macro list	Lists the names of the macros that you have created and can run.
7	Window selection buttons	Open windows for a particular Genotyper document, and provides access to GenBase and GenoPedigree data.  Main window Dye/lane List window Plot window Category window Table window GenoPedigree GenBase
8	Step list	Contains the list of steps for the current Step Log or the macro selected in the Macro list.

Viewing Genotyper Document Windows.

Introduction

From the Main window, you can open windows that allow you to view parts of a Genotyper Document related to specific kinds of data.

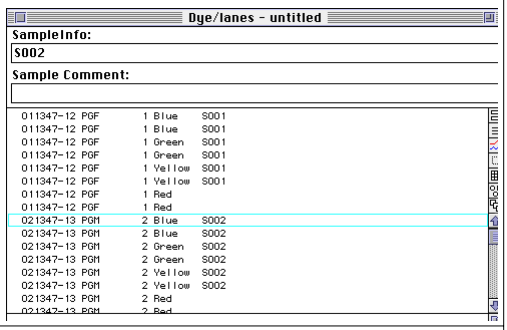
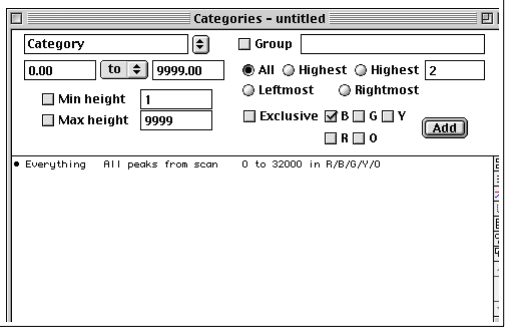
Opening Document Windows

Document windows display the different parts of a Genotyper Document.

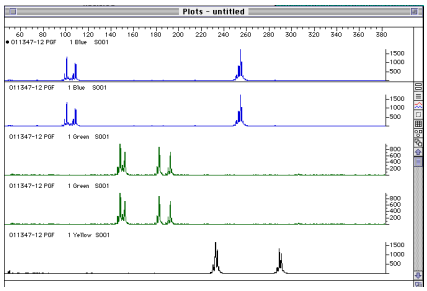
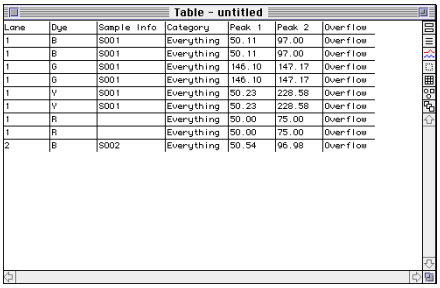
Example

If you want to view details of plot data, you can open up the Plot window.

To use the selection buttons to open Document windows:

To see the...	Click...	And Genotyper displays...
Dye/lane window		
Category window		

To use the selection buttons to open Document windows: *(continued)*

To see the...	Click...	And Genotyper displays...
Plot window		
Table window		

Saving Documents

You can save a Genotyper Document as a file. Although the different types of document windows for a document are not saved individually, all of the data for the document is saved in one file regardless of which windows are open at the time you save the document.

Importing GeneScan Files

Introduction

Importing GeneScan files supplies peak data for all Genotyper tasks. A GeneScan file can be a Sample file (GeneScan version 2.0 or later), or a Results file (GeneScan 1.x versions).

IMPORTANT You cannot import GeneScan Gel files.

Process

When you import a GeneScan file in Genotyper, Genotyper extracts Sample file information and generates one Dye/lane list entry for each dye color of each lane. Each Dye/lane list entry contains size, quantity, and sample information for all fragments labeled with a single dye color and electrophoresed in a single lane.

For more information on how Genotyper generates dye/lanes from imported GeneScan data see, “Where Dye/lanes Come From” on page 5-2.

Ways to Import GeneScan Files

You can either import GeneScan data all at once or in batches. There are advantages and disadvantages to both approaches.

The advantages and disadvantages of importing GeneScan data all at once or in batches:

Way to Import	Advantages	Disadvantages
All GeneScan data at once. Useful when you are importing data from a few gels or approximately 36 lanes or capillaries.	More convenient. You can see all results after performing the command just once.	♦ Requires that you allocate more memory to Genotyper for more than 36 lanes. ♦ Inefficient processing will slow down the operation.
In batches. Useful when you are importing data from many gels or more than 36 lanes or capillaries.	♦ More GeneScan data can be processed more efficiently. ♦ Does not require as much memory allocated to Genotyper.	You cannot see all dye/lanes at one time.

Storing GeneScan Files

When setting up and running gels, the number of gels you run and the number of lanes in each gel determines the number of GeneScan sample files in a project.

Example

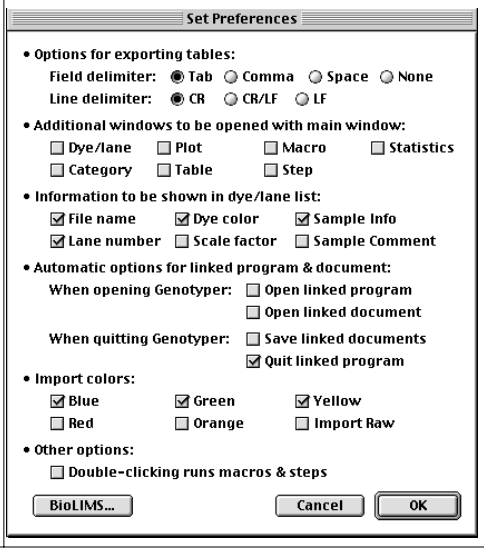
Number of Gels x Number of lanes = Number of GeneScan sample files

Note You usually do not need to refer to Gel files once Sample files have been generated from them.

You can optimize the performance of the ABI PRISM Genotyping Software System if you store all GeneScan sample files in one location, for example one shared disk on a file server, and process all data from that storage location. If you move GeneScan sample files around, then GenBase will not be able to locate them if you want to refer to them at a later time.

How to Import GeneScan Files

To import GeneScan files:

Step	Action
1	Choose Edit: Set Preferences... The Set Preferences dialog box opens. 

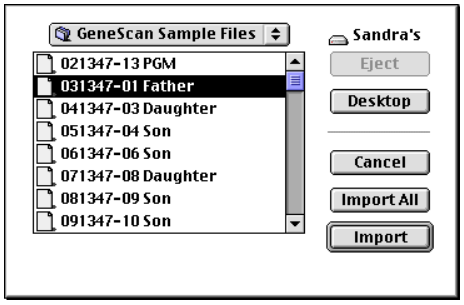
To import GeneScan files: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Select the appropriate checkboxes for import options.

<table>
<tr><th>If you select...</th><th>Then Genotyper imports...</th></tr>
<tr><td>Import raw data</td><td>Unprocessed collection data, as well as data processed by GeneScan.

Note Not selecting the Import raw data checkbox will improve system performance when importing large numbers of GeneScan files.</td></tr>
<tr><td>Import colors</td><td>Imports only sample fragment data labelled with the dye color you select.</td></tr>
</table></td></tr>
<tr><td>3</td><td>Choose Import GeneScan Files...from the FIle menu.

The Import GeneScan Files dialog box appears.

 
</td></tr>
<tr><td>4</td><td>Locate and select the GeneScan Sample file, or Results file that you want to import.</td></tr>
<tr><td>5</td><td>Click Import.

If you want to import all GeneScan files in a folder, select one file in the folder, then click Import All.

Genotyper remembers the last folder from which you imported files.</td></tr>
</table>

Importing Project Files

You can import all files in a GeneScan Project by selecting the project file and clicking import.

Import All does not include Project files. If you want to import from more than one project file, you must select each project file one at a time.

Editing Document Lists

Introduction

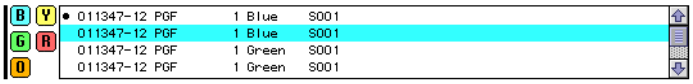
Genotyper supplies a number of general editing tools that you can use to modify items in Genotyper Document Dye/lane lists, Category lists, or Macro lists.

Making a List Active

A vertical bar to the left of a list indicates that list is active. This means that all the edit commands apply only to this list.

To make a list active, press the tab key repeatedly until the thin vertical bar appears to the left side of the list you want to make active.

Vertical bar



Selecting Items in a List

To edit entries for any list item in Genotyper, you must first select the items you are going to edit.

To select items in a list:

If you are selecting...	Then...
A single item	♦ Click on the item in the list. The item is highlighted, indicating that it is selected.
A range of continuous items	♦ Click on an item in the list. ♦ Hold down the shift key and click on the last item in the range. All the items in the range are highlighted.
Discontinuous items in a list	♦ Click on an item in the list. ♦ Hold down the command key and click on another item. ♦ Repeat the previous step for each item you want to select. Only the items selected are highlighted.

Editing Document Windows

Using the Cut, Copy, and Paste Commands

The Cut, Copy, and Paste commands available from the Edit menu allow you to transfer the same type of information from one window to another. For example, if you copy an item in a Macro list in one window and paste it into a Macro list in another window, all of the macro data will be transferred. However if you paste the same information onto another application, only the text is transferred; no data is transferred.

IMPORTANT Copying or pasting many dye/lanes from one document to another requires a lot of memory. Therefore, we recommend that you copy or paste only a few dye/lanes at a time.

Using the Clipboard

Items you copy from Genotyper Document windows will appear on the Clipboard.

To view the Clipboard, choose Show Clipboard in the Edit menu.

Note The Clipboard stores only the last item (or group of items) copied.

Copying Windows

You can use the Copy Window command to copy a picture of the Main window or the Plot window.

To copy a window, choose Copy Window in the Edit menu.

A picture of everything in the active window (except the title bar) is copied to the Clipboard. You can now paste this image into a paint or draw program to edit it.

Locking and Unlocking Documents

Introduction

After you have created a set of categories, or macros, you can lock them to prevent accidental modification. Categories, macros, and steps that are locked cannot be edited or cleared until they are unlocked.

How to Lock Documents

Choose Lock from the File menu.

Padlock icons appear next to the locked panes and in the File menu Lock is checked.

How To Unlock Documents

Choose Unlock from the File menu.

The padlock icons disappear and in the File menu Unlock is checked.

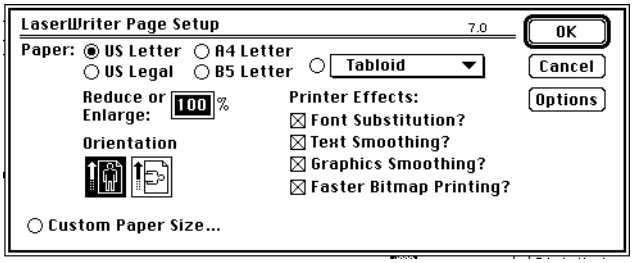
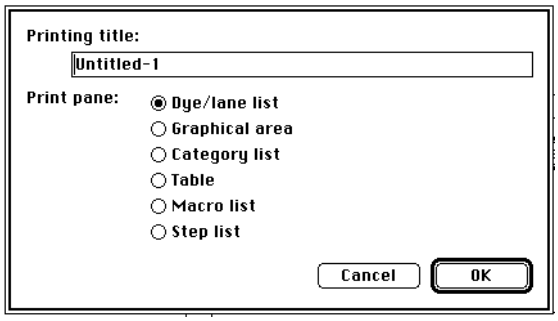
Printing Genotyper Document Windows

Introduction

You can print any active Genotyper window in a Genotyper Document.

How to Print a Document Window

To print the Genotyper Document Window:

Step	Action
1	Select the Genotyper Document Window that you want to print.
2	Choose Page setup...from the File menu. Your printer's Page Setup dialog box appears. 
3	Select the appropriate checkboxes and options.
4	Click OK.
5	Choose Print...from the File menu. The Print dialog box appears. 

To print the Genotyper Document Window: *(continued)*

Step	Action
6	Enter a printing title. If you are printing when the Main window is active, the Print dialog box prompts you to specify which part of the document that you want to print.
7	Select the appropriate radio button.
8	Click OK. The Print dialog box appears.
9	Click Print to begin printing. **Note** To print multiple dye/lane plots, choose Show Plot Window before printing.

Automating Genotyping Procedures 4

Chapter Overview

Introduction Genotyper can automatically perform all calculations, comparative analyses, and peak labeling activities once you specify appropriate settings and issue the appropriate sequence of commands. Using Genotyper macros, and templates, you can automate the setting of analysis parameters, and issuing of commands to perform either a particular analysis procedure, or all of the procedures for an entire genotyping application.

In This Chapter This chapter contains the following topics:

Approaches to Automating Procedures

Introduction

Genotyper uses templates, macros, and Apple Scripts to automate procedures for an application.

The macros and templates supplied with Genotyper automate procedures presumed necessary to complete particular genotyping applications. They serve as examples of actual procedures you might want to perform in your application. You must modify them for use with your particular Genotyper application.

Definitions

Definitions of terms used:

Term	Definition
Template	Templates are ready-to-use Genotyper applications. A Genotyper template contains the Category list and macros necessary for automatic analysis of particular applications.
Macro	A macro is a sequence of commands or steps that you can run to perform a particular analysis procedure.
AppleScript	AppleScript is a programming language that you use to create Scripts, lists of Genotyper commands for your computer to perform.

Automation Options

Genotyper supplies customizable templates for automating complete genotyping applications, and macros for automating individual procedures.

Options for automating genotyping applications and procedures:

If you want to automate a Genotyping...	And...	Then See Topic
Application	You know that a template for a similar application exists,	"How to Create Templates" on page 4-17.
Procedure within an application	You know that a macro exists for that procedure,	"How to Run Macros" on page 4-5.
	You know that a macro does not exist for that procedure,	"How to Create a New Macro" on page 4-12.

Running Macros

Introduction

Running a macro executes a pre-defined set of Genotyper commands and functions that automates a procedure. Genotyper provides several macros that can serve as examples of the kinds of macros you can run to automate analysis tasks.

In addition to running supplied macros, you can create your own macros from the Step list, save them and run them.

For more information on creating your own macros, see “Recording Steps in the Step List” on page 4-7.

Supplied Macros

Genotyper supplies a number of sample macros on the Tutorials disk that you can select and run to perform Genotyper procedures.

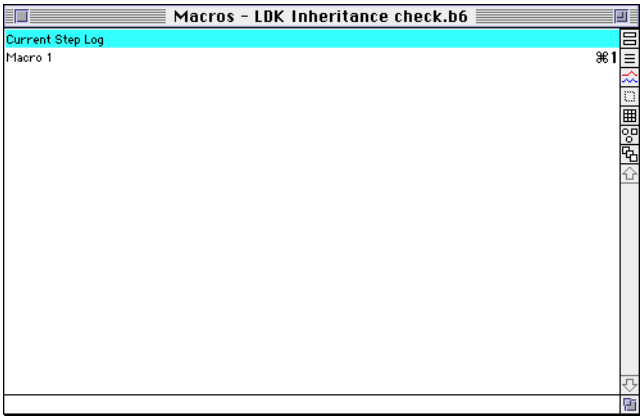
Tips for Running Macros

You can set preferences so that you can run macros by double-clicking a macro after selecting it. Choose Set Preferences from the Edit menu, and select the Double-clicking runs macros & steps checkbox.

How to Show the Macro Window

The Macro window shows macros that are stored in the active Genotyper Document or Template.

To show the Macro window:

Step	Action
1	Open the Main window
2	From the Views menu, select Show Macro window. The Macro window appears. 

How to Run Macros

You can run any macro that is displayed in the Macro list.

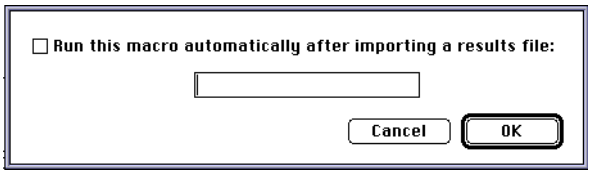
To run a macro:

Step	Action
1	From the Macro List, select a macro.
2	Choose Run Macro from the Macro menu. All of the steps in the selected macro are run automatically. **Note** You can assign a command key combination when a macro is created to run the macro. If a command key is assigned, simply press ⌘-[assigned key] to run the macro.

How to Run a Macro After Importing GeneScan Data

You can use the Set Import Macro command to specify a macro that you want to run immediately after you have imported GeneScan data into Genotyper.

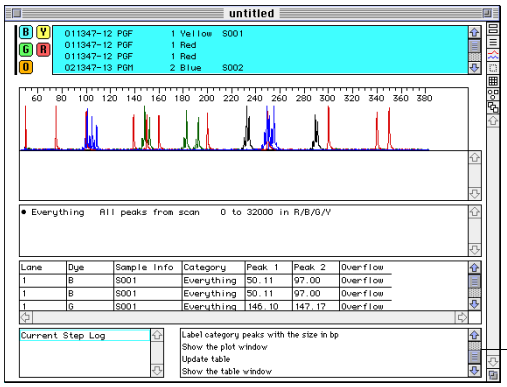
To use the Set Import command:

Step	Action
1	Choose Set Import Macro from the Macro menu. The Set Import Macro dialog appears. 
2	Type in the name of the macro that you want to run immediately after you import GeneScan data.
3	Select the checkbox to run the macro automatically, and click OK. The name of the macro selected to run automatically appears in the Import GeneScan data dialog box.

Recording Steps in the Step List

Definition The Step list records many of the Genotyper commands after you issue them. You can create macros from the steps you record in the Step list.

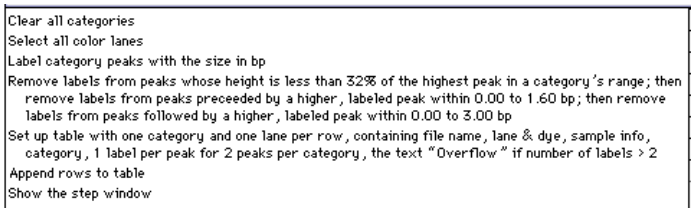
Location of Step Window The Step list appears in the Step window, located in the lower right-hand corner of the Main window.



How to Show the Step Window

The Step window shows the current list of recorded steps, or the steps that make up a macro.

To display the Step window:

Step	Action
1	Open the Main Window.
2	Select a macro in the Macro list of the Macro window.
3	From the Views menu, select Show Step window. The Step window showing the steps for the currently selected macro, appears. 

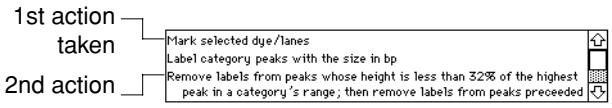
Displaying Commands issued in Documents

When you select Current Step Log in the Macro list of the Macro window, and show the Step window, Genotyper displays commands you have issued since you last cleared the Step Log.

How to Record Steps

Genotyper records most tasks you perform or commands you issue as a step in the Step list.

To record steps in the Step list:

Step	Action
1	Pull down the Macro menu and verify that Record Steps is checked, If it's not checked, click it to activate it.
2	Actions you perform in Genotyper, such as marking, labeling, and filtering are recorded as steps in the Step list. 

How to Turn Off Step Recording

If you are not making a macro, and do not want to fill up the Step list, you can turn off step recording.

To turn off the recording of steps:

Step	Action
1	Pull down the Macro menu.
2	Click Record Steps to deactivate it.

Editing the Step List

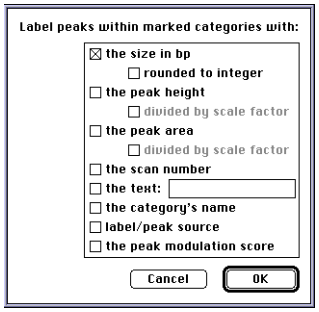
Introduction

You can edit the contents of your current Step list before or after creating a macro.

How to Edit a Step

You can edit steps in the Step list, and change the parameters set when you performed the step.You can only edit steps in the Step list that involve making selections from a dialog box.

To edit steps in the Step list:

Step	Action
1	Select a step from the Step list.
2	Choose Edit Step...from the Macro menu. The dialog box for that step appears. This figure shows an example of the dialog box that appears if you edit the step for a Label Peaks...command. 
3	Change any of the parameter settings in the dialog box that appears.
4	Click Replace. The step with edited parameter settings replaces the original step. **Note** You cannot change a step to a completely different type of step.

Copying and Pasting from the Step List

You can Cut, Copy and Paste steps to change the sequence of steps in the Step list.

To copy and paste steps in the Step list:

Step	Action
1	Select a step in the Step list.
2	Choose Copy from the Edit menu.
3	Click on the step that precedes the place you want the copied step to be inserted.
4	Choose Paste from the Edit menu. The step is pasted in the place you selected in the Step list.

How to Run a Step

If you want to find out what genotyping task a step performs, you can run that step from the Step list.

To run a step in the Step list:

Step	Action
1	Select a step in the Step list.
2	Choose Run Step from the Macro menu. This runs the step you selected, and repeats that step in the Step list. **Note** If you have set preferences appropriately, you can double-click on a step to run it.

Creating Macros from the Step List

Introduction

If a macro does not already exist for a particular genotyping procedure that you plan on running repeatedly, you can create a macro that performs all of the steps in the procedure, and run it each time you want to repeat that procedure for a different set of GeneScan data.

How to Clear the Step List

Before you record steps for a macro, you will want to clear the Step list of steps that have been previously recorded.

To clear the Step list:

Step	Action
1	Make sure that you have selected the Current Step Log in the Macro window.
2	Choose Clear Step Log from the Macro menu.

How to Create a New Macro

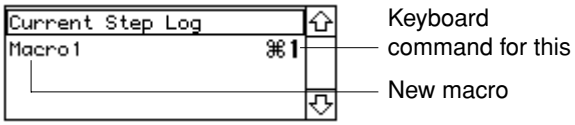
Once you have cleared the Step list of all previously recorded steps, you can create a new macro for a genotyping procedure by performing all of the steps in the procedure once. Genotyper records each of the steps in the Step list.

Note You can select more than one macro at a time from the Macro list to Cut, Copy, and Paste. If more than one macro is selected, nothing appears in the Step list.

To create a new macro:

Step	Action
1	Pull down the Macro menu and verify that Record Steps is checked, If it's not checked, click it to activate it.
2	Perform all of the steps in the genotyping procedure for which you want to create a macro. Check the Step list to make sure that each step has been recorded.

To create a new macro: *(continued)*

Step	Action
3	Choose Save Step Log...from the Macro menu. The New Macro name dialog box appears 
4	Enter the name of the new Macro in the text box.
5	Click the checkbox and enter the key you want to press with the command key to run this Macro from the keyboard.
6	Click OK.
7	The new Macro appears in the Macro list. 

How to Change a Macro Name

You can change the name of any macro.

IMPORTANT If any other macros refer to the macro whose name was changed, you must edit those other macros to use the new name.

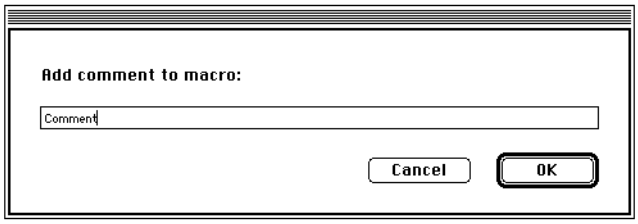
To change the name of a macro:

Step	Action
1	Select a macro from the Macro list.
2	Choose Change Macro Name...from the Macro menu. The New macro name dialog box appears with the name of the macro in the text box.
3	Enter the new macro name.
4	Click OK. The new macro name should now appear in the Macro list. The comment step at the beginning of a macro does not change when you change the name of the macro.

How to Add a Comment to a Macro

You can add a comment about any macro in the Macro list, and make the comment appear in the macro.

To add a comment to a macro:

Step	Action
1	Choose Add Comment...from the Macro menu. The Add Comment dialog box appears. 
2	Type in a comment that you want to append to the macro.
3	Click OK. The comment appears in the Current Step Log.
4	To make the comment appear in a macro, you can cut and paste it into the macro.

Using and Creating Templates

Definition

Genotyper provides a number of templates that automate all genotyping and analysis tasks for specific applications. Each template is designed for a specific Genotyper application and uses sample GeneScan data to demonstrate how to use it.

Supplied Templates

Genotyper supplies templates containing procedure examples for different kinds of Genotyper applications. The template name describes the kind of application it automates.

How to Create Templates

To create a template, you modify any existing template for your specific application.

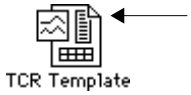
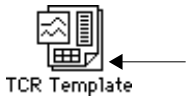
To create a template for your specific application:

Step	Action
1	Make a copy of a supplied template that describes the kind of application that you are running.
2	In the copy, delete the Category list.
3	Make a new Category list specific for your application.
4	Choose Save As....
5	Name the new template.

Saving Templates as Stationery Pads

Saving a template as a stationary pad prevents it from being changed accidentally. A stationary pad can be opened and used, but any changes made can only be saved as a separate document.

To save a template as a stationary pad:

Step	Action
1	Click on a template icon in the Finder to select it. 
2	Choose Get Info from the File menu. The Template info window appears.
3	Click the Stationary pad checkbox at the bottom of the info window.
4	Click the Close box in the upper left-hand corner of the window.The Template is now saved as a stationary pad. The bottom right corner is folded. 

Using AppleScript Scripts

Introduction

You can use the AppleScript programming language to create Scripts that can combine the capabilities of Genotyper with related analysis applications. Using Scripts, you can give your computer a list of things you want it to do, and let the computer do everything on your list.

System Requirements

You can use Apple Scripts on Power Macintosh computers that use system software version 7.5.3 or later.

How to Create Scripts

Like all programs that can be customized with Scripts, Genotyper has an AppleScript dictionary. Use the AppleScript editor provided with the system software to create Scripts for automating Genotyper procedures.

To create Scripts for your specific application:

Step	Action
1	Turn on the Script Editor's recorder and perform a set of Genotyper procedures. AppleScript keeps a list of what you do while the recorder is turned on.
2	Turn the recorder off. You can see the recorded Script.
3	When you run the Script, your computer repeats your actions automatically.

See the AppleScript documentation supplied with your Apple system software, for instructions about how to use AppleScript Scripts.

Working with Dye/lane Lists

5

Chapter Overview

Introduction Dye/lanes contain sample information for electrophoresed nucleic acid fragments. They provide the source data for all Genotyper procedures. Genotyper Documents contain a list of all dye/lanes related to that document. This chapter discusses how you can view, search, and use the information contained in Dye/lane lists to perform particular genotyping analysis tasks.

In This Chapter This chapter contains the following topics:

Where Dye/lanes Come From

Introduction

Dye/lane lists contain entries that correspond to imported GeneScan files. You use information contained in Dye/lanes for all Genotyping comparison and analysis tasks.

Phases of the Process

Genotyper generates from one to four dye/lanes for each lane of each GeneScan file you import. The following diagram shows three phases in the process of generating Dye/lane list entries from electrophoresed dye-labeled nucleic acid fragments (Figure 5-1).

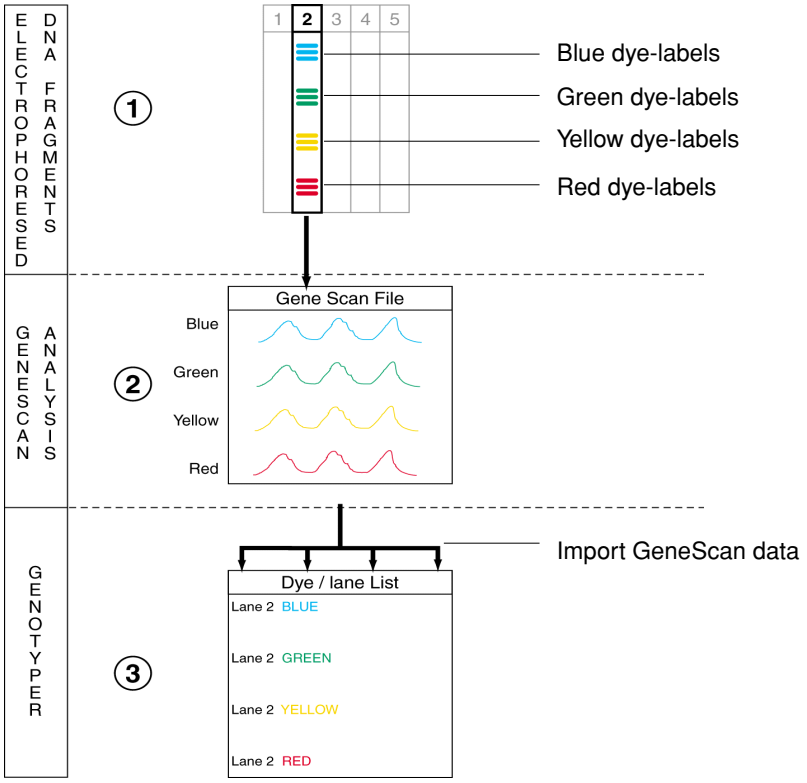


Figure 5-1 Where Dye/lane lists come from

What Happens in Each Phase

What happens in each phase of the dye/lane generation process.

Phase	Process
1	Nucleic acid fragments are labeled with one to four different dye colors (blue, green, yellow, red), and electrophoresed in a single lane (lane 2) of a gel based automated DNA sequencer.
2	GeneScan extracts fragment information from lanes or capillaries and generates one GeneScan file per lane or capillary. Each GeneScan file contains size and quantity information for each dye/labeled fragment.
3	When you import a GeneScan file in Genotyper, Genotyper extracts file information, and generates one dye/lane list entry for each dye color. Each dye/lane list entry contains size, quantity, and sample information for all fragments labeled with a single dye color and electrophoresed in a single lane.

Viewing Dye/lane Lists

Definition

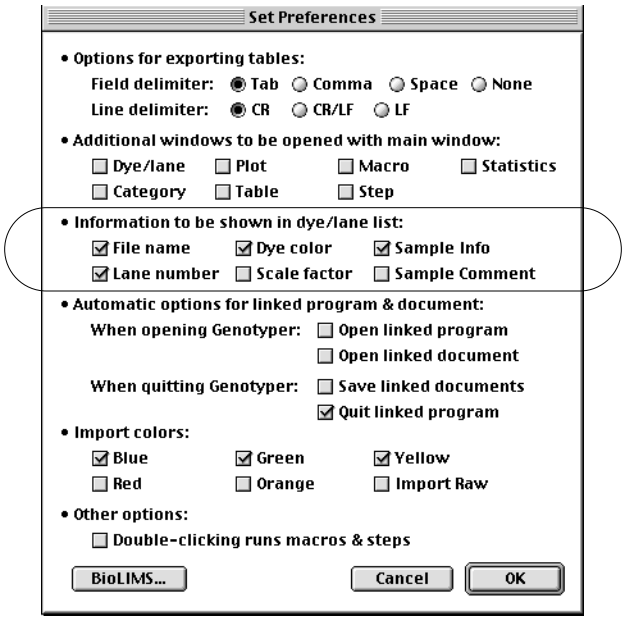
The Dye/lane list is a list of all dye/lanes in the GeneScan data that you have imported into Genotyper. Dye/lanes are added to the list as you import them from GeneScan.

Settings in the Dye/lane sorting dialog box determines the sort order of the list. For more information on sorting dye/lane lists see “Searching and Sorting Through Lists” on page 5-9.

Setting Viewing Preferences

You can set preferences for the information that appears in the Dye/lane list when you view it. Preference settings apply to all open Genotyper Documents, not just the active document, and are saved in the Genotyper Preferences file.

To set Dye/lane list viewing preferences:

Step	Action
1	Choose Set Preferences...in the Edit menu. The Set Preferences dialog box appears. 

To set Dye/lane list viewing preferences: *(continued)*

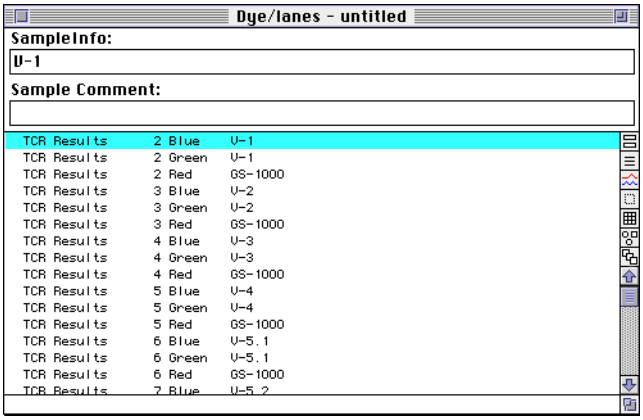
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Under the bullet “Information to be shown in dye/lane list:”, select the checkboxes for what you want to view in the Dye/Lane list.

<table>
<tr><th>If you click...</th><th>Then the Dye/Lane list displays...</th></tr>
<tr><td>File Name</td><td>The name of the associated GeneScan file.</td></tr>
<tr><td>Dye color</td><td>The dye color of sample fragment labels.</td></tr>
<tr><td>Sample Info</td><td>Contents of Sample Info field of the GeneScan Sample Sheet.</td></tr>
<tr><td>Lane number</td><td>The lane number, or injection number for ABI PRISM 310 samples, in which sample fragments were electrophoresed.</td></tr>
<tr><td>Scale factor</td><td>Normalization factor that you can apply to dye/lane peaks.</td></tr>
<tr><td>Sample Comment</td><td>Comments entered in the GeneScan Sample Sheet.</td></tr>
</table>
</td></tr>
</table>

Viewing the Dye/Lanes Window

The Dye/lanes window displays the Dye/lanes list, and also contains associated Sample Information and Sample Comments from GeneScan files.

To view the Dye/Lanes window:

Step	Action
1	From the Main Window, click the Dye/lane window icon. The Dye/lanes window appears. 
2	Click on the dye/lane of interest, for example, the first one. This displays Sample Information, and Sample Comments for the selected Dye/lane, and allows you to edit these items. These fields contain the same information that was entered in the Sample Sheet for associated GeneScan files. **! WARNING ! Information in the Dye/lanes window is not saved back into the original GeneScan files. The edited data in the Sample Info and Sample Comment fields applies only to the current Genotyper Document.**

Clearing the List

Imported GeneScan data are appended to the Dye/lanes list. Therefore, if you are beginning a new Genotyping session, you might want to clear existing GeneScan data from the list

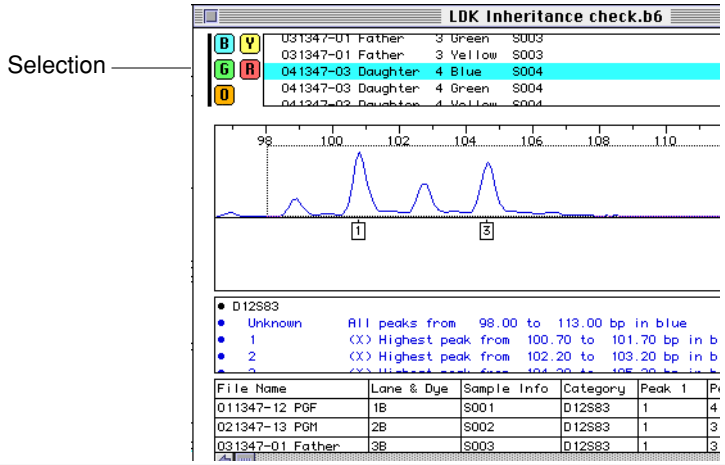
To clear the Dye/lane list:

Step	Action
1	Choose Clear Dye/Lane List in the Analysis menu. The Dye/Lane list is cleared. **Note** If you want to undo this command, choose Undo in the Edit menu. The Undo command must be the next command.

Selecting Dye Colors

Using the Dye Selection buttons you can select those lanes that have sample fragments labeled with the dye color you select.

To display Dye/lanes by the color of the dye-label on fragments in the lane:

Step	Action
1	If the Main window is not already displayed, choose Show Main Window from the Views menu. The Main Window appears. 

To display Dye/lanes by the color of the dye-label on fragments in the lane: *(continued)*

Step	Action
2	Click one of the four Dye Selection buttons to select only dye/lanes labeled with the selected dye colors; B (blue), G (green), Y (yellow), R (Red). To add to your selection, hold down the Shift key and click another Dye Selection button.

Searching and Sorting Through Lists

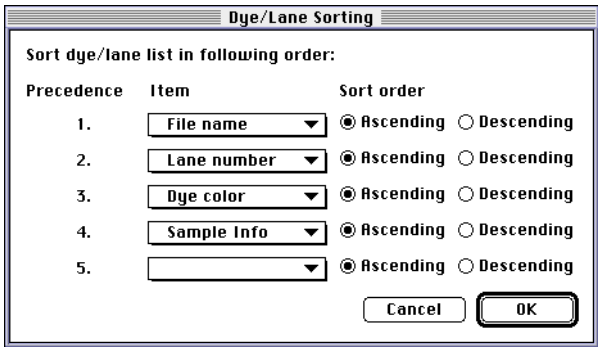
Introduction

For many Genotyper applications, particularly ones in which you import a large number of GeneScan files, you will often want to locate dye/lanes for particular GeneScan Sample files, or Results files. Genotyper offers several search and sorting features that make it easy for you to locate the particular dye/lane or GeneScan file.

How to Sort Dye/lanes

You can sort the Dye/lanes by various items. You can choose a different sorting order for each Genotyper Document.

To change the sort order of the Dye/lane list:

Step	Action
1	If the Main window is not already displayed, choose Show Main Window from the Views menu. The Main Window appears.
2	Choose the Dye/lane sorting...command in the Views menu. The Dye/lane Sorting dialog box appears. 
3	From the pop-up menus, select items that you want Genotyper to sort next to the precedence number in which you want Genotyper to sort them. **Example** If you choose File name after Precedence 1, and Lane number after precedence 2, Genotyper first sorts the list by File name, then sorts that list by lane number.

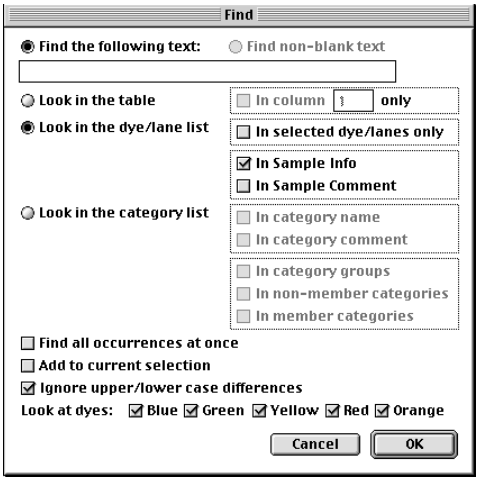
To change the sort order of the Dye/lane list: *(continued)*

Step	Action
4	Click the radio button for how you want items in the list sorted, either in ascending order or descending order. Dye colors are sorted in the following ascending order: blue, green, yellow and red. **Note** If you use the Overlapped dyes, separate lanes option, the dye/lanes must be sorted by File name first and then Lane number.
5	Click OK to apply the sorting order to the active Genotyper Document. Click Cancel to cancel the sorting selections you have made.

How to Specify Search Criteria

You can specify search criteria for finding one or more dye/lanes in the Dye/lane list. For example, you might specify all colors with the text "001101" in Sample Information. Find commands are recorded and can be used as steps in a Genotyper macro.

To specify a search criteria:

Step	Action
1	Choose Find...(⌘-F) in the Edit menu. The Find dialog box appears. 
2	Click the Find the following text radio button.
3	Type in the text you want to locate.

To specify a search criteria: *(continued)*

Step	Action
4	Click the Look in the dye/lane list radio button.
5	Select the checkboxes for how and where you want Genotyper to search for the text string that you have typed (see “How to Search for Dye/lanes” on page 5-12). **Example** If you entered the text “001101” and selected the checkbox for In Sample Comment, Genotyper would search for the specified text in the Sample Comment fields of all dye/lanes.
6	Click OK.

How to Search for Dye/lanes

Once you have defined search criteria (see page 5-10), choose how you want to search for dye/lanes in the list. The following table shows you how you can search for dye/lanes.

If you are...	Then...
Searching for all occurrences at once	Click the Find all occurrences at once checkbox. All dye/lanes with the designated text are selected.
Adding dye/lanes to the current selection	Click the Add to current selection checkbox. Dye/lanes already selected remain selected. Dye/lanes located by this command are also selected.
Restricting the search to currently selected dye/lanes	Click the In selected dye/lanes only checkbox. This is useful for narrowing a selection by repeated use of the Find command.
Searching for the next occurrence of a selection	Choose Find Next (⌘-G) from the Edit menu. The Find Next command repeats the last Find command, using the same options that were used in the last Find command.
Searching for text strings without regard to case	Choose Ignore upper/lower case differences. The Find Next command locates all occurrences of the text string you have entered, ignoring the case of any letters you have entered.

How to Locate GeneScan Data

You can locate GeneScan data associated with a specific dye/lane.

To locate GeneScan data:

Step	Action
1	Select a single dye/lane.
2	Choose Locate GeneScan File in the File menu. The folder with the GeneScan sample file associated with that dye/lane is opened and the file is selected in the Finder. **Note** If the file is located on an unmounted disk, a dialog box appears and asks you to insert the disk that the file is on. **IMPORTANT** This feature works only if the dye/lanes are imported from GeneScan sample files. It will not work if the dye/lanes are imported from a BioLIMS database. You will need to open the Collection Browser to locate the GeneScan sample file in BioLIMS manually.

Editing List Contents

Introduction

If you want to change the information about any item in the Dye/lane list, you can edit related sample information in the Dye/lane window. These changes will affect only the Genotyper Document. The original GeneScan file remains unchanged.

Note You can make changes to only one dye/lane item at a time. If more than one dye/lane is selected or if none is selected, Sample information and Sample comments are grayed out.

How to Edit Sample Information

You can edit the sample information in the Sample Info field of the Dye/lanes window.

To edit the Sample Info field:

Step	Action
1	Open the Dye/lanes window.
2	Select the item in the Dye/lanes list that you want to edit. You can use the arrow keys on the keyboard to scroll up and down the items in the list.
3	Use the Tab key until the cursor goes to the Sample Info text box, or click in the box.
4	Type in any changes you want to make to the Sample Information.

How to Edit Sample Comments

You can edit the Sample Comments in the Sample Comment field of the Dye/lanes window.

To edit the Sample Comment field:

Step	Action
1	Open the Dye/lanes window.
2	Select the item in the Dye/lanes list that you want to edit. You can use the arrow keys on the keyboard to scroll up and down the items in the list.
3	Use the Tab key until the cursor goes to the Sample Comments field.
4	Type in any changes you want to make to the Sample Comments.

Using Scale Factors for Quantitative Applications

Definition

You can use dye/lane scale factors to normalize the height or area of peaks.

Why Use Scale Factors

You can use scale factors for quantitative applications in which you are labeling peaks by height or area, and defining minimum and maximum peak heights in categories.

By using scale factors you can normalize peak heights which helps correct for variations in starting quantities of nucleic acid samples, or variations in amount of samples initially loaded.

How to See Scale Factors

When visible, scale factors appear next to each dye/lane in the Dye/lane list.

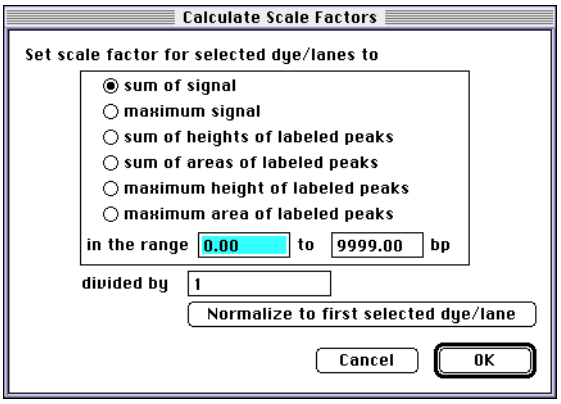
To show Scale Factors in the Dye/lane list:

Step	Action
1	Choose Set Preferences...in the Edit menu. The Set Preferences dialog box appears.
2	Under the bullet “Information to be shown in dye/lane list:”, select the Scale factor checkbox. Scale factors now appear next to each dye/lane in the dye/lane list. The default scale factor is one.

How to Calculate Scale Factors

To calculate scale factors, determine parameters for the calculation, and then scale dye/lanes based on those parameters.

To calculate scale factors:

Step	Action
	Determine parameters for calculation
1	In the Dye/lane list, select the dye/lane to which you want to scale other dye/lane peaks.
2	Choose Calculate Scale Factors from the Analysis menu. The Calculate Scale Factors dialog box appears. 
3	Select the radio button corresponding to how you want to scale peaks.
4	Optionally, you can restrict the calculation of scale factors to a particular range of peak sizes by typing in the peak sizes in the text boxes provided.
5	Click the Normalize button to set the scale factor of the first selected dye/lane peaks to 1.0. **IMPORTANT** Record the number that fills in the divided by field after you click Normalize to first selected dye/lane. You will use it when you scale dye/lanes to defined parameters.
6	Click OK.
	Scale dye/lanes to defined parameters
1	In the Dye/lane list, select all the dye/lanes that you want to scale to the parameters you just determined.

To calculate scale factors: *(continued)*

Step	Action
2	Choose Calculate Scale Factors from the Analysis menu. The Calculate Scale Factors dialog box appears (see figure in step 2 of "How to Sort Dye/lanes" on page 5-9).
3	In the Calculate Scale Factors dialog box, enter the same parameters that you entered when determining parameters for calculation, including the number in the divided by field. **IMPORTANT** Do not click normalize to first selected dye/lane again.
4	Click OK.

Normalizing to the First Dye/lane

If you are normalizing all dye/lanes to the first one in your selection, you do not have to perform the four steps listed under "Scale dye/lanes to defined parameters". Instead, select all dye/lanes and perform the first six steps listed under "Determine parameters for calculation".

How to Reset Scale Factors to One

The default Scale Factor is one. To reset Scale Factors to one, choose the Clear All Scale Factors command from the Analysis menu.

Applying Scale Factors to Other Peaks

Once you have calculated scale factors for all peaks, you can apply the scale factors you have defined to any peaks in the Dye/lane list, not just the range you originally used to calculate the factor.

Defining Categories and Labeling 6

Chapter Overview

Introduction You can label the fragment peaks that appear in plot displays with information such as fragment size, quantity, scan numbers, or customized text. Peak labels appear in the lower pane of the Main window. You can label peaks with more than one kind of label.

In This Chapter This chapter contains the following topics:

Approaches to Labeling

Example of a Labeled Fragment Peak

Figure 6-1 shows an example of a dye/lane fragment peak labeled with the size of the fragment in base pairs. Fragment size is one kind of label you can assign to peak data.

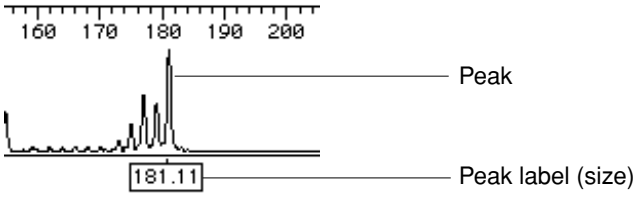


Figure 6-1 Example of a fragment peak labeled with the size in base pairs

Why Label Fragment Peaks

By labeling fragment peaks, you can:

- Visualize size and quantity information for analyzed sample fragments.
- Discover which samples contain fragments in related categories.
- Identify relationships between sample fragments.
- Make decisions about how to configure comparison and analysis tables.
- Make decisions about which samples to export to tables in GenBase.

Ways to Label Fragments

Genotyper provides you with two ways to label fragment peaks: automatically and manually.

The two ways you can label fragment peaks and where you can find detailed instructions for each labeling method.

Labeling Method	Description	See Page
Automatic	Simultaneously labels all peaks within selected dye/lanes with specified criteria.	6-26
Manual	Places defined labels on individual peaks in plot displays when you click a peak. **Note** When two or more electropherogram plots are superimposed, click-labeling is disabled.	6-33

Automatic Label Filtering

Genotyper's automated labeling process screens out peaks resulting from PCR-related artifact fragments detected during electrophoresis. You define the stringency of this filtering process by setting filter parameters that will remove labels from artifact peaks.

For more information on filtering labels, see "Filtering Labels" on page 6-29.

Defining Categories for Labeling

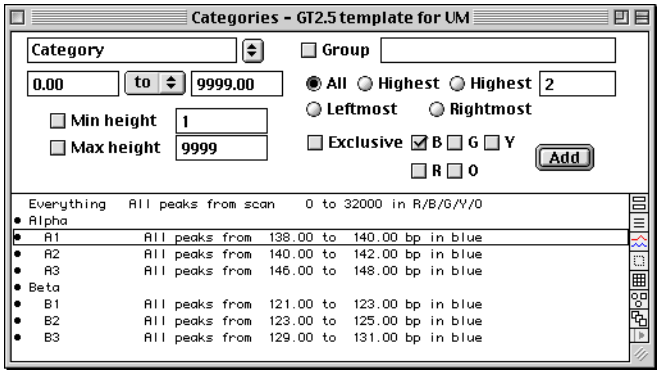
Definition

Categories define which peaks in selected dye/lanes Genotyper will label, and how those peaks will be labeled. You can also use defined categories to specify the contents of tables.

Genotyper uses category information to label appropriate peaks as described in “Automatic Peak Labeling” on page 6-26.

The Categories Window

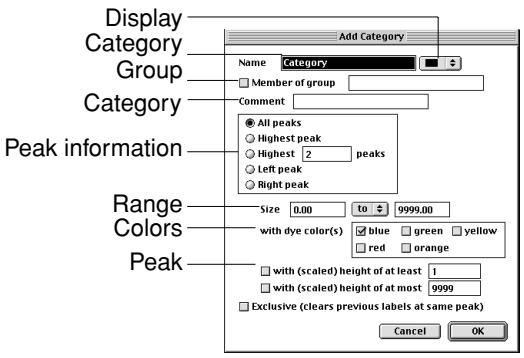
The Categories window shows a list of all defined categories for select dye/lanes in the current Dye/lane list, as well as an abbreviated version of the Add Category dialog box.



Adding Categories

You can add categories that will define how peaks will be labeled when you automatically label peaks of interest.

To add categories:

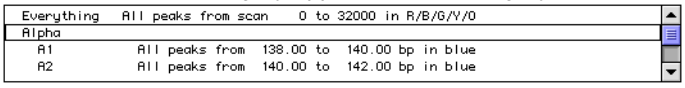
Step	Action
1	Choose Add Category...from the Category menu. The Add Category dialog box appears.  **Figure 6-2** Add Category dialog box
2	Enter the range limits in base pairs for fragments that you want to label. **If you want to...** / **Then...** Automatically fill in range limits / Select a rectangular area in the plot area, and choose the Add Category command. Specify starting and ending coordinates in the range / Use the pop-up menu to select to. Type in the starting and ending sizes in the range. For example, 120 to 140. Specify a center coordinate and range / Use the pop-up menu to select ±. Enter a tolerance For example 120 ± 1.5.

To add categories: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Click a radio button to specify which peaks to label within this category:

<table>
<tr><th>If you want to label...</th><th>Then click...</th></tr>
<tr><td>All peaks</td><td>All peaks.</td></tr>
<tr><td>The highest peak</td><td>Highest Peak.</td></tr>
<tr><td>The highest “n” peaks (where “n” is an integer)</td><td>Highest “n” Peaks, and type an integer for “n”.</td></tr>
<tr><td>Left most peak in a range</td><td>Left Peak.</td></tr>
<tr><td>Right most peak in a range</td><td>Right Peak.</td></tr>
</table></td></tr>
<tr><td>4</td><td>Click the dye color checkboxes for the colors you want labeled.</td></tr>
<tr><td>5</td><td>Optionally, define height requirements for peaks you want to label:

<table>
<tr><th>If you want to define...</th><th>Then click...</th></tr>
<tr><td>A minimum height for labeled peaks</td><td>With (scaled) height of at least, and type a number for the minimum peak height.</td></tr>
<tr><td>A maximum height for labeled peaks</td><td>With (scaled) height of at most, and type a number for the maximum peak height.</td></tr>
</table>
Note If the dye/lane scale factor is 1.0, then the scaled height of a peak is the same as the peak height. If a different scale factor has been calculated for a dye/lane then the scaled height of a peak is equal to the peak height divided by the Dye/lane’s scale factor.</td></tr>
<tr><td>6</td><td>Enter a name for the Category. For example, Alpha.</td></tr>
<tr><td>7</td><td>Optionally, choose a display color for the Category. The entry in the Category list for this Category will appear in this color. Labels for the Category can optionally be displayed in this color.</td></tr>
<tr><td>8</td><td>Optionally, enter a descriptive comment about the Category. The comment will appear in the Category window after the name.</td></tr>
<tr><td>9</td><td>Click OK.

The name of the category appears in the Category list.
 
</td></tr>
</table>

Exclusive Peak Labeling

The Exclusive checkbox in the Add Category dialog box is a priority labeling feature. When you select the Exclusive checkbox, any existing labels on a peak, besides those defined by the “Exclusive” category, are removed.

For more details on using exclusive peak labeling see, “Using Exclusive Peak Labeling–An Example” on page 6-10.

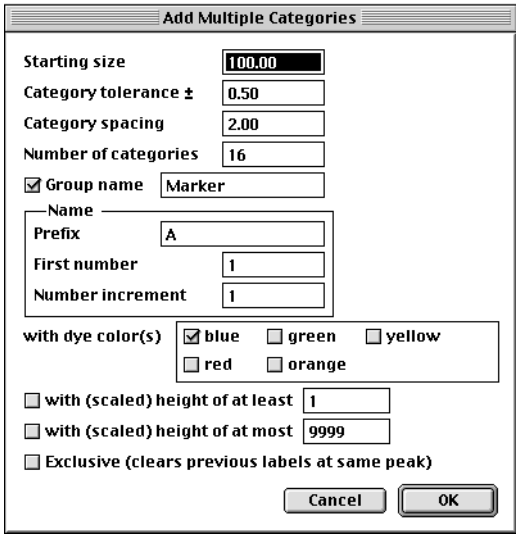
Adding Multiple Categories

You can create multiple categories at once by using the Add Multiple categories command. This can be useful if you are performing applications such as microsatellite repeats where you want to label a large number of peaks that differ by multiples of 2 base pairs.

There are three stages to this process:

- Defining categories
- Choosing optional parameters
- Naming the categories

To define categories:

Step	Action
1	Choose Add Multiple Categories...from the Category menu. The Add Multiple Categories dialog box appears. 

To define categories: *(continued)*

Step	Action
2	In the Starting size field, enter the starting size for fragments that you want to include in the first category.
3	In the Category width ± field, enter the range of fragment sizes that you want to be included in the category. For example, 100 ±.50 labels fragments that are between 99.5 to 100.5 base pairs in length as a single category.
4	In the Category spacing field, enter the number of base pairs between each category. For example, if you enter 2, then the second category will begin for all fragments that are between 101.5 and 102.5 base pairs.
5	In the Number of Categories field, enter the number of categories that you want to create.
6	Select the dye color check boxes for the dye colors that you want included in the categories.

To choose optional parameters:

Step	Action
1	Select the Group Name checkbox and type a name to include all categories under a single group name.
2	Select the checkbox for “with (scaled) height of at least”, and enter a number if you want to limit the categories to only those peaks that generate a signal intensity of at least a particular height.
3	Select the checkbox for “with (scaled) height of at most”, and enter a number if you want to limit the categories to only those peaks that generate signal intensity of at most a particular height.
4	Select the checkbox for Exclusive, if you want to clear any existing labels on peaks.

To name the category:

Step	Action
1	In the Prefix field, type a 1 to 15 character alpha numeric prefix for the category names.

To name the category: *(continued)*

Step	Action
2	In the First number field, enter the number to follow the prefix for the first category name.
3	In the Number increment, enter the number by which you want to increment each successive category number.
4	Click OK.

Example of Category Naming

How Genotyper names categories based on parameters you enter:

If you define...	Then categories are named...
Prefix: AB First number: 10 Number increment: 3	AB10, AB13, AB16,...

Using Exclusive Peak Labeling–An Example

Introduction

The Exclusive option is a priority labeling feature. When the Exclusive check box is marked in the Add Category window, all other labels at peaks in the "Exclusive" category are cleared and the peak(s) is labeled with the desired information.

In general, category ranges should not overlap, but the Exclusive option allows you to use overlapping categories in special cases.

Example Application

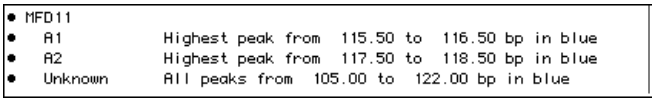
In this example, the peaks of a marker occur between 106 and 122 bp. Two particular alleles, named A1 and A2, are known to occur around 116 bp and 118 bp. If either of these alleles are present, we would like them to be labeled by name, but if any others are present, we would like them to labeled with the text, "Unknown."

In the first part of the example, we will see what happens when the Exclusive option is not used. In the second part of the example, we will see how the Exclusive option allows us to obtain the desired results.

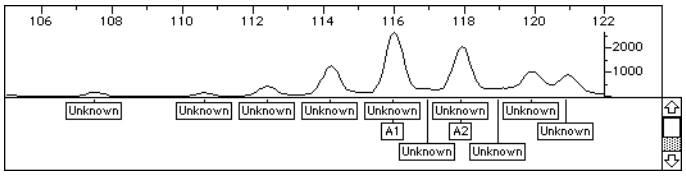
Labeling without the Exclusive Option

In the first part of this example, we will see what happens when we label peaks in the example application without using the Exclusive option.

To label peaks without the Exclusive option:

Step	Action
1	Assume categories have been created so that the Category list looks like the list below (note there are no exclusive peak labels). 
2	Assume peaks have been labeled with the category name checkbox selected and all others de-selected.

To label peaks without the Exclusive option: *(continued)*

Step	Action
3	View the Plot window, which should now look like the plot below. 

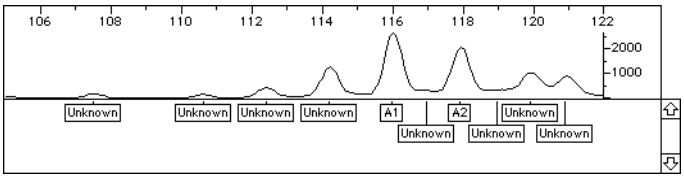
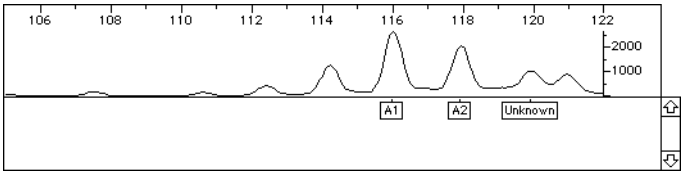
Result of Specifying Overlapping Category Ranges

Note that the peaks at 116 bp and 118 bp each have two labels. This is one of the undesirable consequences of specifying overlapping category ranges.

How to Use Exclusive Labeling

For this example, you can use the “A1” or “A2” labels exclusively for these peaks, by using the Exclusive option.

To label peaks using the Exclusive option:

Step	Action
1	Assume that you have defined Categories like those shown below. MFD11 Unknown All peaks from 105.00 to 122.00 bp in blue A1 (X) Highest peak from 115.50 to 116.50 bp in blue A2 (X) Highest peak from 117.50 to 118.50 bp in blue
2	Label peaks.
3	View the resulting plot (shown below); known peaks are labeled correctly A1 and A2, others are labeled “Unknown”. 
Filter Stutter Peaks	
1	Choose Filter Labels… from the Analysis menu.
2	Click OK, to use the default filtering parameters. The plot area now shows the two known alleles, and a third, spurious peak labeled “Unknown.” 

Creating Category Groups—an Example

Introduction

Category groups may be useful when you want to identify individual alleles within the range of a marker category.

IMPORTANT If Category groups in the same dye color overlap, some commands may not perform as expected

In this example, you will make two sets of categories and specify the groups they are associated with. You will also collapse one of those sets of categories into a single entry in the Category list.

Note These categories are only for illustration and have no biological relevance.

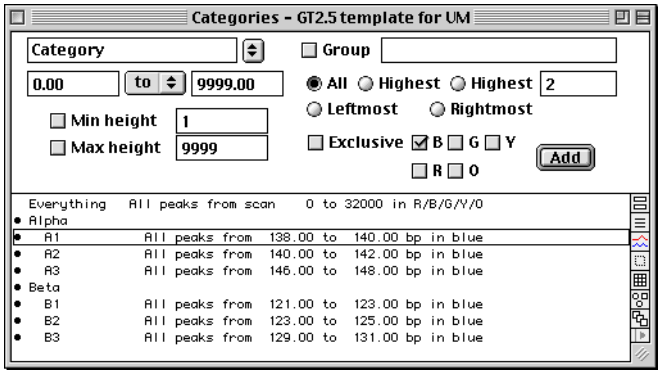
Create a Category Group

Category groups organize groups of similarly defined categories under a single name.

To create two sets of categories and specifying the groups with which they are associated:

Step	Action
1	Choose Add Category...from the Category menu. The Add Category dialog box appears (see figure in step 1 of "Adding Categories" on page 6-5).
2	Name the first Category "A1."
3	Click Member of group checkbox and enter "Alpha".
4	Click the All Peaks radio button.
5	Enter 138 and 140 for the "Size" range limits.
6	Click the blue checkbox and de-select the checkboxes for the other colors.
7	Click OK.

To create two sets of categories and specifying the groups with which they are associated: *(continued)*

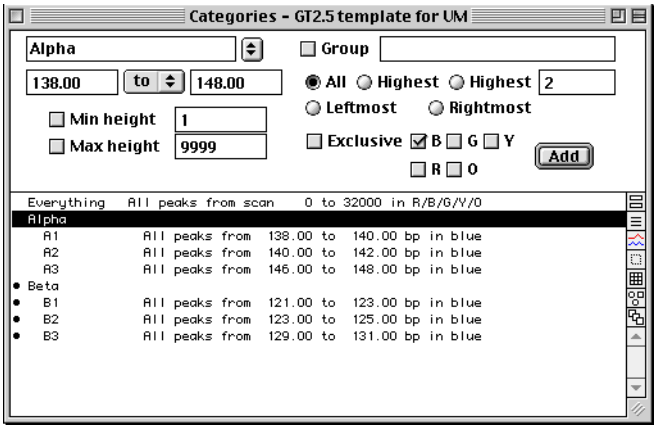
Step	Action
8	Repeat steps 2-7 using the following values: ♦ A2; Alpha; All peaks, 140-142; blue ♦ A3; Alpha; All peaks, 146-148; blue ♦ B1; Beta; All peaks, 121-123; blue ♦ B2; Beta; All peaks, 123-125; blue ♦ B3; Beta; All peaks, 129-131; blue You have created two groups: Alpha and Beta, with three Categories in each group.
9	Choose Show Categories Window from the Views menu. The Categories window shows the two marked Category groups you created. 

Unmark a Group of Categories

Using Category groups allows you to conveniently mark or unmark all entries in that group at the same time, rather than marking or unmarking each one individually.

For more information on marking and unmarking Categories, see “Marking or Unmarking Categories” on page 6-26.

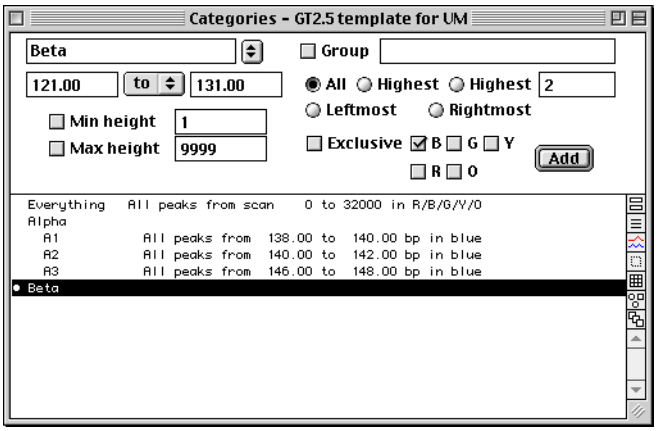
To unmark the alpha category group:

Step	Action
1	Select “Alpha” in the Categories window.
2	Choose Unmark (z-U) from the Edit menu. All three Categories in the Alpha group are now unmarked.  **Note** If all Categories in a group are marked, the group will have a bullet next to its name. If some Categories in a group are marked, and some unmarked, the group name will have a dash (-) next to its name.

Collapse a Group of Categories

Collapsing a group of categories can make viewing of the Category list easier, by reducing the members of a group to a single entry in the Categories list.

To collapse the beta category to a single entry in the categories list:

Step	Action
1	Select the category named Beta in the Categories window.
2	Choose Collapse Categories (z-]) from the Views menu.  The Beta group of categories is collapsed into a single line.

Making Category Members

Introduction

You can make members of categories that represent a distribution of peak height ranges within a particular category. This allows you to categorize marker data based on a distribution of fragment quantities for applications such as AFLP.

For complete details for running AFLP applications look for information soon to be available on the Applied Biosystems web site.

Example of Category Members

Figure 6-3 shows an example of the distribution of peak heights or fragment quantities for five category members (B,C,H,D,A). For example, the peak shown here belongs to category member “B”.

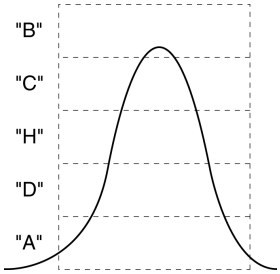


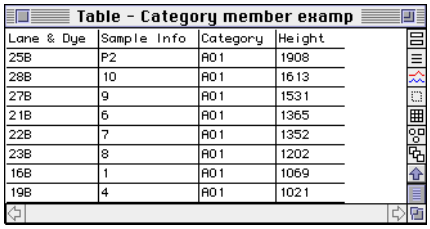
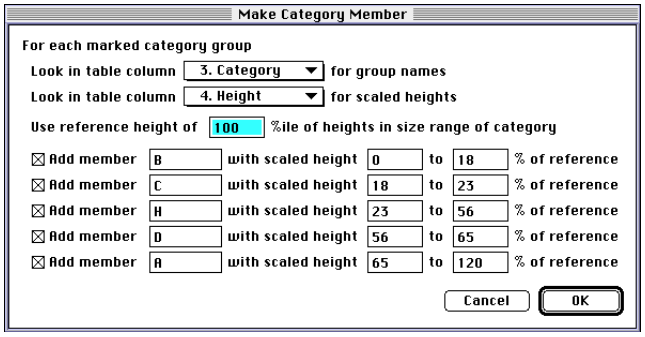
Figure 6-3 Example of peak height distribution for category members

How to Make Category Members

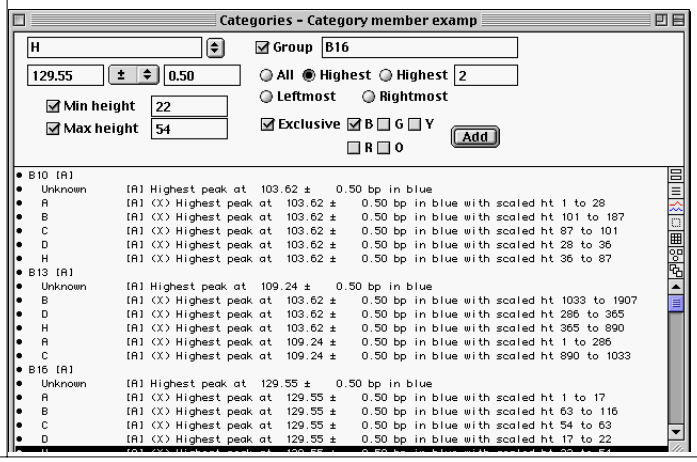
In order to use this command you have to have already created a table.The table must have columns defined for categories and peak height.

Note The following steps refer to an AFLP example soon to be available on the Applied Biosystems web site.

To make category members:

Step	Action
1	Choose Show Table window from the Views menu. This displays a table with rows of peak height and category data such as that shown in this figure. 
2	Choose Make Category Members...from the Category menu The Make Category Members dialog box appears. 
3	Choose the category column from the Look in table column for group names pop-up menu. This defines chosen categories as groups.
4	Choose the peak height column from the Look in table column for scaled heights pop-up menu.

To make category members: *(continued)*

Step	Action
5	For each marked category group, Genotyper calculates the distribution of peak heights (for the group) by looking at the appropriate rows in the table. Type in the percentile of this distribution that you want to use for the “reference height.” For example, if you want the largest height to be the reference height, type in “100” for the percentile.
6	Select from one to five checkboxes for the number of members you want to add to each marked category, and type in a name, and a range that is a percentage of the defined reference height.
7	Click OK. For each box that you checked, Genotyper adds a member, that has a height range that is a certain percentage of the referenced height that you calculated.
8	Choose Show Categories Window from the Views menu. The Categories window appears and displays new members for each category with varying scaled height ranges as shown in this figure. 

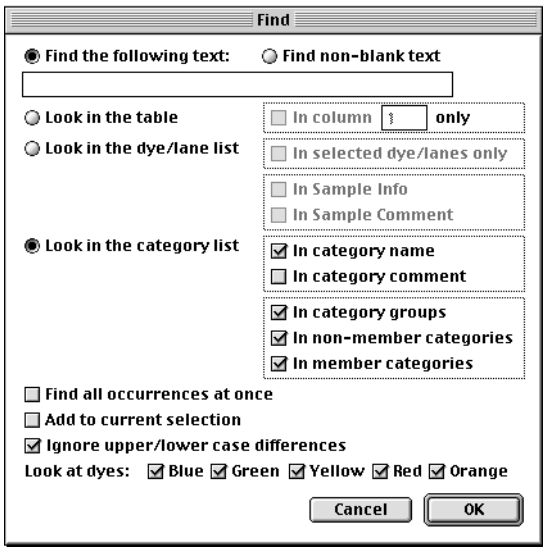
Searching for Categories

Introduction

You can define search criteria for categories and locate a particular category or categories in the list of defined categories.

How to Specify Search Criteria

To specify a search criteria:

Step	Action
1	Choose Find...(⌘-F) in the Edit menu. The Find dialog box appears. 
2	Click the Find the following text radio button.
3	Type in the text you want to locate.
4	Click the Look in the category list radio button.
5	Select the checkboxes for where you want Genotyper to search for the text string that you have typed.
6	Select the checkboxes for how you want Genotyper to search for the text string that you have typed (Table 6-1 on page 6-21).
7	Click OK.

How to Search for Categories

Once you have defined search criteria, choose how you want to search for dye/lanes in the list. The following table shows you how you can search for categories.

Table 6-1 Ways to search for categories:

If you are...	Then...
Searching for all occurrences at once	Click the Find all occurrences at once checkbox. All categories with the designated text are selected.
Adding categories to the current selection	Click the Add to current selection checkbox. Categories already selected remain selected. Categories located by this command are also selected.
Searching for the next occurrence of a selection	Choose Find Next (⌘-G) from the Edit menu. The Find Next command repeats the last Find command, using the same options that were used in the last Find command.
Searching for text strings without regard to case	Click Ignore upper/lower case differences. The Find Next command locates all occurrences of the text string you have entered, ignoring the case of any letters you have entered.
Restricting the search to categories defined for particular dye colors only	Click the checkboxes for the particular dye colors you want to search.

Sorting and Editing Categories

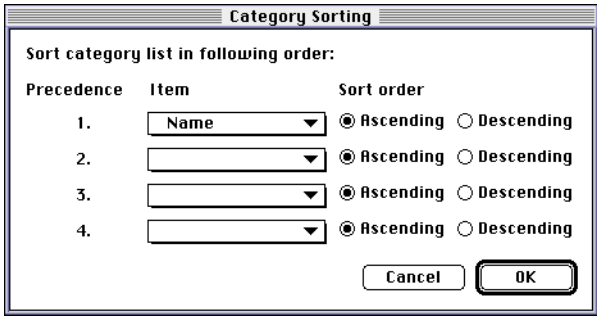
Introduction

Once you create a number of different categories, you can easily sort the category list, edit existing categories, and create new categories from existing ones.

Sorting the Categories List

You can change the sort order of the Category list.

To sort the category list:

Step	Action
1	Choose Category Sorting...from the Views menu. The Category Sorting dialog box appears. 
2	Choose the precedence of sorting the items (name, size/scan, minimum height, comment, or dye color) by clicking and holding down the pop-up menus.
3	Choose the sort order of these items in ascending or descending order by clicking the appropriate radio buttons. The “Everything” category always appears first in the Category list. Exclusive categories are sorted after non-exclusive categories, within their groups.

How to Edit Category Parameters

Once you have added a category, you can edit the parameters for that category at any time.

To edit category parameters:

Step	Action
1	Select a category in the Category list.
2	Choose Edit Category...from the Category menu. The Edit Category dialog box appears. This dialog is identical to the Add Category dialog box, except for the title (see figure in step 1 of “Adding Categories” on page 6-5).
3	Modify parameter settings. **Note** You cannot edit a member of a group to be a member of another group. A group contains categories, each of which applies to the same set of dye colors.
4	Click Replace. The parameters you changed will replace the previous settings for this category.

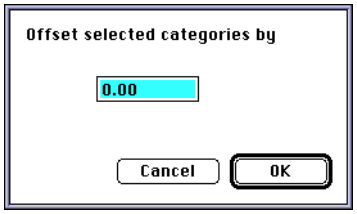
Offsetting Categories

Introduction

You can use either the Offset Category, or the Calculate an Offset... command to temporarily shift the size range of a category.

How to Offset Categories

To use the Offset Category command:

Step	Action
1	Select one or more categories from the Category list in the Main window.
2	Choose Offset Categories from the Category menu. The Offset Categories dialog box appears. 
3	Enter a number, positive or negative, for how many base pairs you want to offset the current size range defined for a given category.
4	Click OK. For the selected category in the Category list, the number you entered appears in parentheses next the size range. For example, if you entered –0.2, (–0.2) appears next to the size range, and the start and end point of the size range is decreased by –0.2 base pairs.

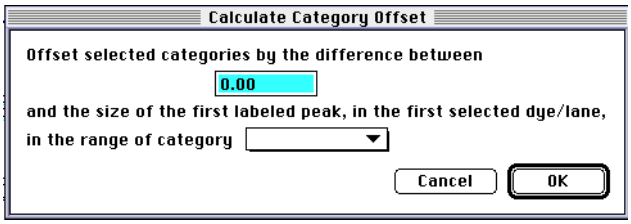
When to Calculate an Offset

The Calculate Offset command automates the Offset Category command. Use the Calculate Offset command if you have run the same samples in the same lanes of two or more different gels or capillaries. Calculating an offset can help eliminate run-to-run variability of fragment size values in categories.

The Calculate Offset command is particularly useful for genotyping applications that make use of allelic ladders.

How to Calculate an Offset

To use the Calculate Offset command:

Step	Action
1	For GeneScan data from each gel, establish a standard set of category values for peak data.
2	Import all GeneScan data that you are using in your application.
3	Select one or more categories from the Category list in the Main window.
4	Choose Calculate Offset... from the Category menu. The Calculate Offset... dialog box appears. 
5	Enter the number for the size in base pairs of your reference peak.
6	From the pop-up menu, select the category from which you want to calculate the offset.
7	Click OK.

Automatic Peak Labeling

Introduction

Automatic peak labeling allows you to label peaks in selected dye/lanes using criteria defined in marked categories, and the Label Peaks dialog box.

Note Repeated use of the Label Peaks...command will produce duplicate labels of the same type at a peak. Use the Clear All labels command to remove all previously added labels.

Peak Label Limit

No more than 500 peaks should be labeled in any one dye/lane. If more than 500 peaks are labeled, then some commands (such as Filter Labels) may not be available.

Marking or Unmarking Categories

When you add a category to the Category list, it is marked. Marked categories are used for automatic labeling of peaks. Unmarked categories are ignored.

To mark or unmark Categories:

Step	Action
1	Select one or more Categories from the Category list.
2	Choose Mark (⌘-M) or Unmark (⌘-U) from the Edit menu, or double-click a single category to toggle between a marked and unmarked state. A bullet appears to the left of categories, indicating the categories are marked.

How to Label Peaks Automatically

To automatically label peaks:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Mark categories that define how you want peaks labeled.</td></tr>
<tr><td>2</td><td>Select the dye/lane or dye/lanes that contain the peaks you want to automatically label.</td></tr>
<tr><td>3</td><td>Choose Label Peaks...from the Analysis menu.</td></tr>
<tr><td>4</td><td>Click the appropriate checkboxes for what you want to appear on labels:
<table>
<tr><th>If you want to label Peaks with...</th><th>Then click...</th></tr>
<tr><td>Fragment size in base pairs

Note Size can be rounded to nearest integer.</td><td>the size in bp.</td></tr>
<tr><td>Height in units defined by GeneScan

Note Height can divided by Dye/lane scale factor.</td><td>the peak height.</td></tr>
<tr><td>Area in units defined by GeneScan

Note Area can be divided by Dye/lane scale factor.</td><td>the peak area.</td></tr>
<tr><td>Number of scans required to detect the peak</td><td>the scan number.</td></tr>
<tr><td>A pre-defined text description</td><td>the text, and type in a peak label.</td></tr>
<tr><td>A text box that you can annotate after putting a blank label on a peak

Note For click labeling only.</td><td>the requested text.</td></tr>
<tr><td>Either Manual or Auto depending how the peak was labeled</td><td>label/peak source.</td></tr>
<tr><td>A score for each peak that indicates how well the peak image resolves with respect to the background</td><td>the peak modulation score.</td></tr>
</table></td></tr>
<tr><td>5</td><td>Click OK.</td></tr>
<tr><td>6</td><td>To view the peak labels, click the Plot window icon, and show the Plot data window.</td></tr>
</table>

When to Round Integers

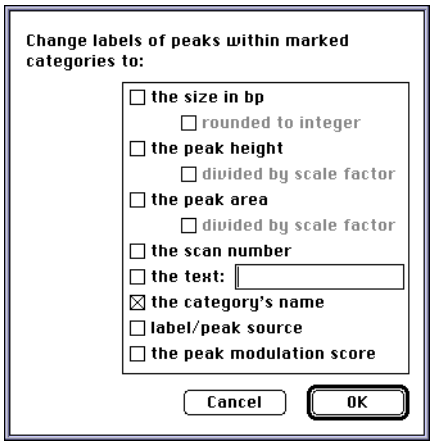
When setting parameters for how you want to automatically label peaks, select the round integers checkbox only if you are labeling large sized fragments where the numbers to the right of the decimal point can be ignored.

IMPORTANT Do not round size labels to the nearest integer if you are performing a microsatellite application. You will not obtain satisfactory results.

Changing Existing Labels

The Change Labels command enables you to change labels within marked categories on currently selected dye/lanes. If you are running a genotyping application that uses genetic marker allele designations, you can change existing labels, by renaming allele labels from size in base pairs to a category or allele name.

To change existing labels:

Step	Action
1	Choose Change Labels...from the Analysis menu. The Change labels dialog box appears. 
2	Click the checkboxes for what you want to now appear on peaks with existing labels.
3	Click OK.

Filtering Labels

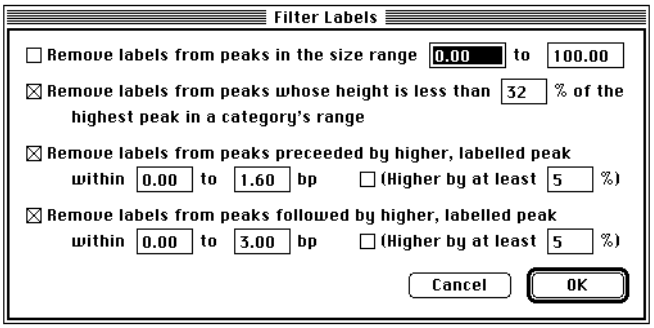
Definition

Genotyper may label some peaks that, for various reasons, you may not want to be labeled. You can use Genotyper's filtering feature to remove these unwanted labels.

How to Filter Labels

Note When viewing by scan, only the first option in the Filter labels dialog box is available. The other options are intended to be used only when viewing by size.

To filter labels:

Step	Action
1	Choose Filter Labels...from the Analysis menu. The Filter Labels dialog box appears.  Generally, the default settings in the Filter Labels dialog box remove most of the "stutter" bands and noise from electropherograms. These filtering parameters are designed for dinucleotide microsatellite data repeats. **Note** The filtering operations listed in the dialog box are performed one at a time, in the order they are listed. You can isolate the effect of each filtering operation by performing only one operation at a time.
2	You can change settings to remove labels from peaks that do not represent significant fragment data (see figure in step 1).

Kinds of Peaks You Can Filter

Figure 6-4 shows the kinds of peaks for which you can filter labels and remove them from plot displays.

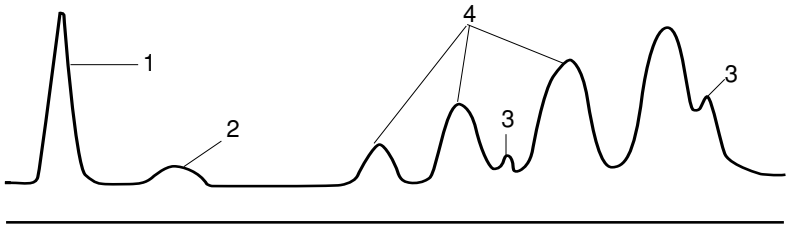


Figure 6-4 Kinds of peaks you can filter

Description of each of the numbered peaks in Figure 6-4:

Peak Number	Description
1	Spurious peaks at known locations.
2	Small peaks.
3	Small peaks on the shoulders of stutter peaks (“+A” peaks).
4	Stutter peaks.

How to Remove Spurious Peak Labels

Spurious peaks are often large thin peaks that appear on the far left of plot displays. They can result from primers, excessive salt in samples, or from pooling samples during PCR preparation.

To remove spurious peak labels at known locations:

Step	Action
1	In the Filter Labels dialog box, click the first checkbox and enter the size range of the spurious peaks that occur in selected electropherograms plots.
2	De-select the other checkboxes.
3	Click OK.

How to Remove Labels from Small Peaks

Small peaks close to the baseline are referred to as *background noise*, and can result from spectral overlap or other GeneScan matrix file problems.

To remove small peaks:

Step	Action
1	In the Filter Labels dialog box, click the second checkbox and type in a percentage of the height of the highest peak, for which peaks that are less than this percentage will be removed.
2	De-select the other check boxes.
3	Click OK.

How to Remove Labels from Small Peaks on Peaks

One of the most common errors in automated genotyping results from the tendency of Taq DNA Polymerase to add an additional (non-templated) nucleotide, usually an A, to the end of the extending strand. This results in the production of PCR fragments one nucleotide longer than the true allele product which display as small peaks on allele peaks in Genotyper. These “+A” peaks can display on either the left or right side of the true allele peak, usually 1 nucleotide in length.

IMPORTANT When the peak height of the “true” allele product and that of the +A allele are similar, Genotyper may recognize the +A bands as the true allele, resulting in a genotyping error of about 3-5%.

To remove small peaks on the shoulders of allele peaks:

Step	Action
1	In the Filter Labels dialog box, click the third checkbox from the top, and enter the peak range, for peaks you want removed.
2	De-select the other check boxes.
3	Click OK.

In addition to using the Filter Labels command, you can define categories so that only the left or right peak in a pair of peaks is labeled, and the peak that results from the +A artifact remains unlabeled.

How to Remove Stutter Peaks

Stutter peaks can occur when genotyping microsatellite samples, and can be caused by slippage of the polymerase enzyme during PCR.

To remove stutter peaks:

Step	Action
1	In the Filter Labels dialog box, click the fourth checkbox and enter the peak range of peaks to include in the filtering process.
2	Click the Higher by at least checkbox, and type the percentage height that a preceding peak must be to removed.
3	De-select the other checkboxes.
4	Click OK.

Manually Putting Labels On Peaks

Introduction

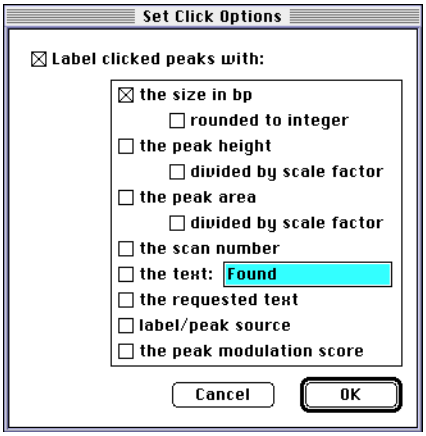
You can label individual peaks in plot displays by locating the peak or peaks of interest, and then clicking on the peak. When you click on the peak a second time, the label is removed.

You can label peaks with more than one label, for example, size and height. Peak labels appear in the lower pane of the Main window.

How to Manually Label Fragment Peaks

To label fragment peaks manually, define what you want to appear in the label and then click on the peaks that you want to label.

To manually label peaks:

Step	Action
1	Choose Set Click Options... from the Analysis menu. The Set Click Options dialog box appears. 

To manually label peaks: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Click the appropriate check boxes for what you want to appear on labels:

<table>
<tr><th>If you want to label Peaks with...</th><th>Then click...</th></tr>
<tr><td>Fragment size in base pairs</td><td>the size in bp.</td></tr>
<tr><td>Height in units defined by GeneScan</td><td>the peak height.</td></tr>
<tr><td>Height divided by scale factor</td><td>divided by scale factor.</td></tr>
<tr><td>Area in units defined by GeneScan</td><td>the peak area.</td></tr>
<tr><td>Area divided by scale factor</td><td>divided by scale factor.</td></tr>
<tr><td>Number of scans required to detect the peak</td><td>the scan number.</td></tr>
<tr><td>A Pre-defined text description</td><td>the text, and type in a peak label.</td></tr>
<tr><td>A text box that you can annotate with different text each time you click a peak</td><td>the requested text.</td></tr>
<tr><td>Either Manual or Auto depending how the peak was labeled</td><td>label/peak source.</td></tr>
<tr><td>A score for each peak that indicates how well separated the peak is from background.</td><td>the peak modulation score.</td></tr>
</table>
</td></tr>
<tr><td>3</td><td>Click OK.</td></tr>
<tr><td>4</td><td>Select a dye/lane that contains peaks you want to label.</td></tr>
<tr><td>5</td><td>Move the cursor in the electropherogram part of the Plot area until the vertical line jumps to the peak that you want to label.</td></tr>
<tr><td>6</td><td>Click the peak with the mouse button.

A label for that peak appears in the lower pane of the plot area.</td></tr>
</table>

Customizing Text in Labels

Introduction

Once you have assigned labels to peaks, you can customize the text in the labels.

Note Text and color customizations you make to labels apply only to the labels in the active (frontmost window) Genotyper Document. However, each document can have its own independent customization.

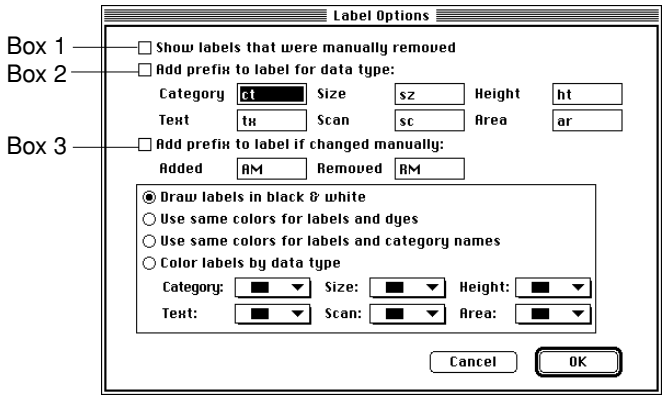
How to Show Labels that Have Been Manually Removed

You can show labels that have been manually removed to provide an audit trail of adjustments to labels that have been automatically assigned to peaks.

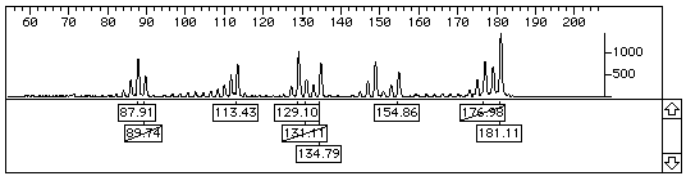
For more information on removing labels from peaks, see “Removing Labels” on page 6-42.

For more information on automatically labeling peaks, see “Automatic Peak Labeling” on page 6-26.

To display labels that have been manually removed:

Step	Action
1	Choose Plot Options in the Views menu, then choose the Label Options...submenu. The Label Options dialog box appears.  **Figure 6-5** Label Options dialog box

To display labels that have been manually removed: *(continued)*

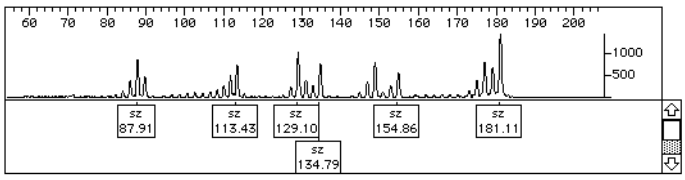
Step	Action
2	Labels that have been manually removed are shown with slashes in plot displays. 

How to Add Data Type Prefixes

If you want to identify the data type of a peak label, you can add a prefix to labels that identify the type of the label. Data Types and associated default prefixes include:

- Category (ct)
- Size (sz)
- Height (ht)
- Text (tx)
- Scan (sc)
- Area (ar)

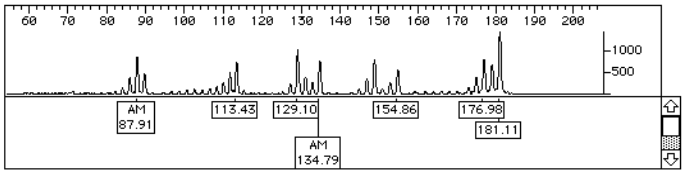
To add data type prefixes to labels:

Step	Action
1	Click Box 2 in Label Options dialog box (see Figure 6-5 on page 6-35).
2	Enter the prefixes you want or use the default prefixes.
3	Click OK. The labels are assigned prefixes for data type. This figure shows an example of labels with size prefixes (sz). 

How to Add a Prefix to Manually Changed Labels

You can add a prefix to labels to mark labels that were either added or removed manually.

To add a prefix to labels that were added or removed manually:

Step	Action
1	Click Box 3 in the Label Options dialog box (see Figure 6-5 on page 6-35).
2	Enter the prefixes you want or use the default prefixes.
3	Click OK. The prefix “AM” appears on the labels that were added manually; the prefix “RM” appears on the labels that were removed manually. 

Customizing the Color of Labels

Introduction

To distinguish between different types of labels you can customize the color of the labels.

How to Draw Labels in Black and White

If you are planning to print results data, or display labels on a black and white monitor, you may want to draw labels in black and white.

To draw labels in black and white:

Step	Action
1	Select Plot Options in the Views menu and choose Label Options.... The Label Options dialog box appears (see Figure 6-5 on page 6-35).
2	Select the Draw labels in black and white radio button in the Label Options dialog box.
3	Click OK.

How to Use the Same Colors for Labels and Dyes

You can make peak labels the same color as their associated peaks. For example, blue electropherograms will have all blue labels and green electropherograms will have all green labels.

To color labels the same as associated peaks:

Step	Action
1	Select Plot Options in the Views menu and choose Label Options.... The Label Options dialog box appears (see Figure 6-5 on page 6-35).
2	Select the Use same colors for labels and dyes radio button.
3	Click OK.

How to Use the Same Colors for Labels and Category Names

The Add Category dialog box (Figure 6-2 on page 6-5) allows you to associate a color with a category for display purposes. The name of the category in the Category list will appear in this color. This option allows you to display labels that have the same color as a particular category.

To color labels the same as associated categories:

Step	Action
1	Select Plot Options in the Views menu and choose Label Options.... The Label Options dialog box appears (see Figure 6-5 on page 6-35).
2	Select the Use same colors for labels and category names button.
3	Click OK.

How to Choose Color Labels by Data Type

You can color labels according to the type of data that the label describes. Data types include:

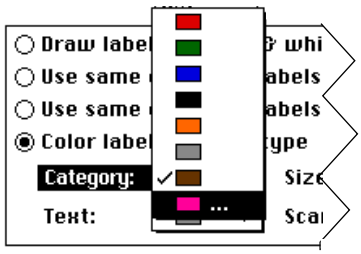
- Category
- Peak Size
- Peak Height
- Label Text
- Scan number
- Peak Area

To color labels according to data type:

Step	Action
1	Select Plot Options in the Views menu and choose Label Options.... The Label Options dialog box appears (see Figure 6-5 on page 6-35).
2	Select the Color labels by data type radio button.
3	Click OK.
4	Click and hold down the pull-down menu for color selection by data type.
5	Select a color.
6	Repeat step 5 for each data type label you want to color. The data types appear in the selected colors in the Plot window.

How to Select a Custom Color for a Data Type Label

To select a custom color for a data type:

Step	Action
1	Select Plot Options in the Views menu and choose Label Options.... The Label Options dialog box appears (see Figure 6-5 on page 6-35).
2	Select Color labels by data type radio button.
3	Click OK.
4	Click and hold down the pull-down menu for color selection by data type.
5	Select the last color in the list (the one with the ellipsis (...)).  **Note** Selecting a custom color may temporarily change the highlight color used by the Finder. The Pick a color dialog box appears.
6	Place the cursor on the color wheel and click on the color of your choice.
7	Click OK.
8	Repeat steps 5-7 for each data type color you want to change.

Removing Labels

Introduction

During a genotyping session, you will often want to remove fragment labels from peaks.

Ways to Remove Labels

You can remove all labels from peaks, select specific kinds of labels and remove those labels automatically, or remove individual labels by locating them in plot displays and clicking on the labels you want removed.

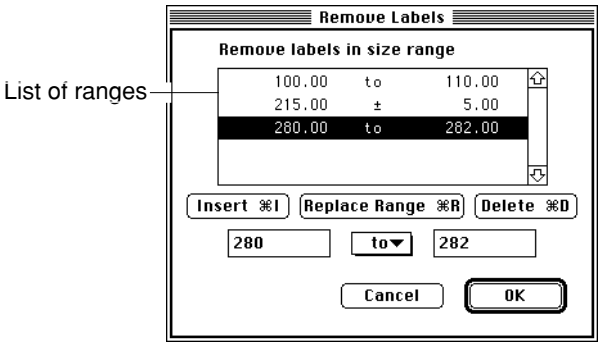
Removing All Labels

If you want to remove all peak labels in all dye/lanes (whether selected or not), choose Clear All Labels from the Analysis menu.

How to Remove Specific Labels

You can specify the range of peaks for which labels will be removed.

To remove labels within a specified size range:

Step	Action
1	Select the dye/lanes from which you wish to remove labels.
2	Choose Remove Labels...from the Analysis menu. The Remove Labels dialog box appears. 
3	Type in the range in base pairs, then click Insert (⌘-I). The size range appears in the list of ranges. **Note** You can also specify the size range by choosing "±" from the pop-up menu, for example, 105 ± 10.
4	Repeat Step 2 for each range you want to include.

To remove labels within a specified size range: *(continued)*

Step	Action
5	Click OK. Labels in the specified ranges in all currently selected dye/lanes are cleared.

How to Correct Errors in the Size Range

To correct size range errors in the Remove Labels dialog box (see step 2 of "How to Remove Specific Labels" on page 6-42):

Step	Action
1	Select the range from the range list.
2	Type in the new range.
3	Click Replace Range (⌘-R).
4	Click OK.

Removing a Range From the Range List

To remove a range from the range list in the Remove Labels dialog box (see step 2 on page 6-42):

Step	Action
1	Select the range from the range list.
2	Click Delete (⌘-D).
3	Click OK.

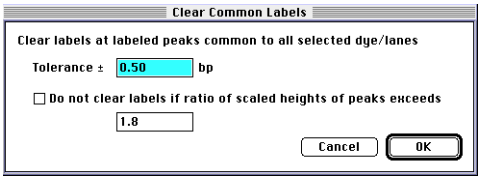
Removing Common Labels

Removing common labels is useful for genotyping applications such as AFLP where you have many labeled peaks of which you are only interested in labels that represent a polymorphism.

To remove common labels:

Step	Action
1	Select two or more dye/lanes. **Note** If only one dye/lane is selected, all labels for that dye/lane will be cleared.

To remove common labels: *(continued)*

Step	Action
2	Choose Clear Common Labels...from the Analysis menu. The Clear common labels dialog box appears. 
3	Enter the tolerance in base pairs. Two peaks are considered to be at the same location if their peaks are within the specified tolerance.
4	If you do not want to clear labels when the peaks in different dye/lanes have a significant height difference, select the checkbox.
5	Click OK. Labels are cleared at those peaks that are labeled in *all* of the currently-selected dye/lanes. For example, if a peak at a particular location is labeled in five out of six lanes, none of the labels will be cleared; only if labels are present for six out of six lanes will they all be cleared.

Labeling Normalized Peaks—an Example

Introduction

In quantitative applications, where relative peak height is important, you can normalize peak heights relative to the height of a control peak.

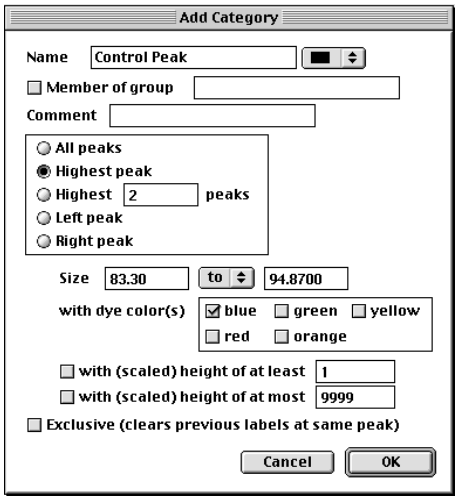
This example shows you how to perform the three procedures required for labeling normalized peaks:

- Defining a control peak
- Normalizing peaks to the control peak
- Labeling normalized peaks

Define a Control Peak

The first procedure in labeling normalized peaks is to define a control peak.

To define a control peak:

Step	Action
1	Select a dye/lane.
2	In the Plot pane, select a range that includes the control peak.
3	Choose Add Category...() from the Analysis menu. The Add Category window appears.
4	Click the Highest peak radio button.
5	Name the Category Control Peak. 

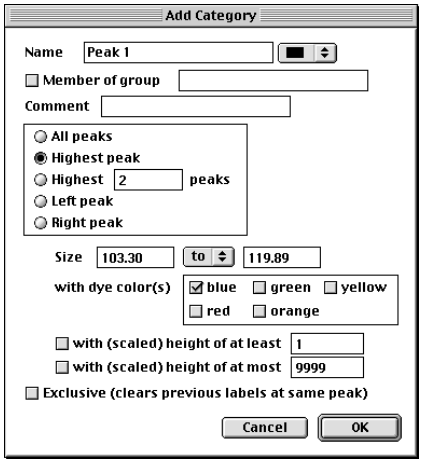
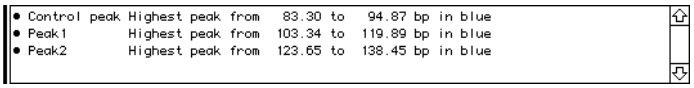
To define a control peak: *(continued)*

Step	Action
6	Click OK.

Normalize Peaks to the Control Peak

Once you define a control peak, normalize the heights of other peaks to the height of your control peak, which serves as a reference.

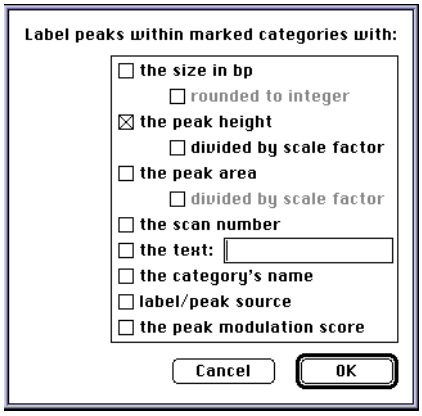
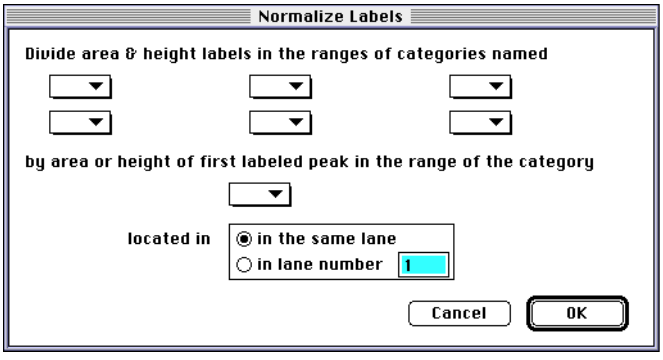
To normalize peaks to the control peak:

Step	Action
1	In the plot pane, select a range that contains a peak to be normalized.
2	Choose Add Category....
3	Name the peak “Peak 1”.
4	Click the Highest Peak radio button. 
5	Click OK.
6	Repeat this procedure for another peak to be normalized. The Category list should now look like this figure. 

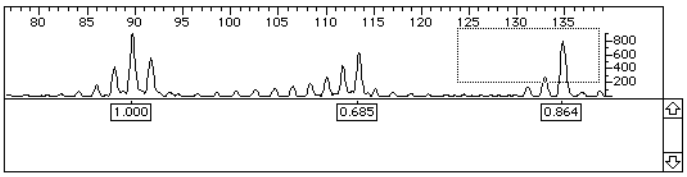
Label Normalized Peaks

Once you have defined a control peak and the other peaks to be normalized, you can label them.

To label peaks:

Step	Action
1	Choose Label Peaks...from the Analysis menu. The Label Peaks dialog box appears. 
2	Click the peak height and/or peak area check box.
3	Click OK.
4	Choose Normalize Labels... from the Analysis menu. The Normalize Labels dialog box appears. 

To label peaks: *(continued)*

Step	Action
5	From the upper set of pop-up menus, choose the Categories to be normalized, for example, Control peak, Peak1, and Peak2.
6	From the middle pop-up menu, choose the name of the Category you defined as a control, for example Control Peak.
7	Click the “in the same lane” radio button.
8	Click OK. The plot pane of the Main window now displays peaks with normalized labels. 

Making Categories from Labels

Introduction

For genotyping applications that require making categories from one distinct set of peaks, you can make categories from defined labels. For certain kinds of applications, this process of defining Categories will be easier than defining them as described in “Defining Categories for Labeling” on page 6-4.

When to Make Categories from Labels

Genotyping applications for which making categories from labels may be useful include:

- AFLP applications
- Allelic ladders

For most microsatellite genotyping applications, you will not want to make categories from labels. Usually you want to define categories while looking at the distribution of several allele peaks, not just one.

For more information on defining categories while viewing allele distributions, see “Editing Categories in Histograms” on page 9-22.

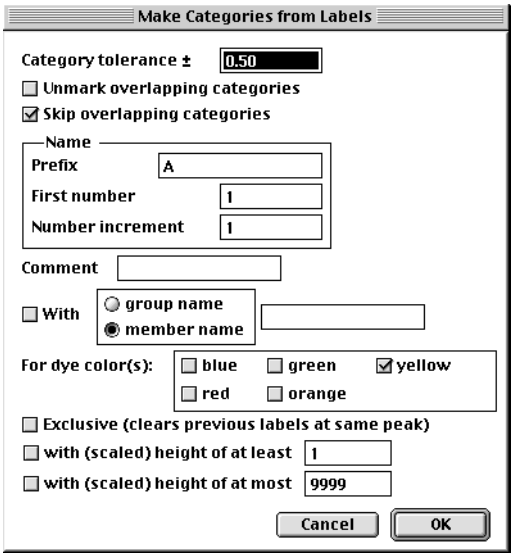
How to Make Categories from Labels

For select dye/lanes, you can make a separate category for each labeled peak according to what you have specified for each peak label.

To make categories from labels:

Step	Action
1	Import GeneScan data, for either allelic ladders or AFLP studies.
2	Select dye/lanes for which you want to make categories from labeled peak data.
3	Label peaks for which you want to create categories.

To make categories from labels: *(continued)*

Step	Action
4	Choose Make from Labels... from the Category menu. The Make from Labels... dialog box appears. 
5	Type in the Category tolerance.
6	Select checkboxes for either including, or skipping overlapping categories.
7	Name categories that will be created. For information on how Genotyper names categories see “Example of Category Naming” on page 6-9.

To make categories from labels: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>8</td><td>Optionally, select “with” and make created categories a group, or members of a group:
<table>
<tr><th>If you want to make each category...</th><th>Then click...</th><th>And...</th></tr>
<tr><td>A group to which you can add member categories</td><td>member</td><td>type in the name of the first member of the group.</td></tr>
<tr><td>A member of a group</td><td>group name</td><td>type in the name of the group.</td></tr>
</table></td></tr>
<tr><td>9</td><td>The remaining category definition checkboxes have the same meaning as those described for the “Add Category dialog box” on page 6-5.</td></tr>
<tr><td>10</td><td>When you are satisfied with all your choices for defining categories, click OK.

Genotyper makes a category for each peak that has a label.</td></tr>
</table>

Working with Plot Data 7

Chapter Overview

Introduction Genotyper Documents display dye/lane data as electropherogram plots. This chapter explains how to make use of the many options Genotyper offers for viewing, interpreting, and customizing plot displays within a Genotyper Document.

In This Chapter This chapter contains the following topics:

Viewing Plots of Imported Dye/Lanes

Introduction

Genotyper generates plot data from imported dye/lanes in the form of electropherograms (see Figure 7-1).

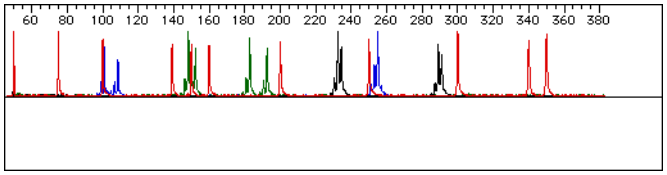


Figure 7-1 Example of an electropherogram

Electropherograms are peak representations of the size and quantity data from dye-labeled nucleic acid fragments that have been electrophoresed on an ABI PRISM instrument and analyzed in GeneScan.

The Plot Window

The Plot window provides an expanded full screen view of the plot area, allowing you to view each selected dye/lane as an individual electropherogram.

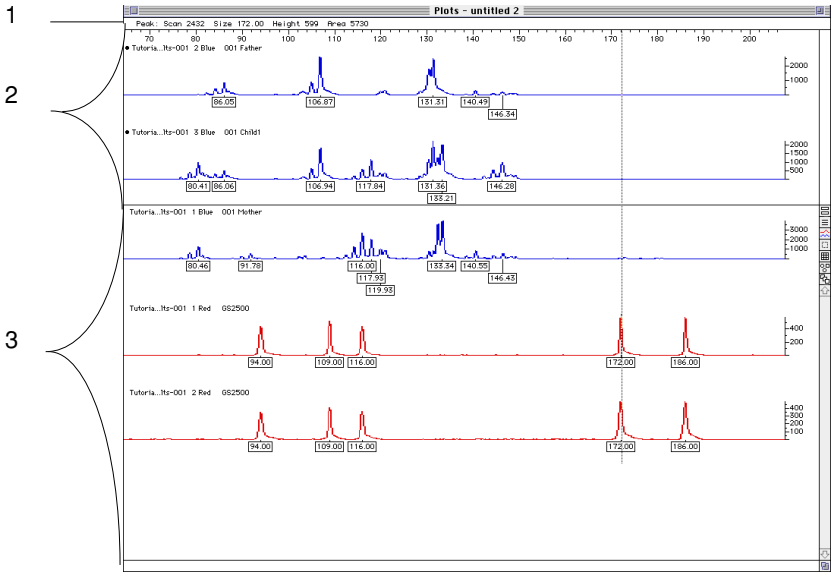


Figure 7-2 The Plot window

Note In the Main window, labels are shown only if one dye/lane is selected. If more than one dye/lane is selected, choose Show Plot Window to view all the labels.

Parts of the Plot Window

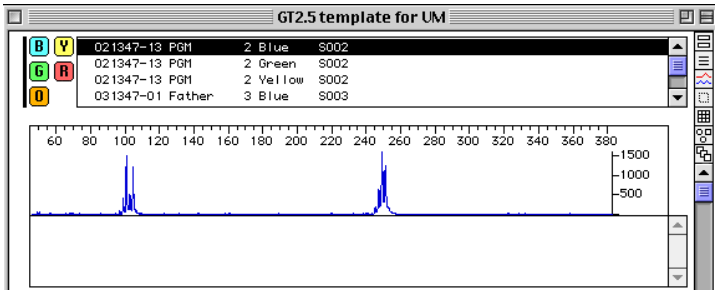
Parts of the Plot window:

Item	Name	Description
1	Peak Description Area	Displays information about scan line number, size, height, area, and category for peaks the cursor is on.
2	Upper Pane	A reference area where you can display one or more “reference” plots.
3	Lower Pane	Plot area where you can display one or more plots. You can scroll this area and visually compare these plots to those in the Upper Pane.

How to View Plots of a Single Dye/lane

You can view electropherogram plots of any imported dye/lane that appears in the Dye/lane list.

To view plot data of imported dye/lanes:

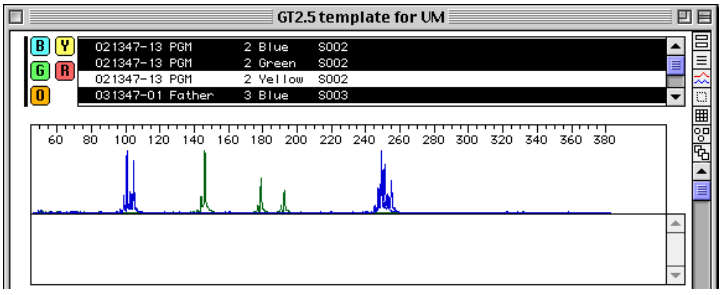
Step	Action
1	In the Dye/lane list, locate the dye/lane for which you want to display plot data.
2	Select the dye/lane in the Dye/Lane list. An electropherogram plot showing peaks for each analyzed nucleic acid fragment in the sample appears in the Plot Area.  The vertical scale on the right is relative peak height. The default horizontal scale at the top is fragment length.
3	Click the Plot window icon to display an expanded view of the electropherogram in the Plot window (Figure 7-2 on page 7-3).

How to View Plots of Multiple Dye/lanes

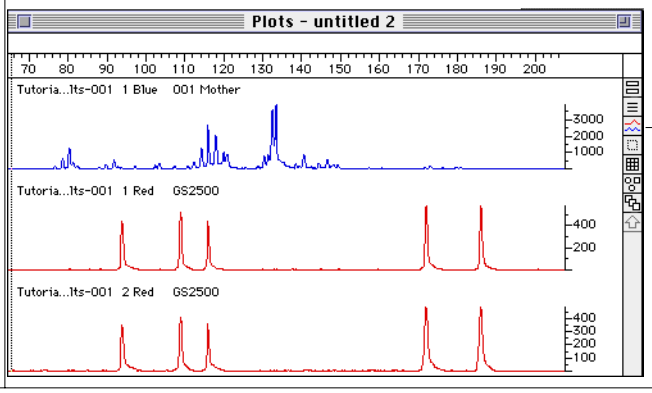
You can view an electropherogram plot showing all detected peaks for multiple dye/lanes.

Note If you have selected many dye/lanes to be shown, a light gray background, instead of the electropherogram plots, will appear briefly in the plot area. This means Genotyper is processing the electropherograms in the background and will draw them when they are ready. You may continue to run other Genotyper commands or change the dye/lane selections during this time.

To show plot data for multiple dye/lanes.

Step	Action
1	In the Dye/lane list, locate the dye/lane or dye/lanes for which you want to display plot data.
2	Select the dye/lanes in the Dye/lane list. An electropherogram plot appears showing overlapping peaks for each selected dye/lane in the Plot Area. The highest peak fills the available area. The vertical scale disappears because each dye/lane plot is scaled independently to occupy the full height available. 

To show plot data for multiple dye/lanes. *(continued)*

Step	Action
3	To view each selected dye/lane as an individual electropherogram plot, click the Plot window icon. The Plot window opens, displaying individual electropherograms for each selected dye/lane. This figure shows a plot window for multiple dye/lanes. 

The Order of Plot Displays

For plots derived directly from the dye/lane list, the order that the plots are displayed is determined by the dye/lane list order preferences (determined by the Dye/lane Sorting command).

For plots derived from table rows, the order of plots is determined by the table row order.

Low Memory Warning

If a dark gray background appears in the plot area, Genotyper is running low on memory. Save your work as soon as possible, quit from Genotyper, then allocate more memory to Genotyper.

For more information on allocating memory to Genotyper, see “Setting Memory Allocation” on page 1-11.

Viewing and Interpreting Peak Data

Introduction

Peak data in electropherograms can show you size and quantity data for imported GeneScan files. Following are some key terms for interpreting peak data.

Definitions of terms:

Term	Definition
Peak Height	A representation of the quantity of sample for a given fragment. The height of a peak is determined by the intensity of signal at the highest point that fluoresces for each dye-labeled fragment.
Scan Number	For automated gel data collection software, the laser samples data each time it scans across the gel. Each sampling is stored as a data point. The scan number describes the location of the data point.
Base Pairs	A unit of fragment size. The number of base pairs indicates the estimated length of a nucleic acid fragment, relative to the size standard.

How to View GeneScan Peak Data

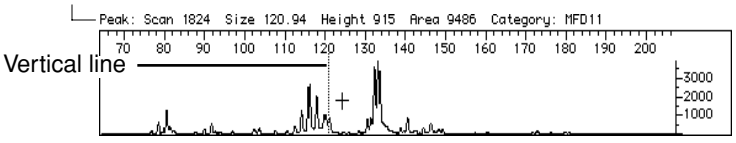
You can view the following data for peaks resulting from GeneScan analysis of electrophoresed sample fragments:

- Scan number
- Peak size
- Peak height
- Peak area
- Genotyper Category

To view peak data for imported GeneScan files:

Step	Action
1	Select dye/lanes of interest, and view plot data.

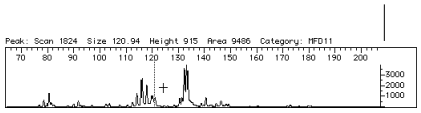
To view peak data for imported GeneScan files: *(continued)*

Step	Action
2	Use the mouse to move the cross hairs along the plot. The vertical line "jumps" from peak to peak. Information about each peak appears above the horizontal scale. Peak information 

How to View Relative Peak Size and Quantity

The horizontal and vertical scales for plot data can inform you of the approximate size and quantity of fragment peaks.

To view approximate size and quantity values for peak data:

If you want to know...	Then read...
The relative quantity of a dye/lane fragment	The vertical scale. It displays fragment quantity in terms of peak height. Vertical scale 
The relative length of a dye/lane fragment in base pairs	The Default horizontal scale at the top is fragment length in base pairs.
Relative time required for a dye/lane fragment to be detected	The Default horizontal scale at the top is fragment length in base pairs. You can change this scale to Scan number.

How to Change the Size and Quantity Scale

You can change the scale Genotyper uses for the horizontal axis.

To change the peak sizing scale:

Step	Action
1	Select dye/lanes of interest, and view plot data.
2	From the Views menu, choose how you want to display size and quantity information for peak data.

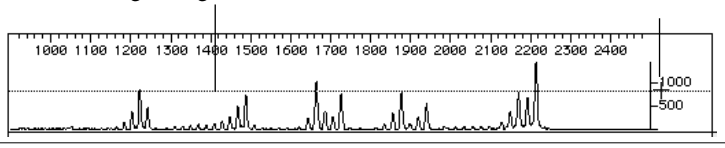
If you choose...	Then the horizontal scale displays...
Display by Size	Base pairs.
Display by Scan	Number of scans required to detect sample fragment data.

Note If you did not use GeneScan to call fragment sizes, or if no sized peaks were found in a dye/lane, then you will not be able to view the electropherogram when you choose DIsplay by Size. The text No Size Data will appear in the Plot area. To view the electropherogram choose Display by Scan.

How to Compare Peak Heights

For qualitative comparisons of fragment quantities in select dye/lanes, you can compare peak heights in a plot view.

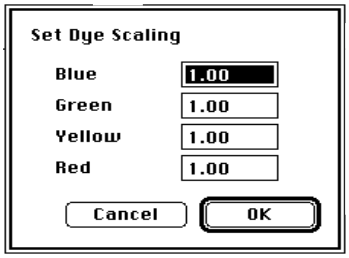
To compare peak heights of select dye/lanes:

Step	Action
1	Select dye/lanes of interest, and view plot data.
2	Use the mouse to move the “cross hairs” to the vertical scale area. A horizontal line appears across the length of the plot. You can use this line as a “straight edge” to compare relative peak heights. “Straight edge” “Cross hairs” 

How to Adjust Peak Heights

You can adjust the signal height of peaks by choosing a scaling factor for each of the four dye colors. Dye scaling affects only the appearance of the electropherogram plots. All other data, such as the values appearing in the cursor information line above the plot, remain unchanged.

To adjust the height of dye-colored peaks:

Step	Action
1	Select dye/lanes of interest, and view plot data.
2	Choose Plot Options from the Views menu.
3	Drag the arrow pointer to the submenu, and choose Dye Scaling... The Dye Scaling dialog box appears.  Signal heights for each color are multiplied by the indicated factor before being plotted. The vertical scale reflects the adjusted heights.
4	Choose the peak colors that you want to scale, and type in a number for the percent by which you want to scale the peak height. **Example** If you want all the blue peaks to display at half of their current height, type in .50.
5	Click OK.

Zooming In and Out

Introduction

Zooming in and out of a plot view, allows you to view particular peaks closer up by zooming in, or see a wider range of peaks by zooming out.

How to Zoom in on the Plot Area

For a closer view of particular peaks, or a group of peaks, you can zoom in on the Plot Area.

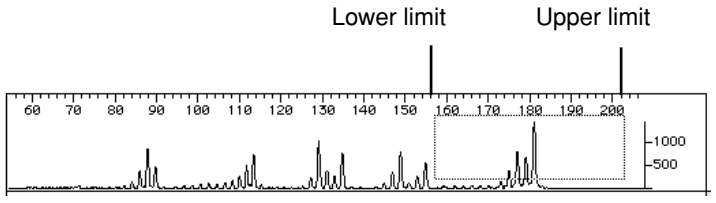
To zoom in on the entire Plot Area:

Step	Action
1	Select dye/lanes of interest, and view plot data.
2	Choose Zoom In from the Zoom submenu in the Views menu. You can now view the middle 50% of the plot.

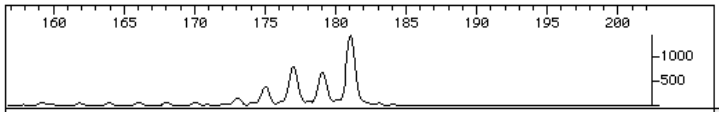
How to Zoom in on a Selected Range

For a closer view of a group of peaks, you can zoom in on a particular region, or range of the Plot Area.

To zoom in on a selected range of the Plot Area:

Step	Action
1	Select dye/lanes of interest, and view the Plot Area.
2	Drag the cross hairs across the region you want to zoom in on. The vertical bar becomes a dotted rectangle that indicates the lower and upper limits of the area you selected. 

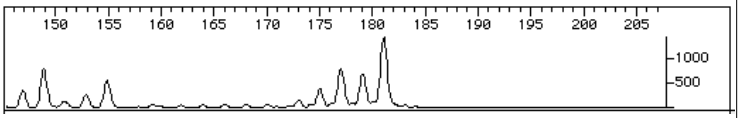
To zoom in on a selected range of the Plot Area: *(continued)*

Step	Action
3	Choose Zoom In (Selected Range) in the Views menu. The range you selected is magnified to fill the Plot Area.  **Note** Only the left and right boundaries of the selection rectangle apply to the Zoom In (Selected range) command. The top and bottom boundaries are not used.

How to Zoom Out

For a view of a wider range of peaks in the Plot Area, you can zoom out.

To partially zoom out for a broader view of the plot:

Step	Action
1	Select dye/lanes of interest, and view plot data.
2	Choose Zoom Out (⌘- –) from the Zoom submenu in the Views menu. You can now view about 50% more of the plot. 

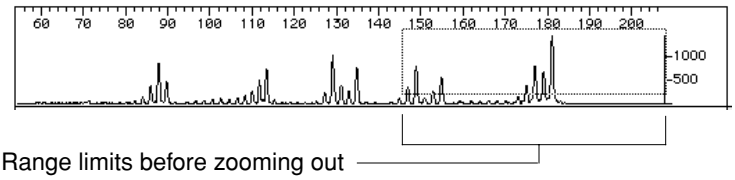
How to Zoom Out to Full Range

To view all of the peaks in select dye/lanes you can zoom out to full range.

To zoom out:

Step	Action
1	Select dye/lanes of interest, and view plot data.

To zoom out: *(continued)*

Step	Action
2	Choose Zoom Out (Full Range) (⌘-H) from the Zoom submenu in the Views menu. You can now view the entire plot. The dotted rectangle in the plot area indicates the range limits that existed before you zoomed out to full range.  Range limits before zooming out

How to Zoom to a Specific Range

You can specify a range of peaks that you want to view in the Plot Area. The table below shows you how to zoom to ranges that you specify.

If you want to zoom to...	Then...
A specific size range of fragments	a. Choose Zoom to...from the Zoom submenu. b. The Set Plot dialog box appears. c. Enter the plot range (from size__to size__). d. Click OK.
The range of one or more Categories	a. Select one or more categories in the Categories list. b. Choose Zoom to Category in the Zoom submenu. c. You can now view the range that includes the range of the selected categories.
The range of the next marked, unselected Category	a. Choose Zoom to Next Category in the Zoom submenu. b. The next marked Category in the plot is selected automatically.

Customizing Plot Areas

Introduction

By default, the upper graphical pane in the Main window is the electropherogram view area; the lower graphical pane is reserved for peak labels. However, you can customize the settings to make these areas serve different purposes. Likewise, you can customize the upper and lower panes of the Plot window.

Note You can set the Plot Options for the Plot window only when the Plot window is displayed.

The Main Window Plot Area

The upper pane and lower pane of the Main window plot area.

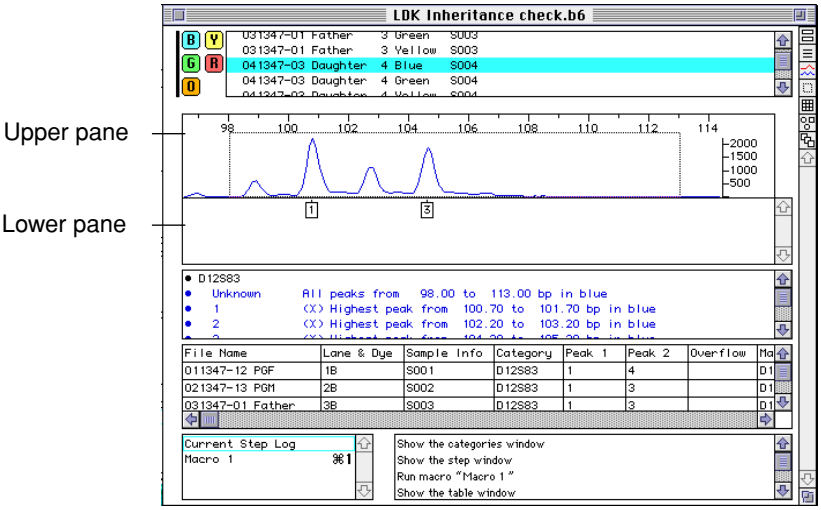


Figure 7-3 Location of upper and lower panes in the Main window

The Plot Window Panes

The upper pane and lower pane of the Plot window.

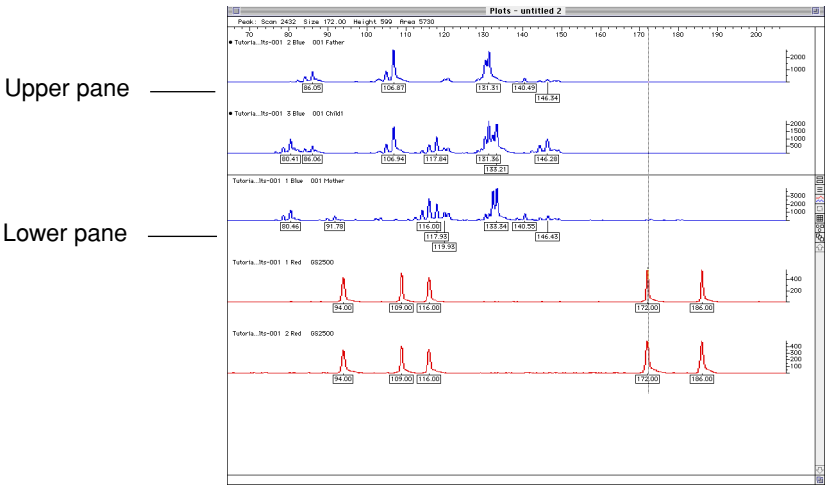


Figure 7-4 Location of upper and lower panes in the Plot window

How to Customize Plot Displays

You can customize the plot display in the upper and lower panes of either the Main window (Figure 7-3) or the Plot window (Figure 7-4).

To customize plot displays:

Step	Action
1	Choose the kind of plot display that you want to customize.

If you want to customize...	Then...
The Main window	
Upper pane	Select Upper Pane, Main window from the Plot Options sub-menu of the Views menu.
Lower pane	Select Lower Pane, Main window from the Plot Options sub-menu of the Views menu.
The Plot window	
Upper pane	Select Upper Pane, Plot window from the Plot Options sub-menu of the Views menu.
Lower pane	Select Lower Pane, Plot window from the Plot Options sub-menu of the Views menu.

To customize plot displays: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Select the checkboxes for the kind of plot that you want to view.

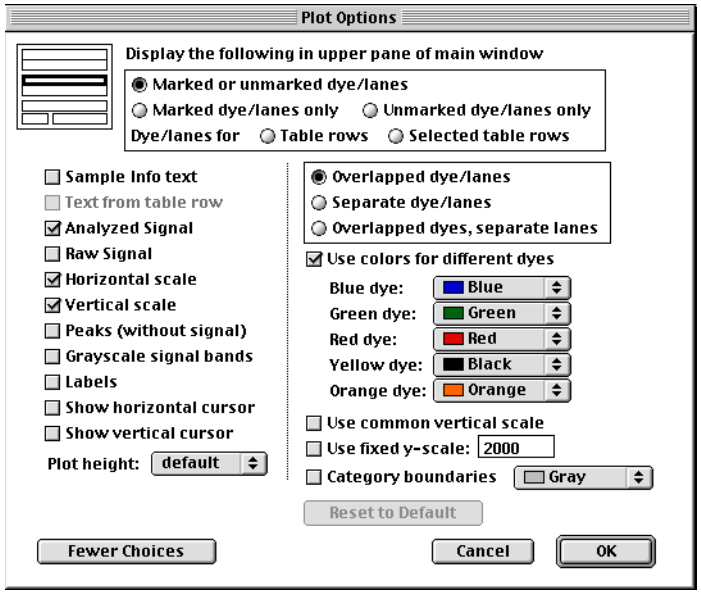
<table>
<tr><th>If you click...</th><th>Then the plot displays...</th></tr>
<tr><td>Analyzed signal</td><td>Peaks that have been baselined and analyzed by GeneScan.</td></tr>
<tr><td>Raw signal</td><td>Peaks from fragments that have not been baselined or analyzed by GeneScan.</td></tr>
<tr><td>Grayscale signal bands</td><td>A display that looks like autoradiography signals, but is derived from the electropherogram.</td></tr>
<tr><td>Labels</td><td>Size and quantity labels on peaks.</td></tr>
<tr><td>Use colors for different dyes</td><td>Electropherograms in color; if unchecked, electropherograms will be drawn in black.</td></tr>
<tr><td>Use fixed y-scale</td><td>Draws all plots to a specified vertical scale.</td></tr>
<tr><td>Category boundaries</td><td>Boundaries around peaks in a category.

Note Not recommended for Main window displays or overlapped displays.</td></tr>
</table>
</td></tr>
</table>

Adding More Detail to the Plots

You can add more details to your plot displays.

To add more detail to customized plot displays:

Step	Action
1	From the Plot Options dialog box, click More Choices. This displays a dialog box that offers you more choices of what you can display for the plot area you have chosen to customize. 

To add more detail to customized plot displays: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Choose the radio buttons for the kind of plot you want to view.

<table>
<tr><th>If you click...</th><th>Then the plot area displays...</th></tr>
<tr><td>Marked or unmarked dye/lanes</td><td>Electropherogram plots for all selected dye/lanes. This is the default.</td></tr>
<tr><td>Marked dye/lanes only</td><td>Only plots for dye/lanes that are both selected, and marked.</td></tr>
<tr><td>Unmarked dye/lanes</td><td>Only plots for dye/lanes that are both selected, but not marked.</td></tr>
<tr><td>Dye/lanes for Table rows</td><td>Plots for all rows in the associated table.</td></tr>
<tr><td>Selected table rows</td><td>Plots for table rows you select.</td></tr>
<tr><td>Overlapped dye/lanes</td><td>Plots for each selected dye/lane on top of each other in the pane.</td></tr>
<tr><td>Separate dye/lanes</td><td>Plots for each selected dye/lane separate from one another.</td></tr>
<tr><td>Overlapped dyes, separate lanes</td><td>All dye colors in a lane superimposed on each other, but each lane appears separately from the others.</td></tr>
</table>
</td></tr>
</table>

To add more detail to customized plot displays: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Select the checkboxes for kind of data to include in each plot

<table>
<tr><th>If you click...</th><th>Then the plot area displays...</th></tr>
<tr><td>Sample Info text</td><td>Text for associated Sample Info field.</td></tr>
<tr><td>Analyzed Signal</td><td>Plots of peaks that have been baselined and analyzed by GeneScan.</td></tr>
<tr><td>Raw Signal</td><td>Fluorescent signal before GeneScan analysis.</td></tr>
<tr><td>Horizontal scale</td><td>A horizontal scale.</td></tr>
<tr><td>Vertical scale</td><td>A vertical scale.</td></tr>
<tr><td>Peaks (without signal)</td><td>a vertical line for each peak.</td></tr>
<tr><td>Grayscale signal bands</td><td>Bands similar to a Autoradiograph.</td></tr>
<tr><td>Labels</td><td>Any labels put on peaks.</td></tr>
<tr><td>Show horizontal cursor</td><td>A horizontal cursor.</td></tr>
<tr><td>Show vertical cursor</td><td>A vertical cursor.</td></tr>
<tr><td>Use common vertical scale</td><td>All plots are drawn to the same vertical scale. When this box is unchecked (the default), each plot fills the amount of vertical space available.</td></tr>
<tr><td>Use fixed y-scale</td><td>All plots drawn to the specified vertical space.

Note When selected, then Use common vertical scale is disabled.</td></tr>
<tr><td>Use colors for different dyes</td><td>All dye colors in a lane superimposed on each other, but each lane appears separately from the others.</td></tr>
</table>
</td></tr>
</table>

To add more detail to customized plot displays: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>In the Plot height pop-up menu, you can adjust the height of plots that display in the Plot Area.

<table><tr><th>If you choose...</th><th>Then the Plot Area...</th></tr><tr><td>small</td><td>Fits more plots in the window.</td></tr><tr><td>default</td><td>Shows a medium-sized plot.</td></tr><tr><td>large</td><td>Shows more detail in the plot.</td></tr></table></td></tr>
<tr><td>5</td><td>Click OK to accept your selections.</td></tr>
</table>

Viewing Table Data in Plots

Introduction

You can view information from tables in plot displays.

For more information about working with Tables, see “Parts of the Plot Window” on page 7-4.

Plot Data Associated with Dye/lanes

Genotyper generates all plots from data in dye/lanes. Do not delete dye/lanes that contain data shown in your table or you will not be able to view plots of rows that contain that data.

How to Show Table Data in Plots

When you display plot data for select dye/lanes, you can include information from related tables in the plot display.

To show table row text in plot displays:

Step	Action
1	Open the Genotyper Document that contains the table information you want to display in the Plot window.
2	Open the Plot window from the Main window.
3	Open the Table window.
4	Select the rows in the table for which you want to display corresponding plots. **Note** A row is considered selected if any cell in the table is selected.
5	Choose the Plot Options from the Views menu.
6	Select the Plot window, lower pane plot option. The Plot window, lower pane dialog box appears.
7	Click the More Choices button. The More Choices dialog box appears. See figure in step 1 of “Adding More Detail to the Plots” on page 7-18.
8	Choose one of the radio buttons after “Dye/lanes for”: **If you choose...** / **Then the Plot window displays...** Table rows / Plots for all rows in the table. Selected table rows / Plots for table rows you select.
9	Select the Text from table row checkbox if you want to display the row text in the corresponding plot display.

To show table row text in plot displays: *(continued)*

Step	Action
10	Click OK. The Plot Window displays plots and associated information for selected table rows.

Automatic Scrolling

When you select a row in a table, the plot automatically scrolls to the corresponding peak data in the plot display.

Comparing Plot Data to Reference Plots

Introduction

You can designate selected dye/lanes as reference plots, and display one or more of these reference plots in the upper pane of the Plot window (Figure 7-2 on page 7-3).

Once you have set up a reference plot, you can display plots for one or more dye/lanes in the lower pane of the Plot window and compare their plots to the reference plots in the upper pane.

Setting Up Reference Plots

You can compare plot information from the reference pane to scrollable plot data in a lower pane.

To set up reference plots:

Step	Action
1	In the Dye/lane list, locate the dye/lane or dye/lanes for which you want to display reference plots.
2	Choose the Mark Command from the Edit menu, or double-click the dye/lanes, marking them with a bullet. The bullet signifies that plots for those dye/lanes are reference plots.
3	Select the dye/lanes you marked as reference plots, and any dye/lanes that you want to compare to the reference plots.
4	Click the Plot window icon to display an expanded view of the Plot window (Figure 7-2 on page 7-3). The upper pane of the Plot window displays reference plots, and the lower pane displays plots for the dye/lanes that you want to compare to the reference plots. You can scroll plot data in the lower pane. **Note** The upper pane does not scroll, so you will probably only want one or two dye/lanes displayed in this pane.

8 Working with Tables

Chapter Overview

Introduction Putting your results data into a table allows you to organize it in a manner meaningful to your genotyping application. You can use tabular data for comparison analysis, as well as export results to a database.

In This Chapter This chapter contains the following topics:

Setting Up a Table

Sources of Table Data

Table contents are generated from labeled fragment peaks within select dye/lanes. Before setting up a table make sure that the appropriate dye/lane peaks are labeled with the kind of information that you want to present in your table.

For more information on how to label fragment peaks in dye/lanes, see Chapter 6, “Defining Categories and Labeling”.

Choosing a Table to Create

The following table shows examples of tables you can create for different genotyping applications.

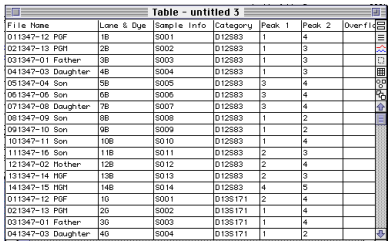
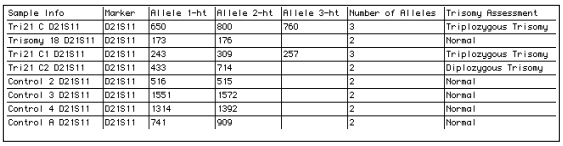
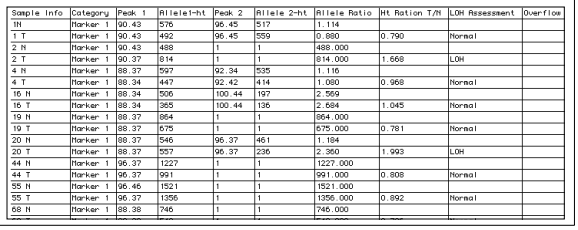
For this genotyping application...	You can create this kind of table...
Linkage Mapping	
Gene Expression Profiling	
Loss of Heterozygosity	

Table - untitled 3

File Name	Lane & Dye	Sample Info	Category	Peak 1	Peak 2	Overfl
011347-12 PGF	1B	S001	D12S83	1	4	
021347-13 PGM	2B	S002	D12S83	1	3	
031347-01 Father	3B	S003	D12S83	1	3	
041347-03 Daughter	4B	S004	D12S83	1	3	
051347-04 Son	5B	S005	D12S83	3	4	
061347-06 Son	6B	S006	D12S83	3	4	
071347-08 Daughter	7B	S007	D12S83	3	4	
081347-09 Son	8B	S008	D12S83	1	2	
091347-10 Son	9B	S009	D12S83	1	2	
101347-11 Son	10B	S010	D12S83	1	4	
111347-16 Son	11B	S011	D12S83	2	3	
121347-02 Mother	12B	S012	D12S83	2	4	
131347-14 MGF	13B	S013	D12S83	2	3	
141347-15 MGM	14B	S014	D12S83	4	5	
011347-12 PGF	1G	S001	D13S171	2	4	
021347-13 PGM	2G	S002	D13S171	1	4	
031347-01 Father	3G	S003	D13S171	1	4	
041347-03 Daughter	4G	S004	D13S171	1	2	

Sample Info	Marker	Allele 1-ht	Allele 2-ht	Allele 3-ht	Number of Alleles	Trisomy Assessment
Tri21 C D21S11	D21S11	650	800	760	3	Triplozygous Trisomy
Trisomy 18 D21S11	D21S11	173	176		2	Normal
Tri21 C1 D21S11	D21S11	243	309	257	3	Triplozygous Trisomy
Tri21 C2 D21S11	D21S11	433	714		2	Diplozygous Trisomy
Control 2 D21S11	D21S11	516	515		2	Normal
Control 3 D21S11	D21S11	1551	1572		2	Normal
Control 4 D21S11	D21S11	1314	1392		2	Normal
Control A D21S11	D21S11	741	909		2	Normal

Sample Info	Category	Peak 1	Allele1-ht	Peak 2	Allele 2-ht	Allele Ratio	Ht Ration T/N	LOH Assessment	Overflow
1N	Marker 1	90.43	576	96.45	517	1.114			
1 T	Marker 1	90.43	492	96.45	559	0.880	0.790	Normal	
2 N	Marker 1	90.43	488	1	1	488.000			
2 T	Marker 1	90.37	814	1	1	814.000	1.668	LOH	
4 N	Marker 1	88.37	597	92.34	535	1.116			
4 T	Marker 1	88.34	447	92.42	414	1.080	0.968	Normal	
16 N	Marker 1	88.34	506	100.44	197	2.569			
16 T	Marker 1	88.34	365	100.44	136	2.684	1.045	Normal	
19 N	Marker 1	88.37	864	1	1	864.000			
19 T	Marker 1	88.37	675	1	1	675.000	0.781	Normal	
20 N	Marker 1	88.37	546	96.37	461	1.184			
20 T	Marker 1	88.37	557	96.37	236	2.360	1.993	LOH	
44 N	Marker 1	96.37	1227	1	1	1227.000			
44 T	Marker 1	96.37	991	1	1	991.000	0.808	Normal	
55 N	Marker 1	96.46	1521	1	1	1521.000			
55 T	Marker 1	96.37	1356	1	1	1356.000	0.892	Normal	
68 N	Marker 1	88.38	746	1	1	746.000			

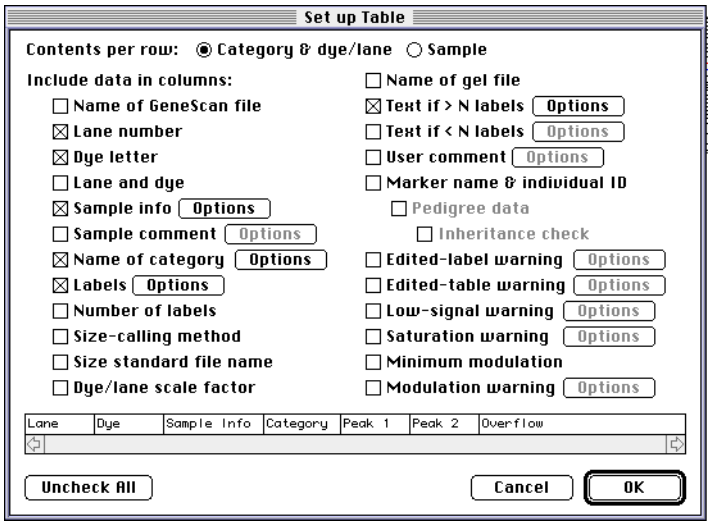
Column Number Limit

There is a limit of 128 columns in a table.

How to Determine Row Contents

Genotyper generates row contents from dye/lanes in the Dye/lane list and categories in the Category list.

To determine row contents in your table:

Step	Action
1	Mark those Categories in the Category list that define the kind of peak data that you want to include in the table.
2	From the table menu, choose Set Up Table... The Set up Table dialog box appears.  **Figure 8-1** The Set up Table dialog box
3	In the Contents per row field, click one of the two radio buttons. **If you want each row to correspond to... / Then Click...** Marked categories and selected dye/lanes — Category & dye/lane. Sample Information entered in the Sample Info field for a dye/lane entry — Sample.

How to Determine Column Contents

You can select the column contents for the rows in your table from a list of checkboxes in The Set Up Table dialog box (“The Set up Table dialog box” on page 8-3). The order in which you select the checkboxes determines the order in which the column contents will appear from left to right in your table.

To determine column contents for each table row:

Step	Action
1	From the Table menu, choose Set Up Table.... The Set Up Table dialog box appears (Figure 8-1 on page 8-3). As shown at the bottom of the dialog box, the checkboxes selected beneath the “Include Data in Columns” heading are the current settings for column headings.
2	Click OK to accept the current selections for the column contents, or click the Uncheck All button to clear all the selections. Checkboxes that you can select for column headings depend on the kind of contents per row you defined. The text of unavailable checkboxes appears in gray. **Note** To change the column heading text, see “Editing Table Cells and Column Headings” on page 8-28.

To determine column contents for each table row: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Select checkboxes under the Include Data in Columns heading:
<table>
<tr><th>If you want to define a column for...</th><th>Then click...</th></tr>
<tr><td>Name of an imported GeneScan file</td><td>Name of GeneScan file.</td></tr>
<tr><td>The lane number of a dye/ lane</td><td>Lane number.</td></tr>
<tr><td>The color of the dye for the dye/lane in a row</td><td>Dye letter.</td></tr>
<tr><td>The lane number and dye color of the dye/lane containing peak data</td><td>Lane and dye.</td></tr>
<tr><td>Contents of Sample Info field</td><td>Sample info.</td></tr>
<tr><td>Contents of Sample comment field in Sample Sheet, and Dye/lane window</td><td>Sample comment.</td></tr>
<tr><td>The name of a selected category</td><td>Name of category.</td></tr>
<tr><td>Labeled Peak data</td><td>Labels.</td></tr>
<tr><td>The number of labels on peaks in the category, dye/lane, or sample</td><td>Number of labels.</td></tr>
<tr><td>GeneScan size-calling method</td><td>Size-calling method.</td></tr>
<tr><td>GeneScan size standard</td><td>Size standard file name.</td></tr>
<tr><td>Scale factors, if defined</td><td>Dye/lane scale factor.</td></tr>
<tr><td>The name of associated Gel files</td><td>Gel file name.</td></tr>
<tr><td>Text, when more than a specified number of labels are detected in a category, dye/lane, or sample</td><td>Text if > N labels.</td></tr>
<tr><td>Text, when less than a specified number of labels are detected in a category, dye/lane, or sample</td><td>Text if < N labels.</td></tr>
<tr><td>Your own comments</td><td>User comment.</td></tr>
<tr><td>Data for, and results of inheritance check</td><td>Marker name & Individual ID.</td></tr>
<tr><td>A warning when labels are edited</td><td>Edited-label warning.</td></tr>
<tr><td>A warning when cell contents edited</td><td>Edited-table warning.</td></tr>
<tr><td>A warning for low dye/lane signal</td><td>Low-signal warning.</td></tr>
<tr><td>A warning for intensity of signal</td><td>Saturation warning.</td></tr>
<tr><td>Lowest modulation score value</td><td>Minimum modulation.</td></tr>
<tr><td>A warning for low modulation scores</td><td>Modulation warning.</td></tr>
</table></td></tr>
</table>

To determine column contents for each table row: *(continued)*

Step	Action
4	Click OK when you have defined all the columns that you want to include in your table.

How to Append Rows to a Table

Once you have created a table, you can append rows to the table.

To append rows to a table:

Step	Action
1	If it is not already open, open the Genotyper Document that contains the table to which you want to append rows.
2	Select those dye/lanes that have sample information that you want to add to the table. **IMPORTANT** Genotyper puts all information from dye/lanes into the existing format of rows in the current table. All rows in a table must have the same number of columns and the same column headings.
3	From the Table menu, choose Append to Table. A row containing sample information from select dye/lanes is added to the bottom of the table.

Re-importing Dye/lanes

If you have made a table and deleted or cleared all dye/lanes, select a table cell, then choose Re-import Dye/lane from the File menu.

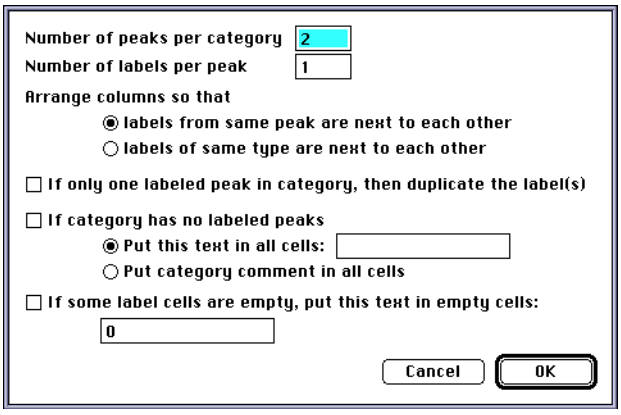
Arranging Columns of Labeled Peak Data

Introduction

For each row in a table, you can order columns of labeled peak data according to the kind and number of labels defined by selected categories.

How to Arrange the Order of Peak Label Columns

To specify the order of peak columns that contain peak data labels:

Step	Action
	Select Source of Peak Labels
1	Make sure that you mark the categories that define the kind of labeling you want to include in the table.
2	From the Table menu, choose Set Up Table.... The Set Up Table dialog box appears (“The Set up Table dialog box” on page 8-3).
	Arrange Columns of Peak Labels
1	Select the Labels checkbox.
2	Click the Options button. The Label Options dialog box appears. 

To specify the order of peak columns that contain peak data

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Select the radio button for how you want to arrange the order of columns containing peak label data:

<table>
<tr><th>If you want to put columns of...</th><th>Then click...</th></tr>
<tr><td>Labeled data from the same peaks next to each other (for example: size, height, size height)</td><td>labels from same peak are next to each other.</td></tr>
<tr><td>The same type of labeled peak data next to each other (for example: size, size, height, height)</td><td>labels of same type are next to each other.</td></tr>
</table>
</td></tr>
<tr><td>4</td><td>Modify columns based on kinds of peak labels in select categories:

<table>
<tr><th>If you want to...</th><th>Then Select..</th></tr>
<tr><td>Duplicate labels, when only one is found in a category

Note When working with homozygote STRs, you assume that any single peak is a result of a homozygotic state.</td><td>If only one labeled peak in category, and duplicate the label(s).</td></tr>
<tr><td>Display a text message when no labeled peaks are found in a category</td><td>If category has no labeled peaks, click Put this text in all cells, type a text message.</td></tr>
<tr><td>Display a pre-defined category comment in all label cells of a row when no labeled peaks are found in a category</td><td>Select If category has no labeled peaks, click Put Category comment in all cells.</td></tr>
<tr><td>Display a text message when no labeled peaks are found in cells defined as columns for peak label data</td><td>If some label cells are empty, put this text in empty cells, type in a text. message.</td></tr>
</table>
</td></tr>
<tr><td>5</td><td>Click OK to accept all of your selections.</td></tr>
</table>

Specifying Columns for Number of Labels in a Row

Introduction

You can specify that columns with specified text appear when the number of labels for a particular row is greater than or less than a specified number.

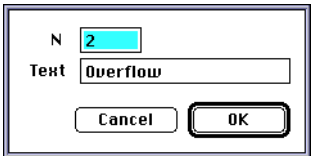
How to Specify Columns for Label Detection

In the Set Up Table dialog box, you can specify that columns with customized text are appended to all rows where more or less labels are detected than the number that you specify.

To specify columns for labels in a row:

Step	Action
1	In the Dye/lane list, select the dye/lanes whose labels you want to put into a table.
2	Make sure that you mark the Categories that define the kind of labeling you want to include in the table.
3	From the Table menu, choose Set Up Table... The Set Up Table dialog box appears (“The Set up Table dialog box” on page 8-3).
4	Choose what kind of column and text you want to display for the number of labels detected for a specific set of peak labeled data:

If you want to display text when the number of labels is...	Then click...
More than a specific number	Text if > N labels.
Less than a specific number	Text if < N labels.

Step	Action
5	Click the Options button. The Label detection dialog box appears. 
6	For N, type in the number of labels for which you want to display a message if more or less than that number are detected in a specified row.

To specify columns for labels in a row: *(continued)*

Step	Action
7	Type in the warning text that you want to appear in the column appended to the end of affected rows.
8	Click OK to accept all of your selections.

Specifying Warning Columns for Edited Tables

Introduction

You can select checkboxes in the Set Up Table dialog box (Figure 8-1 on page 8-3) that specify that columns with specified text will appear to warn you when any row in a table, or any peak label in a row has been edited.

How to Specify Warnings for Edited Labels

When setting up a table, you can specify that Genotyper append a column containing warning text when a peak label has been manually edited in a category and dye/lane before the table was made.

To specify warnings for edited labels:

Step	Action
1	In the Dye/lane list, select the dye/lanes whose labels you want to put into a table.
2	Make sure that you mark the Categories that define the kind of labeling you want to include in the table.
3	From the Table menu, choose Set Up Table.... The Set Up Table dialog box appears (“The Set up Table dialog box” on page 8-3).
4	Select the Edited-label warning checkbox.
5	Click the Options button. The Edited-label warning dialog box appears. 
6	Type in the warning text that you want to appear in the column.
7	Click OK to accept all selections.

How to Specify Warnings for Edited Tables

When setting up a table, you can specify that Genotyper append a column containing warning text to the end of any row that contains cells that have been edited after initial creation of the table.

To specify warnings for rows containing edited table cells:

Step	Action
1	In the Dye/Lane list, select the dye/lanes whose labels you want to put into a table.
2	Make sure that you mark the categories that define the kind of labeling you want to include in the table.
3	From the Table menu, choose Set Up Table... The Set Up Table dialog box appears (“The Set up Table dialog box” on page 8-3).
4	Select the Edited-table warning checkbox.
5	Click the Options button. The Edited-table dialog box appears. 
6	Type in the warning text that you want to appear in the column.
7	Click OK.

Specifying Modulation Warning Columns

Introduction

When setting up a table, you can specify that Genotyper append a column containing warning text in any row that contains peaks that have a modulation score lower than what you specify to be adequate for your application.

What is Modulation

Modulation refers to the degree to which peak data resolves with respect to its immediate background.

Modulation Scores

Modulation scores measure the quality of peak resolution. The following figure shows an example of peaks labeled with modulation scores. Note the correlation between modulation scores and the degree of separation of individual peaks from neighboring valleys. Higher scores show a greater degree of separation from the background.

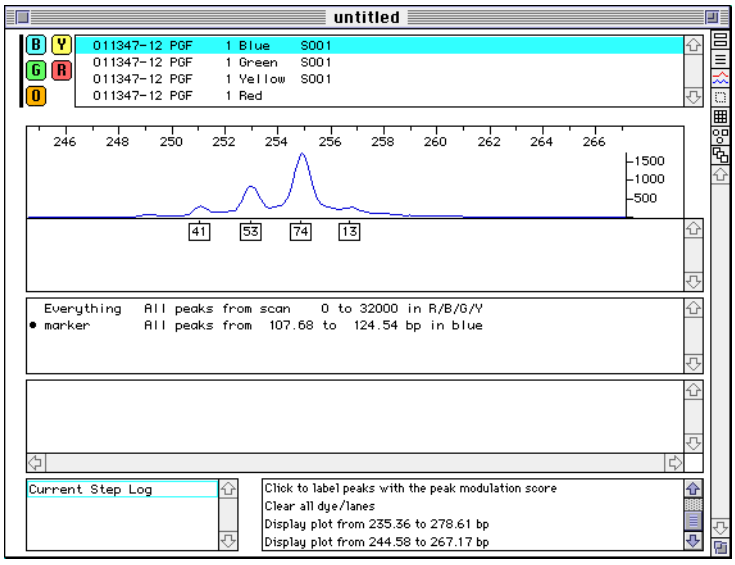
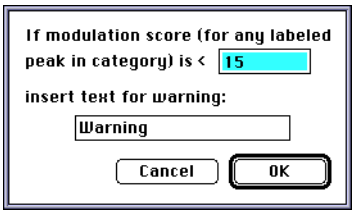


Figure 8-2 Peak modulation scores

How to Specify a Modulation Warning

To specify warnings when peaks do not meet specified modulation scores:

Step	Action
1	In the Dye/Lane list, select the dye/lanes whose labels you want to put into a table.
2	Make sure that you mark the categories that define the kind of labeling you want to include in the table.
3	From the Table menu, choose Set Up Table... The Set Up Table dialog box appears (Figure 8-1 on page 8-3).
4	Select the Modulation warning checkbox.
5	Click the Options button. A dialog box appears. 
6	Type in the modulation score for the minimum acceptable degree of peak modulation for your Genotyper application.
7	Type in the warning text that you want to appear in the column, if peaks are found that are less than the modulation score you have specified.
8	Click OK to accept all your selections.

Specifying Low-signal Warning Columns

Introduction

When setting up a table, you can specify that Genotyper append a column containing warning text to the end of any row that contains peaks that have a fluorescent signal lower than what you specify to be adequate for your application.

What Causes a Low-Signal

A weak fluorescent signal is often caused by problems during sample preparation; in particular problems with PCR, or errors during loading of samples on your ABI PRISM instrument.

Low Signal Example

Figure 8-3 shows an example of a Genotyper plot display showing the vertical axis maximum set at 150 for peak heights. The peaks in the display are labeled with peak heights. If you create a table using the default value for low signal warning of less than 200, Genotyper will append a column containing warning text to the end of the row containing these peak labels.

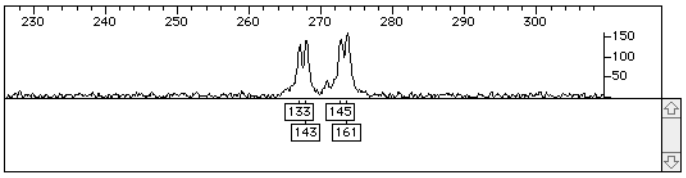


Figure 8-3 Low signals in Genotyper

How to Specify a Low-signal Warning Column

To specify warnings when peaks data has been generated from a signal defined as low:

Step	Action
1	In the Dye/Lane list, select the dye/lanes whose labels you want to put into a table.
2	Make sure that you mark the categories that define the kind of labeling you want to include in the table.
3	From the Table menu, choose Set Up Table... The Set Up Table dialog box appears (Figure 8-1 on page 8-3).
4	Select the Low-signal warning checkbox.
5	Click the Options button. A dialog box appears. 
6	Type in the minimum amount of signal data acceptable before issuing a low signal warning for the associated labeled peak data.
7	Type in the warning text that you want to appear in the column, if peak data has been generated from fluorescent signals less than the amount you specified in the previous step.
8	Click OK to accept all of your selections.

Specifying Saturation Warning Columns

Introduction

When setting up a table, you can specify that Genotyper append a column containing warning text to the end of any row that contains peaks that have a fluorescent signal higher than what you specify to be adequate for your application; a saturation warning.

What Causes Saturation

When you import raw data from GeneScan files, you may also import saturated signals. Saturated signals result when the fluorescent signal from an excess of PCR product exceeds the detection limit of your ABI PRISM instrument. Consequently, if left undetected, Genotyper will count resulting artifact peaks, such as primer peaks, as actual sample peaks. By identifying peaks caused by saturated signals, you can prevent erroneous peak identification.

Saturation Example

Figure 8-4 shows an example of imported dye/lanes displaying peaks with saturated signals.

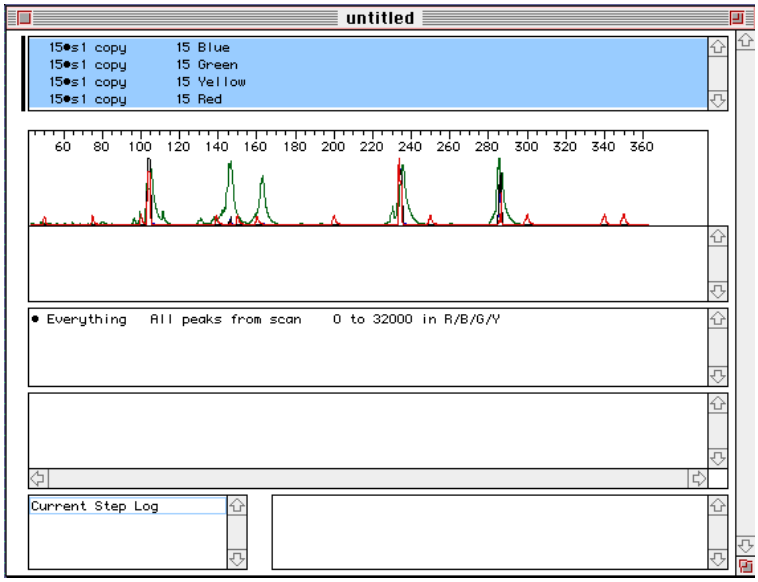
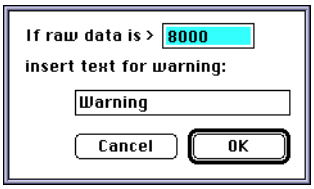


Figure 8-4 Saturated signals in Genotyper

How to Specify a Saturation Warning Column

How to specify warnings when peaks data has been generated from a saturated signal:

Step	Action
1	In the Dye/Lane list, select the dye/lanes whose labels you want to put into a table.
2	Make sure that you mark the categories that define the kind of labeling you want to include in the table.
3	From the Table menu, choose Set Up Table... The Set Up Table dialog box appears (Figure 8-1 on page 8-3).
4	Select the Saturation warning checkbox.
5	Click the Options button. The Saturated signal dialog box appears. 
6	Type in the maximum amount of raw signal data acceptable before issuing a saturated signal warning for the associated labeled peak data.
7	Type in the warning text that you want to appear in the column, if peak data has been generated from fluorescent signals greater than the amount you specified in the previous step.
8	Click OK to accept all of your selections.

Calculating Results from Table Data

Introduction

You can use the Calculate in Table... command to perform numerical calculations of table cell contents. You can review results of calculations in a results column you define when setting up the table.

For an example of how the Calculate in Table command is used for a genotyping application, see “Using Analyze and Calculate in Table Commands–An LOH Example” on page 8-26.

Setting Up Results Columns

Genotyper puts results of table data calculations in a column you specify. The column for the results must already exist. So, if you plan to calculate results from table data, create some extra columns for your results when setting up columns.

For more information on setting up table columns, see “Setting Up a Table” on page 8-2.

Reading the Text Box

The key to using the Calculate in Table... command is to read the text box at the bottom of the dialog box as you enter parameters. The text box explains the calculation and what the result will be. It is easier to read the text box then it is to review settings in the dialog box.

Kinds of Calculations

Table 8-1 shows the kinds of calculations you can perform after specifying values in The Calculate in Table...dialog box. “Fields you can use” refers to those fields shown in the table in “How to Calculate Results From Table Data” on page 8-21.

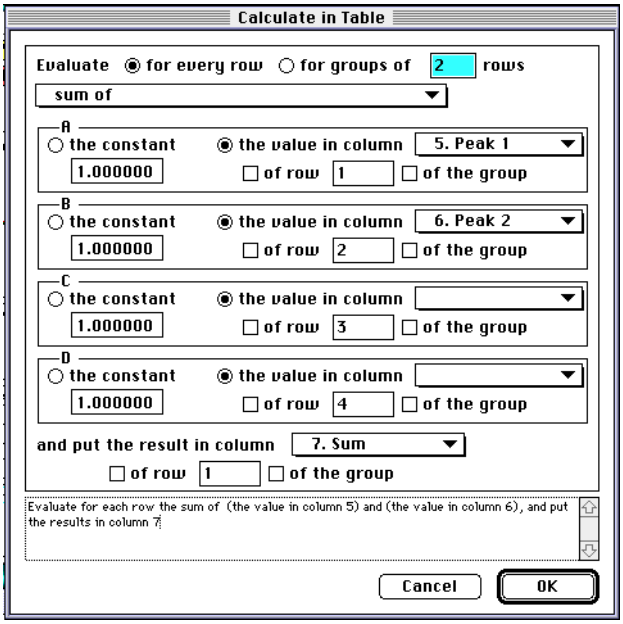
Table 8-1 Kinds of Calculations you can perform on table data

Kind of Calculation	Description	Fields you can use
Sum of	Sum of values defined. Useful for many quantitative applications	A,B,C,D
Difference (A - B)	A minus B. Useful for applications such as HMA where the difference in mobility for a given fragment determines the degree of similarity or difference.	A, B
Absolute difference |A -B|	Absolute value of A minus B	A,B
Ratio (A/B)	A divided by B. Useful for Loss of heterozygosity applications.	A,B
Average of	Average of values chosen	A,B,C,D
Product of	Product of values chosen.	A,B,C,D
Member for category (A) and size/scan (B)	Treat the text in column A as the name of a category group; look through the members of that group and see which one involves size or scan value in column B. The result is the name of the category member.	A, B (must be column value only).
Difference squared (A -B) * (A - B) of	Square of the difference between value of A and value of B.	A, B
Square root (A) of	Square root of the value of A.	A

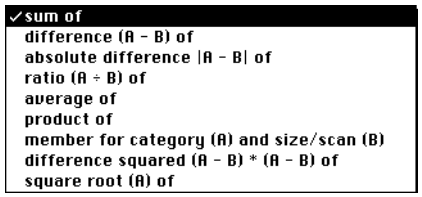
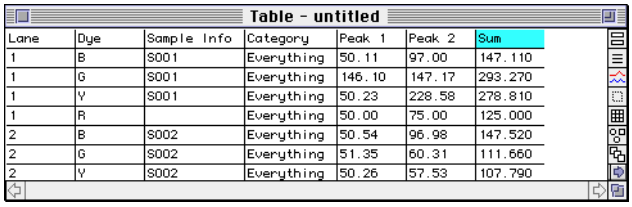
How to Calculate Results From Table Data

Use the Calculate in Table... command to calculate results from table data.

To calculate results from table data:

Step	Action
1	Open the Table window from the Main window.
2	Choose Calculate in Table...from the Table menu. The Calculate in Table dialog box appears. 
3	Choose the radio button for the rows that you want to include in your calculation. **Note** If you choose “for every row”, Genotyper counts row 1 as the title row, and row 2 is the first row in the table that contains data. However, Groups do not count the title row, and row 1 is the first row that contains data.

To calculate results from table data: *(continued)*

Step	Action
4	From the first pop-up menu, choose the kind of calculation that you want to perform. 
5	In fields A-D, choose the table data on which you want to perform the calculation. Type in constants, or choose the column number from the pop-up menus. For example, if your table has data for peaks in columns 5 and 6, and you want to calculate the sums of the those two peaks, choose column 5 in field A, and column 6 in field B.
6	In the field “and put the result in column”, choose the column and optionally the row or group, where you want to put the calculated result.
7	Review the text box at the bottom of the dialog box which explains the calculation you’ve specified. If this is what you intend to do, click OK.
8	Check the Table window to verify that results have been calculated in the table. For example, if you specified that results from the calculation described in step 5 be placed in column 7 of your table, the resulting table will look like. 

Lane	Dye	Sample Info	Category	Peak 1	Peak 2	Sum
1	B	S001	Everything	50.11	97.00	147.110
1	G	S001	Everything	146.10	147.17	293.270
1	Y	S001	Everything	50.23	228.58	278.810
1	R		Everything	50.00	75.00	125.000
2	B	S002	Everything	50.54	96.98	147.520
2	G	S002	Everything	51.35	60.31	111.660
2	Y	S002	Everything	50.26	57.53	107.790

Analyzing Data in Tables

Introduction

Genotyper provides some of the functionality found in spreadsheet applications such as Excel. For example, you can select rows in tables, and perform comparison analysis of cell contents. You can review results of analysis algorithms in a results column you define when setting up the table.

Reading the Text Box

The key to using the Analyze Table...command is to read the text box at the bottom of the dialog box as you enter conditional parameters in the Analyze Table dialog box (Figure on page 8-24). The Text Box provides a verbal explanation of the calculation and its results. It is often easier to read the text box then to review settings in the dialog box.

Example Text Box

For every row, examine columns 3-5; if exactly 2 of these cells in the row are not empty, and if the values in at least 2 cells are greater than 1, then put “Normal” in column 2.

Error Message in Text Box

If the word ERROR appears in the text box, there is a logical inconsistency in the conditional parameters that have been entered. For example, if you specify that Genotyper analyze the cell contents of *two* columns in a row, but also specify that *three* of these cells not be empty, Genotyper will issue an error message in the text box.

Examples of Applications

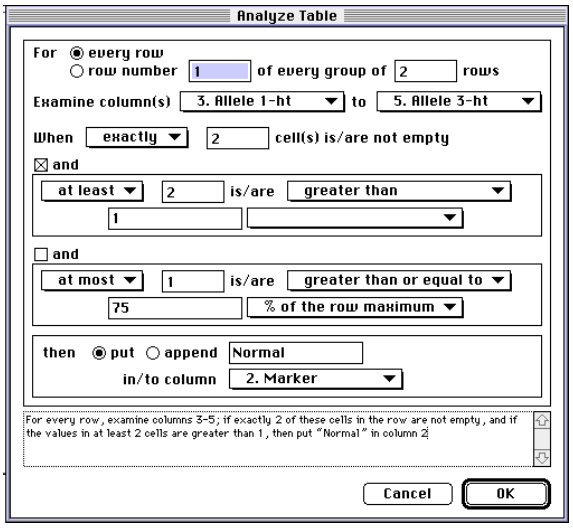
Examples of applications for which you can use the Analyze Table command include:

- Trisomy
- DMD Analysis
- Loss of Heterozygosity
- Gene Quantitation

How to Analyze Data in Tables

Use the Analyze Table command to specify conditions for logical comparisons of table cell contents. If the analysis conditions you specify are met, Genotyper writes a message you specify into a results column.

To analyze data in tables:

Step	Action
1	Choose Analyze Table from the Table menu. The Analyze Table dialog box appears. 
2	Choose the radio button for the rows that you want to include in your analysis. **Note** Groups do not count the title row, and row 1 of the group is the first row that contains data.
3	In the Examine column(s) pop-up menus, choose the range of columns that you want to include in your analysis.
4	In the When pop-up menu, choose the comparison conditions for the columns you are analyzing. **Note** "at least zero", means for every cell.
5	Optionally select the "and" checkboxes if you want to add conditions to your analysis. Choose relational operators from the pop-up menus, and type in peak size criteria.

To analyze data in tables: *(continued)*

Step	Action
6	In the “then” field, type in the message text that you either want to “put” or “append” in a select column if the conditions you have specified are met.
7	Review the text box at the bottom of the dialog box which explains the conditions you’ve specified. If this is what you intend to do, click OK.
8	Check the Table Window to verify that the results of your analysis have been added to your table.

Clearing Columns

You can clear entire columns by specifying that if at least zero cells are not empty then clear the column. At least zero, means for every cell.

Example Text Box

For every cell examine column 5. Whether or not the cell is empty, put (blank) in column 4.

In other words, clear column 4.

Using Analyze and Calculate in Table Commands–An LOH Example

What is LOH?

Loss of Heterozygosity (LOH) is the loss of polymorphic DNA markers in tumors compared with normal cells, and often indicates somatic deletion of tumor suppressor genes. LOH detection has application to a wide range of cancers involving tumor suppressor genes.

Genotyper Commands Used to Detect LOH

You can use the Calculate in Table and Analyze Table commands to assess the presence or absence of LOH for labeled DNA fragment data from patient samples.

Example LOH Table

Figure 8-5 shows a table that was created using the Calculate in Table Command. Allele peaks were identified, labeled, filtered, and a table was created with columns for allele size and height.

Sample Info	Category	Peak 1	Allele1-ht	Peak 2	Allele 2-ht	Allele Ratio	Ht Ration T/N	LOH Assessment	Overflow
1N	Marker 1	90.43	576	96.45	517	1.114			
1 T	Marker 1	90.43	492	96.45	559	0.880	0.790	Normal	
2 N	Marker 1	90.43	488	1	1	488.000			
2 T	Marker 1	90.37	814	1	1	814.000	1.668	LOH	
4 N	Marker 1	88.37	597	92.34	535	1.116			
4 T	Marker 1	88.34	447	92.42	414	1.080	0.968	Normal	
16 N	Marker 1	88.34	506	100.44	197	2.569			
16 T	Marker 1	88.34	365	100.44	136	2.684	1.045	Normal	
19 N	Marker 1	88.37	864	1	1	864.000			
19 T	Marker 1	88.37	675	1	1	675.000	0.781	Normal	
20 N	Marker 1	88.37	546	96.37	461	1.184			
20 T	Marker 1	88.37	557	96.37	236	2.360	1.993	LOH	
44 N	Marker 1	96.37	1227	1	1	1227.000			
44 T	Marker 1	96.37	991	1	1	991.000	0.808	Normal	
55 N	Marker 1	96.46	1521	1	1	1521.000			
55 T	Marker 1	96.37	1356	1	1	1356.000	0.892	Normal	
68 N	Marker 1	88.38	746	1	1	746.000			
68 T	Marker 1	88.38	549	1	1	549.000	0.736	Normal	
79 N	Marker 1	88.37	683	96.37	593	1.152			
79 T	Marker 1	87.37	208	95.39	185	1.124	0.976	Normal	
80 N	Marker 1	90.36	803	1	1	803.000			
80 T	Marker 1	92.71	244	1	1	244.000	0.304	LOH	

Figure 8-5 Example of table created to assess LOH presence

Using the Calculate in Table Command

To create the table shown in Figure 8-5, we used the Calculate in Table Command to calculate:

- A ratio of allele 1 to allele 2 for each sample.
- The ratio of the tumor signal to that of the normal signal (T1/T2 over N1/N2). This value is called the *Allelic Imbalance* or AI.

Using the Analyze in Table Command

To create the table shown in Figure 8-5, we used the Analyze Table Command to calculate the ratios in Ht Ratio T/N column for presence or absence of LOH. For example, if the ratio is less than 0.67 or more than 1.35, then LOH is entered into a column named Assessment, otherwise Normal is entered in the same column.

Editing Table Cells and Column Headings

Introduction

Once you have created a table, you can edit the contents of some of the cells in each row, or change the names of any of the column headings. However, all rows in a table must have the same column headings.

IMPORTANT Changes you make to the contents of table cells affect only the table you are editing. For example, if you edit the peak label information in a table cell, the corresponding information in other parts of the Genotyper Document such as dye/lanes or plot displays remains unaffected.

Marking Rows as Edited

When setting up a table, you can select a checkbox in the Set Up Table dialog box (Figure 8-1 on page 8-3), that enables Genotyper to notify you of any row in a table that has been edited. After any cell in a row is edited, Genotyper enters a text string that you specify in a column at the end of the row.

For more information on setting up table features such as this, see “Specifying Warning Columns for Edited Tables” on page 8-11.

Kinds of Cell Data You Can Edit

There are the three kinds of data you can manually edit in table cells.

They are:

- Column headings
- Peak label information
- User comments

How to Edit Table Cells

To edit cells in a table:

Step	Action
1	Open the Genotyper Document that contains the table that you want to edit.
2	Optionally, open the Table window from the Main window.
3	Select the table cell that you want to edit.
4	Choose Edit Cell from the Edit menu.
5	Type in your changes. When you save the Genotyper Document, the changes will be saved in the table.

Recording Steps For Editing Table Cells

If you are making a macro, the Step list records edits of individual cells as “selected cells”. If you run the macro, the macro will change whatever you have selected to include the text you enter.

How To Edit Column Headings

To edit column headings in tables:

Step	Action
1	Open the Genotyper Document that contains the table that you want to edit.
2	Optionally, open the Table window from the Main window.
3	Select the table cell that you want to edit.
4	Choose Edit Cell from the Edit menu.
5	Type in your changes. When you save the Genotyper Document, the changes will be saved in the table.

Recording Steps For Editing Column Headings

If you are making a macro, the Step list records edits of column heading cells as applying to a specific column number of the first row.

Sorting the Rows in a Table

Introduction

Rows in tables are initially not sorted; they appear in the order in which they were added to a table. However, you can sort the rows on command.

The Concept of Precedence

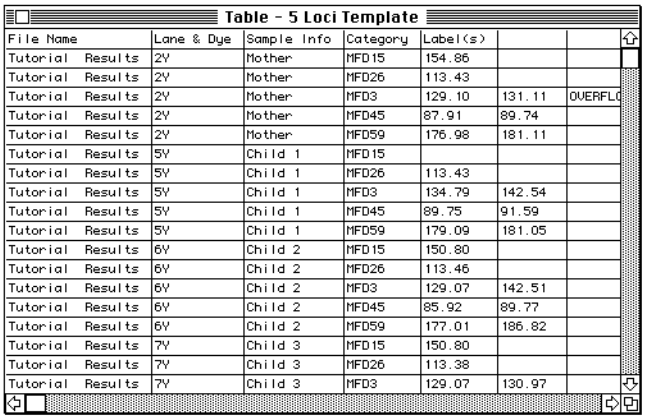
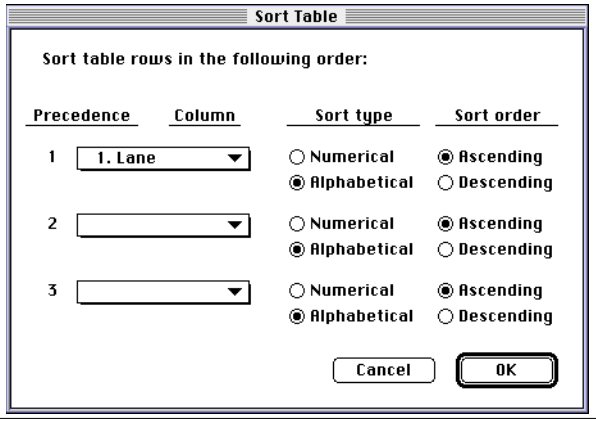
The concept of precedence is important when sorting rows in a table. The precedence for a column number establishes a priority when sorting rows.

Example

If you choose the Category column as a precedence 1, and Sample Info as a precedence 2, and the sort type is alphabetical, rows will first be sorted in alphabetical order according to category name, and then, within categories, sort them in alphabetical order according to entries in the Sample Info column.

How to Sort Rows

To sort rows in multiple columns of a table:

Step	Action
1	To view the Table window more easily, choose Show Table Window from the Views menu. The Table window appears. 
2	Choose Sort Table...from the Analysis menu. The Sort Table dialog box appears. 
3	Under Column number, you can choose from 1 to 3 different columns to sort, by choosing the appropriate pop-up menu.

To sort rows in multiple columns of a table: *(continued)*

Step	Action
4	Under Sort Type, choose the radio button for how you want each column to be sorted, in Alphabetical, or Numerical order.
5	Under Sort Order, choose either ascending or descending order.

Searching for Table Entries

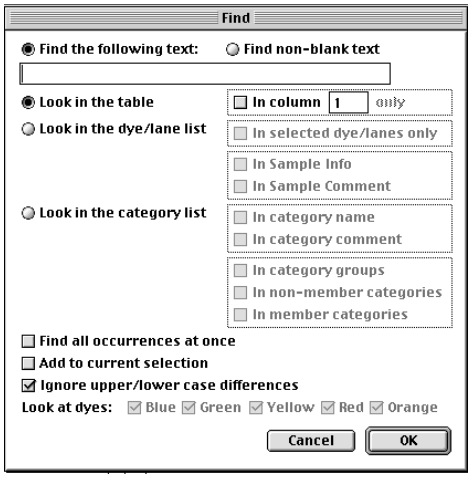
Introduction

You can use the Find command to locate one or more entries in a table.

How to Find a Table Entry

You can search for table entries by alphanumeric text string.

To locate a table entry using the Find command:

Step	Action
1	Make the table active to make it easier to view the results of this command. Tab until the vertical bar is on the left of the table. If the table is not active, the selection will be outlined, not highlighted. Or, open the Table window.
2	Choose Find.. .(⌘-F) in the Edit menu. The Find dialog box appears. 
3	In the Text box, enter the alphanumeric text you want to find.
4	Click the Look In the table radio button.

To locate a table entry using the Find command: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>5</td><td>Select checkboxes to specify how you want to search for table entries:

<table>
<tr><th>If you want to...</th><th>Then click...</th></tr>
<tr><td>Restrict the search to a particular column</td><td>Look in column, and enter the number of the column where you want to search.</td></tr>
<tr><td>Find all entries in the table with the designated text</td><td>Find all occurrences at once.</td></tr>
<tr><td>Select additional table cells located by the command</td><td>Add to current selection.</td></tr>
<tr><td>Ignore case differences in text searches</td><td>Ignore upper/lower case differences.</td></tr>
</table>
</td></tr>
<tr><td>6</td><td>Click OK.</td></tr>
</table>

How to Find the Next Occurrence

You can use the Find Next command to repeat the last Find command using the same options as the last Find command. The Find Next command is equivalent to the most recently used Find command, but eliminates the need to click the OK button in the Find dialog box.

Choose Find Next (⌘-G) from the Edit menu.

The next occurrence of the text is selected.

Updating Tables

Introduction

If you have created a table, and made changes to peak labels, you can update the corresponding information in your table.

IMPORTANT The Update Table command should only be used to update label data in the table. If you change any other information that can appear in the table, such as the sample information or the name of a category, then you should use the Clear Table command and start over with a new table.

How To Update Tables

Update tables after making changes to information in other parts of your Genotyper Document.

To update table contents:

Step	Action
1	Select dye/lanes that have labels that have been changed.
2	Choose Update Table from the Table menu. Table cells that contain data that has been changed are automatically updated to match the labels.

Deriving a Second Table from an Existing Table

Introduction

You can create Derived tables by copying the contents of a table and saving it as a Derived table. You can compare the contents of the Derived table to the original table, and export the Derived table.

How to Derive Tables

You can derive a second table from an existing table. A Derived table is not linked to dye/lanes or categories like the table from which it was derived.

To derive a second table:

Step	Action
1	Open the Genotyper Document that contains the table for which you want to derive a copy.
2	Open the Table window from the Main window.
3	Choose Show Derived table from the Views menu. A blank Derived table window appears.
4	Choose Derive table from the Analysis menu.
5	Choose the Copy Table submenu. The table in the Table window is copied to the Derived table window.

Checking for Mendelian Inheritance

Introduction

Performing a Mendelian inheritance check on Genotyper table data verifies that alleles from members of a family follow Mendelian inheritance.

Example Applications

Examples of applications for which performing a Mendelian inheritance check can help you verify genotyping results include:

- Microsatellite analysis
- Disease gene mapping
- Forensic research
- Paternity testing

Why Perform a Mendelian Inheritance Check

Mendelian inheritance checks can help you identify samples which do *not* follow expected patterns of Mendelian inheritance. Consequently, you can investigate where possible errors occurred in sample handling during your genotyping experiment, as well as verify non-paternity in paternity testing.

Using GenBase to Access Pedigree Data

To perform an inheritance check you must be able to access pedigree data from GenBase. GenBase can store actual pedigree structures that show family member relationships. For example, each individual in a family study has an ID assigned.

GenoPedigree can export pedigree information to GenBase. GenBase places this information in its pedigree table. You can then access this pedigree data from Genotyper.

For information on how to access GenBase from Genotyper, see “Communicating with GenBase” on page 10-1.

Start with a Table

Figure 8-6 shows an example of the kind of table you must first create before you can perform a Mendelian inheritance check in Genotyper.

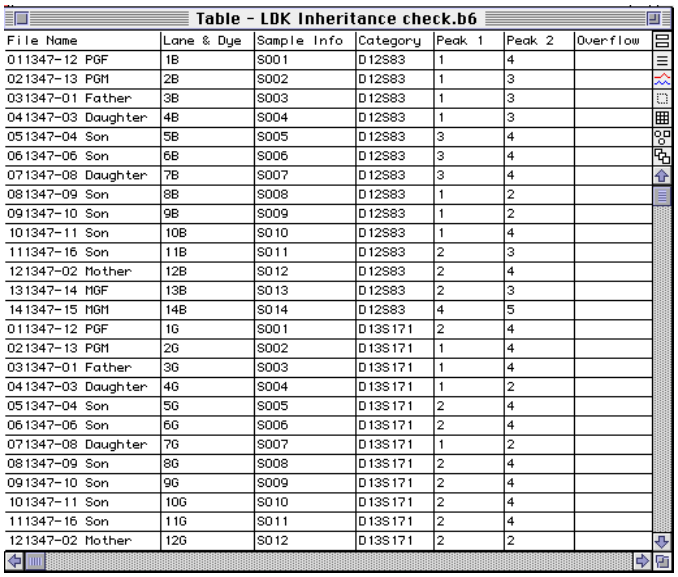


Table - LDK Inheritance check.b6

File Name	Lane & Dye	Sample Info	Category	Peak 1	Peak 2	Overflow
011347-12 PGF	1B	S001	D12S83	1	4	
021347-13 PGM	2B	S002	D12S83	1	3	
031347-01 Father	3B	S003	D12S83	1	3	
041347-03 Daughter	4B	S004	D12S83	1	3	
051347-04 Son	5B	S005	D12S83	3	4	
061347-06 Son	6B	S006	D12S83	3	4	
071347-08 Daughter	7B	S007	D12S83	3	4	
081347-09 Son	8B	S008	D12S83	1	2	
091347-10 Son	9B	S009	D12S83	1	2	
101347-11 Son	10B	S010	D12S83	1	4	
111347-16 Son	11B	S011	D12S83	2	3	
121347-02 Mother	12B	S012	D12S83	2	4	
131347-14 MGF	13B	S013	D12S83	2	3	
141347-15 MGM	14B	S014	D12S83	4	5	
011347-12 PGF	1G	S001	D13S171	2	4	
021347-13 PGM	2G	S002	D13S171	1	4	
031347-01 Father	3G	S003	D13S171	1	4	
041347-03 Daughter	4G	S004	D13S171	1	2	
051347-04 Son	5G	S005	D13S171	2	4	
061347-06 Son	6G	S006	D13S171	2	4	
071347-08 Daughter	7G	S007	D13S171	1	2	
081347-09 Son	8G	S008	D13S171	2	4	
091347-10 Son	9G	S009	D13S171	2	4	
101347-11 Son	10G	S010	D13S171	2	4	
111347-16 Son	11G	S011	D13S171	2	4	
121347-02 Mother	12G	S012	D13S171	2	2	

Figure 8-6 Example of table created for Mendelian inheritance check

The table in Figure 8-6 contains rows for categories and samples. The numbers in the peak 1 and peak 2 columns are the allele names.

For detailed instructions on how to create the table shown in Figure 8-6, see Part 2 of the Microsatellite tutorial, in the *ABI PRISM Genotyper Applications Tutorials*. The steps used to create the table in that part of the tutorial are the same as you would use to set up a table for most kinds of Mendelian inheritance checks.

Sorting the Table

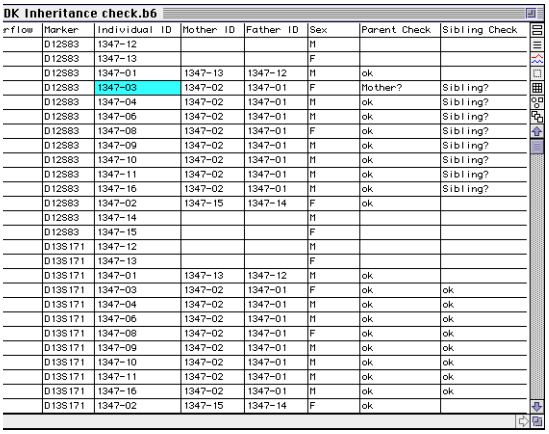
For ease of viewing, you can sort the table using the Sort table command. Sorting by markers, you can group all the family members and identify presence or absence of alleles in particular family members.

Typically, you would sort the table by category name first, and then by Sample Information. For more information on how to sort tables, see “Sorting the Rows in a Table” on page 8-30.

How to Add Columns for Inheritance Checking

Once you have set up a table such as that shown in Figure 8-6, you must add additional columns to the table before performing the inheritance check.

To add columns to your table:

Step	Action
1	Clear the table.
2	Select the Set up Table command, this displays the settings for the original table shown in Figure 8-6.
3	Select checkboxes for Marker name & individual ID, Pedigree data, and Inheritance check. This adds seven columns to the original table.  **Note** The columns shown above already have inheritance check data in them. This data is not added until you perform the inheritance check.
4	Choose Append to Table from the Table menu. This appends rows to the table, adding the allelic information to the table.
5	Sort table by category name and file name.

DK Inheritance check.b6

rflow	Marker	Individual ID	Mother ID	Father ID	Sex	Parent Check	Sibling Check
	D12S83	1347-12			M		
	D12S83	1347-13			F		
	D12S83	1347-01	1347-13	1347-12	M	ok	
	D12S83	1347-03	1347-02	1347-01	F	Mother?	Sibling?
	D12S83	1347-04	1347-02	1347-01	M	ok	Sibling?
	D12S83	1347-06	1347-02	1347-01	M	ok	Sibling?
	D12S83	1347-08	1347-02	1347-01	F	ok	Sibling?
	D12S83	1347-09	1347-02	1347-01	M	ok	Sibling?
	D12S83	1347-10	1347-02	1347-01	M	ok	Sibling?
	D12S83	1347-11	1347-02	1347-01	M	ok	Sibling?
	D12S83	1347-16	1347-02	1347-01	M	ok	Sibling?
	D12S83	1347-02	1347-15	1347-14	F	ok	
	D12S83	1347-14			M		
	D12S83	1347-15			F		
	D13S171	1347-12			M		
	D13S171	1347-13			F		
	D13S171	1347-01	1347-13	1347-12	M	ok	
	D13S171	1347-03	1347-02	1347-01	F	ok	ok
	D13S171	1347-04	1347-02	1347-01	M	ok	ok
	D13S171	1347-06	1347-02	1347-01	M	ok	ok
	D13S171	1347-08	1347-02	1347-01	F	ok	ok
	D13S171	1347-09	1347-02	1347-01	M	ok	ok
	D13S171	1347-10	1347-02	1347-01	M	ok	ok
	D13S171	1347-11	1347-02	1347-01	M	ok	ok
	D13S171	1347-16	1347-02	1347-01	M	ok	ok
	D13S171	1347-02	1347-15	1347-14	F	ok	

How to Import Pedigree Data

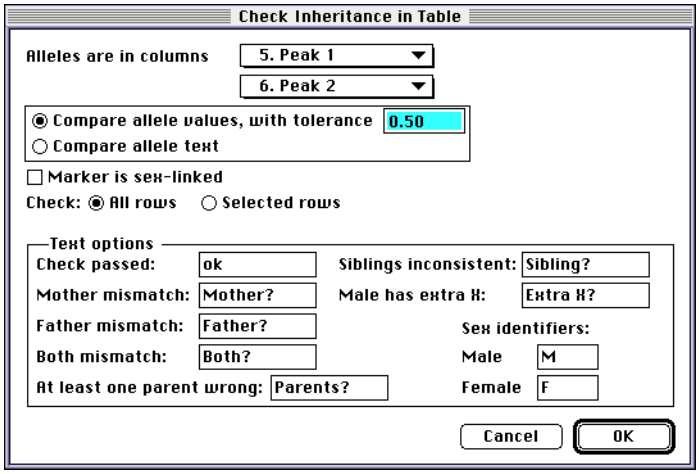
To import pedigree data from GenBase:

Step	Action
1	Choose Import Inheritance Data from GenBase. This imports the pedigree data from GenBase.
2	A dialog box appears and prompts you for the structure ID that specifies the particular set of pedigree data to use. The ID's are imported and placed in columns right next to the appropriate family member sample data.

How to Check for Mendelian Inheritance

Genotyper uses the pedigree IDs to check and see if siblings have inherited alleles according to Mendelian principles.

To check Mendelian inheritance in tables:

Step	Action
1	Choose Check Inheritance...from the Table menu. The Check Inheritance dialog box appears. 
2	Select the columns that contain allele information from the Alleles are in columns pop-up menus.
3	Click the radio button for how you want to compare alleles: **If you want to compare...** / **Then click...** Allele values — Compare allele values, with tolerance, and type in a tolerance. Allele text — Compare allele text. **Note** Select Marker is sex-linked checkbox if you know you are working with sex-linked alleles.

To check Mendelian inheritance in tables: *(continued)*

Step	Action
4	Click the radio button for the rows you want to check: **If you want to check...** / **Then click...** All rows in the table / All rows. Only rows you have selected in the table / Selected rows.
5	In the Text Options text boxes, type in messages that you want to appear when certain conditions are met after the inheritance check.
6	Click OK. Genotyper performs an inheritance check on family member data in the table. OK should appear in the column next to siblings that have inherited alleles from parents according to Mendelian principles.
7	You can export the table to GenBase send results to GenBase for storage and possibly later retrieval.

Evaluating Results

For any table cells that contain family members that have been labeled as possible mismatches, you can select the table cell for that individual and display the associated plot view for that individuals allelic information. This allows a graphical depiction of the potential mismatch.

Parents Inconsistent Check

This check verifies that an individual is the child of the identified parent. The check verifies that children:

- Have one allele in common with each known parent for each locus.
- Not have any alleles not found in the parents.

Siblings Inconsistent Check

This check verifies that the set of individuals identified as siblings are in fact siblings. This check does not depend on the parents. If the check fails, then all purported siblings are labeled with the warning text. This check verifies that:

- No more than four alleles per locus are allowed among the siblings.
- A single allele cannot be paired with more than two other alleles for a locus.
- If one sibling is homozygous, no more than three alleles for that locus are allowed.
- If two siblings are homozygous for different alleles, no more than two alleles for that locus are allowed.

Formatting Tables for Export

Introduction

Some third party applications require that you re-format tables before exporting them from Genotyper. To format tables so that they are compatible, you can use the Flip Table command and flip tables before exporting them.

How to Format Tables for Export

To flip and format tables for export:

Step	Action
1	Choose Derive Table from the Analysis menu.
2	Choose the Flip Table submenu. The table in the Table window is flipped, copied and placed in the derived table. See Figure 8-7 for an example of how the Flip Table command reformats a table.

Example of Flipped Table

Figure 8-7 shows an example of a table before and after flipping

Table - flip table examp

Lane	Dye	Sample Info	Category	Peak 1	Peak 2	Overflow
1	B	001 Mother	Everything	76.71	78.54	Overflow
1	R	GS2500	Everything	94.00	109.00	Overflow
2	B	001 Father	Everything	82.27	84.16	Overflow
2	R	GS2500	Everything	94.00	109.00	Overflow

Derived Table - flip table examp

Lane	1	1	2	2
Dye	B	R	B	R
Sample Info	001 Mother	GS2500	001 Father	GS2500
Category	Everything	Everything	Everything	Everything
Peak 1	76.71	94.00	82.27	94.00
Peak 2	78.54	109.00	84.16	109.00
Overflow	Overflow	Overflow	Overflow	Overflow

Figure 8-7 Table before (top) and after (bottom) using Flip Table command

Exporting Tables

Introduction

You can export the contents of tables to databases, linked programs, and other Genotyper System applications.

How to Export Tables

The following table explains where you can find information for how to export tables to different target applications.

To Export Tables to...	See
GenBase	“Exporting Tables to GenBase” on page 10-10.
GenoPedigree	The *ABI PRISM GenoPedigree User’s Manual.*
Linked Programs	“Exporting and Copying Tables” on page 11-8.
Files	“Exporting and Copying Tables” on page 11-8.

How to Export Derived Tables

There is a different command to export derived tables, than there is to export tables.

IMPORTANT You can only export derived tables as text files.

To export derived tables:

Step	Action
1	Use the Derive Table command to copy the table you want to export. For instructions on how to use the Derive Table command, see “How to Derive Tables” on page 8-36.
2	Choose Export Derived Table from the Analysis menu. A file selection dialog box appears.
3	Choose the folder location for where you want to export the table.
4	In the Export Table as field, type in the name of the exported table.

Working with Statistical Data

9

Chapter Overview

Introduction This chapter discusses how you can generate and view statistical data and histograms for peak data in Genotyper Documents.

In This Chapter This chapter contains the following topics:

Generating Statistical Data

Introduction

Genotyper generates statistical data from select parts of the active Genotyper Document. You can display generated statistical data in tables or histograms.

Kinds of Statistical Data

The following table describes the kinds of statistical data Genotyper can calculate for Genotyper Document contents.

Information appearing in the Statistics window:

Kind of Statistic	Description
Source	Within a Genotyper Document, the source of data from which statistics are generated.
Value Type	The kind of data for which Genotyper generates statistics. Possible Value Types are size in base pairs, scan number, peak height, peak area, and label text.
Number of Data Points	The total number of counts.
Minimum	The minimum value for a range of quantities you define by the Value Type you specify.
Maximum	The maximum value for a range of quantities you define by the Value Type you specify.
Mean	The average value for a set of values defined by the Value Type you specify.
Median	The center value of a series of quantities you define by the Value Type you specify.
Standard Deviation	The standard deviation from the mean of a set of values defined by the Value Type you specify.
Bin	The range of Value Types over which Genotyper calculates a count and frequency.
Count	The number of Value Types found within a Bin.
Frequency	The number of Value Types found within a Bin divided by the total number of data points.

Setting Statistics Options

Genotyper generates statistical data and histograms based on settings you make in the Set Statistics Options dialog box.

To set statistics options, and where you can find instructions for each step:

Step	Action	See Page
1	“Choosing the Source of Data”	9-4
2	“Choosing a Value Type”	9-9
3	“Determining the Bin Size”	9-13

Choosing the Source of Data

Introduction

You can specify the source of data in a Genotyper Document for which Genotyper generates statistics. There are two sources of data:

- Labeled peaks
- Tables

IMPORTANT For labeled peak sources, calculated statistics do not depend on the particular type or contents of a label, only whether or not a peak has a label.

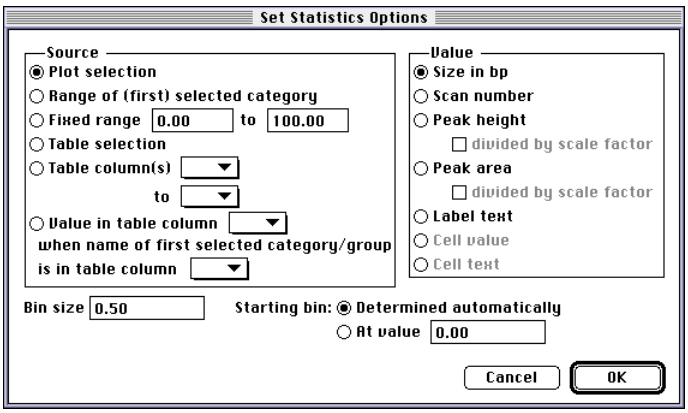
Labeled Peak Data from Plot Selections

Calculate statistics for a range of labeled peaks you select in plot displays.

To calculate statistics from plot selections of data:

Step	Action
1	In the Dye/lane list, select the dye/lane or dye/lanes for which you want to calculate and display statistical data. An electropherogram plot showing peaks for each analyzed nucleic acid fragment in the sample appears in the Plot Area.
2	Click the Plot window icon, to open the Plot window. **IMPORTANT** Peaks in the Plot window must be labeled to include them in statistical calculations. If they are not labeled, label them.

To calculate statistics from plot selections of data: *(continued)*

Step	Action
3	From the Analysis menu, choose Set Statistics Options. The Set Statistics Options dialog box appears.  **Figure 9-1** Set Statistics Options dialog box
4	In the Source field, select the Plot selection radio button.
5	Use the mouse and select a rectangular range in the electropherogram plot.
6	See “Choosing a Value Type” on page 9-9, for instructions on selecting the appropriate Value Type radio button in the Set Statistics Options dialog box.

Labeled Peak Data from Categories

You can calculate statistics for the range of peaks defined by a selected category.

To choose categories as the source of peak data:

Step	Action
1	In the Dye/lane list, select the dye/lane or dye/lanes for which you want to calculate and display statistical data. An electropherogram plot showing peaks for each analyzed nucleic acid fragment in the sample appears in the Plot Area.
2	Select the category from the Category list which defines the range of peaks for which you want to generate statistical data.

To choose categories as the source of peak data: *(continued)*

Step	Action
3	From the Analysis menu, choose Set Statistics Options. The Set Statistics Options dialog box appears (see Figure 9-1 on page 9-5).
4	In the Source field, select the Range of (first) selected category radio button.
5	See “Choosing a Value Type” on page 9-9, for instructions on selecting the appropriate Value Type radio button in the Set Statistics Options dialog box.

Labeled Peak Fragment Size

You can specify a range of fragment sizes in base pairs. Genotyper generates statistical data for all peaks in the selected dye/lane of your active Genotyper Document that are within the fragment size range you specify.

To specify a range of fragment sizes as the source of peak data:

Step	Action
1	In the Dye/Lane list, select the dye/lane or dye/lanes for which you want to calculate and display statistical data. An electropherogram plot showing peaks for each analyzed nucleic acid fragment in the sample appears in the Plot Area.
2	From the Analysis menu, choose Set Statistics Options. The Set Statistics Options dialog box appears (see Figure 9-1 on page 9-5).
3	In the Source field, select the Fixed Range radio button.
4	Type in the size range in base pairs for fragments that you want to specify as the source of peak data.
5	See “Choosing a Value Type” on page 9-9, for instructions on selecting the appropriate Value Type radio button in the Set Statistics Options dialog box.

Table Cell Contents from Selection

You can calculate statistics for the contents of selected table cells.

To choose the contents of table cells as the source of data:

Step	Action
1	Select the cell or cells in the table for which you want to generate statistics.
2	From the Analysis menu, choose Set Statistics Options. The Set Statistics Options dialog box appears (see Figure 9-1 on page 9-5).
3	In the Source field, select the Table Selection radio button.
4	See “Choosing a Value Type” on page 9-9, for instructions on selecting the appropriate Value Type radio button in the Set Statistics Options dialog box.

Table Cell Contents from Column Selection

You can specify a range of table columns to include as a source of data.

To specify a range of table columns as the source of peak data:

Step	Action
1	From the Analysis menu, choose Set Statistics Options. The Set Statistics Options dialog box appears (see Figure 9-1 on page 9-5).
2	In the Source field, select the range of Table columns for inclusion as source data.
3	From the table column pop-up menus select the columns that define the beginning and ending range or conditions for source peak data.
4	See “Choosing a Value Type” on page 9-9, for instructions on selecting the appropriate Value Type radio button in the Set Statistics Options dialog box.

Table Cell Contents from Category and Column Selection

You can specify categories in table columns as a source of data.

To specify a category in a table column as the source of data:

Step	Action
1	Select the cell or cells in the table for which you want to generate statistics.
2	From the Analysis menu, choose Set Statistics Options. The Set Statistics Options dialog box appears (see Figure 9-1 on page 9-5).
3	In the Source field, select when name of first selected category/group is in table column.
4	From the table column pop-up menus select table column containing the category or group that you want to define as the source of your data.
5	See “Choosing a Value Type” on page 9-9, for instructions on selecting the appropriate Value Type radio button in the Set Statistics Options dialog box.

Choosing a Value Type

Introduction

The Value Type is the kind of data for which Genotyper generates statistics. Possible Value Types are size in base pairs, scan number, peak height, peak area, and label text.

Size in Base Pairs

When you choose “Size in Base Pairs” as a Value Type, Genotyper generates statistics based on the size of fragments associated with your source peak data.

To choose the Size in Base Pairs as a Value Type:

Step	Action
1	If it is not already open, open the Set Statistics Options dialog box by choosing Set Statistics Options from the Analysis menu. See Figure 9-1 on page 9-5.
2	If you have not already done so, choose a source of peak data. See “Choosing the Source of Data” on page 9-4.
3	In the Value field, select the Size in bp radio button.
4	See “Determining the Bin Size” on page 9-13, for instructions on selecting the appropriate range of fragment sizes, for which Genotyper calculates a count, frequency of occurrence, as well as related statistics.

Scan Number

When you choose Scan number as a Value Type, Genotyper generates statistics based on the number of scans used by your ABI PRISM instrument to detect the fragments associated with your source peak data.

To choose the Scan number as a Value Type:

Step	Action
1	If it is not already open, open the Set Statistics Options dialog box by choosing Set Statistics Options from the Analysis menu. See Figure 9-1 on page 9-5.
2	If you have not already done so, choose a source of peak data. See “Choosing the Source of Data” on page 9-4.
3	In the Value field, select the Scan Number radio button.

To choose the Scan number as a Value Type: *(continued)*

Step	Action
4	See “Determining the Bin Size” on page 9-13, for instructions on selecting the appropriate range of Scan numbers, for which Genotyper calculates a count, frequency of occurrence, as well as related statistics.

Peak Height

When you choose Peak height as a Value Type, Genotyper generates statistics based on the height of a select source of peak data.

To choose peak height as a Value Type:

Step	Action
1	If it is not already open, open the Set Statistics Options dialog box by choosing Set Statistics Options from the Analysis menu. See Figure 9-1 on page 9-5.
2	If you have not already done so, choose a source for peak data. See “Choosing the Source of Data” on page 9-4.
3	In the Value field, select the Peak height radio button.
4	Optionally, select the checkbox for divided by scale factor.
5	See “Determining the Bin Size” on page 9-13, for instructions on selecting the appropriate range of peak data, for which Genotyper calculates a count, frequency of occurrence, as well as related statistics.

Peak Area

When you choose Peak area as a Value Type, Genotyper generates statistics based on the area of specified source peaks.

To choose peak area as a Value Type:

Step	Action
1	If it is not already open, open the Set Statistics Options dialog box by choosing Set Statistics Options from the Analysis menu. See Figure 9-1 on page 9-5.
2	If you have not already done so, choose a source of peak data. See “Choosing the Source of Data” on page 9-4.
3	In the Value field, select the Peak area radio button.
4	Optionally, select the checkbox for divided by scale factor.

To choose peak area as a Value Type: *(continued)*

Step	Action
5	See “Determining the Bin Size” on page 9-13, for instructions on selecting the appropriate range of peak data for which Genotyper calculates a count, frequency of occurrence, as well as related statistics.

Labeled Text

When you choose Labeled text as a Value Type, Genotyper calculates statistics based on the number of text labels within a specified range of source peak data.

To choose Labeled text as a Value Type:

Step	Action
1	If it is not already open, open the Set Statistics Options dialog box by choosing Set Statistics Options from the Analysis menu. See Figure 9-1 on page 9-5.
2	If you have not already done so, choose a source of peak data. See “Choosing the Source of Data” on page 9-4.
3	In the Value field, select the Label text radio button. **Note** For the Labeled text Value Type, Genotyper only calculates the Number of Data Points, count, and frequency of text labels located within a specified range.

Cell Value

When you choose Cell value as a Value Type, Genotyper generates statistics based on the numerical value in table cells.

To choose Cell value as a Value Type:

Step	Action
1	If it is not already open, open the Set Statistics Options dialog box by choosing Set Statistics Options from the Analysis menu. See Figure 9-1 on page 9-5.
2	If you have not already done so, choose a source of data. See “Choosing the Source of Data” on page 9-4.
3	In the Value field, select the Cell value radio button.

To choose Cell value as a Value Type: *(continued)*

Step	Action
4	See “Determining the Bin Size” on page 9-13, for instructions on selecting the appropriate range of peak data for which Genotyper calculates a count, frequency of occurrence, as well as related statistics.

Cell Text

When you choose Cell text as a Value Type, Genotyper generates statistics based on the text in table cells.

To choose Cell text as a Value Type:

Step	Action
1	If it is not already open, open the Set Statistics Options dialog box by choosing Set Statistics Options from the Analysis menu. See Figure 9-1 on page 9-5.
2	If you have not already done so, choose a source of data. See “Choosing the Source of Data” on page 9-4.
3	In the Value field, select the Cell Text radio button. **Note** For the Cell Text Value Type, Genotyper only calculates the Number of Data Points, count, and frequency of Cell text labels located within a specified range.

Determining the Bin Size

Definition

The Bin size defines an interval within which Genotyper calculates a count and frequency of each occurrence of labeled peak data that matches criteria defined by the Source and Value Type. Results of the calculations are displayed in the Statistics Window, and Histogram window.

Guidelines for Bin Size Selection

If you choose too small of a number for your Bin size, you will have a large number of bins making it difficult to view a histogram representation of the statistical data.

There is a limit of 5000 bins that can be displayed in the Statistics window.

How to Define the Bin Size

You define the Bin size in the Set Statistics Options dialog box.

To define the Bin size:

Step	Action
1	If it is not already open, open the Set Statistics Options dialog box by choosing Set Statistics Options from the Analysis menu. See Figure 9-1 on page 9-5.
2	If you have not already done so, choose a source of peak data. See “Choosing the Source of Data” on page 9-4.
3	If you have not already done so, choose a Value Type. See “Choosing a Value Type” on page 9-9.
4	In the Bin size field, type in an interval within which you want to calculate the count and frequency of occurrences of the specified Value Type.
5	Determine the starting bin. See “How to Determine the Starting Bin” on page 9-14.

How to Determine the Starting Bin

The Starting bin determines the initial value of the Bin size. You can either determine the Starting bin automatically, or specify a starting value.

To define the Starting bin:

Step	Action
1	If it is not already open, open the Set Statistics Options dialog box by choosing Set Statistics Options from the Analysis menu. See Figure 9-1 on page 9-5.
2	If you have not already done so, choose a source of peak data. See “Choosing the Source of Data” on page 9-4.
3	If you have not already done so, choose a Value Type. See “Choosing a Value Type” on page 9-9.
4	In the Bin size field, type in an interval within which you want to calculate the count and frequency of occurrences of labeled peak data.
5	In the Starting bin field, determine the Starting bin: **If you want to...** / **Then click...** Use the first bin containing one or more counts / Determine automatically. Define the Starting bin size / At value, and type in the Starting bin.
6	When you are satisfied with all the settings in the Set Statistics Options dialog box, click OK.

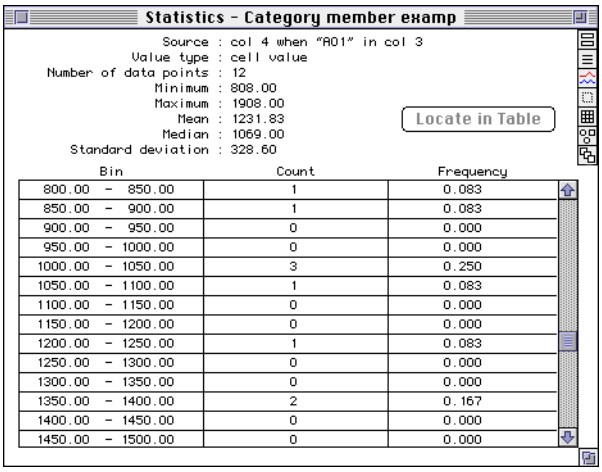
Viewing Statistics

Introduction

Once you have set statistics options, you can show statistics for the kinds of data you have selected.

The Statistics Window

The Statistics window displays the statistics of selected data.



Bin	Count	Frequency
800.00 - 850.00	1	0.083
850.00 - 900.00	1	0.083
900.00 - 950.00	0	0.000
950.00 - 1000.00	0	0.000
1000.00 - 1050.00	3	0.250
1050.00 - 1100.00	1	0.083
1100.00 - 1150.00	0	0.000
1150.00 - 1200.00	0	0.000
1200.00 - 1250.00	1	0.083
1250.00 - 1300.00	0	0.000
1300.00 - 1350.00	0	0.000
1350.00 - 1400.00	2	0.167
1400.00 - 1450.00	0	0.000
1450.00 - 1500.00	0	0.000

Figure 9-2 The Statistics window

How to View Peak Statistics

Once you have set statistics options, you can show the Statistics window at any time.

To view the Statistics window:

Step	Action
1	Complete the Set Statistics Options dialog box (Figure 9-1 on page 9-5).
2	Select the data from the appropriate part of the Genotyper document that corresponds to the source you selected in Set Statistics Options dialog box.
3	Choose Show Statistics window from the Views menu. The Statistics window appears (Figure 9-2 on page 9-15). To see statistics from a different source of data, or of a different Value Type, or of a different Bin size, make changes in the Set Statistics Options dialog box.

How to Locate Bins in Tables

You can use the Locate in Table button to find data in a table that corresponds to bins in the Statistics window. The Locate in Table button is enabled when you are using table columns (not table selection) as the source of data for statistics.

To locate bins in tables:

Step	Action
1	Open the Table window.
2	Select one or more cells in contiguous rows in the Statistics window (Figure 9-2 on page 9-15).
3	Click the Locate in Table button. Cells in the table that correspond to selected bins are highlighted.

Viewing Histograms

Introduction

Histograms show a graphical representation of data shown in the Statistics window.

The Histogram Window

The Histogram window displays a histogram of data based on settings you have made in the Set Statistics Options dialog box.

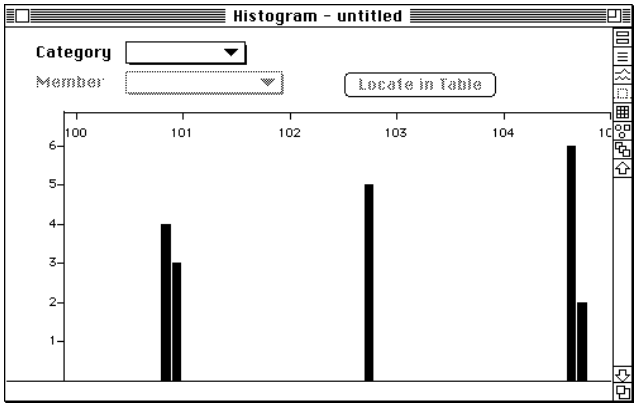


Figure 9-3 The Histogram window

How to View the Histogram Window

You can show the Histogram window at any time once you have completed the Set Statistics Options dialog box.

To view the Histogram window:

Step	Action
1	Complete the Set Statistics Options dialog box (Figure 9-1 on page 9-5).
2	Select the data from the appropriate part of the Genotyper document that corresponds to the source you selected in Set Statistics Options dialog box.

To view the Histogram window: *(continued)*

Step	Action
3	Choose Show Histogram window from the Views menu. The Histogram window appears (Figure 9-3). The bars in the Histogram window represent the defined bin sizes. Putting your cursor on a bar displays Statistical information for that bin at the bottom of the Histogram window. To see a different kind of histogram, make changes in the Set Statistics Options dialog box.

Displaying Bin Statistics

The bars in the Histogram window show statistics for defined Bin sizes. To display Statistical information for that bin at the bottom of the Histogram window, use the mouse to select a range in the Histogram window. Bin statistics for the range you selected display at the bottom of the window (Figure 9-4).

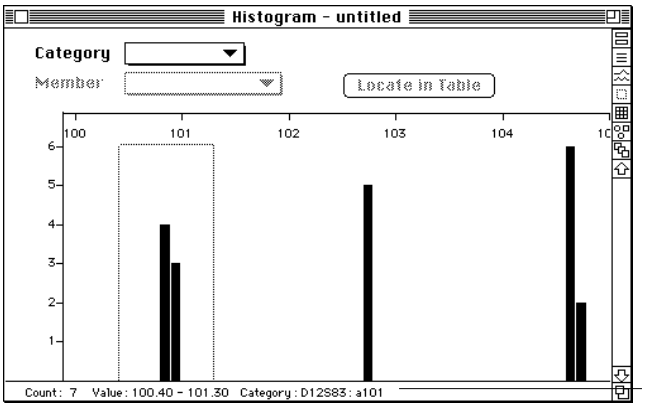


Figure 9-4 Bin statistics for a selected range

Viewing Histograms with Small Bin Sizes

If the text “WARNING - “Bin sizes too small to display” appears at the bottom of the Histogram window, this means that the width of one or more bins is too narrow to be displayed on your computer screen.

To view histograms with small bins sizes, do one of the following:

- Choose Set Statistics Options from the Analysis menu, and increase the Bin size to a value adequate for viewing.
- Zoom in on the region of the histogram that you want to view.
- Increase the size of the histogram window.

Setting Histogram Viewing Options

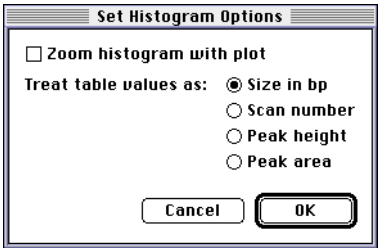
Introduction

You can set options for viewing histograms which allows you to associate histogram data with corresponding data in plot displays and tables.

How to Set Plot Zooming Options

You can associate data in histograms with corresponding Plot displays, so that when you zoom to a region of peaks in the Plot window, the histogram view zooms to the corresponding region of data. Plots views also zoom to corresponding regions of peak data when you zoom to the region in the histogram.

To set display options for histograms:

Step	Action
1	View the Histogram for peak data you want to associate with corresponding plot display.
2	Choose Set Histogram Options from the Analysis menu. The Set Histogram Options dialog box appears. 
3	Select Zoom histogram with plot.
4	Open the Plot window, and select a region of the electropherogram.
5	Choose Zoom to a selected range (⌘-R). The Histogram window zooms to the region in the histogram that contains the corresponding peak data.

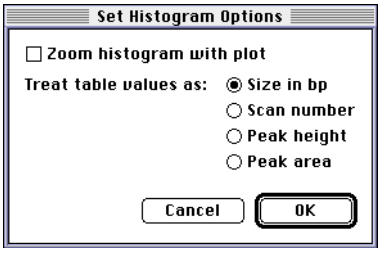
Viewing Histograms of Table Data

If you want to view histograms of data from tables, you need define the value type of the data in the table.

For example, if your table contains peak sizes and peak heights, and you have chosen to make a histogram of the peak heights by selecting table columns, then select the Peak height radio button in the Set Histogram Options dialog box(Figure).

For more information on choosing a source of data for histograms see“Choosing the Source of Data” on page 9-4.

To set display options for histograms:

Step	Action
1	View the Histogram for the table you defined as a source of peak data.
2	Choose Set Histogram Options from the Analysis menu. The Set Histogram Options dialog box appears. 
3	In the “Treat table values as:” field, click the value type of the data in your table.
4	Click OK. The Histogram window should now display the same type of peak data as the associated table.

Editing Categories in Histograms

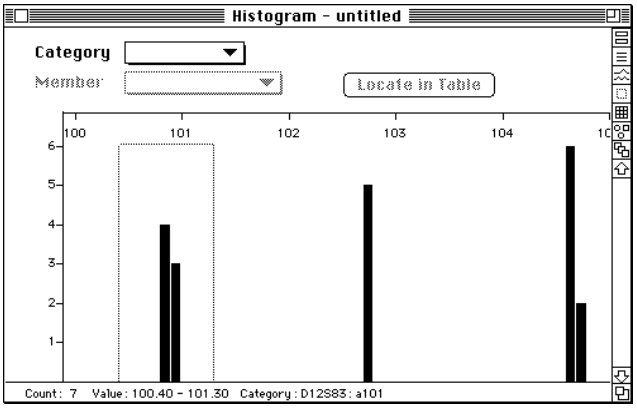
Introduction

The Histogram window provides a graphical display of peak size ranges. When viewing the Histogram window you can define new categories or edit existing ones.

How to Define the Range for a New Category

You can define the range of peak sizes in the Histogram window to include in a new category.

To define the range for a new category:

Step	Action
1	View the appropriate histogram by showing the Histogram window.
2	Place the cursor on the part of the histogram that displays peak sizes for the start point of the range of peak sizes that you want to include in your new category.
3	Drag the mouse across the histogram display drawing a box around the range of peak sizes that you want to include in your new category. 
4	Choose Add Category...from the Category menu. The Add Category dialog box appears. A size range of from/to will be defined by the Add Category dialog box. If you instead hold down the Shift key when choosing Add Category, the range will be ± a fixed number centered on the weighted average of bins in the selected range (or a center value if no bins have been selected).

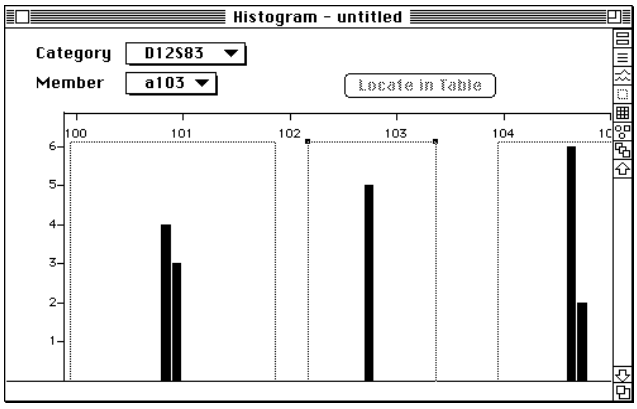
To define the range for a new category: *(continued)*

Step	Action
5	Type in the name of the new category. The name of the newly defined category is added to the Category pop-up menu of the Histogram window.

How to View Category Size Ranges

You can edit existing categories from the Histogram window, redefining the range of peak sizes included in the category.

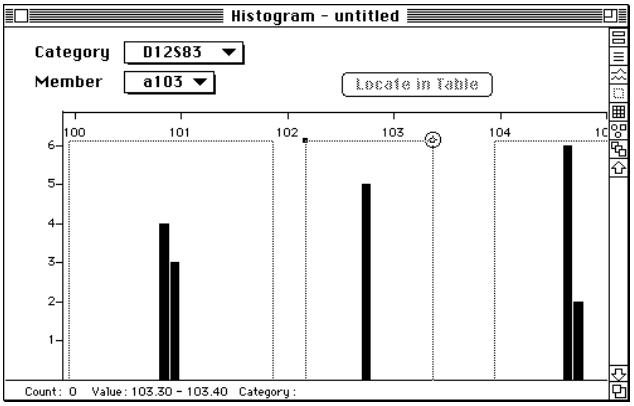
To view category size ranges in histograms:

Step	Action
1	Generate a histogram of peak sizes.
2	Choose a category from the Category pop-up menu. All category groups and non-member categories will have ranges shown with dashed boxes. The selected category will have handles. 
3	To view ranges for category members, choose a member in the Member pop-up menu. All members of selected groups will have ranges shown with dashed boxes. Selected members will have handles.

How to Change Category Size Ranges

You can change the size range of categories from the Histogram window.

To change the category size ranges in histograms:

Step	Action
1	Generate a histogram of peak sizes.
2	Show the Histogram window.
3	Choose a category or a member from the appropriate pop-up menu.
4	Move the cursor to the handle of selected category. 
5	Using the mouse, drag the handle on the box until the size range is modified to your specifications. **Note** The range will be changed to align with bin boundaries. Overlapping categories will not be allowed. If the text “WARNING - Bin sizes too small to display” appears at the bottom of the Histogram window, you will not be able to modify the categories by this method. Use the Set Statistics Options command to increase the bin size to an adequate value.

How to Select Another Category

After you have adjusted the size range of one category, you can select another category from the Histogram window, and modify its size range as well. There are four different ways that you can select another category:

- Select another category from the Category pop-up menu. The handles move to the dashed box of the selected category.
- Click within the dashed box representing the size range of a category. The category name of your selection changes in the Category pop-up menu.
- Make your selection from the Category list.
- Press ⌘-J, the Zoom to next category command.

Communicating with GenBase 10

Chapter Overview

Introduction

This chapter discusses how you can expand your genotyping research efforts by communicating with GenBase, a database of genotyping results data. Once you establish a link to GenBase, you can import data relevant to your research into your Genotyper Documents, or export your results data to GenBase for storage or later retrieval. GenBase also provides you access to data exported from GenoPedigree and other linkage analysis applications.

In This Chapter

This chapter contains the following topics:

Linking to GenBase

What is GenBase

GenBase is a database application that enables you to create large databases of genotyping results data without reformatting. In GenBase, you can manipulate genotyping results data and other information related to Genotyper Documents, GeneScan files, or GenoPedigree drawings.

For detailed information about interacting with GenBase to perform any of the procedures discussed in this Chapter, refer to the *ABI PRISM GenBase User's Manual*.

Why Link to GenBase

By linking to GenBase, you can exchange Genotyper results data with relevant research data stored in GenBase tables.

Some reasons why you might want to communicate with GenBase include:

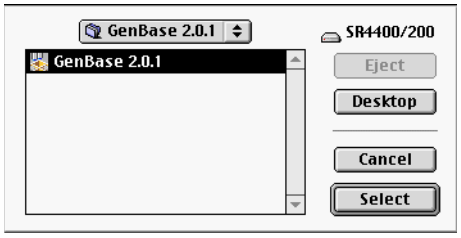
- Importing or exporting genotypes or genotyping data such as sample, patient, pedigree information.
- Accessing genetic disease research information such as penetrance data and patient sample information relevant to your applications.
- Improving error checking by maintaining an audit trail of previous genotyping activities.

  By linking to GenBase, you can retrieve information about sample types, gel conditions, or patient related information such as pedigree data, patient ID from previous genotyping applications and compare it to your most recent data.
- Creating a repository of allelic bin settings.
- Calculating frequency of markers used in previous studies similar to your application.
- Performing Mendelian inheritance checks.

How to Link to GenBase

After you link to GenBase, you can import or export results data from Genotyper. You only need to perform the following steps for linking to GenBase once. After you have established a link, the next time you start Genotyper, you only have to click the GenBase icon from the Main Window to access the database.

To link to GenBase from Genotyper:

Step	Action
1	Install GenBase on the same computer as Genotyper.
2	Select Links from the File menu.
3	Select Choose GenBase. An application selection dialog box appears. 
4	Highlight the version of GenBase to which you want to connect.
5	Click Select and Genotyper is connected to GenBase. Once you've established a link to GenBase, each time you want to access the database, click the GenBase icon on the Main window. 

Importing and Exporting Results Data

Introduction

GenBase stores all results data in a Genotype Record. Once you have linked to GenBase from Genotyper, you can import information from the Genotype Record or export Genotyper results data to the Genotype Record. Using GenBase commands, you can manipulate the exported Genotyper data.

IMPORTANT Once you begin communicating with GenBase, do not attempt to switch to another GenBase data file while Genotyper is still running. Quit both programs first, before switching GenBase to a different data file. If you save a Genotyper document that contains dye/lanes or a table, do not attempt to use this file with a different GenBase data file other than the one originally used with the document, even if you quit both programs first.

The Genotype Record

The Genotype Record window in GenBase shows all of the fields in a Genotype Record.

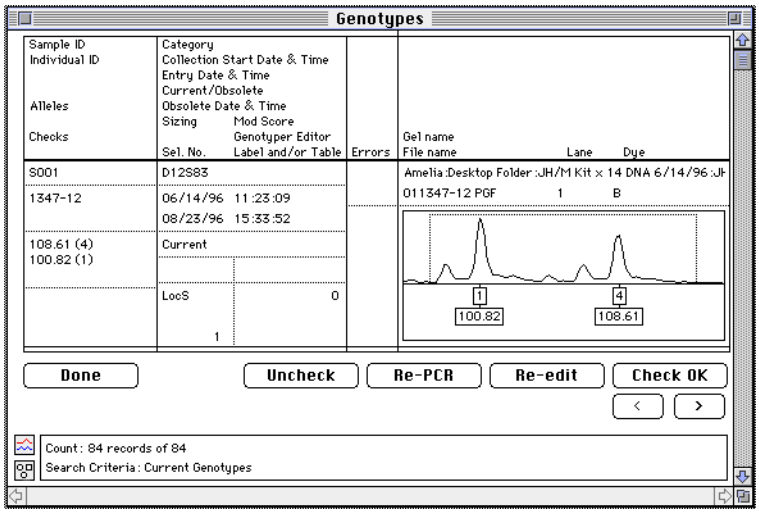


Figure 10-1 The Genotype Record

For a detailed description of fields in the Genotype Record, see the *ABI PRISM GenBase User's Manual*.

Kinds of Results Data You Can Exchange

Genotyper can exchange results data for tables and categories with GenBase. The following table shows which pages to refer to for instructions on how to either import or export the specified results data.

Kinds of results data Genotyper and GenBase exchange:

If you want to...	See Page
Import tables	10-6
Export tables	10-10
Import categories	10-15
Export categories	10-15

Importing Tables from GenBase

Introduction

GenBase organizes all genotyping results data into function-specific tables. For example, data for populations, samples, and allele definitions are organized in separate tables. You can import data from GenBase tables into Genotyper for modification or inclusion in Genotyper tables.

Kinds of GenBase Tables

GenBase tables are stored in the Genotyper Record. This table shows the kind of GenBase tables that contain data appropriate for import and use in Genotyper.

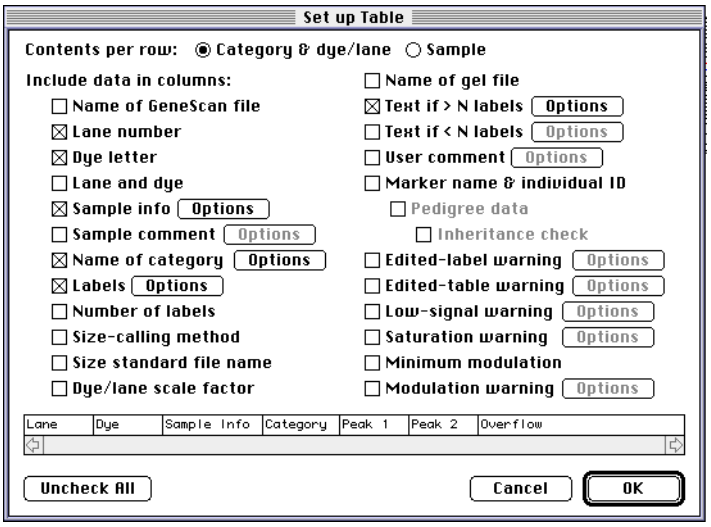
Kinds of GenBase tables:

GenBase Table Name	Description
Category Members	Stores category definitions from Genotyper.
Genotypes	Stores genotyping results from Genotyper.
Individuals	Keeps track of information on every individual in your study and provides convenient cross-references for sorting and searching through patient populations.
Markers	Defines markers. You can specify map locations (for a particular map) and alternate names (for a particular Alternate Marker Name Set).
Pedigrees	Provides information that is imported from GenoPedigree. Can be set up manually if you don't have GenoPedigree.
Pedigree Structures	List of names of hypotheses for pedigree relationships.
Samples	Assigns IDs to your nucleic acid samples, and provides cross-references to individuals and their samples.

How to Specify the Format

Before you import data from a GenBase table into Genotyper, use the Set Up Table... command in Genotyper to specify formats for imported GenBase data.

To import GenBase tables into Genotyper:

Step	Action
1	Link to GenBase.
2	Select Clear Table from the Analysis menu.
3	Select Set Up Table from the Table menu. The Set Up Table dialog box appears. 

To import GenBase tables into Genotyper: *(continued)*

Step	Action
4	Select the following checkboxes: ♦ Sample Info ♦ Name of category ♦ Labels The following fields are optional: ♦ Name of GeneScan file ♦ Lane number ♦ Dye letter ♦ Lane and dye ♦ Text if >N labels ♦ Edited-label warning ♦ Edited-table warning **Note** Other fields are not available for import.
5	Click OK.

How to Import Tables

You can import data from GenBase tables, and further analyze it with Genotyper commands.

To import GenBase tables into Genotyper:

Step	Action
1	Link to GenBase.
2	Open the Genotypes Summary Table in GenBase.
3	Select and show records that you want to import, and append to your Genotyper table.
4	In Genotyper, set up the kind of table to which you want to append rows of Genotype Records. For more information on setting up tables in Genotyper, see “Setting Up a Table” on page 8-2.
5	From the Table menu, choose Append from GenBase. A dialog box appears telling you how many records, or rows Genotyper will append to your table.

To import GenBase tables into Genotyper: *(continued)*

Step	Action
6	Click OK and Genotyper imports select records and appends them as rows to your table. **Note** If you plan to edit the table data in Genotyper and then export the table to GenBase, you must use the Re-import Dye/lane command to import the associated dye/lanes into the Dye/lane list before exporting the table. For more information on using the Re-import Dye/lane command, see “Re-importing Dye/lanes” on page 8-6.

Exporting Tables to GenBase

Introduction

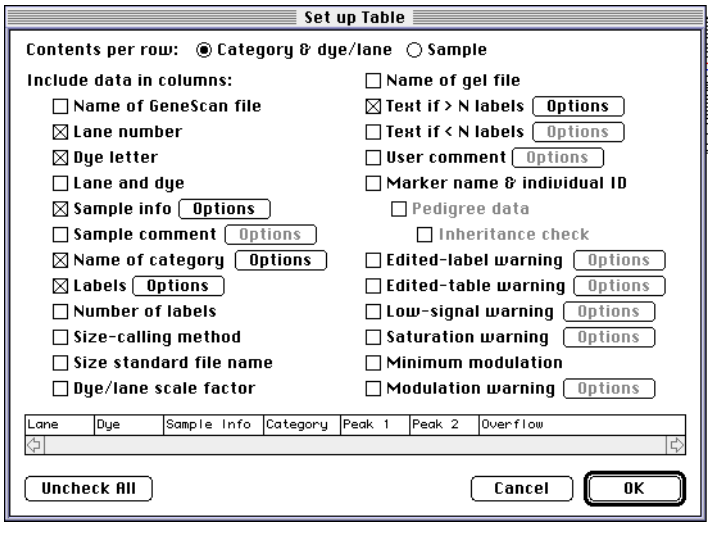
GenBase organizes all genotyping results data into function specific tables. For example, data for populations, samples, and allele definitions are organized in separate tables. You can export data from Genotyper tables for inclusion in specific GenBase tables.

IMPORTANT The label data must be set up correctly in the table for the Export to GenBase...command to give satisfactory results. If the table is not set up correctly in Genotyper, then the data in GenBase may end up in the wrong format.

How to Specify the Format

Before you export data from a Genotyper table into GenBase, use the Set Up Table command in Genotyper to specify formats for exported GenBase data.

To specify format of tables for export to GenBase:

Step	Action
1	Link to GenBase.
2	Select Clear Table from the Analysis menu.
3	Select Set Up Table from the Table menu. The Set Up Table dialog box appears. 

To specify format of tables for export to GenBase: *(continued)*

Step	Action
4	Select the following checkboxes: ♦ Sample Info ♦ Name of category ♦ Labels The following fields are optional, and will only be sent if checked: ♦ Size-calling method ♦ Text if >N labels ♦ Low-signal warning ♦ Saturation warning ♦ Minimum modulation ♦ Modulation warning The following fields are sent automatically and do not need to be checked: ♦ Name of GeneScan file ♦ Lane number ♦ Dye letter ♦ Name of Gel file ♦ Edited-label warning ♦ Edited-table warning **Note** Other fields are not available for export.

To specify format of tables for export to GenBase: *(continued)*

Step	Action
5	Choose the kind of label data that you want to send to GenBase: **If your labeled peaks have...** / **Then...** Size labels only / Choose one label per peak in the labels options of the Set Up Table...command. Sizes and allele names / Choose two labels per peak in the labels options of the Set Up Table...command. **Note** Category member names correspond to allele names. **Note** If you choose sizes and allele names, make sure that every labeled peak has two labels. For a description of how to set up your categories to insure that all peaks are labeled with either the correct allele name or with the name "Unknown" to indicate a new allele has been detected, see "Using Exclusive Peak Labeling–An Example" on page 6-10.
6	Click OK.

How to Export Tables

You can export data from Genotyper to specific GenBase tables stored in the Genotype Record.

IMPORTANT The Export to GenBase command sends data from the dye/lanes as well as the table. If associated dye/lanes have been deleted or are not present in dye/lane list, do not export the table to GenBase.

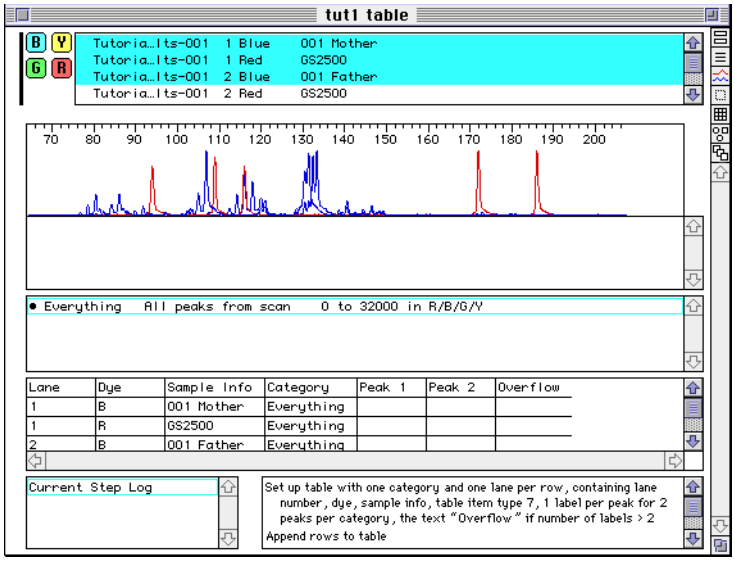
To export Genotyper tables to GenBase:

Step	Action
1	Establish a link to GenBase.
2	Open the Genotyper Document that contains the data that you want to export. **Note** If a document does not already exist, import the GeneScan files that contain the sample data that you want to export.

To export Genotyper tables to GenBase: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>
<table>
<tr><th>If the Genotyper Document...</th><th>Then...</th></tr>
<tr><td>Contains a Table that you want to export</td><td>Go to step 7.</td></tr>
<tr><td>Does not contain a Table you want to export</td><td>Choose Set Up Table from the Table menu, and specify the format for the table you want to export.</td></tr>
</table>

For more information on setting up tables in Genotyper, see “Setting Up a Table” on page 8-2.
</td></tr>
<tr><td>4</td><td>Select the dye/lanes from the Dye/lane list that you want to include in the Table.</td></tr>
<tr><td>5</td><td>Select the category that specifies the kind of peak data you want to include from select dye/lanes.</td></tr>
<tr><td>6</td><td>Select Append to Table from the Table menu, and the selected dye/lane peak information appears in the Table view of the Main Window.

 
</td></tr>
</table>

To export Genotyper tables to GenBase: *(continued)*

Step	Action
7	Select Export to GenBase from the Table menu.

How to Update Tables in GenBase

If you have appended rows to a Genotyper table from a GenBase table, and then edited the contents of that table either by editing cells or by clicking on labels and updating the table, you may want to update the corresponding table in GenBase with your edited table.

To update tables in GenBase with table data edited in Genotyper, choose Update Table in GenBase from the Table menu.

Note Remember that the associated dye/lanes must be present in the Dye/lane list in order for you to update the table in GenBase.

Importing and Exporting Categories

Introduction

Once you have linked to GenBase, You can import marker data and categories from GenBase, and Export Genotyper categories to GenBase.

IMPORTANT You can only export members of category groups to GenBase. The first time you attempt to export a non-member category, Genotyper issues an alert. reminding you that such categories will not be sent to GenBase.

A Comparison of Markers and Categories

GenBase stores categories and markers. Although it is not uncommon to use the terms interchangeably, GenBase allows you to make a distinction between the two terms and assign multiple categories to each marker.

How to Import Categories

To import Genotyper categories from GenBase:

Step	Action
1	Link to GenBase.
2	Open the Category Members table in GenBase, and make sure that only those categories that you want to import are currently shown in the table.
3	Choose Import Categories from Database, from the Category menu. **Note** Categories are imported from GenBase and appear in the Category list of your active Genotyper Document.

How to Export Categories

To export Genotyper categories to GenBase:

Step	Action
1	Mark the categories to export from the Category list in the Main window.
2	Select Export Marked Category to Database from the Category menu.

Importing and Exporting GenoPedigree Data

Introduction

Establishing a link to GenBase provides a means of exchanging results data between Genotyper and GenoPedigree, the pedigree drawing software application.

Through GenBase, you can import pedigree drawings, marker and allele data into Genotyper from GenoPedigree, and export table and category information from Genotyper to GenoPedigree.

For detailed instructions on how to import and export GenoPedigree data, see the *ABI PRISM GenoPedigree User's Manual*.

How to Link to GenoPedigree Data

You can link to GenoPedigree from the Genotyper Main window, if you have installed the pedigree drawing program on the same system as Genotyper.

To link to GenoPedigree from Genotyper:

Step	Action
1	Select Links from the File menu.
2	Select Choose GenoPedigree. An application selection dialog box appears.
3	Highlight the version of GenoPedigree to which you want to connect.
4	Click Select and Genotyper is connected to GenoPedigree. Once you've established a link to GenoPedigree, each time you want to access the database, click the GenoPedigree icon on the Main window.

Linking to Programs and Files 11

Chapter Overview

Introduction This chapter discusses how you can expand your genotyping research efforts by linking Genotyper to third-party programs or files and transferring results data.

In This Chapter This chapter contains the following topics:

Planning to Link to Programs and Documents

Introduction

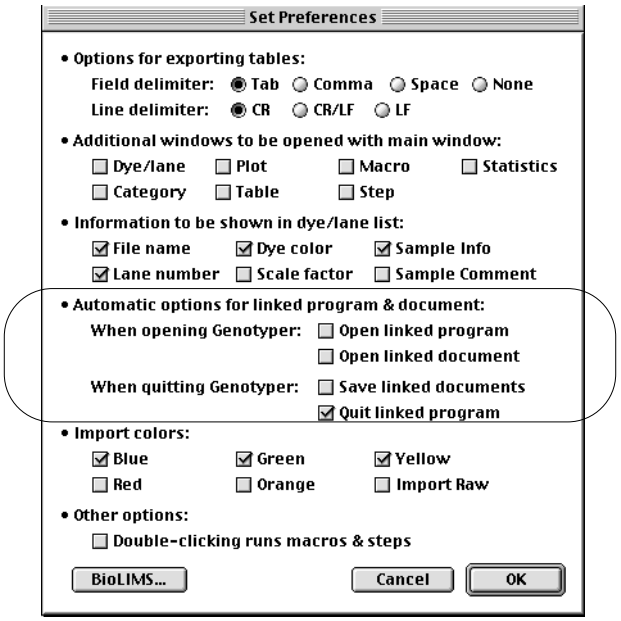
If you are planning to link to third-party programs or spreadsheet applications, after performing genotyping tasks, you need to set up Genotyper so that you can generate results data that is compatible with the program you are planning to use.

How to Set Preferences for Linking to Programs

You can set preferences for opening a linked program or document when you start Genotyper. These linking preferences apply to all open Genotyper Documents, not just the active document. Genotyper remembers all links you have established when you quit Genotyper.

Note Preference settings are saved in the Genotyper preferences file in the System Preferences folder.

To set preferences for linked programs:

Step	Action
1	Choose Set Preferences...in the Edit menu. The Set Preferences dialog box appears. 

To set preferences for linked programs: *(continued)*

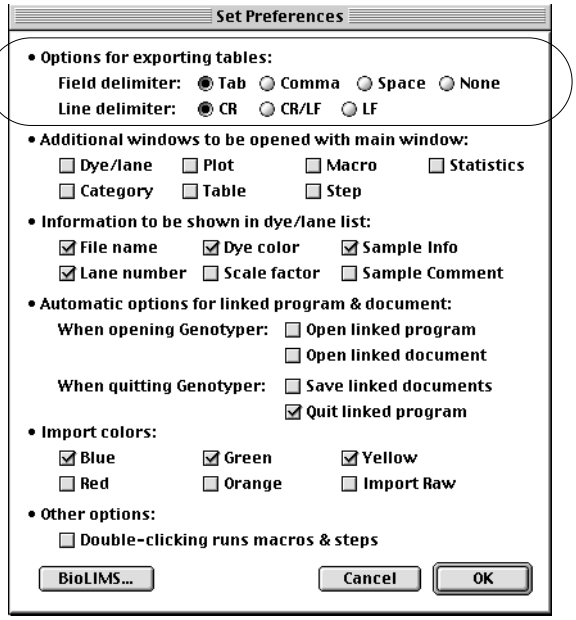
Step	Action
2	Under the bullet “Automatic options for linked program & document:”, select the checkboxes for how you want to link to third-party programs and documents when opening and quitting Genotyper. **Note** These preference settings do not apply to GenBase or GenoPedigree.
3	Click OK.

How to Set Preferences for Exporting a Table to a File

You can export Genotyper tables to a file. Some programs or documents that read this file require the data to be in a specific format; for example, fields must be delimited by tabs or commas. These format options are available in the Set Preferences dialog box.

Note Preference settings are saved in the Genotyper preferences file in the System Preferences folder.

To set preferences for exporting tables to files:

Step	Action
1	Choose Set Preferences...in the Edit menu. The Set Preferences dialog box appears. 
2	Under the bullet “Options for exporting tables:”, click the appropriate radio buttons for field delimiting and line delimiting.
3	Click OK.

Choosing Linked Programs and Documents

Introduction

You can link Genotyper to spreadsheet programs or data base programs that have Apple Event capabilities and use the Apple Event table suite. You may want to link to third-party programs if you have specialized applications that require different analysis procedures than those offered by GenBase or GenoPedigree.

Example Programs

Some examples of third-party programs you might want to link to and transfer your results data include:

- Microsoft Excel
- Claris FileMaker Pro

How to Choose a Linked Program

To choose a program to link to from Genotyper:

Step	Action
1	From the File menu, point to Links and click on Choose Linked Program... The Directory dialog box appears.
2	Locate and select the program to be linked.

How to Choose a Linked Document

You can choose a document in the linked program after you have chosen the linked program. If you do not choose a specified linked document, Genotyper will use the untitled default document, if any, opened by the linked program.

Step	Action
1	From the File menu, point to Links and click on Choose Linked Document... The Directory dialog box appears.
2	Locate and select the document to be linked.

Opening a Linked Program

If you have configured the Set Preferences dialog box to open the linked program automatically, and if the linked program was already opened by Genotyper, then you do not need to use the Open Linked Program command.

To open a linked program:

Step	Action
1	Choose Open Linked Program in the File menu. The linked program opens.

IMPORTANT If a program is already opened, but was not opened using the Open Linked Program command in Genotyper, you still need to choose Open Linked Program.

Opening a Linked Document

To open a linked document:

Step	Action
1	Choose Open Linked Document in the File menu. A specific document in the linked program opens.

Running a Macro or Script in a Linked Program

Introduction

You can run a macro or script from Genotyper that you created in a linked program.

How to Run a Macro/Script in a Linked Program

To run a macro/script in a linked program:

Step	Action
1	Create and name a macro/script in the linked program.
2	In Genotyper, choose Run Linked Macro/Script...in the Macro menu.
3	Enter the name of the macro that was defined in the linked program.
4	Click OK. The macro runs in the linked program.

Examples of Macros in Linked Programs

The following are examples of how you can define macros in different kinds of linked programs:

- FileMaker Pro users enter the name of the FileMaker Pro script.
- Microsoft Excel users enter the following:

  [name of Macro Sheet]![name of macro]()

Example

Macro1!Record()

IMPORTANT The macro will not work for Excel programs without the pair of parentheses at the end.

Exporting and Copying Tables

Introduction

You can export a Genotyper table to a plain text file so that you can read it using a word processing or spreadsheet application. You can open this file in Simple Text to view it.

IMPORTANT Once a table is exported to a file it cannot be imported back into Genotyper. If you want to continue working with the table, save your work as a Genotyper Document by using the Save command. This will save the dye/lanes, categories, or labels, and will allow you to continue your work at a later time.

How to Export a Table

To export a table to a file:

Step	Action
1	Choose Export Table...from the Edit menu. The standard Macintosh Directory dialog box appears.
2	Enter the name of the file in the text box.
3	Designate where you want to place that file (for example, desktop or in another folder).
4	Click Save.

How to Copy Tables

As an alternative to making a table in a linked spreadsheet document, you can copy a table to a spreadsheet document.

To copy tables to spreadsheets:

Step	Action
1	Select a contiguous portion of the Genotyper table.
2	Choose Copy in the Edit menu.
3	Select the spreadsheet program.
4	Choose Paste in the Edit menu.

Menu and Command Reference 12

Chapter Overview

Introduction This chapter provides a reference for the names, locations, and definitions of all menus and commands available in Genotyper 2.5.

In This Chapter This chapter contains the following topics:

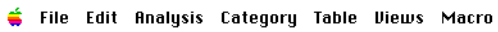
Genotyper Menus

Introduction

You can access all Genotyper commands and options from pull-down menus listed on the menu bar.

The Menu Bar

The menu bar displays across the top of your computer screen after you start the Genotyper application.



Menu Items

Menus you can access from the menu bar:

Menu	Description
The File menu	Lists commands for working with Genotyper Documents and GeneScan files.
The Edit menu	Lists commands for editing Genotyper Documents and table contents.
The Analysis menu	Lists commands for labeling fragment peak data, and determining statistical data for fragment peaks.
The Category menu	Lists commands for defining categories.
The Table menu	Lists commands for setting up Tables and working with tabular data.
The Views menu	Lists commands for opening windows for Genotyper Document windows in the Main window, and for customizing viewing of these windows.
The Macro menu	Lists commands for creating and running macros for automating Genotyper procedures and applications.

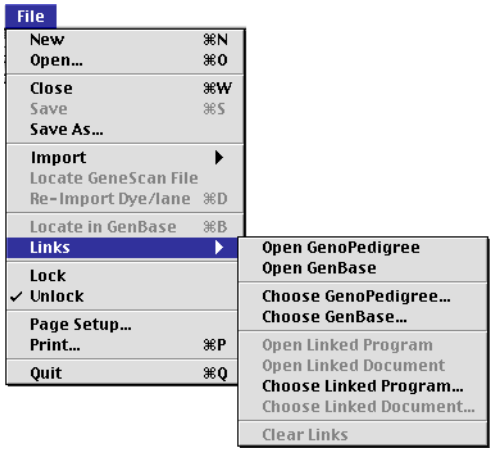
The File Menu

Definition

The File menu contains commands for working with Genotyper Documents.

Menu Options

The figure below shows the list of commands you can access from the File menu.



Commands

Commands in the File menu:

Command	Description
New	Opens a new, untitled Genotyper document.
Open...	Opens a previously saved Genotyper document.
Close	Closes the active window.
Save	Saves the active Genotyper document.
Save As...	Saves the Genotyper document under a new name.
From GeneScan File	Brings up a dialog box which allows you to import a GeneScan file or multiple GeneScan files.

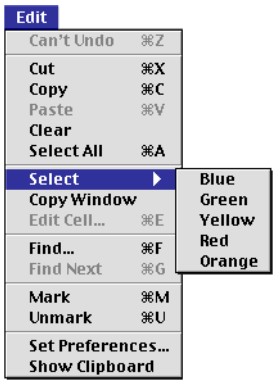
Commands in the File menu: *(continued)*

Command	Description
From BioLIMS	Opens BioLIMS Collection Browser if a database connection has been established.
Locate GeneScan File	When you select an entry in the Dye/lanes list, and then choose this command, the Finder locates the original GeneScan file for that selection and opens its folder.
Re-Import Dye/lane	Re-imports dye/lanes if you have made a table and deleted or cleared all dye/lanes.
Locate in GenBase	Finds specified text in a GenBase database if you are linked to it.
Links	Sub-menu with several choices for linking.
Open GenoPedigree	Provides access to the GenoPedigree program.
Open GenBase	Provides access to GenBase.
Choose GenoPedigree	Provides link to GenoPedigree program.
Choose GenBase	Provides link to GenBase database.
Choose GenBase File...	Provides link to files in GenBase.
Open Linked Program	Opens an application program that is linked to Genotyper.
Open Linked Document	Opens a document that is linked to Genotyper.
Choose Linked Program...	Brings up a dialog box which allows you to choose a linked program.
Choose Linked Document...	Brings up a dialog box which allows you to choose a linked document.

The Edit Menu

Definition The Edit menu contains commands for editing Genotyper documents.

Menu Options The figure below shows the list of commands you can access from the Edit Menu.



Commands Commands in the Edit menu:

Command	Description
Can't Undo	Undoes the last command (whenever possible).
Cut	Cuts the selection and places it on the Clipboard.
Copy	Copies the selection to the Clipboard.
Paste	Pastes the selection at the cursor location.
Clear	Clears the currently selected entries in the Dye/lane list, Categories list, Macro list, or Step list.
Select All	Selects every entry in the selected list or table.
Select	
♦ Blue	♦ Selects all entries in the Dye/lane list that have a blue dye color.
♦ Green	♦ Selects all entries in the Dye/lane list that have a green dye color.
♦ Yellow	♦ Selects all entries in the Dye/lane list that have a yellow dye color.

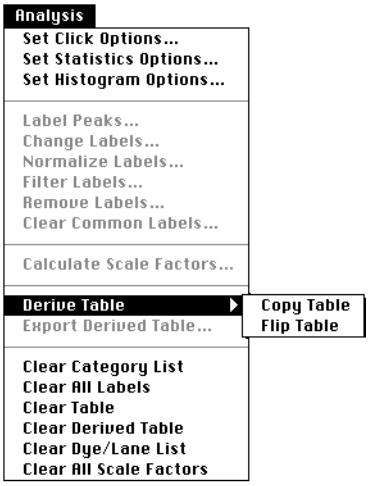
Commands in the Edit menu: *(continued)*

Command	Description
♦ Red	♦ Selects all entries in the Dye/lane list that have a red dye color.
♦ Orange	♦ Selects all entries in the Dye/lane list that have an orange dye color.
Copy Window	Copies the active window to the clipboard.
Edit cell...	Allows you to change the contents of individual cells in tables.
Find...	Shows a dialog box which allows you to locate and select an alphanumeric text in the Dye/lane list, Category list, or the table.
Find Next	Allows you to locate and select the next case of a previously defined alphanumeric text, without the need to repeatedly use the Find dialog box.
Mark	Places a bullet (•) in front of the Categories list or Dye/lane list item. When a dye/lane is not marked, the plot corresponding to that item appears in the upper Plot window. When a category is marked, it is used by labeling and table commands.
Unmark	Removes a bullet (•) from the Category or Dye/lane list item. When a dye/lane is not marked, the plot corresponding to that item no longer appears in the upper Plot window. When a category is not marked, it is not used by labeling and table commands.
Set Preferences...	Shows a dialog box which allows you to set options for exporting tables, define additional windows to be opened with the main window, define information to be shown in the Dye/lane list, and set automatic options for a linked program or document.
Show Clipboard	Shows items that have been cut, or copied, and still reside in the clipboard.

The Analysis Menu

Definition The Analysis menu contains commands for labeling fragment peak data, and determining statistical data for fragment peaks.

Menu Options The figure below shows the list of commands you can access from the Analysis Menu.



Commands Commands in the Analysis menu:

Command	Description
Set Click Options...	Shows a dialog box in which you can set parameters for manual labeling of peaks (for example, labeling by size, height, scan number, or area).
Set Statistics Options...	Shows a dialog box in which you can set parameters for what displays in the Statistics window. Settings include: calculate statistics for peak size, scan number, bin size.
Set Histogram Options...	Shows a dialog box in which you can set parameters for how the histogram window interacts with other parts of a Genotyper Document.

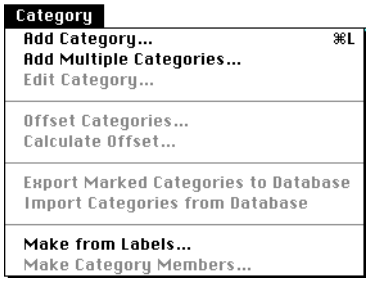
Commands in the Analysis menu: *(continued)*

Command	Description
Label Peaks...	Shows a dialog box which allows you to label peaks for selected dye/lanes that have been defined by marked category parameters.
Change Labels...	Shows a dialog box which, for selected lanes in the Dye/lane list, allows you to change the labels at currently labeled peaks.
Normalize Labels...	Shows a dialog box which allows you to normalize data in several peak labels to data in a specific (control) label.
Filter Labels...	Removes unwanted labels from peaks.
Remove Labels...	Shows a dialog box which allows you to remove labels from specified peak locations of selected dye/lanes in the dye/lane list.
Clear Common Labels...	In currently-selected set of dye/lanes, remove labels for peaks that (within tolerance) are at the same location and are labeled in each of the dye/lanes; shows a dialog box to set the tolerance.
Calculate Scale Factors...	Normalizes the height or area of peaks.
Derive Table...	Enables you to derive a second table from an existing table. A derived table is not linked to dye/lanes or categories like the table from which it was derived.
Copy Table	Copies the table in the Table window to the Derived table window.
Flip Table	Flips the table in the Table window, copies it and places it in the derived table window.
Export Derived Table	Exports derived tables as text files.
Clear Category list	Clears all entries in the Category list.
Clear All Labels	Removes all labels from all peaks (whether selected or not) in the Plot window.
Clear Table	Removes all rows and columns from the table.
Clear Dye/Lane list	Removes all entries from the Dye/lane list.
Clear All Scale Factors	Resets all scale factors from dye/lanes in the Dye/lane list to 1.0.

The Category Menu

Definition The Category menu contains commands for defining categories.

Menu Options This figure shows the list of commands you can access from the Category menu.



Commands Commands in the Category menu:

Command	Description
Add Category...	Shows a dialog box which allows you to set category parameters for new categories.
Add Multiple Categories...	Shows a dialog box which allows you to create multiple categories at once.
Edit Category...	Shows a dialog box which allows you to change the parameters of the selected category.
Offset Categories...	Allows you to adjust the size range for peak labeling for selected categories.
Calculate Offset...	Automatically adjusts the size range for peak labeling for selected categories.
Export Marked Categories to Database	Exports marked Genotyper categories to GenBase.
Import Categories from Database	Imports Genotyper categories from GenBase.
Make from Labels...	Shows a dialog box that allows you to create categories from labels.

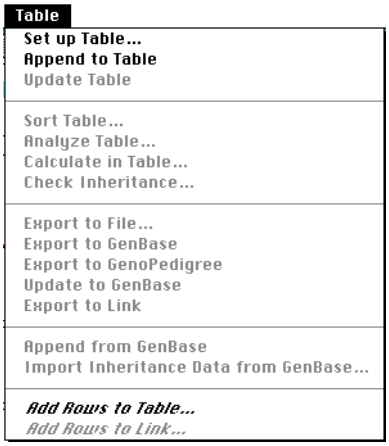
Commands in the Category menu: *(continued)*

Command	Description
Make Category Members...	Shows a dialog box that allows you to create member categories for specialized applications.

The Table Menu

Definition The Table menu contains commands for setting up Tables and working with tabular data.

Menu Options This figure shows the list of commands you can access from the Table menu.



Commands Commands in the Table menu:

Command	Description
Set up Table...	Shows a dialog box which allows you to define the contents and order of rows and column headings for tables.
Append to Table	Adds rows to an existing table.
Update Table	If you have created a table, and made changes to peak labels, updates the corresponding information in your table.
Sort Table...	Shows a dialog box which allows you to sort the rows of a table in a Genotyper document.
Analyze Table...	Allows you to specify conditions for logical comparisons of table cell contents.
Calculate in Table...	Allows you to perform numerical calculations of table cell contents.

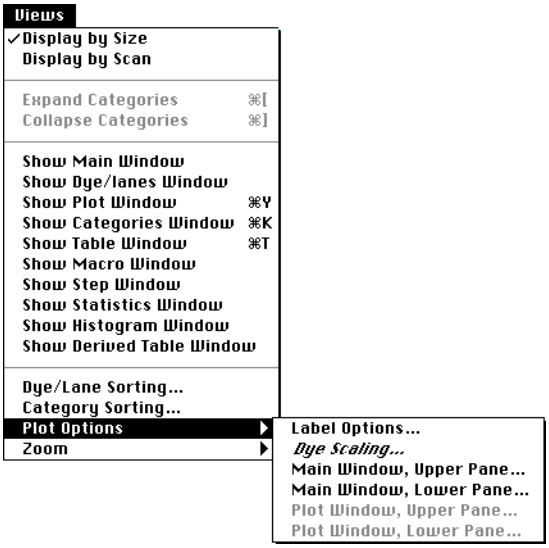
Commands in the Table menu: *(continued)*

Command	Description
Check Inheritance...	Shows a dialog box that allows you to check Mendelian inheritance in the table.
Export to File...	Exports table contents to a text file.
Export to GenBase...	Exports select tables to GenBase.
Update to GenBase...	Updates GenBase tables with information from select Genotyper tables.
Export to Link...	Exports table contents to a linked program (other than GenBase or GenoPedigree).
Append from GenBase...	Allows you to append rows to Genotyper tables from GenBase tables.
Import IDs from GenBase...	Imports data from GenBase for inheritance checking.
Add Rows to Table...	For compatibility with Genotyper versions 1.x only.
Add Rows to Link...	For compatibility with Genotyper versions 1.x only.

The Views Menu

Definition The Views menu contains commands for opening windows for Genotyper Document windows in the Main window, and for customizing viewing of these windows.

Menu Options This figure shows the list of commands you can access from the Views Menu.



Commands

Commands in the Views menu:

Command	Description
Display by Size	Changes the horizontal scale in the plot areas to base pairs.
Display by Scan	Changes the horizontal scale in the plot areas to scan line.
Expand Categories	Displays all categories in a selected category group in the Category list.
Collapse Categories	Displays only the category group name of a selected category in the Category list.

Commands in the Views menu: *(continued)*

Command	Description
Show Main Window	Shows all parts of a Genotyper Document in one window, the Main window.
Show Dye/lanes Window	Opens an additional, larger window that shows only the Dye/lane list.
Show Plot Window	Opens an additional, larger window that shows only the plots and peak labels. The window expands vertically to fill the computer screen.
Show Categories Window	Opens an additional, larger window that shows only the Category list.
Show Table Window	Opens an additional, larger window that shows only the table.
Show Macro Window	Opens an additional, larger window that shows only the Macro list.
Show Step Window	Opens an additional, larger window that shows only the Step list.
Show Statistics Window	Opens window displaying statistics of selected peaks (number of labeled peaks, minimum, maximum, median, standard deviation, count, and bin).
Show Histogram Window	Opens window displaying histogram representation of statistics of selected peaks.
Show Derived Table Window	Opens window displaying Derived table, if one exists.
Dye/Lane Sorting...	Brings up a dialog box which allows you to sort the Dye/lane list by file names, lanes, dye color, sample information, or sample comments in ascending or descending order.
Category Sorting...	Brings up a dialog box which allows you to sort category names, category starting size or scan number, and category dye color in ascending or descending order.
Plot Options	
Label Options...	Brings up a dialog box which allows you to modify and use colors and prefixes in labels.
Dye Scaling...	Brings up a dialog box which allows you to modify signal heights.

Commands in the Views menu: *(continued)*

Command	Description
Main Window, Upper Pane...	Displays the Plot Options dialog box, allowing you to change the display options in the upper pane of the Main window.
Main Window, Lower Pane...	Displays the Plot Options dialog box, allowing you to change the display options in the lower pane of the Main window.
Plot Window, Upper Pane...	When the Plot window is open, displays the Plot Options dialog box, allowing you to change the display options in the upper pane of the Plot window.
Plot Window, Lower Pane...	When the Plot window is open, displays the Plot Options dialog box, allowing you to change the display options in the lower pane of the Plot window.
Zoom	
Zoom In	Zoom in by a factor of two.
Zoom In (Selected Range)	Expands the selected portion of an electropherogram to fill the full plot area.
Zoom Out	Shows a somewhat larger section of the electropherogram.
Zoom Out (Full Range)	Shows the complete electropherogram.
Zoom To...	Brings up a dialog box which allows you to specify a zoom range.
Zoom To Category	Zooms to a range that includes the ranges of currently-selected categories.
Zoom To Next Category	Zoom to the range of the next marked category following the currently-selected category.

The Macro Menu

Definition The Macro menu contains commands for creating and running macros for automating Genotyper procedures and applications.

Menu Options This figure shows the list of commands you can access from the Macro menu.

Commands Commands in the Macro menu:

Command	Description
Record Steps	When this menu item is checked, your steps (commands) will be added to the Current Step Log.
Save Step Log...	Creates a macro from the current step log and brings up a dialog box which allows you to name the macro and choose a keyboard command to run it.
Run Macro	Runs the selected macro.
Change Macro Name...	Brings up a dialog box which allows you to change the name of the selected macro and change the keyboard command which runs it.
Duplicate Macro...	Makes a copy of the selected macro. This is useful if you have a macro that you want to modify slightly, but you do not want to lose the original macro.

Commands in the Macro menu: *(continued)*

Command	Description
Run Linked Macro/Script...	Sends a DoScript Apple event to the linked program.
Edit Step...	Brings up a dialog box which allows you to adjust the values used by the step command. This works only for steps created using a dialog box.
Run Step	Runs the selected step.
Add Comment...	Brings up a dialog box which allows you to add comments at the end of the Current Step log.
Set Import Macro...	Automatically runs a selected macro immediately after import of GeneScan files.
Clear Step Log...	Removes all steps in the Current Step log.

Importing Data from a BioLIMS Database 13

Chapter Overview

New Feature of Genotyper V. 2.5

Genotyper Software version 2.5 has the ability to read ABI PRISM® GeneScan® Analysis data from a BioLIMS® 2.0 database.

- You can now import data from both GeneScan sample files and BioLIMS at the same time into a single document.
- Data imported from BioLIMS can be exported to a GenBase database after being processed in Genotyper.

Note Genotyper v. 2.5 has read-only access to BioLIMS. Hence, Genotyper results cannot be written back to the BioLIMS database. Instead they can be stored in individual Genotyper documents, exported as text files, or stored in a GenBase™ database.

In This Chapter

This chapter describes how to access the BioLIMS® database, how to set the preferences, and how to open or process fragment data that is located in the BioLIMS database.

For information on troubleshooting the BioLIMS database, see Appendix F, “Troubleshooting the BioLIMS Database”, in the *GeneScan Analysis Software Version 3.1 User’s Manual* (P/N 4306157).

Configuring the Macintosh Computer for BioLIMS Database Access

Sybase or Oracle?

This section provides instructions on how to configure the client Macintosh computer (that runs the Genotyper software) for database access.

The BioLIMS database resides on either a Sybase SQL Server or an Oracle Server.

To configure for the…	See…
Sybase SQL Server	"Configuring for Sybase SQL Server Connection" on page 13-2.
Oracle Server	"Configuring for Oracle Server Connection" on page 13-5.

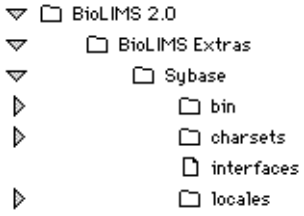
Configuring for Sybase SQL Server Connection

Follow the steps below to configure a Macintosh computer for connection to the Sybase® SQL server.

IMPORTANT The BioLIMS client software for Sybase® must be installed on your Macintosh computer.

IMPORTANT Any time the name, port number, IP address, or host and domain name of the BioLIMS database server is changed, you will need to repeat this procedure.

To configure for Sybase SQL Server connection:

Step	Action
1	Find the interfaces file in the BioLIMS 2.0:BioLIMS Extras:Sybase folder. 
2	Open the file with SimpleText or a similar text editing application.

To configure for Sybase SQL Server connection: *(continued)*

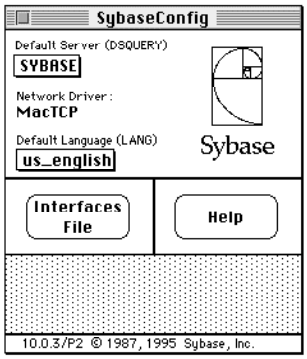
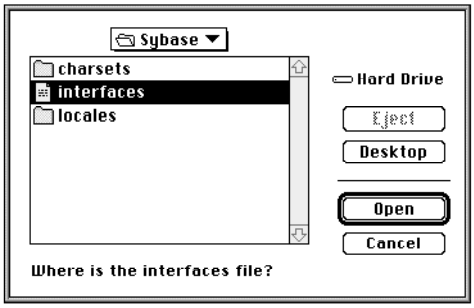
Step	Action

3	Find the lines:
	`SYBASE`
	`query MacTCP mac_ether neuron.apldbio.com 2500`
	and edit them as follows:
	♦ Replace SYBASE by an alias name for the database server (see “About Server Names” on page 13-15).
	♦ Replace neuron.apldbio.com with the IP address or host and domain name of the server machine.
	♦ 2500 is the default port number for the Sybase database. If necessary, replace 2500 with the port number recommended by your BioLIMS database administrator.
	♦ Insert a tab space before the start of the second line if there is no tab space already present.
	You can find this information in the interfaces file on the Sybase Server, or your BioLIMS database administrator can provide the information.

4	If you have access to more than one server, duplicate the two lines and edit them for the other server(s).
	For example, for two servers, one called SYBASE and one called SERVER2, the interfaces file might look like this:
	`SYBASE`
	`query MacTCP mac_ether neuron.apldbio.com 2500`
	`SERVER2`
	`query MacTCP mac_ether 192.135.191.128 2025`

5	Save and close the interfaces file.

To configure for Sybase SQL Server connection: *(continued)*

Step	Action
6	Open the SybaseConfig control panel. This control panel is found in the Control Panels folder in the System folder. 
7	The first time the SybaseConfig control panel is opened, a file browser opens automatically. If a file browser does not open immediately, click the Interfaces Files button to open one. 
8	Use the file browser to locate and open the interfaces file edited in the steps above.
9	Set the Default Language pop-up menu to us_english.
10	Close the SybaseConfig control panel.

Configuring for Oracle Server Connection

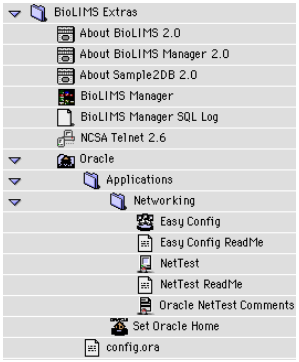
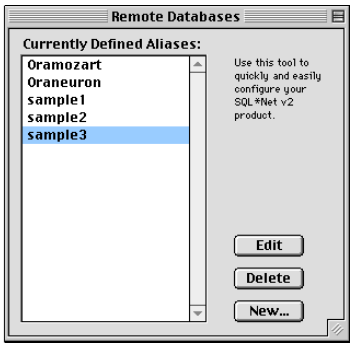
Use the program Easy Config to configure your Macintosh computer for connection to the Oracle Server.

IMPORTANT The BioLIMS client software for Oracle must be installed on your Macintosh computer.

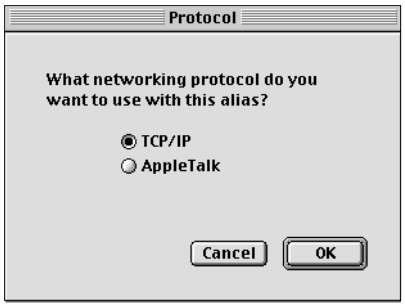
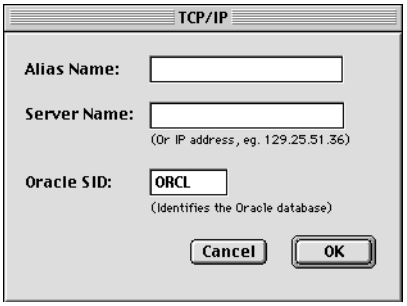
IMPORTANT Any time the name, port number, IP address, or host and domain name of the BioLIMS database server is changed, you will need to repeat this procedure.

Note At installation, Easy Config is placed into the BioLIMS 2.0:BioLIMS Extras:Oracle:Applications:Networking folder.

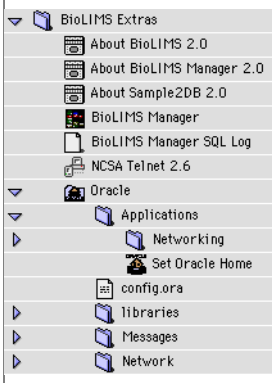
To configure your computer for the Oracle Server connection:

Step	Action
1	Find the Easy Config program in the BioLIMS 2.0:BioLIMS Extras:Oracle:Applications:Networking folder. 
2	Open the Easy Config program. The Remote Databases window appears. 

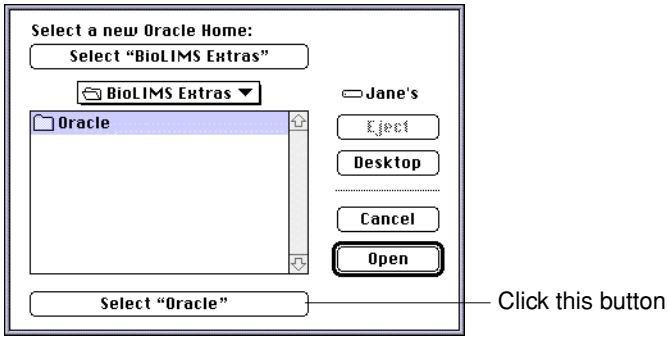
To configure your computer for the Oracle Server connection: *(continued)*

Step	Action
3	Click New. The Protocol dialog box appears. 
4	Select TCP/IP and click OK. The TCP/IP dialog box appears. 

To configure your computer for the Oracle Server connection: *(continued)*

Step	Action
5	Enter text in the fields as follows: ♦ Alias Name: Enter an alias name for the database server. (See “About Server Names” on page 13-15.) ♦ Server Name: Enter the server name. This may be an IP address or host (and domain name) of the server machine. **Note** This field does not scroll horizontally for display even though it accepts characters typed past the end of the field. If the server name is longer than 20 characters, you may want to enter the end characters first and go back or just use the IP address. ♦ Oracle SID: Enter the value of the ORACLE_SID environment variable. You can find this information in the tnsnames.ora file on the Oracle Server, or your BioLIMS database administrator can provide you with the information.
6	Click OK to close the TCP/IP dialog box.
7	From the File menu, choose Save Configuration.
8	From the File menu, choose Quit to exit the Easy Config program.
9	Find the application Set Oracle Home.  The application is contained in your BioLIMS 2.0:BioLIMS Extras:Oracle:Applications folder. 

To configure your computer for the Oracle Server connection: *(continued)*

Step	Action
10	Open the application Set Oracle Home. 
11	Use the file browser to find the Oracle folder in the BioLIMS Extras folder. Click the Select "Oracle" button. Do **not** click Open.

Setting Up Access to the BioLIMS Database

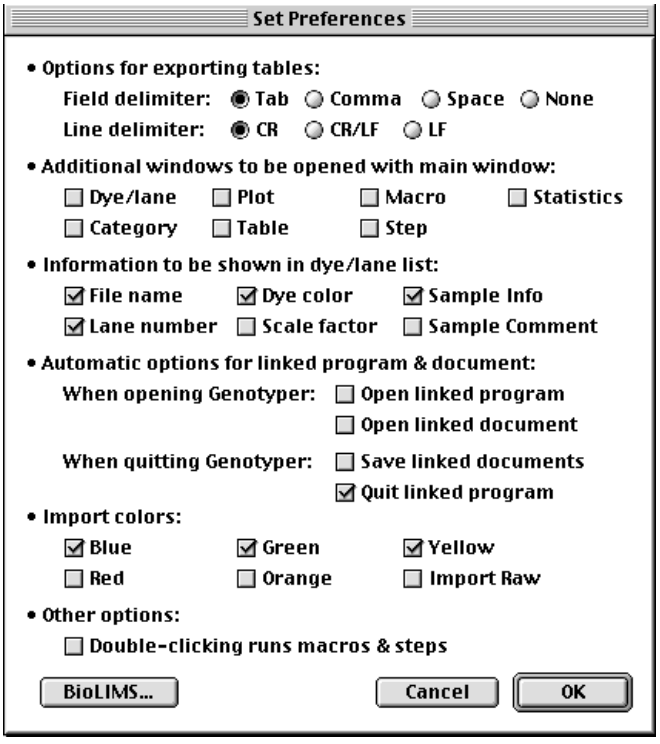
Introduction

The following procedure describes how to access the BioLIMS database by completing the Set Preferences dialog box.

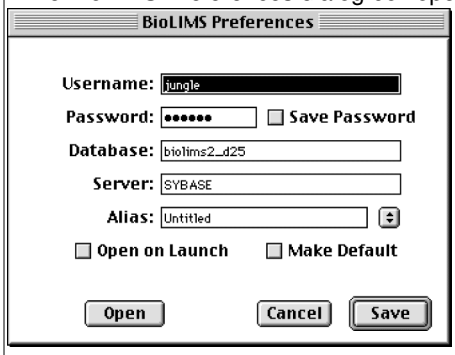
Before you can work with the Genotyper software, you must establish a connection to the BioLIMS database. This connection is made through the BioLIMS Access button in the Set Preferences dialog box.

Using the Preferences Box

To set up your preferences:

Step	Action
1	Launch Genotyper v 2.5.
2	Choose Set Preferences... under Edit in the main menu. The Set Preferences dialog box appears. 

To set up your preferences: *(continued)*

Step	Action
3	Click the BioLIMS button if you wish to open a connection to the database and save the preferences you have made in the session manager. The BioLIMS Preferences dialog box opens.  Enter the requested information and click Open to open a connection. **Note** User entry fields appear gray and will not accept any input if you have already accessed files in BioLIMS through the Choose Channel to Open box (see step 3 on page 13-12). In this case click on the Close button to close the database connection. Now you can re-enter the information into these fields.
4	Click Save to save the information you entered.
5	Click OK in the Set Preferences dialog box.

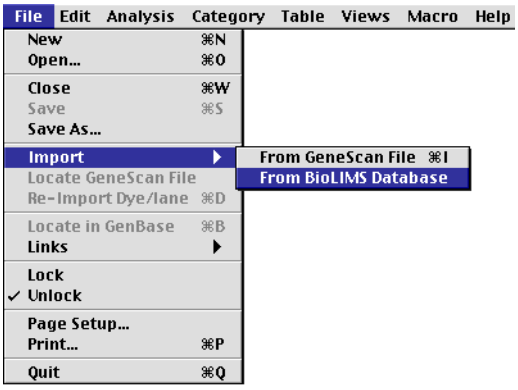
Importing GeneScan Data From BioLIMS

Introduction

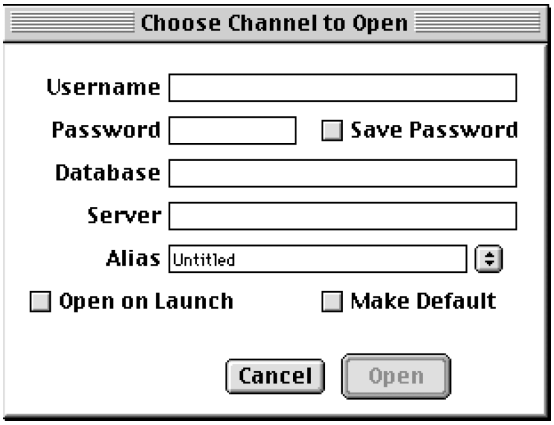
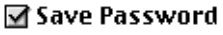
After the client Macintosh has been configured for BioLIMS access, you can access files stored in BioLIMS from the File menu of Genotyper v. 2.5.

Accessing Files in BioLIMS

To access files stored in BioLIMS:

Step	Action
1	Launch Genotyper v. 2.5.
2	From the File menu, point to Import and click on From BioLIMS Database.  The Choose Channel to Open dialog box appears if you have not initiated a connection to the BioLIMS database. Otherwise, the above step will automatically open the Collection Browser window.

To access files stored in BioLIMS: *(continued)*

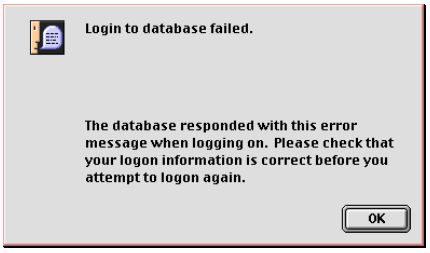
Step	Action
3	Enter the following information requested by the session manager in the Choose Channel to Open box. ♦ Your user name for the database. ♦ Your password for your database account. ♦ The name of the database on the server. (You may have access to more than one database on the server.) **Note** For Oracle, this entry is the schema owner and not the database name. ♦ The server name. This is an alias to the database server which is contained in the interfaces file (Sybase) or in the tnsnames.ora file (Oracle). **IMPORTANT** All of these text boxes are case sensitive. 
4	Click the check box labeled Save Password if you want to: ♦ Save your password so that you do not have to enter it every time you open the connection. ♦ Run AppleScripts that do not contain password information. 

To access files stored in BioLIMS: *(continued)*

Step	Action
5	If you want the database to open automatically when you launch the Genotyper software, click the check box labeled Open on Launch. ☑ Open on Launch **Note** You must also click the check box labeled Save Password if you want the database to open automatically.
6	If you intend to use more than one database or user account, enter an alias for this BioLIMS session information. You can use an alias to connect to the database if no database connection is open. Once you enter an alias, click Open to connect to the database.
7	If you have more than one alias, click the Make Default check box to choose which one appears when you first open the Edit Session dialog box. ☑ Make Default **Note** The default alias is the database that opens if you choose File:Import:From BioLIMS Database. **Note** If both the Make Default and the Save Password boxes are checked, no dialog box will appear when a connection to the server is requested. Since all the information required of the user has been saved, the software will connect to the database automatically.
8	Use the pop-up menu to add, change, or remove aliases.

To access files stored in BioLIMS: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>9</td><td>Click Open and take one of the following actions:

<table>
<tr><th>If the login was...</th><th>Then...</th></tr>
<tr><td>successful</td><td>the Collection Browser window opens. For more information see page 13-19.</td></tr>
<tr><td>unsuccessful</td><td>an alert dialog box appears.
 

Check that:
♦ All the login information was entered correctly and in the correct case.
♦ The interfaces file is correctly configured for a Sybase database (page 13-2).
♦ The tnsnames.ora file is correctly configured for an Oracle database (page 13-5).
♦ If the connection is still not open, consult Appendix F, “Troubleshooting the BioLIMS Database”, in the GeneScan Analysis Software Version 3.1 User’s Manual (P/N 4306157).</td></tr>
</table>
</td></tr>
</table>

About Server Names

Sybase or Oracle?

The BioLIMS Session Manager decides whether you are connected to Sybase SQL Server or to an Oracle Server database by looking at the name in the Server field in the Session Manager dialog box.

How Names Are Recognized

The table below summarizes how names are recognized.

If the Session Manager sees a Server name …	It assumes a …	Example
all in uppercase letters	Sybase SQL Server database connection	MOZART
suffixed by ":s" or ":S"	Sybase SQL Server database connection	Offenbach:S
containing any lowercase letters	Oracle Server database	Oramozart
suffixed by ":o" or ":O"	Oracle Server database	SIBELIUS:O

Sybase SQL Server Examples

Example 1

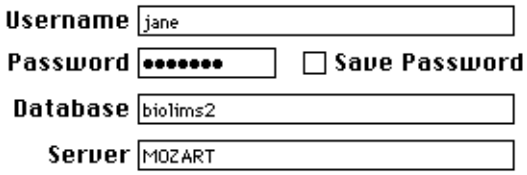
If the interfaces file contains this:

```
MOZART
query MacTCP mac_ether mozart.apldbio.com 2500
```

Note Insert a tab space before the start of the second line if none already exists.

MOZART is recognized as a Sybase SQL Server because the server name is in all uppercase letters.

The Session Manager would look like this:

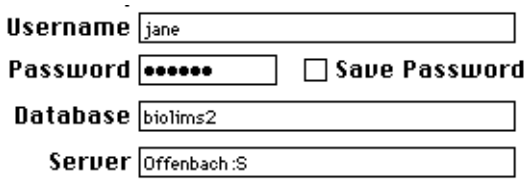


Example 2

If the interfaces file contains this:

```
Offenbach
query MacTCP mac_ether mozart.apldbio.com 2500
```

the Session Manager would look like this:



For Offenbach to be recognized as a Sybase SQL Server, the name in the Server field is suffixed with ":S".

Oracle Server Examples

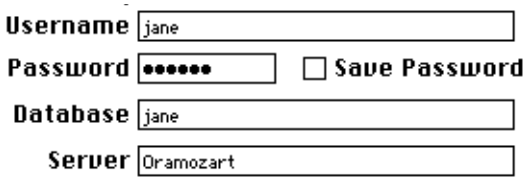
Example 1

If the tnsnames.ora file contains this:

```
Oramozart=(DESCRIPTION=
             (ADDRESS=
              (PROTOCOL=TCP)(host=mozart)(port=1521))
                   (CONNECT_DATA=(SID=WG733)
                  )
           )
```

Oramozart is recognized as an Oracle Server because the server name begins with "O".

The Session Manager would look like this:



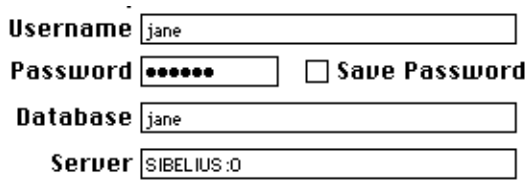
Note The database entry field should contain the name of the schema owner which may be the same as the username.

Example 2

If the tnsnames.ora file contains this:

```
SIBELIUS = (DESCRIPTION=
           (ADDRESS=
           (PROTOCOL=TCP)(host=SIBELIUS)(port=1521))
                  (CONNECT_DATA=(SID=WG733)
                 )
          )
```

the Session Manager would look like this:



For SIBELIUS to be recognized as a Oracle Server, the name in the Server field is suffixed with ":O".

Note The database entry field should contain the name of the schema owner which may be the same as the username.

Using the Collection Browser Window

Applications That Use the Collection Browser Window

The Collection Browser window is common to the following BioLIMS-aware applications.

- AutoAssembler™ DNA Sequence Assembly Software.
- GeneScan® Analysis Software.
- Factura™ Feature Identification Software.
- Sample2DB Software.
- Sequencing Analysis Software.
- Genotyper® Software v. 2.5.

Ways to Search the Database

Using the Collection Browser window from within Genotyper software, you can search the BioLIMS database for specific collections and fragments.

The following table lists ways you can search:

Search by...	See page
Up to 5 collection-specific criteria	13-21
Up to 14 fragment-specific criteria	13-22

In This Section

This subsection includes the following topics.

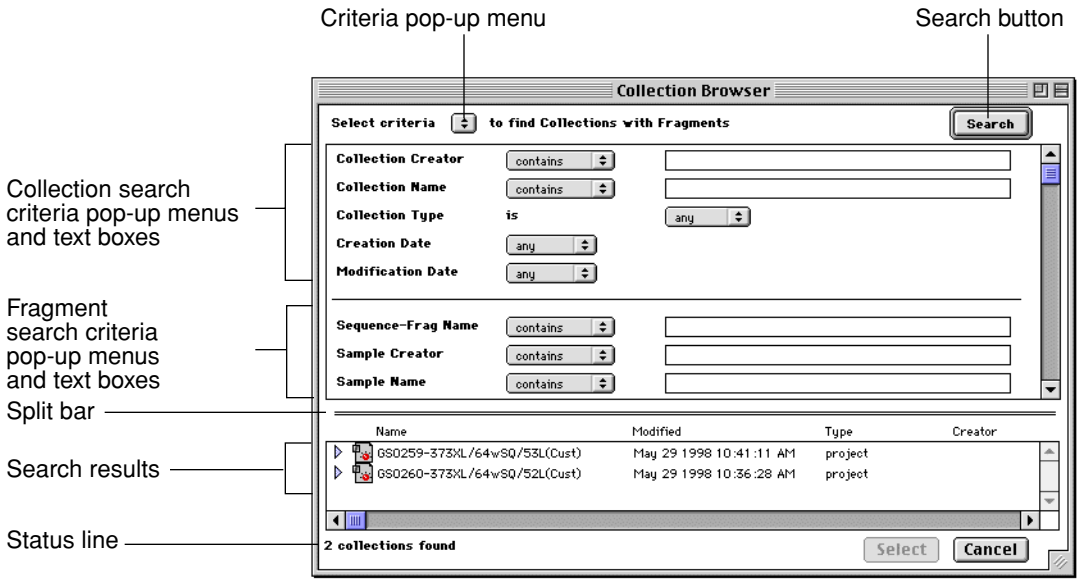
Displaying the Collection Browser Window

To display the Collection Browser window:

If you want to...	Then...	Result
add a fragment to a Genotyper document to view or analyze	choose Import from the File menu and From BioLIMS Database from the submenu.	The Collection Browser window opens if a database connection has been opened. For more information, see “Collection Browser Window Example” on page 13-19.

Collection Browser Window Example

The following is an example of the Collection Browser window.



Parts of the Collection Browser Window

The table below describes the parts of the Collection Browser window that were labeled in the figure above.

Description of the Collection Browser window:

Item	Description
Criteria pop-up menu	Use this pop-up menu to specify the search criteria visible on the Collection Browser window. **Note** If you only intend to use a subset of criteria, setting only that subset visible helps to reduce clutter in the window. The search results are the same whether a criterion is invisible or blank and visible.
Search button	Click this button to query the BioLIMS database. **Note** All collections in the database will be displayed if you click on Search without first specifying any search criteria. **Note** You can also press the Return key to begin a search.
Collection search criteria pop-up menus and text boxes	Use these pop-up menus and text boxes to define the collection criteria of the search. **IMPORTANT** Only those fragments that match each and every criterion you specify are returned. That is, search criteria are combined using the logical AND operation. For more information, see “Collection Search Criteria” on page 13-21.
Fragment search criteria pop-up menu and text boxes	Use these pop-up menus and text boxes to define the fragment criteria of the search. **IMPORTANT** A collection is returned if one or more of the fragments contained in it fulfill all of the specified fragment criteria. **Note** Only fragments meeting search criteria will be displayed in the Collection Browser window. For more information, see “Fragment Search Criteria” on page 13-22.

Description of the Collection Browser window: *(continued)*

Item	Description
Split bar	Drag this bar to alter the relative amount of space allocated to the top and bottom portions of the Collection Browser window.
Search results	After a successful query, found sample files are listed in this area as Name, Modification date, type, and Creator.
Status line	Search results, error messages, and other important information is reported here. For example, the Status Line lists how many collections were returned in a search.

Collection Search Criteria

The table below shows the collection search criteria. The collections returned by the Collection Browser window must match all of the collection criteria and contain at least one fragment that matches all of the fragment criteria.

Allowed Collection Search Criteria:

Criterion	Pop-up Menu Choices	Allowed Text	Description
Collection Creator	♦ is ♦ starts with ♦ ends with ♦ contains	up to 255 characters	Name of the creator/owner of the collection.
Collection Name	♦ is ♦ starts with ♦ ends with ♦ contains	up to 31 characters	Name of the collection.
Collection Type	♦ any ♦ run ♦ project ♦ other	NA	Collection type. Default is any menu item.

Allowed Collection Search Criteria: *(continued)*

Criterion	Pop-up Menu Choices	Allowed Text	Description
Creation Date	♦ any ♦ is ♦ before ♦ after ♦ between	date — set with arrow buttons using the format mm/dd/yy	Date the collection was created.
Modification Date	♦ any ♦ is ♦ before ♦ after ♦ between	date — set with arrow buttons using the format mm/dd/yy	Date the collection was last modified.

Fragment Search Criteria

The table below shows the fragment search criteria. The collections returned by the Collection Browser window must contain at least one fragment that matches all of the specified fragment criteria.

Fragment Search Criteria:

Criterion	Pop-up Menu Choices	Allowed Text	Description
Sequence-Frag Name	♦ is ♦ starts with ♦ ends with ♦ contains	up to 31characters including letters, numbers, and punctuation Cannot use colons (:).	Name of the fragment. This is the file name entered in the Sample Sheet.
Sample Creator	♦ is ♦ starts with ♦ ends with ♦ contains	up to 255 characters including letters, numbers, and punctuation	Name of the person responsible for the run.

Fragment Search Criteria: *(continued)*

Criterion	Pop-up Menu Choices	Allowed Text	Description
Sample Name	♦ is ♦ starts with ♦ ends with ♦ contains	up to 255 characters including letters, numbers, and punctuation	Sample name from the Sample Sheet.
Instrument Name	♦ is ♦ starts with ♦ ends with ♦ contains	up to 255 characters including letters, numbers, and punctuation	Set in the General Settings Preferences of the Data Collection software.
Instrumentation	♦ any ♦ gel ♦ capillary	NA	Whether the sample was run on a gel or capillary instrument.
Start Collect Date	♦ any ♦ is ♦ before ♦ after ♦ between	date— set with arrow buttons using format mm/dd/yy	Date data collection began.
End Collect Date	♦ any ♦ is ♦ before ♦ after ♦ between	date — set with arrow buttons using format mm/dd/yy	Date data collection ended.
Gel Path	♦ is ♦ starts with ♦ ends with ♦ contains	up to 255 characters including letters, numbers, and punctuation	The full path name to the original gel file, e.g., Hard Disk:Data:GelRuns:L28t.

Fragment Search Criteria: *(continued)*

Criterion	Pop-up Menu Choices	Allowed Text	Description
Sample Info	♦ is ♦ starts with ♦ ends with ♦ contains	up to 255 characters including letters, numbers, and punctuation	Sample information from the Sample Sheet.
Sample Comment	♦ is ♦ starts with ♦ ends with ♦ contains	up to 255 characters including letters, numbers, and punctuation	Comment from the Sample Sheet.
Size Data	♦ is present ♦ is not present ♦ does not apply	NA	Is present means that one or more dyes contain sizing information.
			Is not present means none of the dye sample contain sizing information.
Size Calling	♦ done ♦ not done ♦ does not apply	NA	Done means sample file has completed size calling indicated by a size curve.
			Not done indicated by a missing size curve.
% Matched Peaks	♦ any ♦ equal to ♦ less than ♦ greater than ♦ between	0—100	Percentage based on size standard matched peaks divided by size standard defined peaks.

Fragment Search Criteria: *(continued)*

Criterion	Pop-up Menu Choices	Allowed Text	Description
Offscale Data	♦ present ♦ does not apply	NA	Present means the analyzed range contains off-scale dye sample peaks.

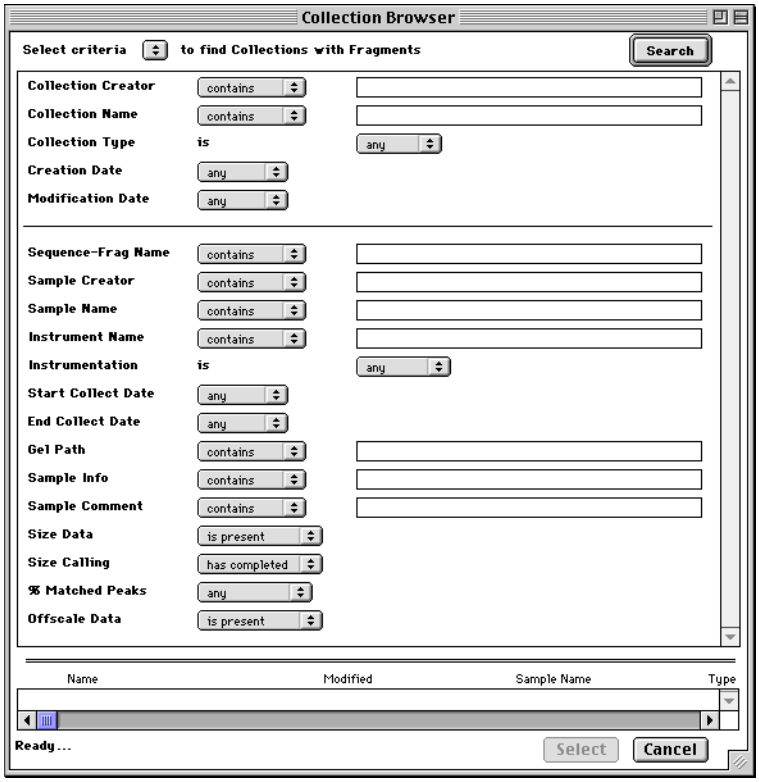
Searching the BioLIMS Database

Follow these steps to use the Collection Browser window to search the BioLIMS database for specific collections and fragments.

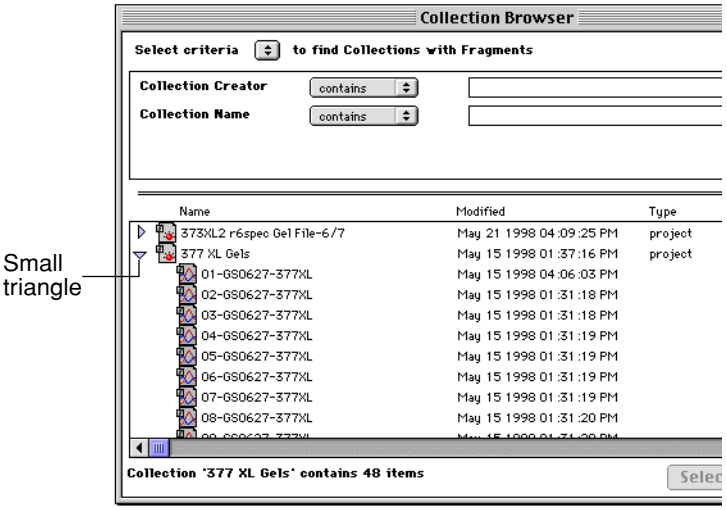
To search the BioLIMS database:

Step	Action
1	**Note** From the Select Criteria pop-up menu, select the criteria by which you want to search **Note** To list all of the items in the BioLIMS database, perform the search with no criteria specified. For large databases, this process may be slow.
	
2	To use the pop-up menu: **Choose menu items...** / **To define the search by...** / **See page** above the horizontal line / Collection Search Criteria / 13-21 below the horizontal line / Fragment Search Criteria / 13-22 **Note** As you choose items from the pop-up menu, a black dot appears next to the item on the menu and the item is added to either the collection search criteria or the fragment name search criteria section of the window.

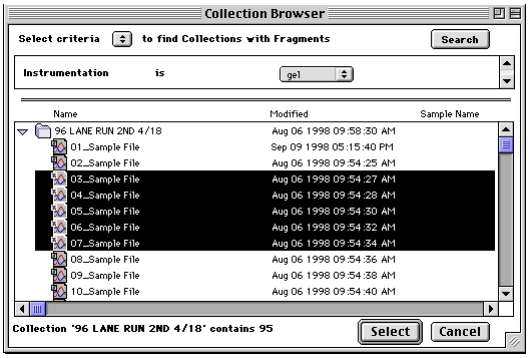
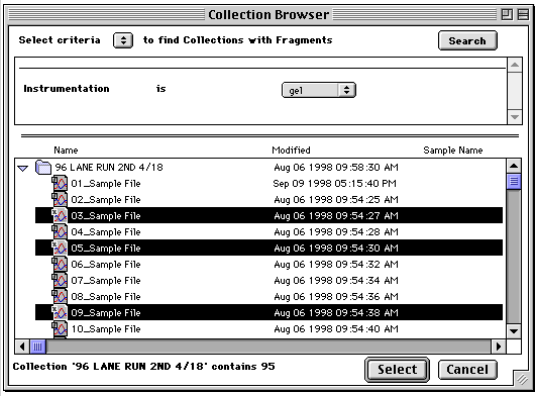
To search the BioLIMS database: *(continued)*

Step	Action
	The following is an example of the Collection Browser window showing all five collection search criteria and all 14 fragment search criteria. 
3	Use the pop-up menus and text fields to define your search query. Refer to "Collection Search Criteria" on page 13-21, and "Fragment Search Criteria" on page 13-22, for details about the search criteria. When you are satisfied with the search, click Search. The results of the search appear in the lower portion of the window. **Note** Collections returned by the Collection Browser window must match all of the collection criteria and contain at least one fragment that matches all of the fragment criteria.

To search the BioLIMS database: *(continued)*

Step	Action
4	To view the fragments contained in the collections, click the small triangle to the left of the collection name. 

To search the BioLIMS database: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>5</td><td>Select one or more fragments. If you wish to select more than one fragment file, refer to the procedure below.
<table>
<tr><th>If...</th><th>Then...</th></tr>
<tr><td>The fragment files you wish to select are listed in sequence
 
</td><td>Hold the shift key and click on the file names.</td></tr>
<tr><td>The fragment files you wish to select are not listed in sequence
 
</td><td>Hold the command key (⌘) and click on the file names.</td></tr>
</table></td></tr>
<tr><td>6</td><td>Click Select in the Collection Browser window or press Return on your keyboard to import the selected files.</td></tr>
</table>

Importing and Processing a 5th Dye 14

Chapter Overview

New Feature of Genotyper V. 2.5

Genotyper Software version 2.5 has the ability to read and process GeneScan sample files containing a 5th dye. Further, Genotyper processed data with a 5th fluorescent dye can be exported to a GenBase database.

Note Reagent kits containing a 5th dye may be available at a future date.

In This Chapter

This chapter describes how to import, view, analyze, and export GeneScan data containing a 5th fluorescent dye.

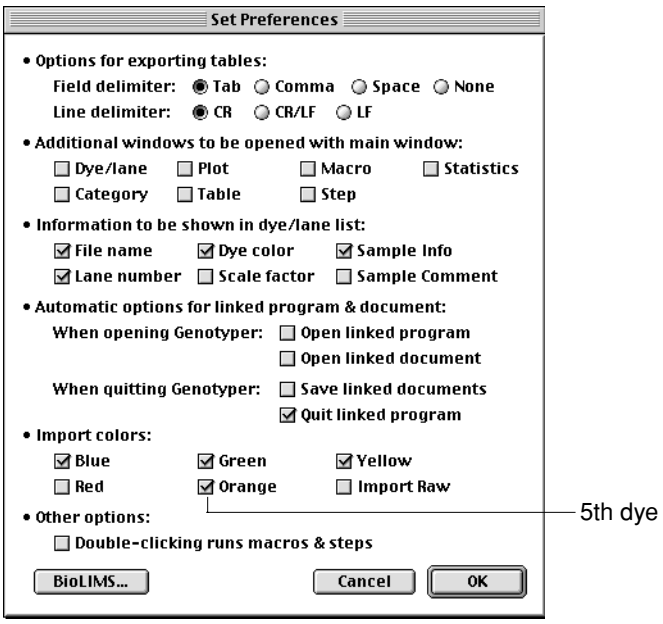
Importing 5th Dye Data

Procedure for Importing 5th Dye Data

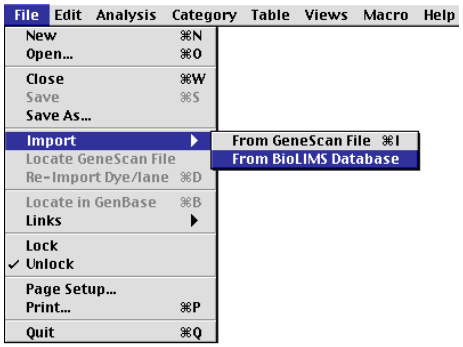
Data containing a 5th dye can be imported, just like data on any of the other four dyes, from either GeneScan sample files or a BioLIMS database.

You must first choose the dyes you wish to import from the Set Preferences dialog box. You can access this dialog box from the Edit pull-down menu.

To import 5th dye data:

Step	Action
1	Select Set Preferences... from the Edit pull-down menu of Genotyper Software v. 2.5. The Set Preferences dialog box appears.
2	Choose the dyes by clicking their respective boxes under Import colors in the Set Preferences dialog box. **Note** The 5th dye is designated Orange. 

To import 5th dye data: *(continued)*

Step	Action
3	Click OK. The Set Preferences box goes away and you are returned to the Main window.
4	From the File menu, point to Import and click on either From GeneScan or From BioLIMS Database. 

Viewing Data

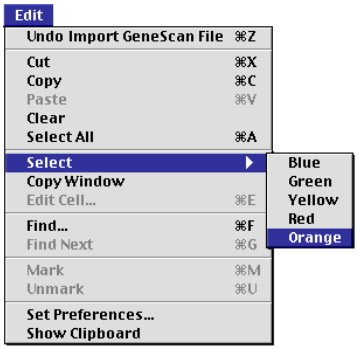
Two Methods to View Data

There are two ways to view dye data in the Main window.

- Use the Menu bar or
- Use the Dye buttons on the Main window.

Using the Menu Bar

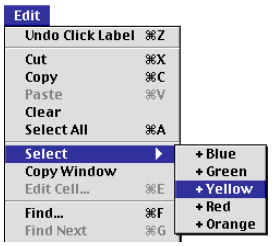
To use the menu bar to view dye data:

Step	Action
1	From the Edit menu, point to Select and click on the dye you wish to view.  Data for that dye are graphically displayed as peaks in the main window.

To use the menu bar to view dye data: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>To display data for more than one dye in the same window,
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>From the Edit menu, point to Select and hold down the shift key.

A + symbol appears to the left of each dye in the menu window. This indicates that data from more than one dye can be selected for viewing.

 
</td></tr>
<tr><td>2</td><td>With the shift key held down, click on a dye.

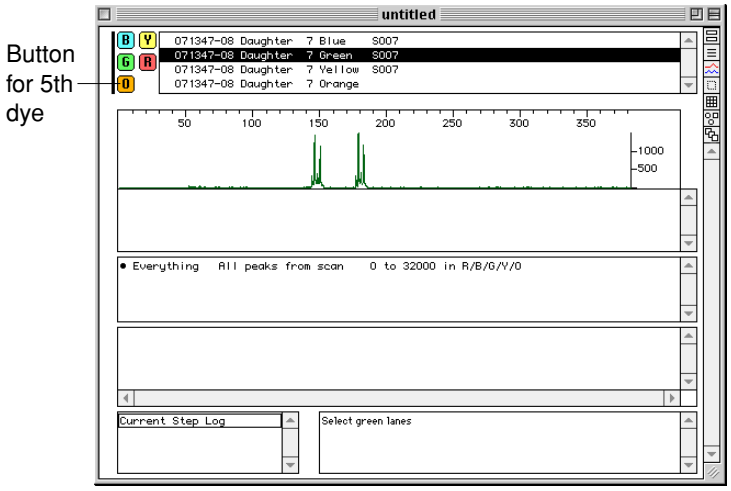
The data for the first dye selected is graphically displayed in the Main Window.</td></tr>
<tr><td>3</td><td>Repeat step 2 to add more dyes to the same Main window.

Data from the each dye selected is graphically displayed in the same window as the first dye.</td></tr>
</table></td></tr>
</table>

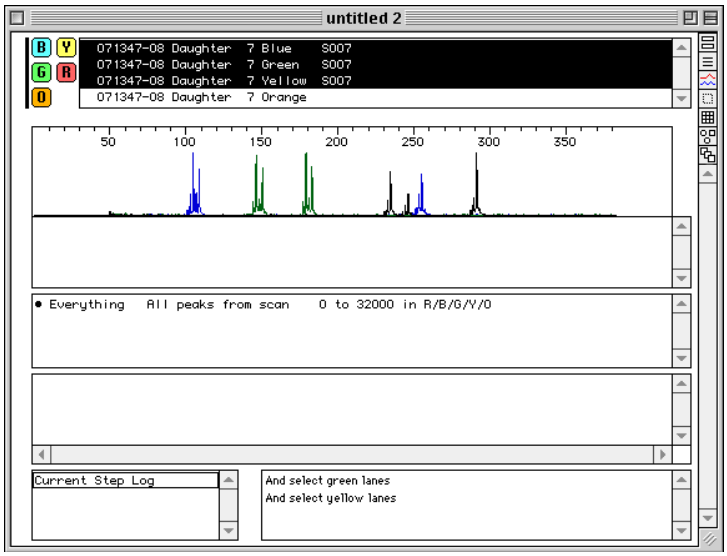
Using the Dye Buttons

To use the dye buttons to view data:

Step	Action
1	Click the dye buttons on the Main window to display the data for each dye.



To use the dye buttons to view data: *(continued)*

Step	Action
2	To display data from more than one dye in the same window, hold down the shift key and click on the buttons of the dyes you want to view. Data for the dyes you selected will be displayed in the same window. 

Analyzing and Exporting Data

Analyzing Data Data containing a 5th dye can be analyzed just like the other four dyes.

Exporting Data The final results from the Genotyper table can be exported either:

- To a GenBase database
- As text files.

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