Genotyper® 3.5 NT Software

Applications Tutorials

PE Biosystems

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Genotyper Software Tutorials 1

Chapter Overview

Introduction This manual is designed to familiarize you with using Genotyper® 3.5 NT software. The manual consists of three tutorials that introduce you to two common genotyping applications:

Application	Description
Microsatellite Analysis Part 1 and Microsatellite Analysis Part 2	Analyze GeneScan data containing microsatellite markers and generate genotype tables for export to linkage analysis applications.
Human Identification Tutorial	Create allelic tables for unknown forensic samples.

In This Chapter This chapter contains the following topics:

Topic	See page
How This Tutorial is Organized	1-2
What You Will Learn	1-3
Guidelines for Running Genotyping Applications	1-4
Guidelines for Using the Genotyper Software	1-5

How This Tutorial is Organized

Introduction

This manual consists of three different tutorials. It is designed as a step-by-step guide learning several features of the Genotyper software. The instructions and illustrations assume that you have followed the tutorial step-by-step.

Parts of the Tutorial

The tutorial is organized into three genotyping applications

- Chapter 2, “Microsatellite Analysis Part 1.”
- Chapter 3, “Microsatellite Analysis Part 2.”
- Chapter 4, “Human Identification Tutorial.”

Assumptions

This tutorial assumes that you are familiar with the operations of the ABI PRISM® DNA Sequencing Instruments and the GeneScan® Analysis software.

Refer to the particular ABI PRISM™ 377 DNA Sequencer Protocols for detailed protocols for setting up the PCR reactions, and for running the amplified fluorescent products on the ABI PRISM 377.

What You Will Learn

Introduction

Each of the tutorials provides a step by step way to learn the features and uses of the Genotyper software. Each chapter emphasizes different features in the software.

Goals

After completing all of the tutorials, you should be able to:

- Run two different kinds of genotyping applications.
- Use features of the Genotyper software.
- Prepare allele information for use in linkage applications.

ABI PRISM Genotyping Application Kits

You will use the following application kits in these tutorials:

- Fluorescent Genotyping Demonstration Kit.
- ABI PRISM linkage mapping panels.
- AmpFℓSTR™ Blue kit.

Features You Will Use

In these tutorials, you will use the following Genotyper software features:

- Importing GeneScan Analysis software files.
- Defining categories and markers.
- Editing categories.
- Labeling allele peaks.
- Filtering unwanted allele peaks.
- Constructing and filling tables.
- Constructing and running macros.
- Exporting Genotyper data.

Guidelines for Running Genotyping Applications

Introduction

In order for you to use this manual and the Genotyper software, it is important to follow basic guidelines when preparing samples and analyzing them using the GeneScan Analysis software.

Preparing Samples

Adjust the pooling conditions to dilute the amplified products that consistently yield off-scale data. Optimal results can be obtained with peak heights of approximately 1000 fluorescent units.

Creating Sample Sheets

Enter sample information in the GeneScan Sample Sheet for each sample so that sample tracking will be easier in the database. It is quite possible that several samples of blood may be taken from the same individual, or that several DNA preps may have been carried out from the single blood sample for an individual.

In order to keep track of this information, it is important to enter the sample information (tube number, DNA number, sample number, and so on) in the Sample Sheet.

Choosing Data-Collection Parameters

Follow these guidelines when choosing data collection parameters:

- Ensure that run parameters are consistent from run-to-run (for example, gel percentage, and Run Module files).
- Choose the right matrix for the right type of gel and run parameters. Using a poor/incorrect matrix will result in off-scale data that is not baselined properly, leading to anomalous peaks being sized.

Choosing Size-Calling Methods

To ensure consistency in size calling, use the same size-calling method for every run. Setting the minimum peak height for red too high may result in size-standard peaks being ignored. In lanes where extraneous bands are being called as size markers, use the “User defined” option to set the value of these peaks to zero.

Guidelines for Using the Genotyper Software

Introduction

Ensure that you set up and follow the procedures in this tutorial step-by-step, and do not introduce any extraneous samples or categories while working through the tutorials. Adhering to the following guidelines will make the tutorial more useful.

Planning out Results Tables

You can simplify the use of the Genotyper software by thinking of how you can best present your results data in tabular format. The earlier in the genotyping process that you can decide what kind of table you want to create, the easier it will be for you to create tables that reveal the significance of your results data.

Recording Genotyper Steps

Steps Recorded in Step List

Most of the steps (commands) used in the Genotyper software will be recorded in the Step List (the bottom-right box in the Main window).

This Step List can later be edited and saved as a macro. Macros are very useful tools for repetitive processing of similar types of data.

Example of Using the Step List

For example, you can set up the categories for all the markers in a linkage project, process the data from the first gel, and make a macro with the common steps that you must go through to process the initial data. You can then create a template containing the categories and the macro that were set up. You can then import the next set of data and process it automatically using the macro and the template without going through all the steps again manually.

In this tutorial, you will create a macro and a template.

Running Macros

To run a macro:

You can either...
press the Ctrl+[assigned key] that you selected when saving the macro.
double-click the macro name. **To Run a Macro or Perform a Step by Double-Clicking** Choose Set Preferences from the Edit menu, and under Other options, select the “Double-clicking runs macros and steps” checkbox.

Viewing Documents

The following table lists how to view full-screen windows that show the part of a Genotyper Document with which you are working.

If...	Then either...
you prefer to see more of the categories at one time	♦ Choose the Show Categories Window command (Ctrl+K) from the Views menu or, ♦ Click on the Categories Window icon ( ) at the right side of the Main window.
you prefer to view more dye/lanes at one time	either: ♦ Choose the Show Dye/lanes command from the Views menu, or ♦ Clicking on the Dye/lanes icon ( ) at the right side of the Main window to open the Dye/lanes window.

If...	Then either...
you want to view more of the plots	either: ♦ Choose the Show Plot Window command (Ctrl+Y) from the Views menu, or ♦ Click on the Plot icon ( ) on the right side of the main window. The Plot window shows all the dye/lanes as separate plots for each color, with all the peaks labeled with the size label. Move this window to one side of the computer screen to see the effects of the next step.

Filtering Labels

When you open this dialog box by choosing the Filter Labels command from the Analysis menu, you will see that the last three of the four boxes for the filtering parameters have already been preselected by default.

The default parameters work well for most dinucleotide repeat markers. The filtering operations listed in this dialog box are performed one at a time, in the order they are listed.

Use the...	To remove labels from...
first checkbox	most of the small (noise) peaks that have a peak height of 32 percent or less than the true alleles.
second checkbox	the +A peaks if their peak height is less than that of the true allele associated with it, and if the +A peak is within 1.6 bp from the true allele.
third checkbox	stutter peaks.

Microsatellite Analysis Part 1 2

Chapter Overview

Introduction This tutorial shows you how to use the Genotyper® 3.5 NT software to analyze patterns of inheritance using family marker data available in the Fluorescent Genotyping Demonstration Kit.

In This Chapter This chapter contains the following topics.

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Overview of Part 1 of the Microsatellite Tutorial

Introduction

In Part 1, you will use the Genotyper software to process Fluorescent Genotyping Demonstration Kit GeneScan® Analysis software files, and create an allelic size table to discriminate between heterozyote and homozygote individuals.

Goals

The goal of Part 1 of the microsatellite tutorial is to learn how to use the Genotyper software features and commands to:

- Process GeneScan Sample files that have been generated from the Linkage Demonstration Kit.
- Create a Genotyper table that contains two columns (one for each allele) for each marker supplied in the kit.

Heterozygote or Homozygote Markers

The following table lists if an individual is a heterozygote or a homozygote for a marker:

If...	Then...
a particular individual is a heterozygote for a marker	there will be two peaks represented with their sizes in bp.
an individual is a homozygote	there will be only one allelic peak represented.

Features You Will Use

You will learn the following features of Genotyper software in this tutorial:

- Creating a new document.
- Creating categories.
- Sorting categories.
- Labeling and filtering peaks.
- Setting up and exporting tables.
- Creating and running macros.
- Saving a document as a template.

Contents of the Fluorescent Genotyping Demonstration Kit

Introduction You will use data from the Fluorescent Genotyping Demonstration Kit for this Microsatellite Analysis tutorial.

Panels The Fluorescent Genotyping Demonstration Kit contains six dinucleotide repeat markers from ABI PRISM Fluorescent Genotyping Panels that are fluorescently labeled with FAM, HEX, and TET amidites. The data from these markers were obtained by typing them on 14 individuals from the CEPH K1347 family.

Markers The Fluorescent Genotyping Demonstration Kit markers used for this tutorial are shown in below.

Demonstration Kit Markers:

Marker Name	Dye Label	Allele Size Range (bp)	Heterozygosity
D12S83	FAM	98-113	0.81
D7S517	FAM	235-261	0.83
D1S220	HEX	226-260	0.82
D3S1266	HEX	281-303	0.81
D2S391	TET	139-153	0.79
D13S171	TET	171-197	0.73

How Fluorescent Genotyping Data Was Preprocessed

Introduction

Fluorescently labeled PCR products were electrophoresed on a ABI PRISM® 377 DNA Sequencer under the following conditions:

- Gel Type – 4%, 36-cm WTR gel.
- Run Module – GS 36C-2400 Run module for two hours.
- Analysis Software – GeneScan Analysis software 3.0.

GeneScan Analysis Parameters

The following table shows the parameters set in the GeneScan Analysis software for sizing sample fragments.

GeneScan Analysis software parameters:

Peak Detection Threshold	Peak Detection Minimum Half-width	Sizing Method	Std. Peak Det. Threshold
50	3	Local Southern	50

Source of DNA Template Samples

Introduction

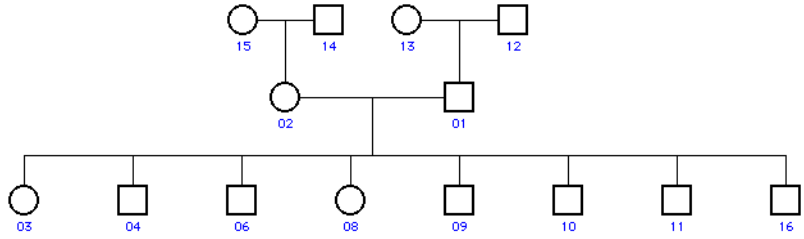
This tutorial provides a pedigree for the samples being used. The pedigree information is part of the sample file name in the GeneScan Analysis software.

Family

The DNA template samples used in this kit are from the CEPH K1347 family, which includes two parents, two sets of grandparents, and eight siblings.

Pedigree Structure

The following figure shows the pedigree structure for the CEPH K1347 family.



Individual IDs

The number under each family member in the “Pedigree Structure” above refers to the individual ID in this family.

Abbreviations of Individuals

Some of the abbreviations used in the sample file names in this data set are as follows:

Abbreviation	Meaning
PGM	Paternal grandmother
PGF	Paternal grandfather
MGM	Maternal grandmother
MGF	Maternal grandfather

Creating a New Genotyper Document

About This Step

To perform genotyping tasks on the Fluorescent Genotyping Demonstration Kit marker data, you must import the related the GeneScan Analysis software files into a Genotyper document.

Procedure

The following steps are used to create a new document:

If...	Then...	Result
Genotyper is already open	select New (Ctrl+N) from the File menu. The Main window appears.	A new “Untitled Genotyper Document appears.
Genotyper is closed	open Genotyper by double-clicking the application’s icon. 	

Defining Categories for Markers

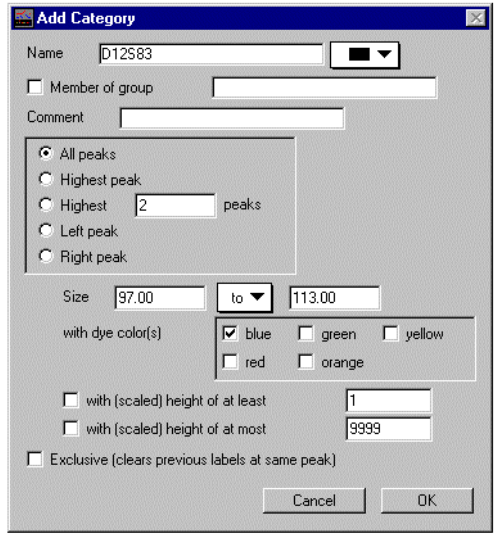
What Is A Category?

Because each GeneScan sample file can contain data from many markers in different size ranges (and up to three different colors) you will need to define the boundaries for each marker. The boundaries are based on the expected allele size range for that marker, and are used to define a category.

Defining Categories

The categories you will be defining are from the Fluorescent Genotyping Demonstration kit (see “Markers” on page 2-3).

To define categories:

Step	Action
1	Choose Clear Category List from the Analysis menu. **Note** This removes the default “Everything” category in the Category window.
2	Choose Add Category from the Category menu (or press Ctrl+L). The following window appears: 
3	Type in the name of the first marker in the Fluorescent Genotyping Demonstration Kit as the Category name (see “Markers” on page 2-3).

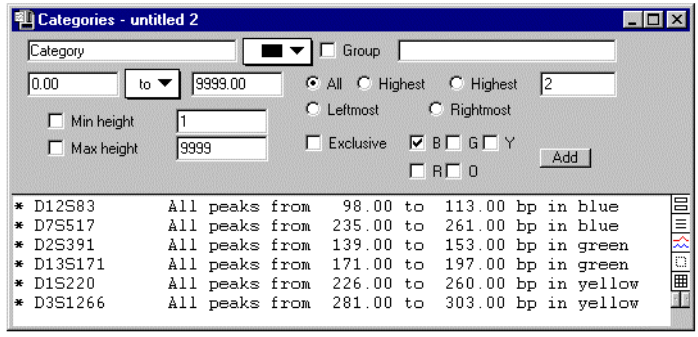
To define categories: *(continued)*

Step	Action
4	In the pop-up menu next to the Category name, select the same color as the dye used to fluorescently label the marker. For example, choose blue for FAM-labeled markers. **Note** Use orange for the HEX markers and green for the TET markers.
5	Click the All peaks radio button.
6	Type in the expected allele size range for the marker name you entered in step 3 on page 2-7. **Note** For marker allele size ranges, see “Markers” on page 2-3.
7	In the dye color checkboxes, select the dye color of the marker.
8	Click OK.
9	Repeat each of these steps for all six markers shown in “Markers” on page 2-3.

Viewing the Category List

The following table lists how to view the Category List.

You can either...	Result
choose Show Categories Window (Ctrl+K) from the Views menu, or	You should now have a Category list that looks like the figure below.
click on the Categories window icon ( ) on the right edge of the Main window	



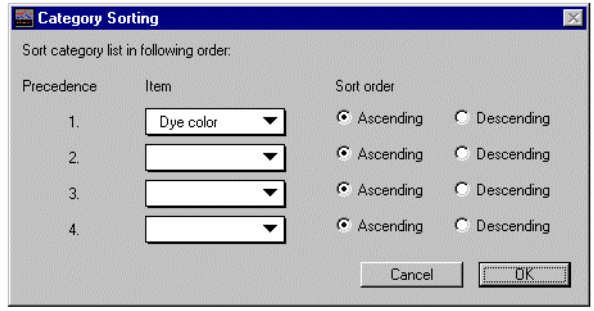
Sorting Categories by Dye Colors

About This Step

The default sort order of the Category list is by the name of the category. You will use the Category Sorting command to sort the categories by dye color.

Procedure

To sort categories by dye color:

Step	Action
1	Choose Category Sorting from the Views menu. The following window appears: 
2	Set the sort order to Dye color in the first pop-up menu.
3	Click OK.

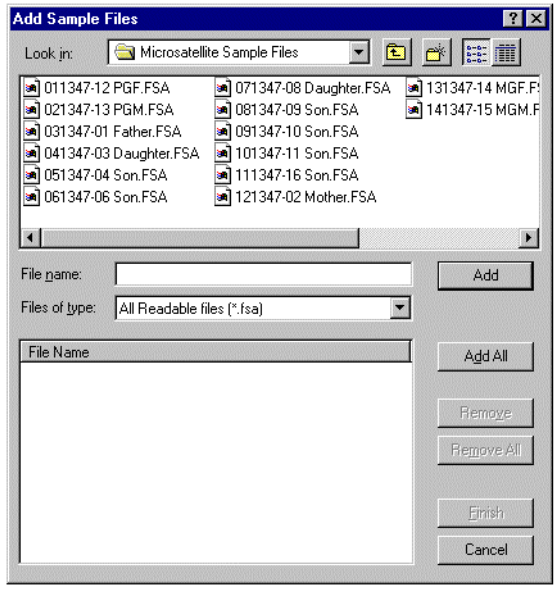
Importing GeneScan Analysis Software Files

About This Step

Once categories are defined, you must import the appropriate GeneScan Analysis software files containing data for these categories.

Procedure

To import GeneScan Analysis software files from the “Microsatellite Sample Files” folder.

Step	Action
1	Choose Import from the File menu and From GeneScan File (Ctrl+I) from the submenu and navigate to the appropriate folder. The Add Sample Files dialog box should appear as follows: 
2	Click Add All to import all the sample files from the folder and click Finish. The Dye/lanes pane should now display all of the sample files.

Viewing GeneScan Files

Once you've imported GeneScan Analysis software files, you can view dye/lanes from the imported files in the Dye/lanes list.

There are two ways to display the Dye/lanes window:

You can either...	Result
click the Dye/lanes window icon ( ) from the Main window, or	The Dye/lanes window appears. The window should now list all of the imported sample files with all three colors. Each sample file is broken down into three dye/lanes, one for each color in that file. Four dye/lanes are displayed for each sample file if you include the red dye in the Import command.
choose Show Dye/lanes Window from the Views menu	

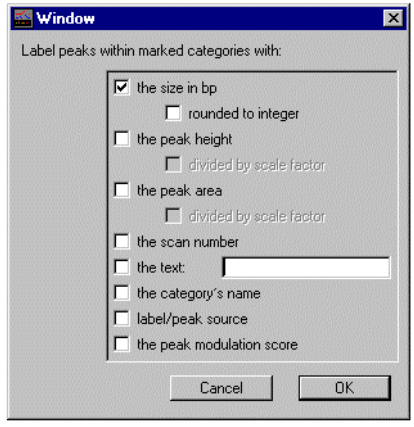
Labeling Allele Peak Data

About This Step

For this step, you must label all the allele peaks for each marker with the respective size in base pairs.

Procedure

To assign labels to all allele peaks in each marker:

Step	Action
1	To make the Dye/lane pane active either: ♦ Click the the pane, or ♦ Tab until a vertical bar appears next to the Dye/lane pane.
2	To select all the dye/lanes in these three colors, either: ♦ Choose Select All (Ctrl+A) from the Edit menu, or ♦ Click on the B, G, Y buttons while holding down the Ctrl+Shift keys. **Viewing More of the Dye/lanes at One Time** You can either: ♦ Choose the Show Dye/lanes Window from the Views menu, or ♦ Click the Dye/lanes icon (☰) located on the right side of the Main window to open the Dye/lane window.
3	Choose Label peaks from the Analysis menu. The following dialog box appears: 

To assign labels to all allele peaks in each marker: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>Check the “Size in bp” box (default) and click OK.

You can also take the following action:

<table>
<tr><th>To view...</th><th>Then...</th></tr>
<tr><td>the labels</td><td>select individual dye/lanes.

Each and every peak that has been sized in GeneScan (including stutters, +A and background peaks) will be labeled with the size in bp.</td></tr>
<tr><td>multiple plots</td><td>Either:

♦ Choose Show Plot Window (Ctrl+Y) from the Views menu.

♦ Click the Plot window icon ( ) in the Main window.</td></tr>
</table>
</td></tr>
</table>

Filtering Peak Labels

About This Step

Since you are interested in placing labels on only those peaks that correspond to the allelic peaks, you must filter out labels from the remainder of the unwanted peaks.

This operation will remove labels from all the unwanted peaks and leave size labels on only the allele peaks of interest for each category. Once this is completed, you are ready to build a table containing the cleaned-up data.

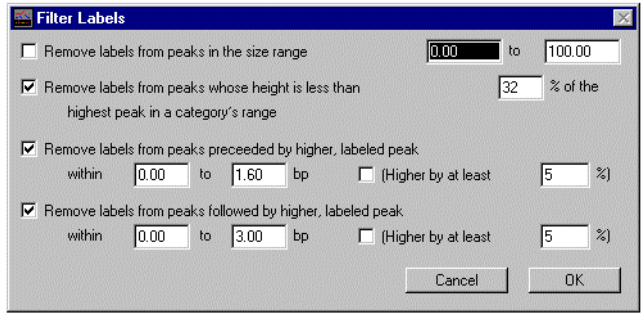
Viewing How Labels Are Filtered

To see how labels are filtered:

You can either...
Choose Show Plot Window (Ctrl+Y) from the Views menu
Click the Plot window icon( ) on the right edge of the Main window

Procedure

To filter peak labels:

Step	Action
1	Select Filter labels from the Analysis menu. The following dialog box appears: 
2	Click OK. **Note** The last three of the four boxes for the filtering parameters have already been pre-selected as default.

Using the Filter Labels Dialog Box

The default parameters work well for most dinucleotide repeat markers. The filtering operations listed in this dialog box are performed one at a time, in the order they are listed.

Use the...	To remove labels from...
first checkbox	most of the small (noise) peaks that have a peak height of 32 percent or less than the true alleles.
second checkbox	the +A peaks if their peak height is less than that of the true allele associated with it, and if the +A peak is within 1.6 bp from the true allele.
third checkbox	stutter peaks.

Setting Up an Allele Table

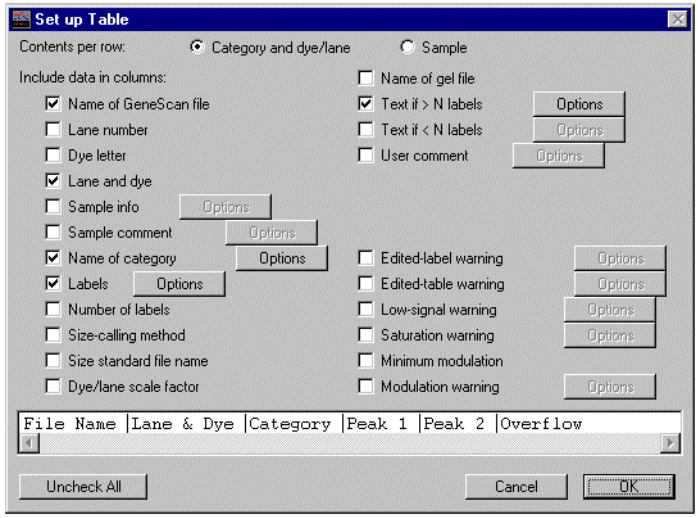
About This Step

For this step, you will create a table containing the following information: file name, sample info, category name, two columns for each allele designation per sample, and an overflow column to warn you if there are too many allele peaks (more than two) detected.

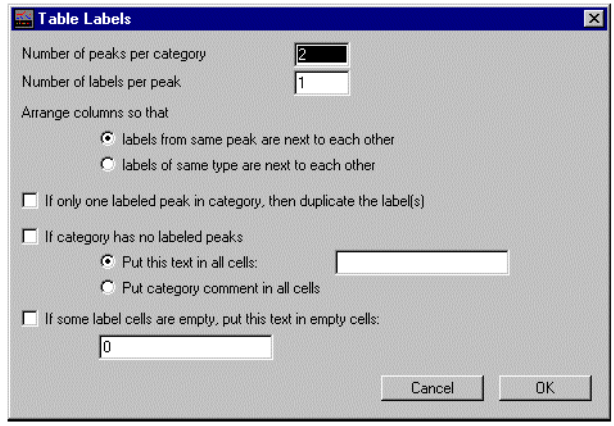
Procedure

To set up the table in the “Column Format of Table Example” on page 2-17.

To set up a table:

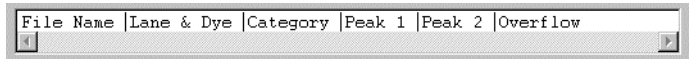
Step	Action
1	Choose Set up Table from the Table menu. The following window appears: 
2	Check the column title boxes exactly as shown here.

To set up a table: *(continued)*

Step	Action
3	Click the Options button next to Labels. The following dialog box appears: 
4	Click OK to accept the defaults. The defaults of the label options create a table that includes a maximum of two peaks (alleles) per category (marker) with both alleles arranged next to each other in the table. **Note** The overflow column will be flagged if there are more than two alleles per category.

Column Format of Table Example

When you have completed the setup for the table, the column format for the table should look like the figure below.



The Concept of Overflow

If there are any anomalies (such as three peaks labeled for a particular individual/marker), a text entry, “Overflow,” will be displayed in the Overflow column, indicating that there is a problem with the data from that particular individual/marker.

Note “Text if > N labels” is the option in the Set up Table window to create an Overflow column.

Appending Rows of Filtered Peak Data

About This Step

For this step, you must append to the table the “cleaned up” data.

Viewing Table Data

To view table data:

Step	Action
1	Select Append to Table from the Table menu.
2	You can either:

You can either...	Result
click the Table button.	This displays a table with rows of peak height and category data.
choose Show Table Window (Ctrl+T) from the Views menu.	

The final table should now appear as follows.

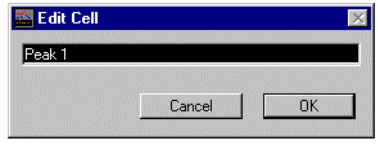
Table - Microsatellite Template.GTA

File Name	Lane & Dye	Category	Peak 1	Peak 2	Overflow
011347-12 PGF.FSA	1B	D12S83	100.82	108.61	
011347-12 PGF.FSA	1B	D7S517	254.88	254.88	
011347-12 PGF.FSA	1G	D2S391	148.24	152.23	
011347-12 PGF.FSA	1G	D13S171	182.74	192.57	
011347-12 PGF.FSA	1Y	D1S220	232.49	234.39	
011347-12 PGF.FSA	1Y	D3S1266	289.08	290.92	
021347-13 PGM.FSA	2B	D12S83	100.93	104.75	
021347-13 PGM.FSA	2B	D7S517	249.11	251.07	
021347-13 PGM.FSA	2G	D2S391	146.18	146.18	
021347-13 PGM.FSA	2G	D13S171	178.86	192.59	
021347-13 PGM.FSA	2Y	D1S220	234.39	244.15	
021347-13 PGM.FSA	2Y	D3S1266	290.94	296.73	
031347-01 Father.FSA	3B	D12S83	100.81	104.62	
031347-01 Father.FSA	3B	D7S517	249.21	254.88	
031347-01 Father.FSA	3G	D2S391	146.18	152.34	
031347-01 Father.FSA	3G	D13S171	178.86	192.59	
031347-01 Father.FSA	3Y	D1S220	234.39	234.39	
031347-01 Father.FSA	3Y	D3S1266	289.01	290.94	

Editing Column Headings

The column headings for the size labels can be edited with different text.

To edit the column headings:

Step	Action
1	Click the column heading you want to edit (Peak 1, for example).
2	Select Edit Cell (Ctrl+ E) from the Edit menu. The following dialog box appears. 
3	Type in a new name in the Edit Cell dialog box. For example, Peak 1 and Peak 2 column headings can be edited to Allele 1 and Allele 2.
4	Click OK.

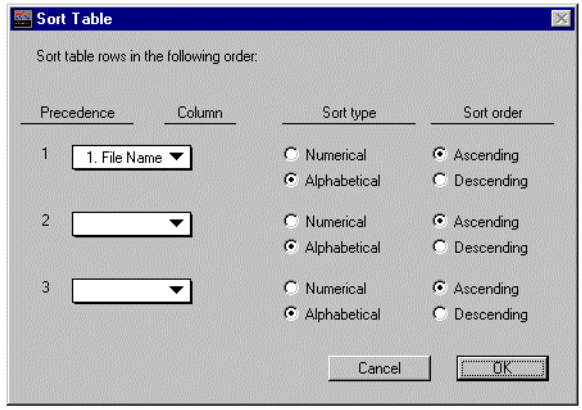
Sorting the Table by Column Heading

About This Step

For this step, you change the contents of the table to be alphanumerically sorted to group the data by any of the column headings.

Procedure

To change the sort order:

Step	Action
1	Choose Sort Table from the Table menu. The following dialog box appears: 
2	Choose Category in the first pop-up and File Name in the second pop-up. **Note** It is easier to view the table if you sort the contents by category and then by file name.
3	Click OK.

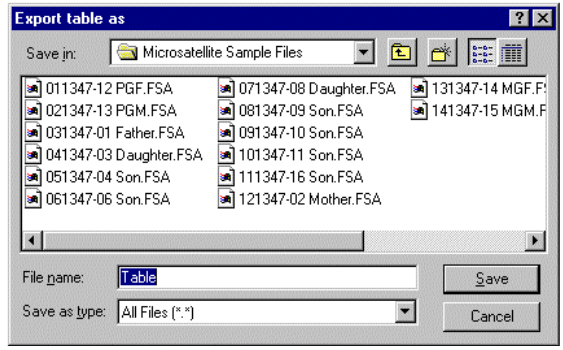
Exporting the Table

About This Step

For this step, you export the table as a separate tab-and-carriage-return-delimited text file.

Procedure

To export the table as a text file:

Step	Action
1	Select Export to File from the Table menu. The Export table as dialog box appears.
2	In the File name text box, assign a name to the table. 
3	Click Save.
4	Choose Clear Table from the Analysis menu.

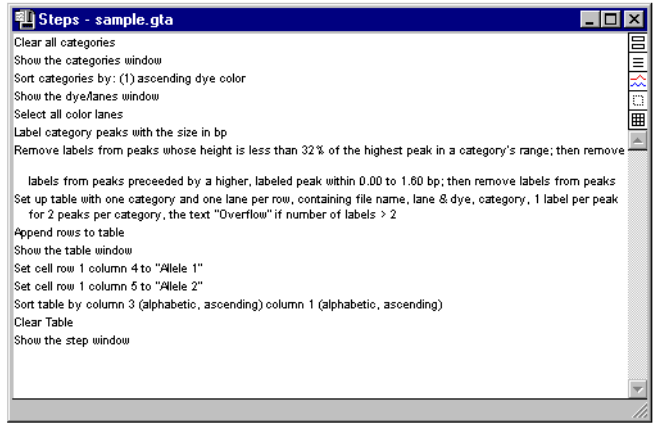
Making a Macro of Tutorial Procedures

Introduction

Most of the steps (commands that you have used so far) have been recorded in the Step List window in the bottom-right corner of the Main window.

Viewing the Steps in the Macro

Choose the Show Step Window command from the Views menu. The following is an example of the Show Step Window.



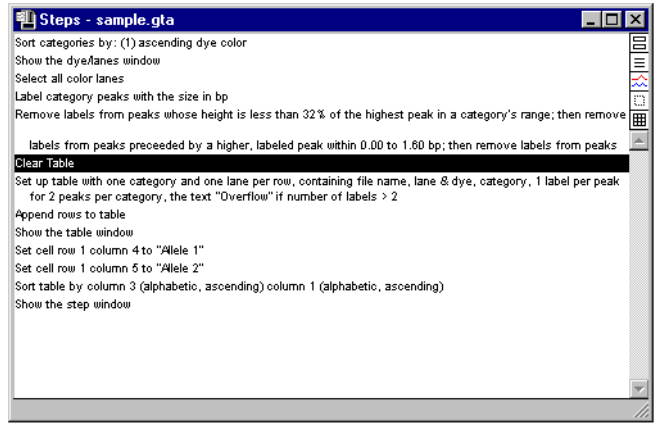
Editing Steps

You can now edit and save this list as a Macro. There are some steps in this list that you do not need to use the next time you want to process more data for the same project.

To edit the steps:

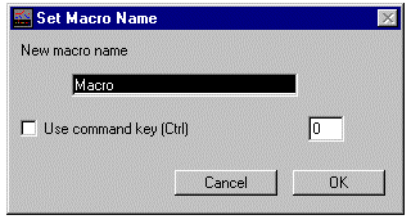
Step	Action
1	While pressing the Ctrl+key, click and select the following steps in the Step window: ♦ Clear all categories. ♦ Show the Categories window. ♦ Other Show commands (optional).
2	To clear these two steps, select Clear from the Edit menu. **Note** The standard Cut (Ctrl+X), Copy (Ctrl+C), and Paste (Ctrl+V), commands also work in this window.

To edit the steps: *(continued)*

Step	Action
3	Select and cut the “Clear Table” step using the Cut (Ctrl+X) command from the Edit menu.
4	Select the “Remove labels from peaks” step and choose the Paste command from the Edit menu. This will insert the step “Clear Table” after the “Remove labels” step and before the “Set up Table” step.
5	Clear from the step list any other commands that you don’t want to be included in the macro. The step list should look like the following figure. 

Creating the Macro

To create a macro:

Step	Action
1	Select Save Step Log from the Macro menu. The following dialog box appears: 
2	Enter a unique name for the macro in the text box.
3	Click the “Use command key (Ctrl+)” checkbox and type in a number for the command key shortcut in the text box.
4	Click OK. **Note** This will clear the Step window.

Running the Macro

To test the macro:

Step	Action
1	Select Clear Dye/Lane List from the Analysis menu.
2	Import the same GeneScan Analysis software sample files you used in the preceding tasks.
3	To run the macro:

You can either...	Result
Press the Command key short-cut (Ctrl+N, where *N* is the number you entered when saving the macro)	The Genotyper software will automatically process the sample files and generate a table containing the allele sizes.
Double-click the macro name in the Macro list window located in the lower-left corner of the Main window. **Note** To run a macro by double-clicking, you must choose Set Preferences from the Edit menu and check the box "Double-clicking runs macros and steps."	

If the Macro Does Not Work

If the macro doesn't generate the table, go back through the preceding steps for generating a macro, and check the steps in the Step window against procedures in this chapter.

Making a Template for the Tutorial

Introduction

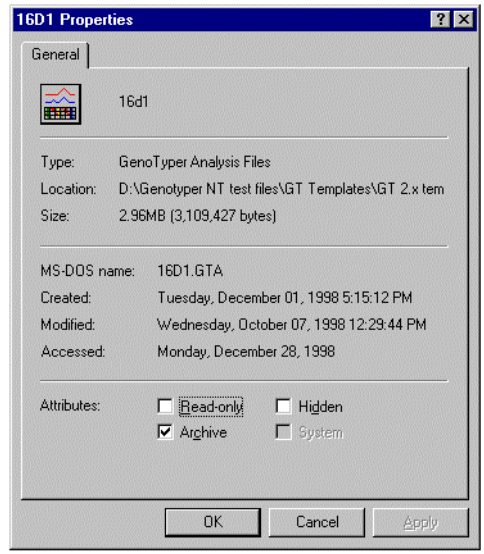
Once you have set up the required categories and created a macro, you can create a template (stationery pad) out of the Genotyper file. You can then use this file to perform large-scale data processing automatically without having to set up the categories or write the macros again.

Creating a Template

To create a template:

Step	Action
1	Select Clear Dye/Lane List and Clear Table from the Analysis menu. You should now have a document that contains only the categories and the macro.
2	Select Current Step Log from the pane in the lower left corner of the Main window and choose Clear Step Log from the Macro menu.
3	Save and close this document.
4	Open the folder that contains the newly saved document.
5	Click once to highlight the document file name.

To create a template: *(continued)*

Step	Action
6	Choose Properties from the File menu. The following dialog box appears. 
7	Check Read-only under the Attributes heading.
8	Close the Properties window. The document is now a read-only template, and will always retain the original categories and macro information. **Note** Such templates are permanent documents whose contents cannot be changed. When you open a read-only template, you can change/modify and save them to a new file only.

Using the Template

To use the template:

You can either...	Result
Double-click the template icon.	A new, untitled document will be opened that cotnains the categories and macros into which you can now import the GeneScan Analysis software files.
a. Choose Open (Ctrl+O) from the File menu and navigate to the folder containing the template. b. Select the template and click the Open button.	

Microsatellite Analysis Part 2 3

Chapter Overview

Introduction This is the second part of the Microsatellite tutorial. This chapter shows you how to use features of the Genotyper® 3.5 NT software to analyze patterns of inheritance using family marker data available in the Fluorescent Genotyping Demonstration Kit.

In This Chapter This chapter contains the following topics.

Topic	See page
Overview of Part 2 of the Microsatellite Tutorial	3-2
Importing GeneScan Analysis Software Files	3-3
Labeling Allele Peaks with Size Labels	3-4
Filtering Peak Labels	3-6
Defining Category Groups for Marker Alleles	3-8
Making New Categories for Alleles	3-11
Building an Allele Table	3-17
Editing Table	3-23
Exporting the Table	3-25

Overview of Part 2 of the Microsatellite Tutorial

Introduction

Most linkage analysis applications accept alleles from genotyping data as numeric integers. For example, alleles must be called as 1,2,3,4...and so on, or can be numbered based on the size of the alleles (for example, alleles that fall in the range of 99.82 to 100.16 can be called 100).

Using the template you created in “Microsatellite Analysis Part 1”, you will use the Genotyper software to create a table that contains allele numbers instead of sizes in base pairs. This kind of table can then be exported as a text file to be used in linkage-analysis applications.

Goals

The goal of the Part 2 of the microsatellite tutorial is to learn how to use the Genotyper software features and commands to

- Use the template you created in Microsatellite Analysis Part 1, and import GeneScan® Analysis software files from the Fluorescent Genotyping Demonstration Kit.
- Create a Genotyper table that contains allele numbers instead of sizes in base pairs using the Histogram window.
- Export the table in a format that can be used by third-party analysis applications.

Features You Will Use

In this tutorial, you will use the following Genotyper software features:

- Importing GeneScan Analysis software data.
- Labeling and filtering peaks.
- Defining Category groups for markers using the Histogram window.
- Creating categories from labels.
- Creating and filling tables.
- Editing tables.
- Changing size labels to allele names.
- Exporting tables as files.

Importing GeneScan Analysis Software Files

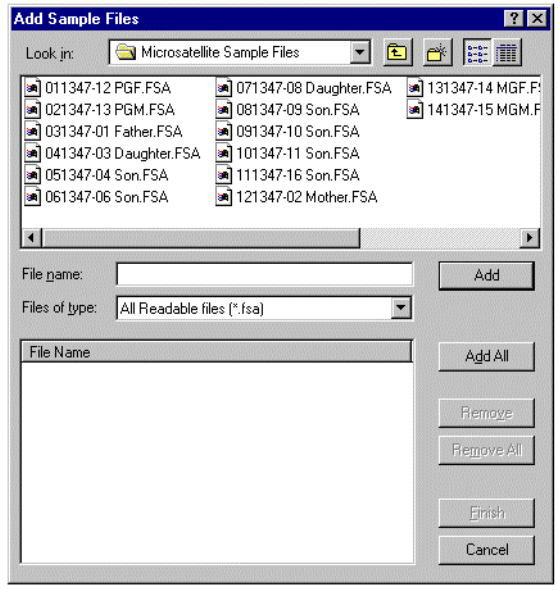
About This Step

For this step, you will be importing GeneScan Analysis software files into the template you created in “Making a Template for the Tutorial” on page 2-26.

If you don’t have that template open already, open it now. For more information, see “Using the Template” on page 2-28.

Procedure

To import GeneScan Analysis software files from the “Microsatellite Sample Files” folder:

Step	Action
1	Choose Import from the File menu and From GeneScan File (Ctrl+I) from the submenu and navigate to the appropriate folder. The Add Sample Files dialog box should appear as follows: 
2	Click Add All to import all the sample files from the folder and click Finish. The Dye/lanes pane should now display all of the sample files.

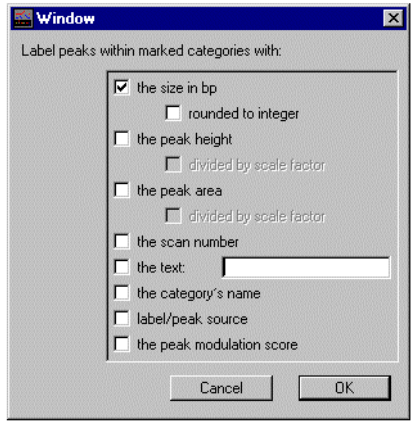
Labeling Allele Peaks with Size Labels

About This Step

For this step, you must label all of the allele peaks for each marker with the respective size in base pairs.

Procedure

To assign labels to all allele peaks in each marker:

Step	Action
1	To make the Dye/lane pane active either: ♦ Click the the pane, or ♦ Tab until a vertical bar appears next to the Dye/lane pane.
2	To select all the dye/lanes in these three colors, either: ♦ Choose Select All (Ctrl+A) from the Edit menu, or ♦ Click on the B, G, Y buttons while holding down the Ctrl+Shift keys. **Viewing More of the Dye/lanes at One Time** You can either: ♦ Choose the Show Dye/lanes Window from the Views menu, or ♦ Click the Dye/lanes icon ( ) located on the right side of the Main window to open the Dye/lane window.
3	Choose Label peaks from the Analysis menu. The following dialog box appears: 

To assign labels to all allele peaks in each marker: *(continued)*

Step	Action
4	Check the “Size in bp” box (default) and click OK. You can also take the following action:

To view...	Then...
the labels	select individual dye/lanes. Each and every peak that has been sized in GeneScan (including stutters, +A and background peaks) will be labeled with the size in bp.
multiple plots	Either: ♦ Choose Show Plot Window (Ctrl+Y) from the Views menu. ♦ Click the Plot window icon ( ) in the Main window.

Filtering Peak Labels

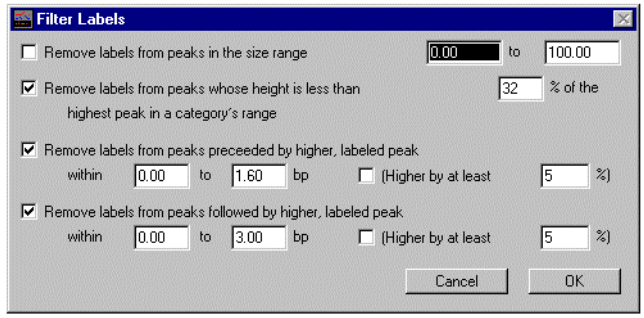
About This Step

For this step, since you are interested in placing labels on only those peaks that correspond to the allelic peaks, you must filter out labels from the remainder of the unwanted peaks.

This operation will remove labels from all the unwanted peaks and leave size labels on only the allele peaks of interest for each category. Once this is completed, you are ready to build a table containing the cleaned-up data.

Procedure

To filter peak labels:

Step	Action
1	Select Filter labels from the Analysis menu. The following dialog box appears: 
2	Click OK. **Note** The last three of the four boxes for the filtering parameters have already been pre-selected as default.

Using the Filter Labels Dialog Box

The default parameters work well for most dinucleotide repeat markers. The filtering operations listed in this dialog box are performed one at a time, in the order they are listed.

Use the...	To remove labels from...
first checkbox	most of the small (noise) peaks that have a peak height of 32 percent or less than the true alleles.
second checkbox	the +A peaks if their peak height is less than that of the true allele associated with it, and if the +A peak is within 1.6 bp from the true allele.
third checkbox	stutter peaks.

Defining Category Groups for Marker Alleles

About This Step

You now must redefine each of the categories from “Defining Categories for Markers” on page 2-7

In Chapter 2 (“Microsatellite Analysis Part 1”), each category referred to a marker. You will now create several category members for each marker and group them under the marker name.

Creating Category Groups for Markers

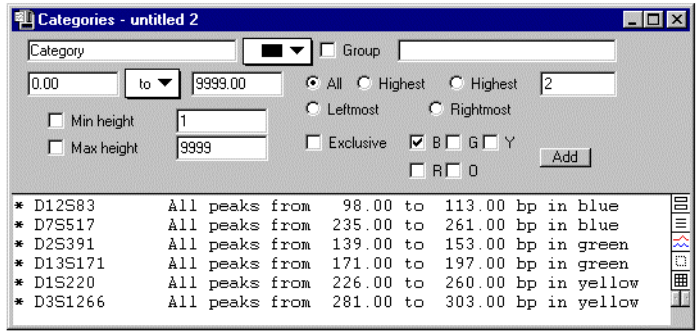
Each of these new members for a particular group (marker) will represent a single allele.

To create category groups for markers:

Step	Action
1	Take the following action:

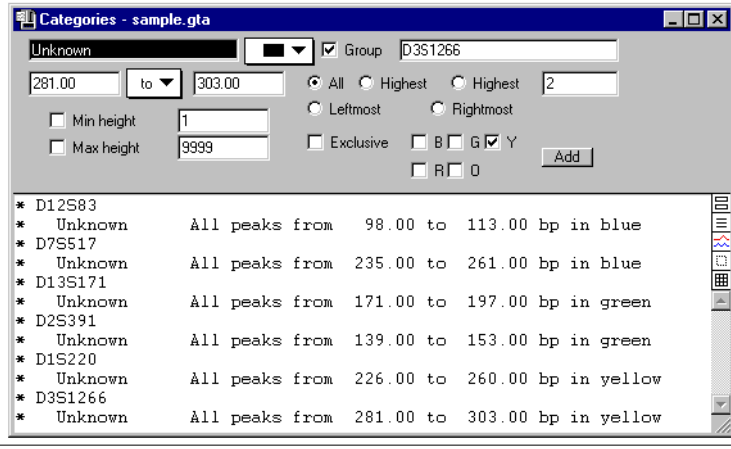
You can either....	Result...
click the Categories button.	The Categories window appears.
choose Show Categories Window (Ctrl+K) from the Views menu.	

The six categories (markers) defined in “Defining Categories for Markers” on page 2-7 are displayed.



2	Click once to select the first category (D12S83).

To create category groups for markers: *(continued)*

Step	Action
3	Choose the Clear command from the Edit menu. The corresponding information (name, size range and so on) for that category will still be displayed in the upper part of the window.
4	Highlight and delete the name of this category (D12S83) and type in a new name (for example, “Unknown”).
5	Click the “Group” checkbox and type in the name of the marker (D12S83) as the group name. **Note** You can use the Cut and Paste commands from the Edit menu in step 4 and step 5.
6	Click the Add button. This will create a single category member called “Unknown” under the Group D12S83.
7	Repeat step 2 through step 6 for the other categories.
8	Verify that the window appears as follows: 
9	Sort categories by Color and Size by choosing Category Sorting from the Views menu.
10	Close Categories window.

Why Create an Unknown Category

The “Unknown” category will find and label any peaks that do not belong to any of the allelic bins, but are still within the size range for that marker. These alleles will be labeled as an “unknown allele.”

Making New Categories for Alleles

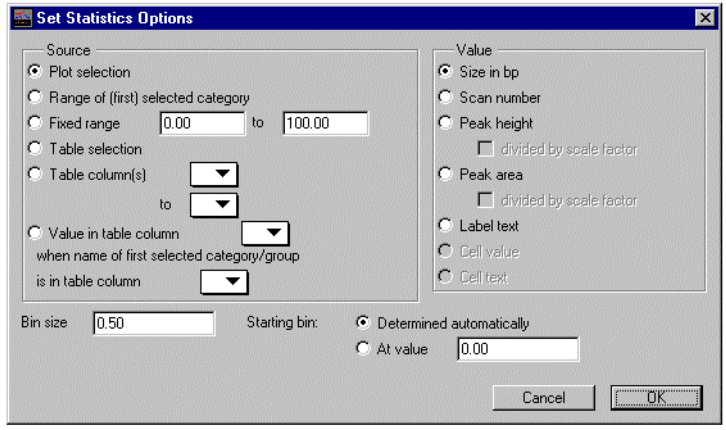
About This Step

Now you will make new categories representing each allele for the selected marker. To do this, you will use the Statistics and Histogram options to bin the alleles for each marker.

You will use the range of peaks with labels in the Plot window to bin the peaks together for each allele, using the information from the Histogram display. The histograms are linked to the Statistics window, and are displayed according to the options that you set up for the statistics. The source of the data for the histograms in this exercise will be the peaks selected in the Plot window. Keep the bin size to 0.1 bp so that you can bin the alleles more precisely.

Setting Up the Statistics Options

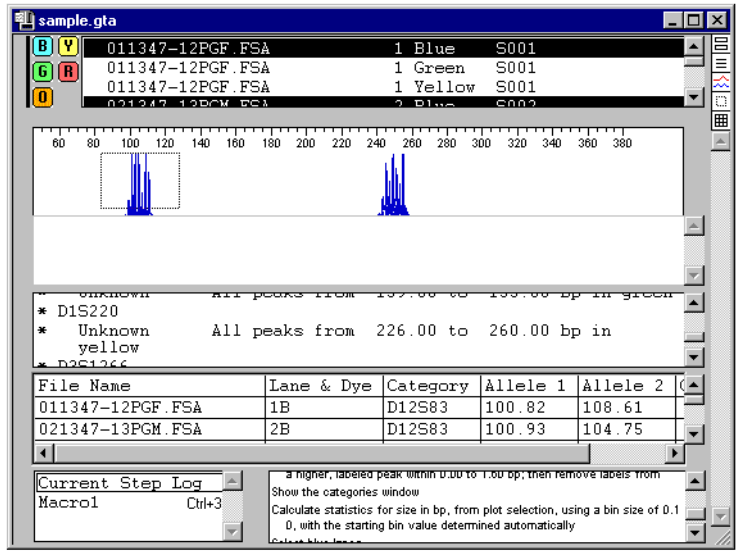
To set the statistics options:

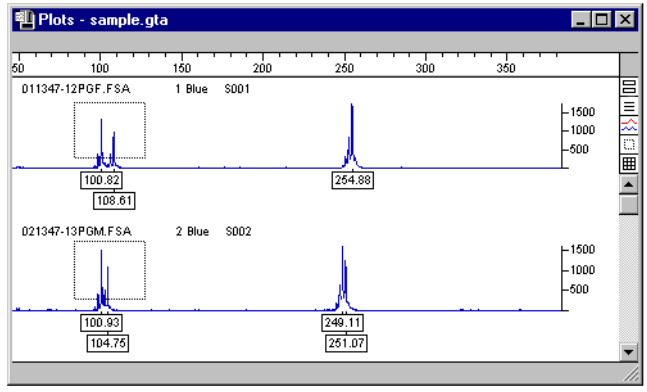
Step	Action
1	Select Set Statistics Options from the Analysis menu. The following window appears.
	
2	Set the Bin Size to 0.1 (default is 0.5).
3	Click OK.

Selecting Peaks for Individual Marker

To select peaks for individual markers:

Step	Action
1	Click the Blue lane button next to the Dye/lane pane to select blue lanes only.
2	Using the cursor, draw a rectangular box that includes all allelic peaks for the first marker/category (D12S83) in the plot displayed in the Main window, or the Plot window, as shown here:

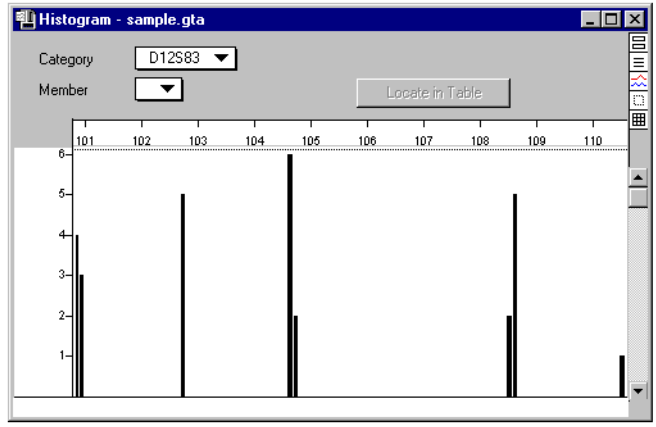




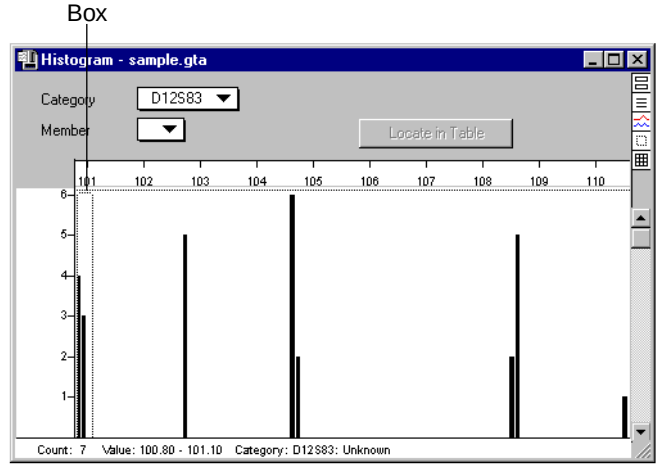
Creating New Category Members for Each Marker

You can now use the Histogram window to select and create new category members.

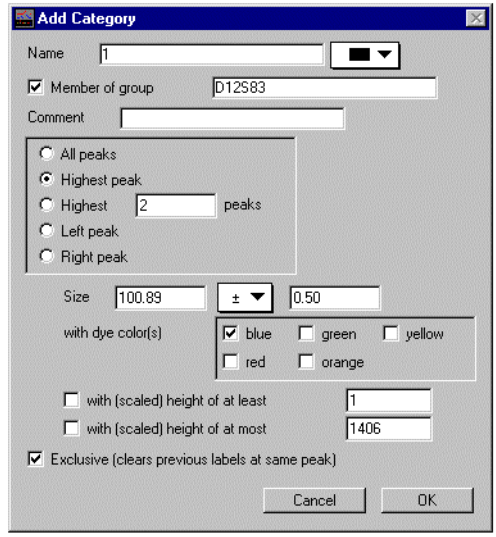
To create new category members:

Step	Action
1	Choose Show Histogram Window from the Views menu. The following window appears: 
2	Verify that the correct category name (in this case D12S83) is displayed in the Category pop-up menu. If not, choose the correct category from the pop-up menu.
3	Choose Zoom from the Views menu and Zoom Out (Ctrl+Minus Sign) from the submenu.
4	Using the cross-hair cursor, draw a box around the first group of peaks. The distance between the left and right edges of the bin should be approximately 1 bp, as shown in the figure below.

To create new category members: *(continued)*

Step	Action
	 The status bar at the bottom of the histogram displays the value (size in bp) of the range selected by the drawn box in increments of 0.1 bp (set in the Set Statistics Option window) as you drag the cursor across the peaks. The count in the status bar displays the number of peaks found within that bin.
5	While pressing the Shift key, choose Add Category from the Category menu. **Note** If you choose Add Category without holding down the Ctrl+Shift keys, the category size will appears as a range (for example, 100.39 to 101.39). If you hold down the Ctrl+Shift keys, the size will appear as a center value and tolerance (for example, 100.89 ± 0.5). The center value will be calculated as the weighted average of the selected bins.

To create new category members: *(continued)*

Step	Action
6	Type in the name or number of the first allele (1) in the following window:  **Note** You can also name the alleles using the midpoint of the bins. For example, an allele falling in the bin 99.8 to 100.8 could be called “100.” Or, you can use an alternate naming system of your choice.
7	Click OK. This will create a new, exclusive category member called “1” which is defined as a member of the group D12S83 with the highest peak in the size range of 100.89 ± 0.5 bp with blue dye.
8	Repeat step 3 through step 6 for the remainder of the grouped peaks (bins) from the same Histogram window. There are a total of five bins (alleles) for this marker and the allele names will be 1 through 5.

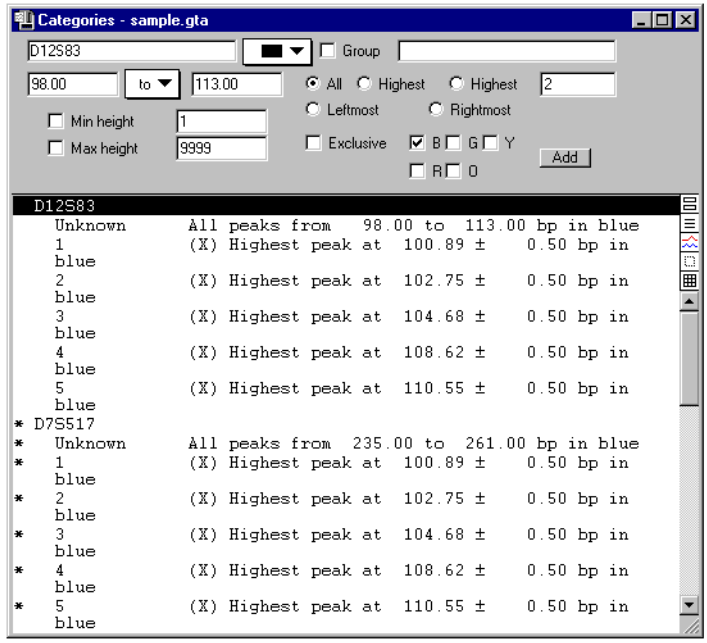
Finishing the Remainder of the Categories

Repeat “Selecting Peaks for Individual Marker,” and “Creating New Category Members for Each Marker,” for the remainder of the markers.

Categories Window Example

The final Categories window should look like the figure below.

Each category group will have several members, including an "Unknown" category member. If a labeled peak does not fall into any of the allelic bins, but is still within the size range of a marker, that particular peak will be labeled "Unknown."



Building an Allele Table

About This Step

You will now build a table containing the number alleles for each marker.

This table will contain information about the samples, markers (categories), and the corresponding numbered alleles for each individual.

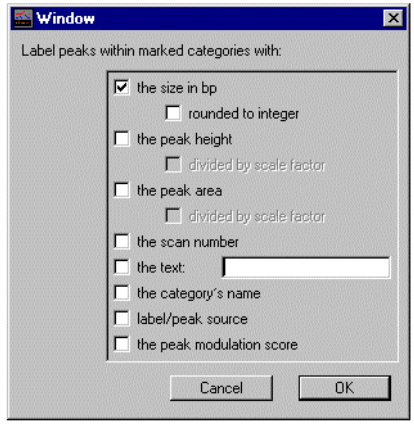
Selecting Dye/lanes

To select dye/lanes:

Step	Action
1	Choose Clear all labels from the Analysis menu. This will remove any labels that may be left behind from previous steps.
2	Select the dye/lane pane. **You can either:** / **Result** click the Dye/lane window icon. — The Dye/lanes window appears. choose Show Dye/lanes Window from the Views menu.
3	Take the following action: **Either...** / **Result** click each individual color button (B, G, Y) on the left side of the Dye/lane pane with the Shift key held down, or — All the dye/lanes are selected. choose Select All from the Edit menu.

Labeling Allele Peaks

To label allele peaks:

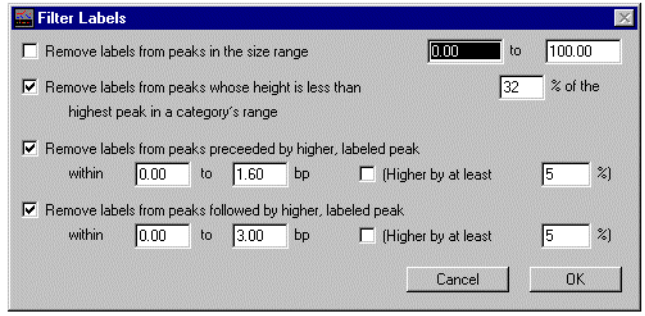
Step	Action
1	Choose Label peaks from the Analysis menu. The following dialog box appears: 
2	Click the checkbox labeled the category's name only.
3	Uncheck all other boxes.
4	Click OK. The following table lists what has happened at this point:

All...	Are labeled...
allelic peaks that fall within the bin for each of the alleles as defined in the Categories window	with the respective allele number.
other peaks (stutters, +A peaks, background and so on) that do not fall in any of the allelic bins, but are still within the size range for that marker	"Unknown".

Filtering Unwanted Labels

This operation will remove labels from all the unwanted peaks, and leave size labels on only the allele peaks of interest for each category.

To filter peak labels:

Step	Action
1	Select Filter labels from the Analysis menu. The following dialog box appears: 
2	Click OK. **Note** The last three of the four boxes for the filtering parameters have already been pre-selected as default.

Using the Filter Labels Dialog Box

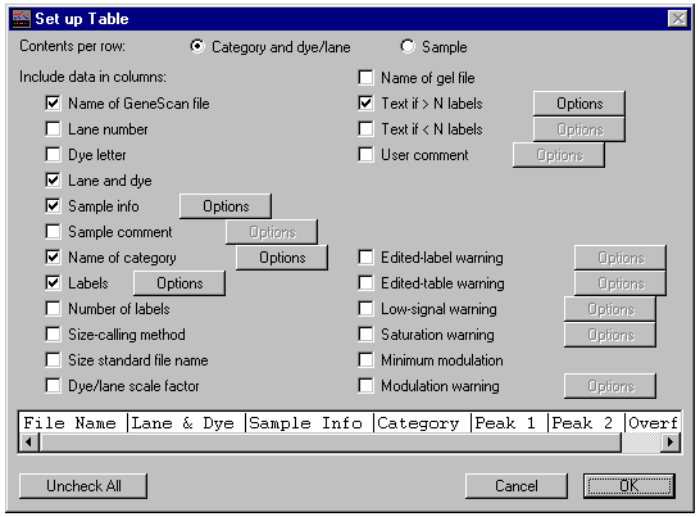
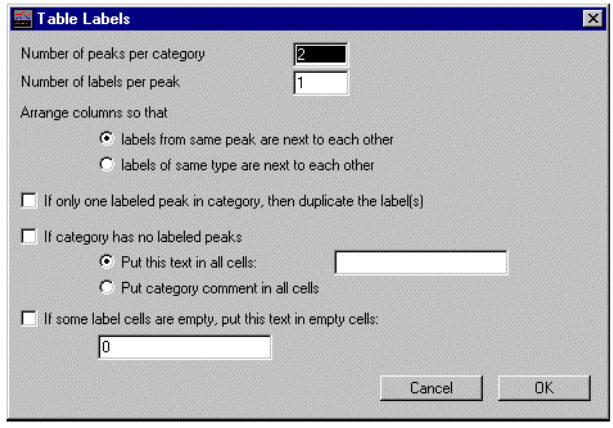
The default parameters work well for most dinucleotide repeat markers. The filtering operations listed in this dialog box are performed one at a time, in the order they are listed.

Use the...	To remove labels from...
first checkbox	most of the small (noise) peaks that have a peak height of 32 percent or less than the true alleles.
second checkbox	the +A peaks if their peak height is less than that of the true allele associated with it, and if the +A peak is within 1.6 bp from the true allele.
third checkbox	stutter peaks.

Setting Up the Table

You are now ready to build a table containing the cleaned-up data.

To set up a table:

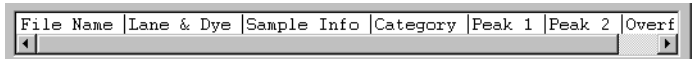
Step	Action
1	Choose Set up Table from the Table menu. The following window appears: 
2	Check the column title boxes exactly as shown here.
3	Click the Options button next to Labels. The following dialog box appears: 

To set up a table: *(continued)*

Step	Action
4	Click OK to accept the defaults. The defaults of the label options create a table that includes a maximum of two peaks (alleles) per category (marker) with both alleles arranged next to each other in the table. **Note** The overflow column will be flagged if there are more than two alleles per category.

Column Format of Table Example

When you have completed the setup for the table, the column format for the table should look like the figure below.



The Concept of Overflow

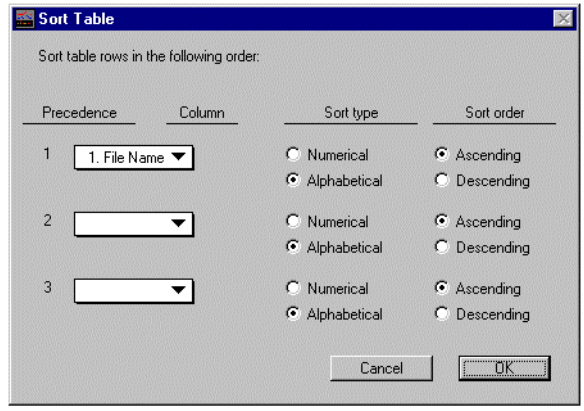
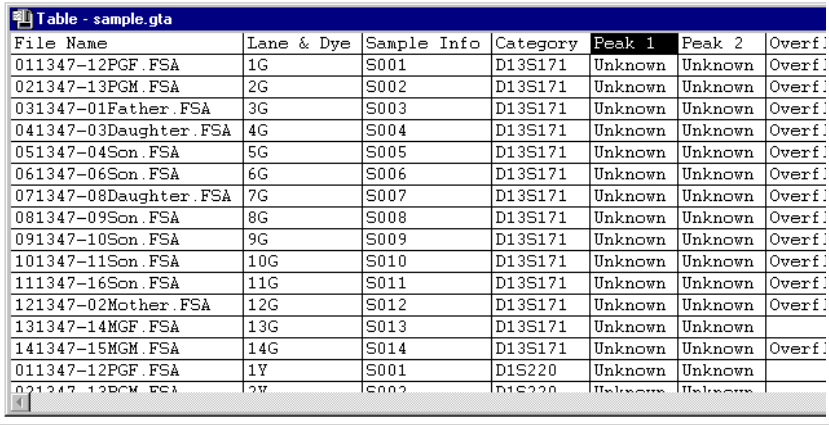
If there are any anomalies (such as three peaks labeled for a particular individual/marker), a text entry, “Overflow,” will be displayed in the Overflow column, indicating that there is a problem with the data from that particular individual/marker.

Note “Text if > N labels” is the option in the Set up Table window to create an Overflow column.

Appending Rows

To append rows to your new table:

Step	Action
1	Choose Append to Table command from the Table menu.
2	Choose Sort Table from the Table menu. The following dialog box appears.

Step	Action
	
3	Choose Category in the first pop-up menu.
4	Choose File Name in the second pop-up menu.
5	Click OK. The window should appear as below:
	

File Name	Lane & Dye	Sample Info	Category	Peak 1	Peak 2	Overf
011347-12PGF.FSA	1G	S001	D13S171	Unknown	Unknown	Overf
021347-13PGM.FSA	2G	S002	D13S171	Unknown	Unknown	Overf
031347-01Father.FSA	3G	S003	D13S171	Unknown	Unknown	Overf
041347-03Daughter.FSA	4G	S004	D13S171	Unknown	Unknown	Overf
051347-04Son.FSA	5G	S005	D13S171	Unknown	Unknown	Overf
061347-06Son.FSA	6G	S006	D13S171	Unknown	Unknown	Overf
071347-08Daughter.FSA	7G	S007	D13S171	Unknown	Unknown	Overf
081347-09Son.FSA	8G	S008	D13S171	Unknown	Unknown	Overf
091347-10Son.FSA	9G	S009	D13S171	Unknown	Unknown	Overf
101347-11Son.FSA	10G	S010	D13S171	Unknown	Unknown	Overf
111347-16Son.FSA	11G	S011	D13S171	Unknown	Unknown	Overf
121347-02Mother.FSA	12G	S012	D13S171	Unknown	Unknown	Overf
131347-14MGF.FSA	13G	S013	D13S171	Unknown	Unknown	
141347-15MGM.FSA	14G	S014	D13S171	Unknown	Unknown	Overf
011347-12PGF.FSA	1Y	S001	D1S220	Unknown	Unknown	

Editing Table

About This Step

You should now edit the table to correct for overflows (more than two called alleles) by removing labels from the incorrectly labeled alleles.

Viewing Plot Data for Overflows

If there are any overflows indicated for any of the individuals, click the overflow cell. The corresponding plot area will be displayed in the Plot pane.

Removing Labels from Overflow Alleles

Making the Appropriate Edits

If...	Then...
a background peak (stutter, +A, and so on) has not been filtered out leading to >2 alleles being called	those labels can be removed.

Removing Labels from a Peak

Step	Action
1	In the Plot window, place the cross-hair cursor on the peak in question. The vertical peak locator line will be aligned with the midpoint of the peak.
2	Click once to remove the label.
3	Choose Update Table command from the Table menu to update the table with the new edits.

Manually Call Alleles

If an allele was not called for some reason, you can manually call that allele (provided that allele is still falls in the bin for one of the alleles).

To manually call an allele:

Step	Action
1	Locate the peak of interest in the Plot window.
2	Place the cross-hair cursor on the peak until the vertical peak locator line is in the middle of the peak.
3	Click once to add the size in bp label to that peak (“Size in bp” is the default for the Click options under Analysis). The Size in bp is the default for the Set Click Options dialog box that is accessed from the Analysis menu.
4	Choose Update Table command from the Table menu to update the table with the new edits.

Changing Size Labels to Allele Names

To change the size label to the corresponding allele name:

Step	Action
1	Select the Dye/lane pane by tabbing or clicking inside the pane.
2	Take the following action: **Either...** / **Result** click each individual color button (B, G, Y) on the left side of the Dye/lane pane with the Shift key held down, or / All the dye/lanes are selected. choose Select All from the Edit menu.
3	Choose Change labels from the Analysis menu.
4	Click “the category’s name” checkbox.
5	Click OK.
6	Choose Update Table command from the Table menu to update the table with the new edits.

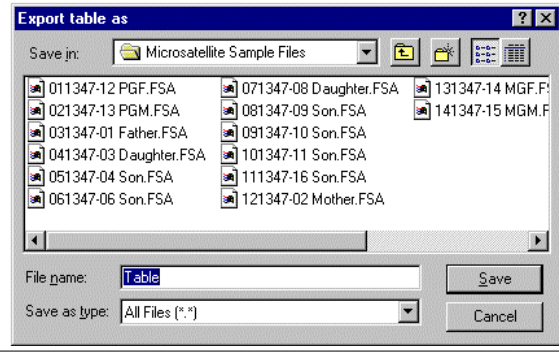
Exporting the Table

About This Step

Once all the edits are finished, and you are satisfied with the contents and format of the table you can export the table as a tab-and-carriage-return-delimited text file.

Procedure

To export the table as a text file:

Step	Action
1	Select Export to File from the Table menu. The Export table as dialog box appears.
2	In the File name text box, assign a name to the table. 
3	Click Save.
4	Choose Clear Table from the Analysis menu.

Human Identification Tutorial 4

Chapter Overview

Introduction This tutorial takes you through a series of steps in the Genotyper® 3.5 NT software that are required to process data obtained from GeneScan® Analysis software using the AmpFℓSTR™ Blue Kit, and create a table containing the allelic information for the unknown forensic samples.

In This Chapter This chapter contains the following topics.

Topic	See page
Overview of The Human Identification Tutorial	4-2
Contents of the AmpFlSTR Blue Kit	4-3
How AmpFlSTR Blue Data Was Preprocessed	4-4
Creating a New Genotyper Document	4-5
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Overview of The Human Identification Tutorial

Introduction

In this tutorial, you will use the Genotyper software to process AmpFℓSTR Blue GeneScan Analysis software files, and create an allelic-size table to discriminate between heterozyote and homozygote individuals.

Goals

The goal of the Human Identification tutorial is to learn how to use the Genotyper software features and commands to:

- Process GeneScan Analysis software Sample files and work with two sets of allelic ladders that have been generated from the AmpFℓSTR Blue Kit.
- Create category groups for each marker supplied, and categories for each allele within a marker group.
- Create a Genotyper Table containing allelic information that will identify 32 unknown forensic samples.

Features You Will Use

In this tutorial, you will use the following Genotyper software features.

- Creating a new document.
- Importing GeneScan data.
- Defining category groups.
- Creating and editing allele categories.
- Labeling alleles with names and numbers.
- Creating tables.

Contents of the AmpFℓSTR Blue Kit

Introduction

A PCR-based DNA typing kit for fluorescent analysis of Short Tandem Repeat (STR) markers is available from PE Biosystems. The AmpFℓSTR Blue Kit includes reagents and protocols for the co-amplification and detection of three STR loci – D3S1358, vWA and FGA.

All three loci are tetranucleotide-repeat containing markers.

One primer from each locus is labeled with FAM that is detected as blue on the ABI PRISM™ Instruments.

Using Allelic Ladders

The allele assignments to the unknown samples are made using a known set of allelic ladders for the three loci in the kit.

The example data in this tutorial consists of GeneScan Analysis software sample files for 32 unknown sample files and two sets of allelic ladders obtained using the ABI PRISM® 377 DNA Sequencer.

Loci Markers

The following table shows the AmpFℓSTR Blue Kit markers used for this tutorial.

AmpFℓSTR Blue Kit markers:

Marker Name	Chromosome Location	Allele Size Range (bp)[a]	Number of Alleles
D3S1358	3	113-141	8
vWA	12	156-196	11
FGA	4	212-268	14

a. The size range and number of alleles refers to the allelic ladder only for each marker.

How AmpFℓSTR Blue Data Was Preprocessed

Introduction

The GeneScan Analysis software files you import to perform procedures in this tutorial are the result of data collection and GeneScan analysis of AmpFℓSTR Blue Kit reagents.

Data-Collection Process

Fluorescently labeled products were electrophoresed on a 5 percent Long Ranger™ gel, 36-cm WTR on the ABI PRISM PRISM 377 DNA Sequencer using the GS36A-2400 Run module for 2.25 hours. The raw data was analyzed using GeneScan version 3.0 software and GS-350 ROX as an internal lane size standard.

Chemical Hazard Warning

! WARNING ! CHEMICAL HAZARD. Long Ranger gel solution contains acrylamide. Acrylamide is a neurotoxin. Avoid skin contact with Long Ranger gel solution because acrylamide can be absorbed through the skin. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves. To receive additional copies of MSDSs, call PE Biosystems at (800) 327-3002.

GeneScan Analysis Parameters

The following table shows the parameters used in the GeneScan analysis.

GeneScan analysis parameters:

Peak Detection Threshold	Peak Detection Minimum Half-width	Sizing Method	Std. Peak Det. Threshold
50	5	Local Southern	150

Creating a New Genotyper Document

About This Step

To perform genotyping tasks on AmpFℓSTR Blue kit marker data, you must import the related GeneScan Analysis software files into a Genotyper Document Document.

Procedure

The following steps are used to create a new document:

If...	Then...	Result
Genotyper is already open	select New (Ctrl+N) from the File menu. The Main window appears.	A new “Untitled Genotyper Document appears.
Genotyper is closed	open Genotyper by double-clicking the application’s icon. 	

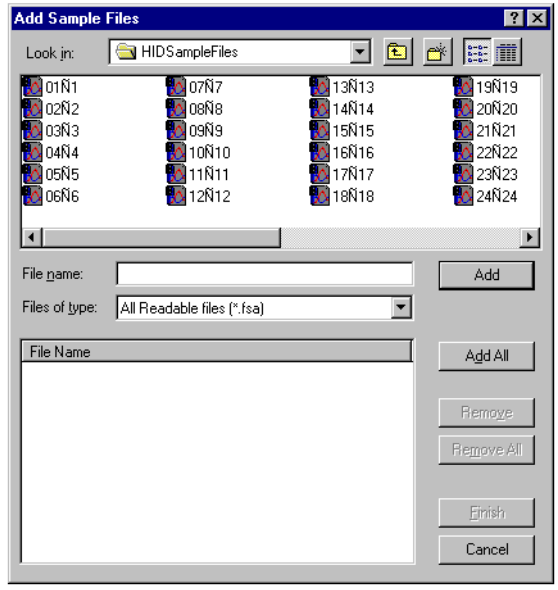
Importing GeneScan Analysis Software Files

About This Step

You must import the appropriate the GeneScan Analysis software files containing data for the categories you define.

Procedure

To import GeneScan Analysis software files from the “HID Sample Files” folder:

Step	Action
1	Choose Import from the File menu and From GeneScan File (Ctrl+I) from the submenu and navigate to the appropriate folder. The Add Sample Files dialog box should appear as follows: 
2	Click Add All to import all the sample files from the folder and click Finish. The Dye/lanes pane should now display all of the sample files.

Defining Category Groups for Allele Markers

About This Step

Each GeneScan Analysis software file contains data from three markers in different size ranges. You will define the boundaries for each marker based on the expected allele size range for that marker. These boundaries define a category group.

About Allelic Ladders

The allelic ladder contains all the common alleles for each locus (marker). The allelic ladder is run in at least one lane of each gel, or in at least one injection in a set of runs on a capillary. Genotypes are assigned to the samples by comparing the sizes obtained for the unknown sample alleles with the sizes obtained for the alleles in the allelic ladder. This method of genotyping normalizes potential run-to-run differences in sizing.

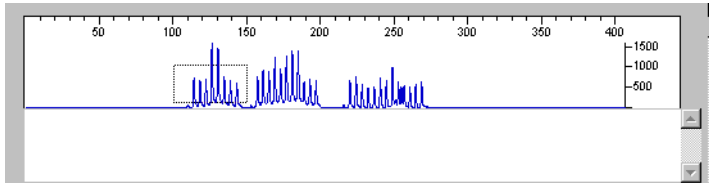
Viewing Allelic Ladders

To view allelic ladders:

Step	Action
1	Choose the Clear Category List command from the Analysis menu to remove the default “Everything” category in the Category list.
2	Click once to select and highlight the first sample file in the Dye/lanes pane. The first Sample file in this list contains the allelic ladder with alleles for all the markers that are displayed in the Plot pane.
3	Choose Zoom from the File menu and Zoom In (Ctrl+=) from the submenu to better view the allelic ladder.

Defining Category Boundaries

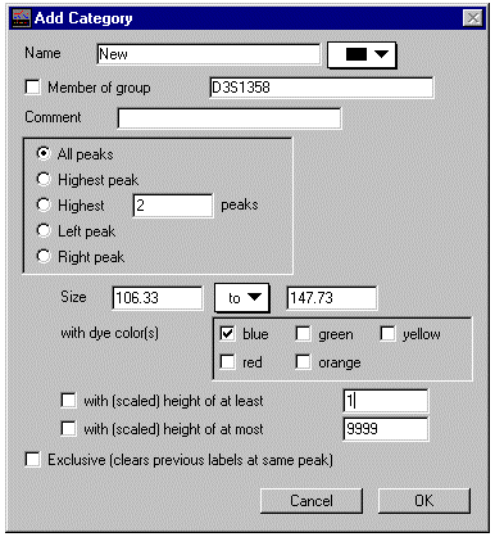
Using the cursor in the Plot pane (which turns into a cross-hair cursor), draw a rectangular box for the first marker. Start a few bp before the leftmost peak, and end a few base pairs after the rightmost peak for that marker as shown in the figure below.



Adding Category Group For Markers

You will now use the cursor in the Plot pane to mark the boundaries of a category group.

To define category groups for marker alleles:

Step	Action
1	Choose Add Category from the Category menu (or press Ctrl+L). The Add Category dialog box appears. 
2	Fill out the Add Category dialog box as follows: ♦ Enter “New” as the category name. (You may also call this category “OL Allele?” where OL means “off ladder”). ♦ Click the “Member of group” checkbox and enter the name of the marker as the group name (D3S1358 in this example). ♦ Leave the following as defaults: All peaks radio button and the “blue” dye color checkbox. The size range in bp displayed is the outer limit of the category, as determined by the rectangular box drawn when defining category boundaries.
3	Click OK. This creates a new category group called D3S138 with a category called New in the size range (106-147 bp).

Creating Allele Categories for Marker Groups

About This Step

Since the allelic ladder for D3S1358 has eight alleles, you need to create additional categories, one for each allele, in the group D3S1358.

Each allele category will have a defined size with a ± 0.5 bp width so that all peaks in the unknown samples within that ± 0.5 bp range will be labeled with the same allele number.

Marking Category Groups

To mark a category group:

Has...	If not...
the group D3S1358 has been marked (that is, has a • mark).	**Step** / **Action** **1** Click and select the group name. **2** Either: ♦ Choose the Mark command (Ctrl+M) from the Edit menu, or ♦ Double click the group name to mark it. **Note** Marking or unmarking a group will automatically mark/unmark all the categories belonging to that group.

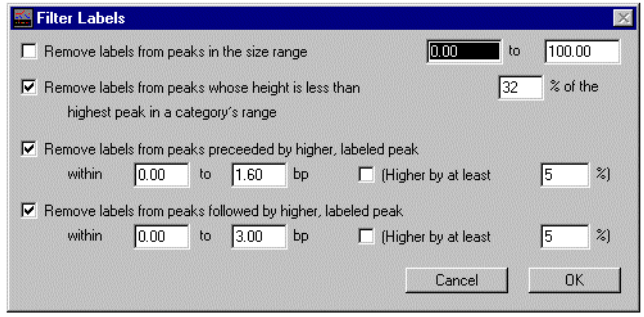
Labeling Peaks for Each Allele

Choose the Label peaks command from the Analysis menu, and label all peaks with the size in bp.

Note In addition to the alleles being labeled, some of the background noise peaks (if present) will also be labeled.

Filtering Unwanted Peak Labels

To remove unwanted labels:

Step	Action
1	Choose Filter labels from the Analysis menu. The following dialog box appears: 
2	Uncheck all the boxes except the second checkbox.
3	Set the filtering parameter to 25%.
4	Click OK to apply filter.

Making Categories from Labels

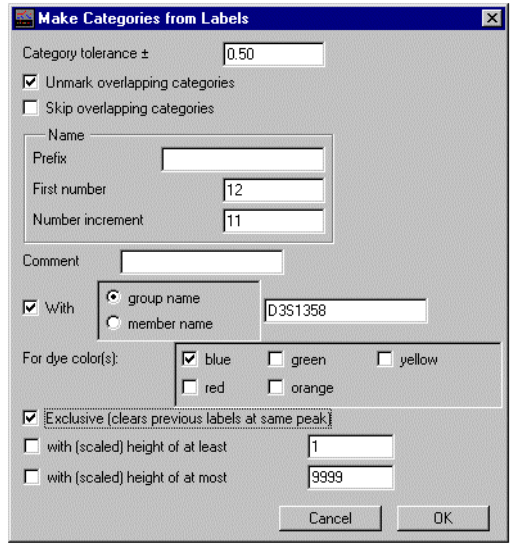
About this Procedure

This procedure creates a set of eight member categories (alleles) for the group “D3S1358” that are numbered sequentially from 12 through 19. The size in bp for each allele is the midpoint of the category with a 0.5 bp window on either side.

All eight categories...	Any peak(s)
will be marked as exclusive (X) categories, meaning that any peak that falls within that window will belong to that particular allele.	that falls outside of this window and does not belong to any of the other seven categories (alleles) will be called as “New” (or “OL Alleles?”) alleles.

Procedure

To make categories from labels:

Step	Action
1	Choose Make from Labels from the Category menu.
2	Choose the options as follows: 
3	Click OK.

Category List Example

The following figure shows the resulting Category list after making allele categories for the D3S1358 category.

```
* D3S1358
*   New          All peaks from   94.36 to  148.29 bp in blue
*   12           (X) Highest peak at  114.09 ±    0.50 bp in blue
*   13           (X) Highest peak at  118.21 ±    0.50 bp in blue
*   14           (X) Highest peak at  122.22 ±    0.50 bp in blue
*   15           (X) Highest peak at  126.24 ±    0.50 bp in blue
*   16           (X) Highest peak at  130.37 ±    0.50 bp in blue
*   17           (X) Highest peak at  134.63 ±    0.50 bp in blue
*   18           (X) Highest peak at  138.77 ±    0.50 bp in blue
*   19           (X) Highest peak at  143.24 ±    0.50 bp in blue
```

Creating Category Groups for Other Markers

Next, you will need to create groups for the remaining two markers.

To create category group for the other markers:

Step	Action
1	Choose Clear all labels from the Analysis menu. **Note** Ensure the other groups are unmarked while creating new categories for each marker.
2	Define category groups for allele markers (see “Defining Category Groups for Allele Markers” on page 4-7).
3	Create allele categories for all marker groups (see “Creating Allele Categories for Marker Groups” on page 4-9). **Note** vWA has 11 alleles numbered from 11 to 21, and FGA has 14 alleles numbered from 18 to 30.

Editing Categories for FGA Alleles

About This Step

Even though most of the alleles in these markers are 4 bp apart, there is a 2 bp variant allele in FGA. To account for this, you must do some manual editing of the categories/alleles for FGA.

Procedure

FGA allele 27 in this example is the 2 bp variant and must be renamed as allele 26.2.

To edit FGA categories:

Step	Action
1	Select the category/allele 27 in the group FGA and choose Edit Category from the Category menu. The Edit Category dialog box appears.
2	Change the name of the category to 26.2 and click Replace.
3	Change the names of the next five alleles to 27, 28, 29, 30 and 31 respectively, by using the Edit Category command.

Editing FGA Categories Example

The following is an example of what the FGA group should look like.

```
* FGA
*    New          All peaks from  214.05 to  274.21 bp in blue
*    12           (X) Highest peak at  157.23 ±    0.50 bp in blue
*    13           (X) Highest peak at  160.95 ±    0.50 bp in blue
*    14           (X) Highest peak at  164.95 ±    0.50 bp in blue
*    15           (X) Highest peak at  168.94 ±    0.50 bp in blue
*    16           (X) Highest peak at  172.93 ±    0.50 bp in blue
*    17           (X) Highest peak at  176.81 ±    0.50 bp in blue
*    18           (X) Highest peak at  180.79 ±    0.50 bp in blue
*    19           (X) Highest peak at  184.77 ±    0.50 bp in blue
*    20           (X) Highest peak at  188.74 ±    0.50 bp in blue
*    21           (X) Highest peak at  192.81 ±    0.50 bp in blue
*    22           (X) Highest peak at  196.88 ±    0.50 bp in blue
*    23           (X) Highest peak at  219.85 ±    0.50 bp in blue
*    24           (X) Highest peak at  224.08 ±    0.50 bp in blue
*    25           (X) Highest peak at  228.20 ±    0.50 bp in blue
*    26           (X) Highest peak at  232.31 ±    0.50 bp in blue
*    26.2         (X) Highest peak at  236.40 ±    0.50 bp in blue
*    28           (X) Highest peak at  240.58 ±    0.50 bp in blue
*    29           (X) Highest peak at  244.64 ±    0.50 bp in blue
*    30           (X) Highest peak at  248.79 ±    0.50 bp in blue
```

Labeling Alleles with Names and Numbers

About This Step Now that all the alleles have been defined for each marker, you can label alleles from both the allelic ladder and the unknown samples with the allele names/numbers.

Procedure To label allele peaks:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Take the following action:

<table><tr><th>You can either....</th><th>Result...</th></tr><tr><td>click the Categories button.
 </td><td rowspan="2">The Categories window appears.</td></tr><tr><td>choose Show Categories Window (Ctrl+K) from the Views menu.</td></tr></table></td></tr>
<tr><td>2</td><td>Choose Select All (Ctrl+A) from the Edit menu.</td></tr>
<tr><td>3</td><td>Take the following action:

<table><tr><th>You can either...</th><th>Result</th></tr><tr><td>choose the Mark Command (Ctrl+M) from the Edit menu, or</td><td rowspan="2">The lanes that you selected are marked with a bullet.

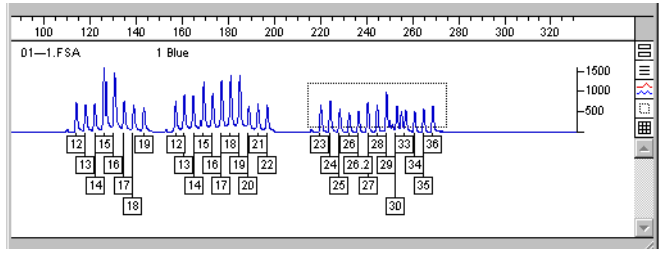
The bullet signifies that plots for those dye/lanes are reference plots.</td></tr><tr><td>double-click the dye/lanes, marking them with a bullet.</td></tr></table>
Close the Categories window if it is still open.</td></tr>
<tr><td>4</td><td>Choose Clear all labels from the Analysis menu to clear any existing labels.</td></tr>
<tr><td>5</td><td>Click the “Blue” button next to the Dye/Lanes pane to select all blue-colored dye/lanes.</td></tr>
<tr><td>6</td><td>Choose Label Peaks from the Analysis menu.

The Label Peaks dialog box appears.</td></tr>
</table>

Step	Action
7	Click “the category name” checkbox and click OK.
8	Choose Filter labels from the Analysis menu and the Filter Labels dialog box appears. Click OK to accept default settings.

Labeled Allelic Ladder Example

The following is an example of the allelic ladder labeled with the allele numbers.



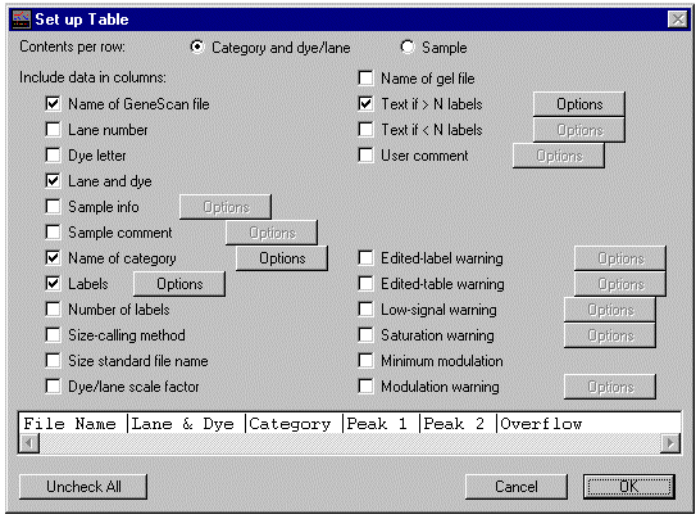
Setting Up an Allele Table

About This Step

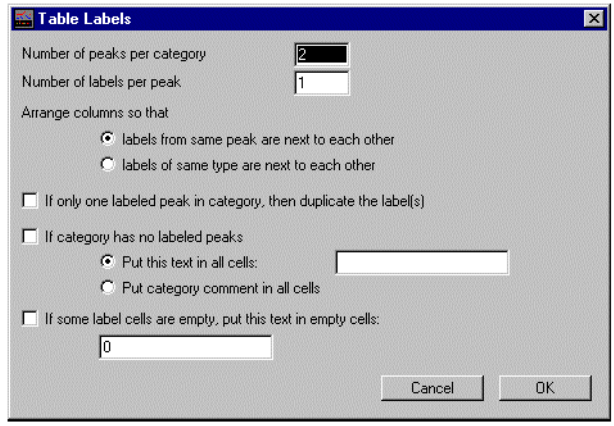
Now you will create a table containing the following information: file name, sample name, category name, two columns for each allele designation per sample, and an overflow column to put “New” alleles in. New alleles are alleles that do not fall within the 0.5bp width of any category within a marker group.

Procedure

To set up a table:

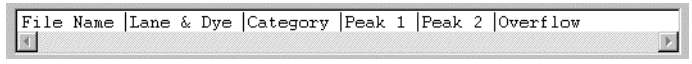
Step	Action
1	Choose Set up Table from the Table menu. The following window appears: 
2	Check the column title boxes exactly as shown here.

To set up a table: *(continued)*

Step	Action
3	Click the Options button next to Labels. The following dialog box appears: 
4	Click OK to accept the defaults. The defaults of the label options create a table that includes a maximum of two peaks (alleles) per category (marker) with both alleles arranged next to each other in the table. **Note** The overflow column will be flagged if there are more than two alleles per category.

Column Format of Table Example

When you have completed the setup for the table, the column format for the table should look like the figure below.



The Concept of Overflow

If there are any anomalies (such as three peaks labeled for a particular individual/marker), a text entry, “Overflow,” will be displayed in the Overflow column, indicating that there is a problem with the data from that particular individual/marker.

Note “Text if > N labels” is the option in the Set up Table window to create an Overflow column.

Appending Rows to Table

About This Step

Next, you can append the allele numbers to the table rows. If you need to edit any of the data in the columns, you can do so with the Edit Cell command (Ctrl+E) from the Edit menu.

Procedure

To append rows to the table you created:

Action	Result
From the Main window, choose Append to Table from the Table menu.	In the table window, the following table appears.

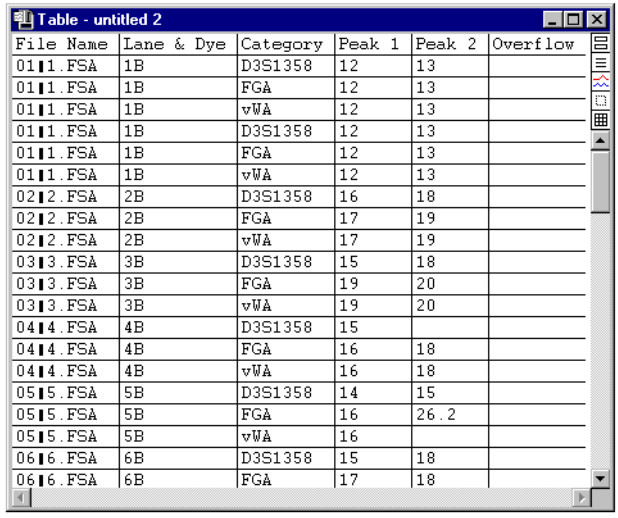


Table - untitled 2

File Name	Lane & Dye	Category	Peak 1	Peak 2	Overflow
01▌1.FSA	1B	D3S1358	12	13	
01▌1.FSA	1B	FGA	12	13	
01▌1.FSA	1B	vWA	12	13	
01▌1.FSA	1B	D3S1358	12	13	
01▌1.FSA	1B	FGA	12	13	
01▌1.FSA	1B	vWA	12	13	
02▌2.FSA	2B	D3S1358	16	18	
02▌2.FSA	2B	FGA	17	19	
02▌2.FSA	2B	vWA	17	19	
03▌3.FSA	3B	D3S1358	15	18	
03▌3.FSA	3B	FGA	19	20	
03▌3.FSA	3B	vWA	19	20	
04▌4.FSA	4B	D3S1358	15		
04▌4.FSA	4B	FGA	16	18	
04▌4.FSA	4B	vWA	16	18	
05▌5.FSA	5B	D3S1358	14	15	
05▌5.FSA	5B	FGA	16	26.2	
05▌5.FSA	5B	vWA	16		
06▌6.FSA	6B	D3S1358	15	18	
06▌6.FSA	6B	FGA	17	18	

Editing Table Cell Contents

Note Editing most commonly involves either removing or adding labels to certain peaks.

To change any of the column headings:

Step	Action
1	Select the cell you want to edit.
2	Select Edit Cell (Ctrl+E) from the Edit menu. The Edit Cell dialog box appears.
3	Type in a new name in the edit box and click OK.

Removing Labels

To remove labels:

Step	Action
1	Select a particular cell. This will display corresponding peaks in the Plot pane.
2	Place the cross-hair cursor on the peak in the Plot pane that you want to edit.
3	Click once to remove the label.

Adding Peaks

To add peaks:

Step	Action
1	Place the cross hairs cursor in the middle peak so that the vertical line locator is in the middle of the peak.
2	Click once. A “size in bp” label will be added to the peak.
3	Choose Change labels from the Analysis menu. The Set Peak labels dialog box appears.
4	Click “the category’s name” checkbox.
5	Click OK.

Updating the Table

To update the table with the new edits, choose Update Table from the Table menu.

Addendum to Human Identification Tutorial

Why Some Peaks Will Not Be Labeled

Some peaks in a couple of the sample files will not be labeled by the Genotyper software in this exercise because they were not sized by the GeneScan Analysis software due to inadequate analysis settings.

Problem Analysis Settings

Some of the analysis settings that could lead to problems are

- Peak Amplitude Threshold set too high.
- Min. Peak Half Width set too wide.
- Different Size Calling methods used for different samples.

Affected Sample Files

The affected sample files are:

Sample file	Description
08•8	A peak at approximately 224 bp was not sized in the GeneScan Analysis software.
18•18	Allele 17 of D3S1358 at approximately 134 bp was not sized in the GeneScan Analysis software.

Why Alleles or Peaks are Not Called

Inadequate GeneScan analysis settings is the most common reason why alleles or peaks are not called in the Genotyper software. Such sample files need to be reanalyzed in the GeneScan Analysis software with the proper analysis parameters and then processed using the Genotyper software.

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A

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PE Biosystems

850 Lincoln Centre Drive
Foster City, California 94404-1128
USA

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