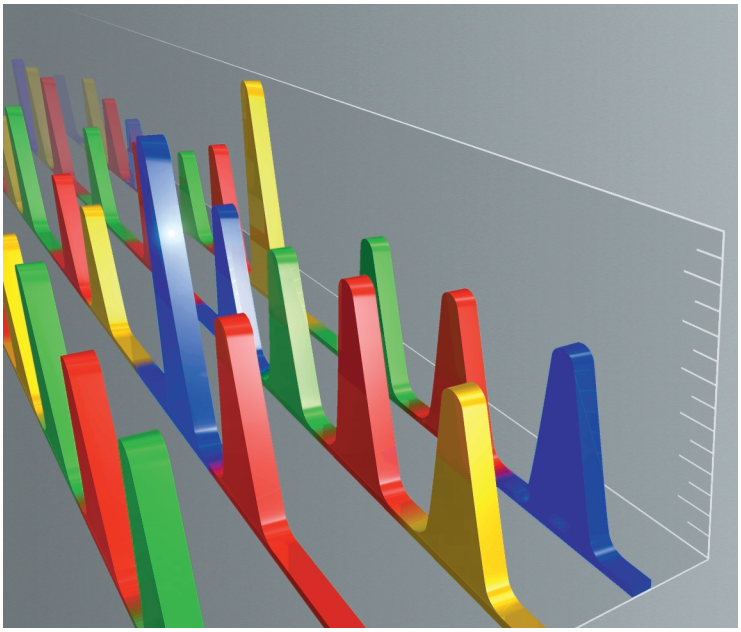

ABI PRISM® GeneScan Analysis Software

Version 3.7 for the Windows NT® Platform

User Guide

Applied Biosystems

ABI PRISM® GeneScan® Analysis Software

Version 3.7 for the Windows NT® Platform

Applied Biosystems

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Glossary

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GeneScan Analysis Software Overview 1

Overview

Introduction This chapter provides a general introduction to the ABI PRISM® GeneScan® Analysis Software, information about the organization of this manual, and instructions on how to get help from Applied Biosystems.

In This Chapter Topics in this chapter include the following:

Topic	See Page
About the GeneScan Analysis Software	1-2
GeneScan Software on the 310 Instrument	1-3
GeneScan Analysis on the 377 Instrument	1-4
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About the GeneScan Analysis Software

What Does the Software Do

The GeneScan Analysis Software performs DNA fragment analysis, which separates a mixture of DNA fragments according to their lengths, provides a profile of the separation, and estimates the lengths and sizes of the fragments.

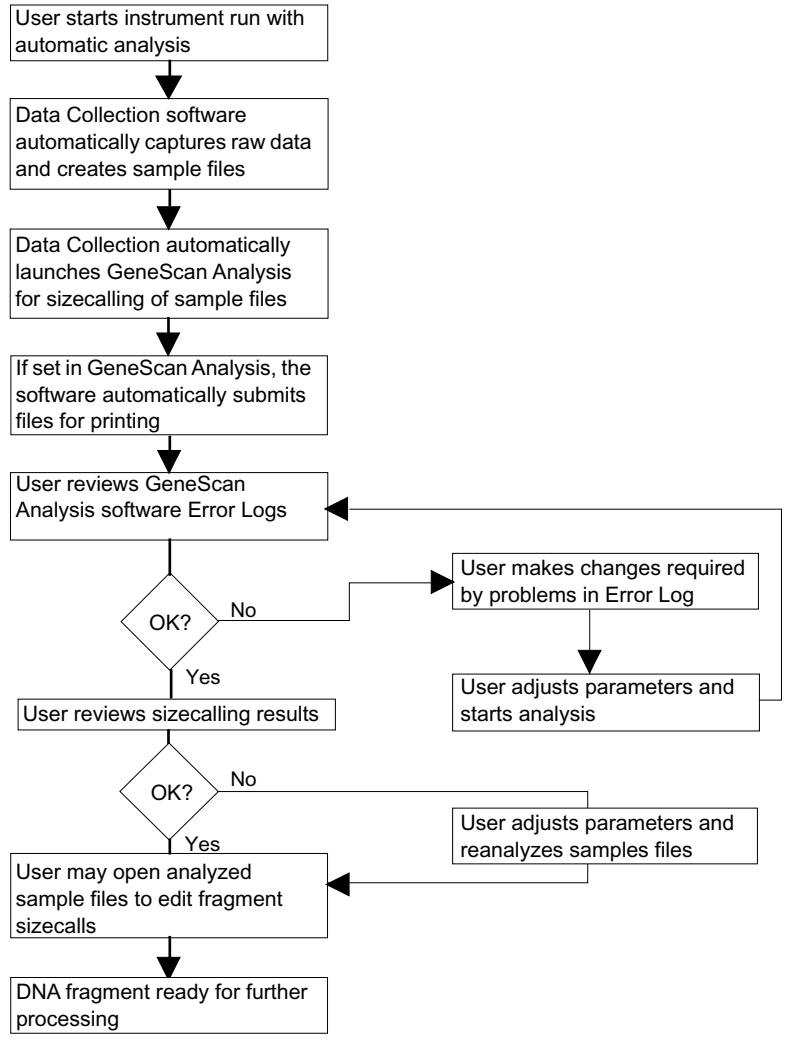
Instruments

GeneScan Analysis Software Version 3.7 works on the following instruments:

- ABI PRISM® 310 Genetic Analyzer
- ABI PRISM® 377 DNA Sequencer
- ABI PRISM® 3100 Genetic Analyzer
- ABI PRISM® 3700 DNA Analyzer

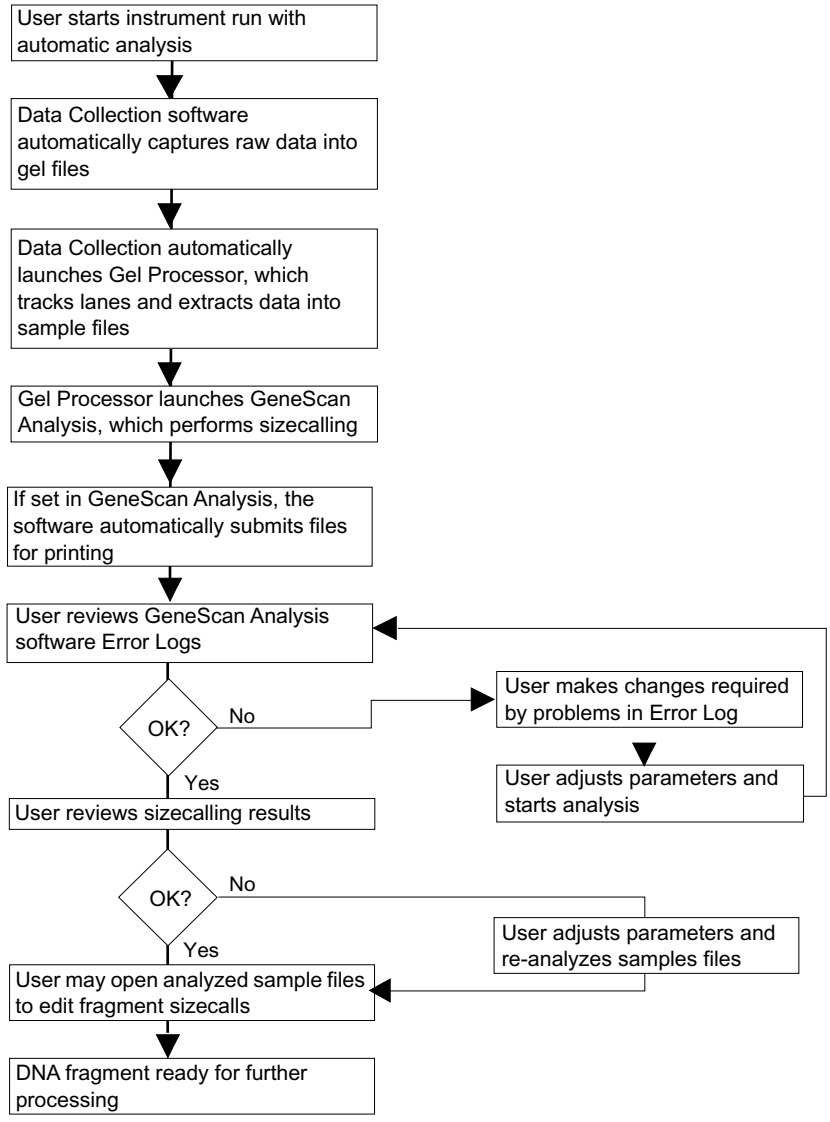
GeneScan Software on the 310 Instrument

Flowchart



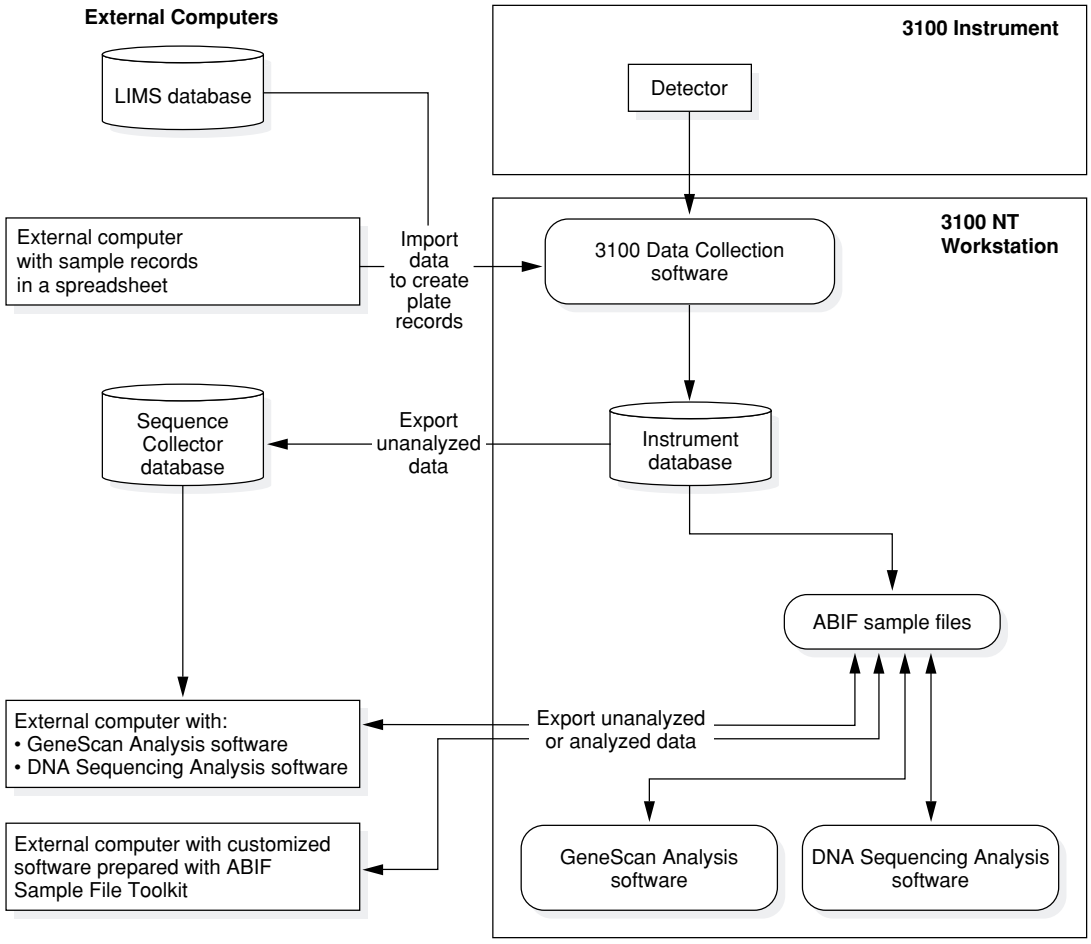
GeneScan Analysis on the 377 Instrument

Flowchart



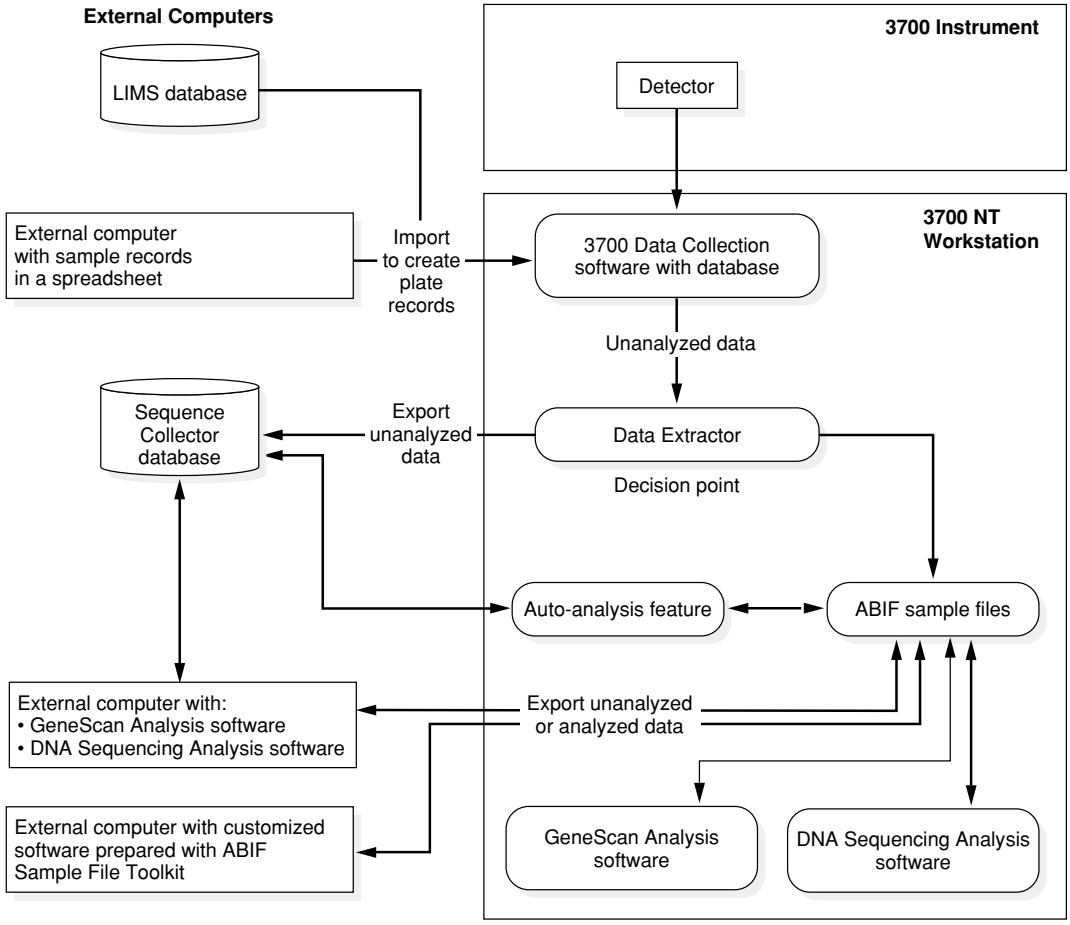
GeneScan Analysis on the 3100 Instrument

Flowchart



GeneScan Analysis on the 3700 Instrument

Flowchart



Sequence Collector Database Option

With the Sequence Collector database system, data is collected by the Windows NT-based computer and exported to a Sequence Collector database on a networked server.

The data can later be viewed, edited, and analyzed using the GeneScan Analysis Software version 3.7 for Windows NT®, either using the same Windows NT-based computer used to collect the data, or a different Windows NT-based computer with access to the Sequence Collector database. The data can also be viewed and edited (but not analyzed) using the GeneScan Analysis Software on a Macintosh® computer.

Related Manuals

GeneScan Analysis Software is part of a suite of Applied Biosystems hardware and software products.

If the information you need is not in this manual, it may be in one of the other manuals listed in the table below.

For more information about…	See…	Part Number
ABI PRISM® 3700 DNA Analyzer	*ABI PRISM* 3700 DNA Analyzer User's Manual	4325941
ABI PRISM® 3100 Genetic Analyzer	*ABI PRISM® 3100 Genetic Analyzer User's Manual*	4315834
ABI PRISM® 377 DNA Sequencer	*ABI PRISM® 377 DNA Analyzer User's Manual*	4325703
ABI PRISM® 310 Genetic Analyzer	*ABI PRISM® 310 Genetic Analyzer User's Manual*	4317588
specific GeneScan chemistry protocols, designing experiments, and preparing samples	♦ GeneScan® Reference Guide, Chemistry Reference for the ABI PRISM 310 Genetic Analyzer, or ♦ The protocols that accompany Applied Biosystems reagent kits.	4303189
attaching the new matrix to an ABI PRISM 377 gel file	*Gel Processor User's Manual*	
accessing and managing a Sequence Collector database	*Sequence Collector User Guide NT*	4319527
installation and administration of Sequence Collector	*Sequence Collector Installation and System Administration Guide*	4319526

Registering the Software

License and Warranty

Before you begin, read Appendix G, “License and Warranty.” This appendix explains your rights and responsibilities regarding the software.

Registering Your Software

To register your copy of the GeneScan Analysis Software, complete the registration card (included in this software package) and return it to Applied Biosystems.

Note Registering the software enables us to send you notification of software updates and any other future information that may be specific to GeneScan Analysis Software owners.

IMPORTANT Your product registration number is located on the Registration card. Be sure to record this number here before you return the Registration card.

Registration Number:

Hardware and Software Requirements

Introduction

The GeneScan Analysis Software can be installed on the Windows NT®-based computer connected to your ABI PRISM® instrument or on any other Windows NT-based computer that meets the minimum requirements stated below. The software can be installed on a computer used for analysis only, and on one used for both data collection and analysis.

Computers Connected to Applied Biosystems Instruments

The following table provides important information about computers connected to Applied Biosystems instruments:

If...	Then...
you received this software with a newly purchased instrument	the GeneScan Analysis Software was installed by your Applied Biosystems Customer Support Engineer as part of the installation and setup of the instrument. The system requirements for that computer are described in the instrument user's manual.
you are updating the GeneScan Analysis Software, or if you are installing the software on a computer other than the one supplied with your instrument	ensure your computer meets the minimum requirements provided below before loading the software. **IMPORTANT** Your computer MUST meet the requirements listed to run the new GeneScan Analysis Software.

System Requirements

Below are the system requirements to run the GeneScan Analysis Software v. 3.7 for Windows NT® platform on your instrument or analysis computer.

IMPORTANT Applied Biosystems strongly recommends using the computer supplied with your instrument for running GeneScan Analysis software. The software was optimized and tested on systems similar to that shipped with your instrument. Running the software on systems that do not meet the following requirements may cause data loss or other significant problems.

Note These are the minimum requirements. In general, the more memory, the larger the screen size, and the more processing power you have, the better.

System Component	System Requirements	
	For use with 310 and 377 Instruments	**For use with 3100 and 3700 Instruments**
Model	Dell 733 GX 100 Medium Desktop	Dell WorkStation 220
Processor Speed/Bus	733 MHz/133 MHz	733 MHz/133 MHz
CD-ROM drive	Any	Any
Operating system	Windows NT v. 4. with Service Pack 5	Windows NT v. 4. with Service Pack 5
RAM	The minimum memory requirement is 128 MB, although 256 MB is recommended.	256 MB RAM

Minimum System Recommendations

System Component	Recommendations
Monitor	A 17-inch monitor or larger with 1024 x 768 resolution
Disk Space	Storage requirements depend primarily on the quantity of data to be generated and stored. It is common to store many sample files on the analysis computer. Each sample file is approximately 150–250 KB.
Printer	A PostScript-compatible color printer

Hard Drive Partitions

Use the following information to determine on which drive to install software. During installation, the hard drive of the Windows NT-based computer was partitioned to create the following drives:

When installing...	Use drive...
programs for general use	C.
♦ Data Collection program database software ♦ GeneScan Analysis software	D.

Installing the GeneScan Analysis Software

Preparation

To prepare for the installation:

Step	Action
1	Check that you have at least 40 MB of free disk space to accommodate the GeneScan Analysis Software.
2	Exit all programs that are running.
3	Turn off any virus protection software that you may have running.

Procedure

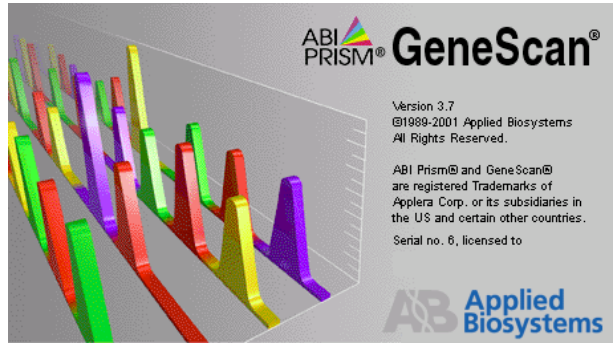
To install the GeneScan Analysis Software from a CD-ROM:

Step	Action
1	Insert the GeneScan v. 3.7 software CD-ROM into the computer's CD-ROM drive. It will automatically launch the setup when you insert the CD and install the software.
2	If it does not run the setup automatically, you can either: ♦ Double-click Setup (.exe) or ♦ Click **Start**, point to **Run**, then browse to the CD-ROM drive and click **OK** Follow the instructions to install the software. The **Setup Complete** window opens when the setup has finished installing the software on your computer.

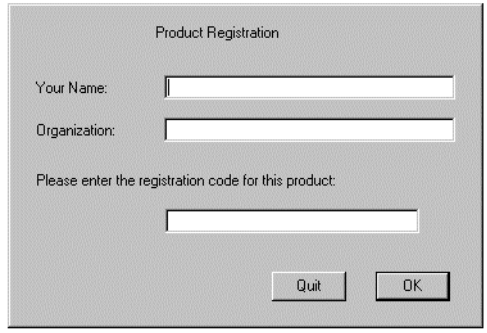
Starting the GeneScan Analysis Software for the First Time

Procedure Follow this procedure when you start the GeneScan Analysis Software the first time or when you start the software after moving the application from the Applied Biosystems folder.

To start the GeneScan Analysis Software for the first time:

Step	Action
1	Before opening the GeneScan Analysis Software for the first time: a. Click **Start**, point to **Settings**, and click **Printers**. b. Right-click the printer you expect to use for GeneScan Analysis Software data. c. Click the **Close** button in the **Printers** window.
2	Go to the D:\AppliedBio\GeneScan\Bin folder and double-click the GeneScan icon to start the software. The GeneScan Analysis Software startup screen opens. 

To start the GeneScan Analysis Software for the first time: *(continued)*

Step	Action
3	The **Product Registration** dialog box opens. Enter your registration information into the three fields and click **OK**. **Note** The registration code is the number you recorded on page 1-8.  This dialog box opens the first time you start the GeneScan Analysis Software, and any time that you move the software to a different disk or partition.
4	When the Product Registration dialog box closes, the GeneScan Analysis Software menu bar opens. **For information about...** — **See Page** Creating a Project — 2-9 Opening Sample Files — 4-7

Removing the Software

This section describes how to remove the GeneScan Analysis Software v3.7 from your Windows NT-based computer. The uninstall process deletes all folders and files installed by the GeneScan Analysis Software Installer.

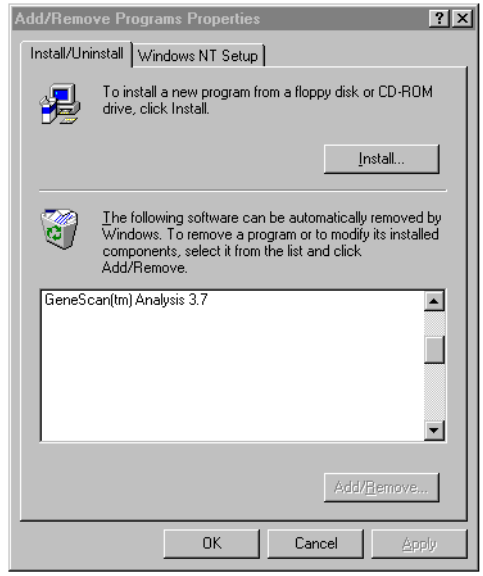
If Files or Folders Have Been Moved

If you have moved the GeneScan Analysis Software files or folders from their original installed locations they may not be found and deleted by the uninstall operation.

Any files that have been added to the application folders, such as those created when the applications are run, are not deleted by the uninstall operation.

Procedure

To remove installed GeneScan Analysis Software:

Step	Action
1	Click **Start,** point to **Settings**, click **Control Panel**, and then double-click **Add/Remove Programs**. The following dialog box opens: 
2	On the **Install/Uninstall** tab, select **GeneScan** and click **Add/Remove**.

To remove installed GeneScan Analysis Software: *(continued)*

Step	Action
3	At the conclusion of the remove operation, an alert box opens with the message whether or not the remove was successful.
4	If files have been moved or added to the GeneScan Analysis Software or AppliedBio folders, the remove operation will be reported as unsuccessful. If this happens do one of the following: • Examine and delete the remaining files yourself, or • On the **Start** menu point to **Remove GeneScan Analysis v3.7**, then select **Programs**, and **Applied Biosystems**.

2 Creating a Project

Overview

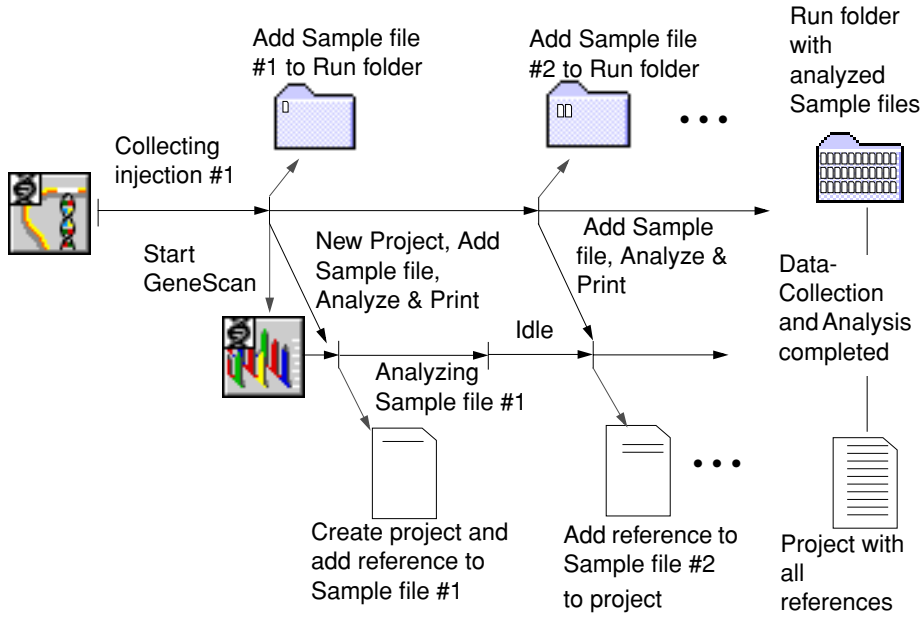
In This Chapter Topics in this chapter include the following:

Topics	See Page
Creating Projects and Auto-Analysis on 310 Instruments	2-2
Creating Projects and Auto-Analysis on 377 Instruments	2-3
Setting Up for Automatic Analysis on 3100 and 3700 Instruments	2-4
Working with Project Files	2-8
Finding Missing Sample Files	2-14

Creating Projects and Auto-Analysis on 310 Instruments

Process Using the 310 Instrument

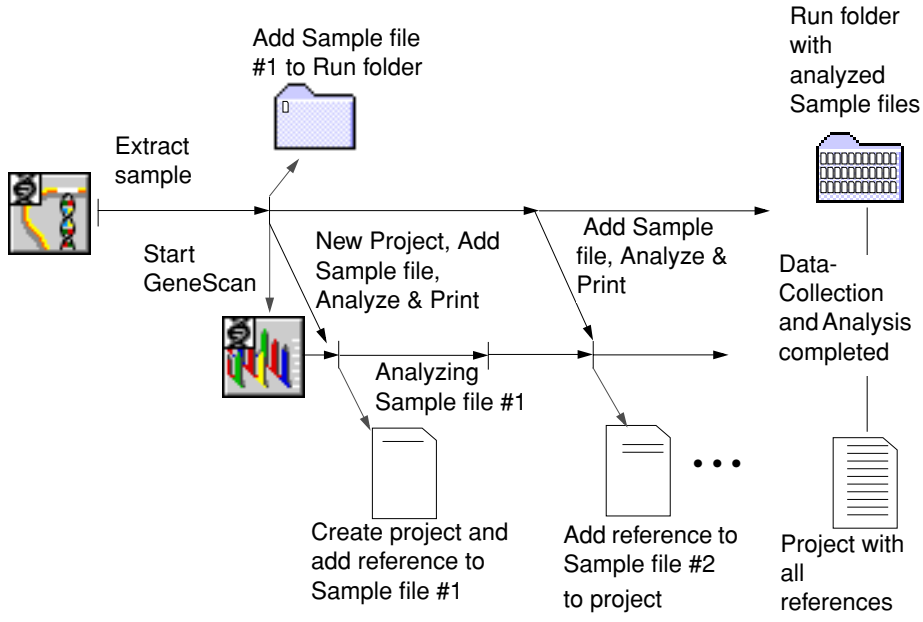
The following diagram illustrates data analysis using the ABI PRISM® 310 Genetic Analyzer.



Creating Projects and Auto-Analysis on 377 Instruments

Process Using the 377 Instrument

The following diagram illustrates the process of automatic analysis and project creation for data from the ABI PRISM® 377 DNA Sequencer.



Setting Up for Automatic Analysis on 3100 and 3700 Instruments

Introduction

To set up the GeneScan® Analysis Software for automatic analysis after data-collection, you must have previously defined analysis parameters and size standards.

For more information refer to the following sections:

- “Chapter 5, “Working with Analysis Parameters.”.
- “Defining the Size Standard” on page 7-3.

Procedure

To set up for automatic analysis after using the 3100 and 3700 Data Collection software:

Step	Action
Complete the following steps in the 3100 and 3700 Data Collection software.	
1	Set the GeneScan Run default preferences to auto-analyze and use the pop-up menu to locate and select the GeneScan Analysis Software.
2	In the GeneScan Sample Sheet, for each sample to be analyzed, do the following: ♦ Enter the sample name. This field must be completed for the samples to be active in the Injection or Run Sheet. ♦ Indicate which dye is the standard. ♦ Select the check box labeled Pres (Present) for each dye/sample you want auto-analyzed. ♦ Select any additional check boxes. **IMPORTANT** If you plan to use the Genotyper® software, you must complete the Sample Info box correctly. For more information, refer to the *Genotyper User’s Manual*.

To set up for automatic analysis after using the 3100 and 3700 Data Collection software: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>In the Injection List or Run Sheet for each applicable sample, select AutoAnalyze, and choose one of the following from one of the appropriate pop-up menus:
♦ Matrix file
♦ Analysis parameters
♦ Size standard
The following table lists considerations when choosing a size standard.
<table>
<tr><th>If you choose a...</th><th>Then GeneScan...</th></tr>
<tr><td>Size Standard definition file in the Collection Injection List or Run Sheet, but you do not specify which dye is the standard in the Sample Sheet</td><td>performs analysis using the dye specified in the Auto-Analysis defaults.</td></tr>
<tr><td>dye as the standard in the Sample Sheet, but do not specify a size standard definition file in the Collection Injection List or Run Sheet</td><td>does not perform size calling.</td></tr>
</table></td></tr>
<tr><td>4</td><td>Select Auto Print to print automatically.</td></tr>
<tr><td colspan="2">Complete the following steps:</td></tr>
<tr><td>1</td><td>If you are using the Auto-Analysis defaults, choose Auto-Analysis Defaults from the Settings menu.
The Auto-Analysis Defaults dialog box opens.
The parameters set in the Auto-Analysis Defaults dialog box apply when you have:
♦ Specified <Analysis Defaults> in the Data Collection software.
♦ Selected the check box labeled Always Override Collection Settings.</td></tr>
</table>

To set up for automatic analysis after using the 3100 and 3700 Data Collection software: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Select the check box labeled Always Override Collection Settings to have the new parameters take precedence over the following files specified in the 3100 and 3700 Data Collection programs:
♦ Size standard
♦ Analysis parameters
♦ Dye standard</td></tr>
<tr><td>3</td><td>Select a new Size Standard if:
♦ Analysis Defaults is specified for the size standard in the data collection settings, or
♦ To override the specified size standard.</td></tr>
<tr><td>4</td><td>Select the Dye pop-up menu to specify the dye that represents the internal size standard you are running with the samples.</td></tr>
<tr><td>5</td><td>Select the analysis parameters you want to use from the Parameters pop-up menu.
To use the default parameters, select Analysis Parameters.</td></tr>
<tr><td>6</td><td>In the Auto-Print section, there are the following options:
<table>
<tr><th>Choose...</th><th>To print...</th></tr>
<tr><td>Show Electropherograms</td><td>electropherograms.
Select the appropriate radio button to specify whether the electropherograms for the four dyes appear:
♦ Together in one panel (overlaid), or
♦ In separate panels (tiled).</td></tr>
<tr><td>Show Tabular data</td><td>tabular data.</td></tr>
</table></td></tr>
<tr><td>7</td><td>Click OK.
The GeneScan Analysis Software is now prepared to perform automatic analysis.
Note The settings that you specify are initial settings. Make two or three trial runs, fine tuning the parameters with each run, to determine which parameters work the best for a particular protocol.</td></tr>
</table>

What Is a Project

A project is a file containing references to a set of sample files that you want to analyze and display together. The project contains Analysis Control (Ctrl+1) and Results Control (Ctrl+2) windows that allow you to analyze specific dye/samples and display the results of analysis.

Why Create a Project

You can create a new project and add any combination of sample files, allowing you to analyze and display samples from different runs.

Adding a sample file to the project sets up a link between the project and the sample file. The file itself is not imported into the project.

For more information, see “Creating a New Project” on page 2-9.

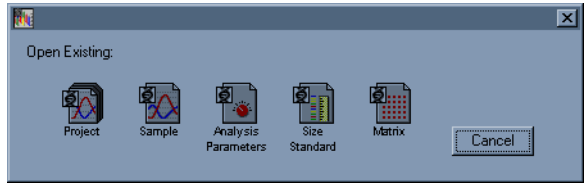
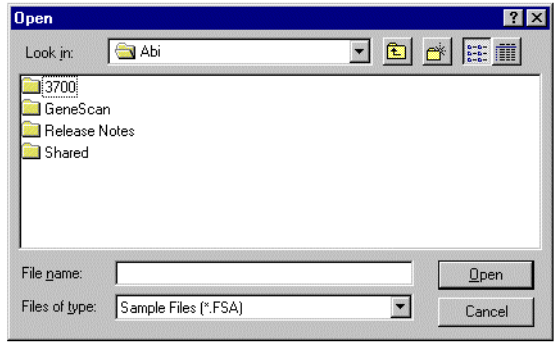
Where to Store Projects

Keep the sample files in the same location on the hard disk relative to the project file so the GeneScan® Analysis Software can locate them when the project is opened.

If...	Then...
sample files are moved and the software does not find them when you open the project	see “Finding Missing Sample Files” on page 2-14.

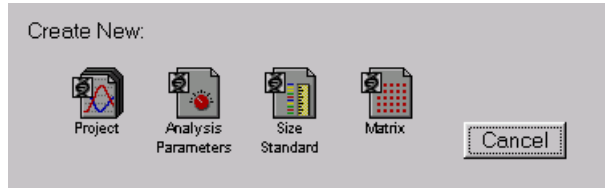
Opening an Existing Project

To open an existing project:

Step	Action
1	Select **Open** (Ctrl+O) from the **File** menu. The **Open Existing** dialog box opens. 
2	Click the **Project** icon. An **Open** dialog box opens. 
3	In the dialog box, find and select the project you want to open.
4	Click **Open**. The project opens in an **Analysis Control** window.

Creating a New Project

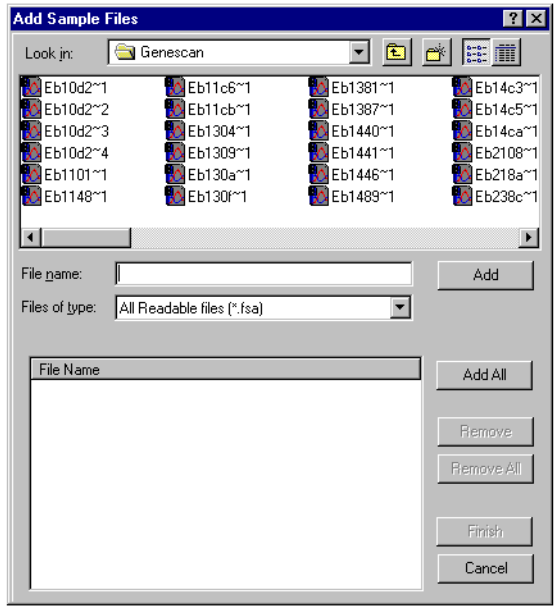
To create a new project:

Step	Action
1	Select **New** (Ctrl+N) from the **File** menu. The **Create New** dialog box opens. 
2	Click the **Project** icon. An untitled **Analysis Control** window opens. **Note** For more information on using the Analysis Control window, refer to page 3-6.
3	There are two ways to add files to a project:

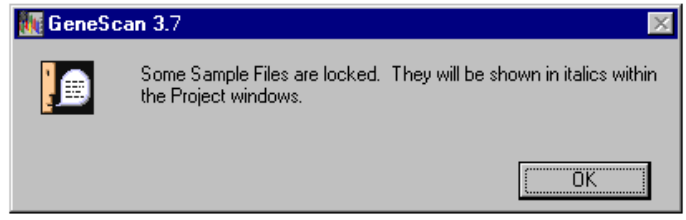
To add...	Select...
sample files you select to the open project	**Add Sample Files** (Ctrl+B) from the **Project** menu. The **Add Sample** dialog box opens (refer to "Using the Add Sample Dialog Box" below).
a sample currently open to the open project	**Add "file name"** from the **Project** menu.

Using the Add Sample Dialog Box

To use the Add Sample dialog box:

Step	Action
1	When the **Add Sample Files** dialog box opens, find the folder containing the samples that you want to add from the **Look in** pop-up menu. 

To use the Add Sample dialog box: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>You can take the following action:
<table>
<tr><th>If you want to...</th><th>Then...</th></tr>
<tr><td>select a single sample file</td><td>double-click the file or select the file and click Add.</td></tr>
<tr><td>select all the sample files</td><td>click Add All.</td></tr>
<tr><td>add a random selection of sample files</td><td>click each file name while pressing the Ctrl key.</td></tr>
<tr><td>add a continuous list of sample files</td><td>a. Click the first sample that you want to add.
b. Press the Shift key and click the last sample you want to add.
All the files between the first and last file are selected.</td></tr>
</table></td></tr>
<tr><td>3</td><td>Click Finish when you have added all the sample files.
<table>
<tr><th>If the...</th><th>Then...</th></tr>
<tr><td>sample files appear in the Analysis Control window</td><td>refer to “Analyzing Sample Files: Using the Analysis Control Window” on page 3-6.</td></tr>
<tr><td>locked files alert appears (see below)</td><td>go to “Unlocking Sample Files” below.</td></tr>
</table>

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

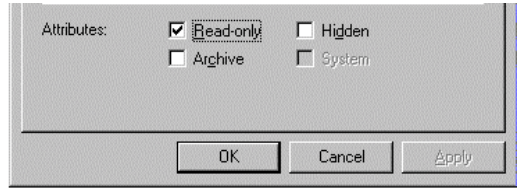
Unlocking Sample Files

Adding Locked Sample Files to a Project

If...	Then...
the sample files added to a project are locked	the GeneScan Analysis Software does not allow changes to them. You cannot analyze locked files. When you add locked files, an alert appears.

Procedure

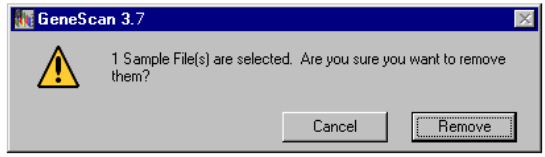
To unlock files:

Step	Action
1	Select **Save Project** (Ctrl+S) from the **File** menu and close the project.
2	Click the **Start** button, and then point to **Programs**.
3	Click **Windows NT Explorer**.
4	Select the applicable sample files and select **Properties** from the **File** menu. The **Properties** dialog box opens.
5	In the **Attributes** section, unselect the **Read-only** check box and click **OK**. 
6	Close the window and reopen the project. **If...** / **Then...** you did not close the project before unlocking the files → once the files are unlocked, close and open the project so the GeneScan Analysis Software recognizes that the sample files are unlocked.

Removing Samples from a Project

Note A removed sample file is not deleted from the hard disk. The reference is removed from the project.

To remove sample files from a project:

Step	Action
1	Select the file or files in the **Analysis Control** window (Ctrl+1) or the **Results Control** window (Ctrl+2) that you want to remove. **Note** Ctrl+click to select multiple files.
2	Select **Remove Sample Files** from the **Project** menu, or press the **Delete** key. A warning dialog box opens. 
3	Click **Remove**.

Finding Missing Sample Files

When Are Files Considered Lost

The project and related sample files are usually located in the Run folder created by the Data Collection software.

If...	Then...
the sample files or the project are moved so they are no longer in the same relative position	the GeneScan Analysis Software might not be able to locate the sample files when the project is opened. Usually, this occurs only if the sample files are moved to another disk drive, another server on a network, or another disk partition on the hard drive.

When an Alert Appears

The following table describes what happens when the software does not locate the sample files associated with a project:

If...	Then...
the GeneScan Analysis Software does not locate the sample files associated with a project	an alert box opens and the sample file names appear dimmed when the project opens.

Searching for Missing Sample Files

You can re-establish the links between the sample files and the project by choosing Find Missing Sample Files from the Project menu and choosing one of the following options from the submenu:

To find missing files:

Choose...	If you...	Description
Fast Search	suspect that the GeneScan Analysis Software could not find the missing sample files because they are located on an unmounted external storage device, or diskette.	Once the volume is mounted, or the diskette containing the files is inserted, Fast Search finds them immediately.

To find missing files: *(continued)*

Choose...	If you...	Description
Search a Folder	know what folder contains the missing sample files.	Specify a folder in which to search for missing sample files. The GeneScan Analysis Software then immediately locates the sample files and re-establishes links to the project.
Exhaustive Search	do not know where any of the specified missing sample files are located.	The GeneScan Analysis Software searches all mounted disk drives, and available servers. When the files are found, the software re-establishes links to the project.

Re-Establishing Links

To re-establish the links with sample files:

Step	Action
1	Click the dimmed file name to select it. When the missing file is selected the **Find "file name"** command becomes active. The name of the missing file appears inside the quotation marks.
2	Select **Find "file name"**. A file dialog box opens.
3	In the file dialog box, locate the proper folder and file.
4	Click **Open** or double-click the file name.

Procedure To set up for automatic analysis after data collection, complete the following steps in the data collection software:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Set the GeneScan Run default preferences to auto-analyze and use the pop-up menu to locate and select the GeneScan Analysis Software.</td></tr>
<tr><td>2</td><td>In the GeneScan Sample Sheet, for each sample to be analyzed, do the following:
♦ Enter the sample name.
This field must be completed for the samples to be active in the Injection or Run Sheet.
♦ Indicate which dye is the standard.
♦ Select the check box labeled Pres (Present) for each dye/sample you want auto-analyzed.
♦ Select any additional check boxes.
IMPORTANT If you plan to use the Genotyper® software, then you must complete the Sample Info box correctly. For more information, refer to the Genotyper User's Manual.</td></tr>
<tr><td>3</td><td>In the Injection List or Run Sheet for each applicable sample, select AutoAnalyze, and choose from the appropriate pop-up menus one of the following:
♦ Matrix file or dye set name
♦ Analysis parameters
♦ Size standard
The following table lists considerations when choosing a size standard.
<table><tr><th>If you choose a...</th><th>Then GeneScan...</th></tr><tr><td>Size Standard definition file in the Collection Injection List or Run Sheet, but you do not specify which dye is the standard in the Sample Sheet</td><td>performs analysis using the dye specified in the Auto-Analysis defaults.</td></tr><tr><td>dye as the standard in the Sample Sheet, but do not specify a size standard definition file in the Collection Injection List or Run Sheet</td><td>does not perform size calling.</td></tr></table></td></tr>
<tr><td>4</td><td>Select Auto Print to print automatically.</td></tr>
</table>

Analyzing Project Files

3

Overview

In This Chapter Topics in this chapter include the following:

Topics	See Page
Analyzing Project Files: About the Analysis Control Window	3-2
Analyzing Sample Files: Using the Analysis Control Window	3-6
Defining Folder Locations	3-16

Analyzing Project Files: About the Analysis Control Window

Introduction

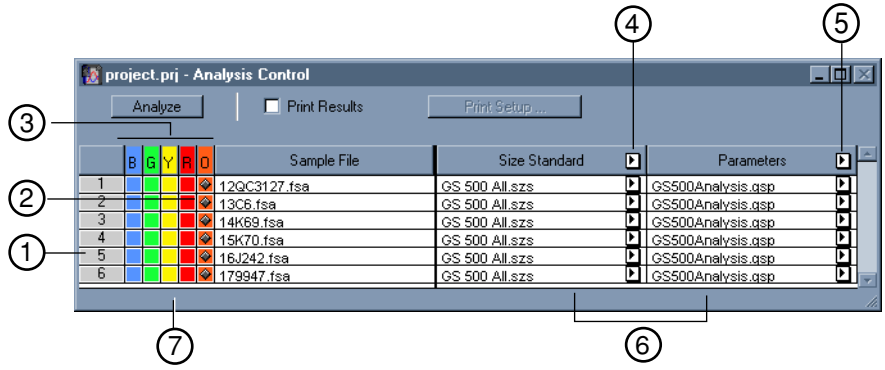
When you open a project, the Analysis Control window opens. The Analysis Control window is the main window of a project. You can use this window to specify the following for each sample in the project:

- Dye that represents the size standard you ran with the sample
- Size standard
- Analysis parameters
- Specific dyes to be analyzed
- Format the document and print the results automatically

For more information, refer to “Analyzing Sample Files: Using the Analysis Control Window” on page 3-6.

Analysis Control Window Example

The following is an example of the Analysis Control window:



Analysis Control Window Callouts Described

The following table describes the callouts shown for the Analysis Control window in the above figure:

Analysis Control window callouts:

Callout	Description
1	Use these columns to choose the dye colors to analyze and to specify which is the size standard.
2	Diamonds mark the standards. For more information, refer to “What Are Size Standards” on page 7-2.
3	Ctrl+click a dye/sample field to specify that dye/sample as the standard.
4	From the arrow pop-up menu , you can: ♦ Choose **Collection Setting**. ♦ Choose a user-defined size standard. ♦ Define a new size standard for that sample. For more information, refer to: **Topic** — **See Page** Defining the Size Standard — 7-3 Using Size Standards — 7-9
5	From the arrow pop-up menu , you can: ♦ Choose **Collection Setting**. ♦ Choose an analysis parameters file. For more information, refer to: **Topic** — **See Page** About the Analysis Parameters — 5-2 Using Analysis Parameter Files — 5-13

Analysis Control window callouts: *(continued)*

Callout	Description
6	Double-click the size standard text field or the analysis parameters text field to edit the size standard or the analysis parameters. For more information, refer to: **Topic** — **See Page** Editing an Existing Size Standard — 7-10 Changing an Existing Analysis Parameters File — 5-17
7	A notation appears in this Information Display field when you move the cursor over a sample file name or over a dye color field. **Note** For more information on how to customize this field, refer to “Displaying Sample and Dye Information” on page 3-11.

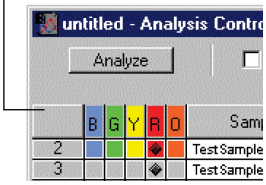
Customizing the Display

The following table explains how you can customize the display by changing the settings:

Note These preferences also apply to the Results Control window. Refer to Chapter 8, “Evaluating Analysis Results.”

To change...	Choose...	For more information
information displayed in the information display field	**Project Options** from the **Settings** menu and **Sample Info Display** from the submenu.	“Displaying Sample and Dye Information” on page 3-11.
the sorting of sample files	**Project Options** from the **Settings** menu and **Sample File Sorting** from the submenu.	“Setting Sample File Sort Order” on page 3-13.
dye indicator code dye color	**Preferences** from the **Settings** menu and **Dye Indicators** from the submenu.	“Setting Dye Indicator Preferences” on page 3-14.

Using the Analysis Control Window

To...	Then...	Result
select all the samples	click the upper-left cell. Click here 	All the columns in the **Analysis Control** window are selected.
select all of one dye color	click the column heading for that dye color.	The column is highlighted for the color selected.
select all dyes for one sample	click the row number.	All the colors in the row are selected.
change all the standards in the size standards column to the same setting	a. Click the arrow in the column heading. b. Select a file from the pop-up menu.	The same size standard is displayed for all the samples.
change a size standard in one of the rows	a. Click the arrow in the row. b. Select a file from the pop-up menu.	The size standard for the selected row changes.
change all the parameters in the parameters column to the same setting	a. Click the arrow in the row heading. b. Select a file from the pop-up menu.	The same parameter is displayed for all the samples.
change a parameter in one of the rows	a. Click the arrow in the row. b. Select a file from the pop-up menu.	The parameter for the selected row changes.
apply a choice to selected fields in the size standards or parameters column	a. Click the row in the column containing the information you want to apply and drag down. b. Select **Fill Down** (Ctrl+D) from the **Edit** menu.	The value in the selected rows changes to the value in the first row selected.

Analyzing Sample Files: Using the Analysis Control Window

Introduction

This section describes using the Analysis Control window to perform the following tasks:

Topic	See Page
Accessing Sample Files	3-6
Analyzing Sample Files	3-7
Specifying the Format for Printed Results	3-8
Displaying Size Standards and Analysis Parameters	3-10
Displaying Sample and Dye Information	3-11
Setting Sample File Sort Order	3-13
Setting Dye Indicator Preferences	3-14

Accessing Sample Files

There are two ways to access sample files contained in a project from the Analysis Control window.

Note Sample files that are dimmed can not be found by the project. To find missing files, refer to “Finding Missing Sample Files” on page 2-14.

<table>
<tr><th>You can...</th><th>Then...</th></tr>
<tr><td>double-click a sample file name</td><td>
<table>
<tr><th>If the sample file is...</th><th>Then that...</th></tr>
<tr><td>open</td><td>Sample File window becomes active.</td></tr>
<tr><td>not open</td><td>sample file opens to its Sample Results view.</td></tr>
</table>
</td></tr>
<tr><td>select a sample file and select one of the five display modes from the Sample menu</td><td>the Sample File window appears in the display mode selected.</td></tr>
</table>

Analyzing Sample Files

The Analysis Control window (Ctrl+1) allows you to analyze multiple samples easily. You choose dyes to analyze, dye standard, size standard, and analysis parameters for each sample file, and then analyze the sample files using these settings.

To analyze sample files:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Click the dye color fields for each sample you want to analyze as follows:
<table>
<tr><th>To...</th><th>Click the...</th></tr>
<tr><td>select a dye for all samples</td><td>colored column header for that dye.</td></tr>
<tr><td>select all dyes for a single sample file</td><td>index number at the left end of the row in which the sample file appears.</td></tr>
<tr><td>all dyes for all samples</td><td>area above the row index numbers.</td></tr>
</table></td></tr>
<tr><td>2</td><td>Identify the sample containing the standard as follows:
<table>
<tr><th>To...</th><th>Ctrl+click the...</th></tr>
<tr><td>identify each sample that contains a size standard</td><td>colored field that represents the size standard.</td></tr>
<tr><td>select the same dye as the size standard for all samples</td><td>colored column heading for that dye.</td></tr>
</table>
A diamond appears in the field to identify the dye color as the size standard.</td></tr>
<tr><td>3</td><td>Select a defined size standard setting from the pop-up menu in the Size Standard column as follows:
<table>
<tr><th>To...</th><th>Refer to...</th></tr>
<tr><td>define a new size standard</td><td>“Using Size Standards” on page 7-9.</td></tr>
<tr><td>edit a size standard</td><td>“Editing an Existing Size Standard” on page 7-10.</td></tr>
</table></td></tr>
<tr><td>4</td><td>To install a new matrix, select a set or all of the samples in the Sample File column and choose Install New Matrix from the Sample menu.</td></tr>
</table>

To analyze sample files: *(continued)*

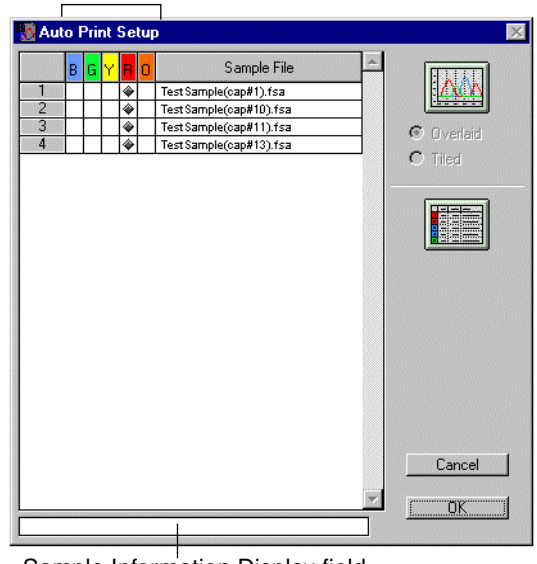
Step	Action
5	Select a parameter setting from the pop-up menu in the **Parameters** column as follows: **To...** / **See...** use the default analysis parameters to specify different parameters / "About the Analysis Parameters" on page 5-2.
6	Select the **Print Results** check box to print the results automatically. For information on print set-up, refer to "Specifying the Format for Printed Results" on page 3-8.
7	Click **Analyze**.
8	To verify the results, refer to "Process of Verifying Results" on page 9-31.

Specifying the Format for Printed Results

To specify the format for printed results:

Step	Action
1	In the **Analysis Control** window (Ctrl+1), select the check box labeled **Print Results**. When this check box is selected, the **Print Setup** button becomes active.

To specify the format for printed results: *(continued)*

Step	Action
2	Click the **Print Setup** button. The **Auto Print Setup** dialog box opens (see below). All the dyes selected for analysis are also selected for printing. Sample Dye color fields  Sample Information Display field
3	Moving the cursor over a Sample File name or over a dye color field, a notation appears in the Sample Information Display field.
4	Click the sample dye color fields to specify any sample you do not want to print as shown above.

To specify the format for printed results: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>5</td><td>Select the format by clicking either or both of the buttons at the right of the window.
<table>
<tr><th>Click this button...</th><th>To print...</th></tr>
<tr><td></td><td>electropherograms for the samples and dyes selected for analysis.

Select the appropriate radio button to specify whether the electropherograms for all the dyes appear:

♦ Together in one panel (overlaid), or

♦ In separate panels (tiled).</td></tr>
<tr><td></td><td>tabular data.</td></tr>
</table></td></tr>
<tr><td>6</td><td>Click OK.</td></tr>
</table>

Displaying Size Standards and Analysis Parameters

Use the Analysis Control window to open, review, or change size standard and analysis parameters.

To display these files:

You can...	Then...	For more information see...
double-click the field containing the size standard or the analysis parameters file	the **Size Standard** or **Analysis Parameter** window opens.	♦ “Using Analysis Parameter Files” on page 5-13. ♦ “Defining the Size Standard” on page 7-3.

Displaying Sample and Dye Information

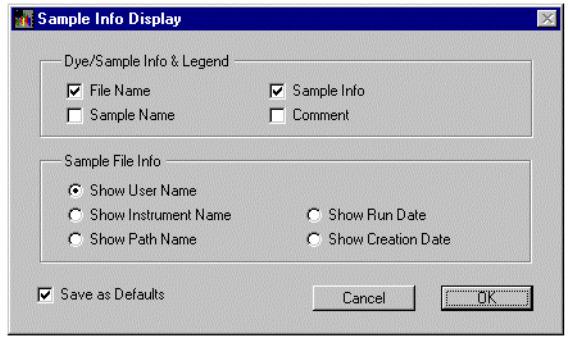
How to Display Sample and Dye Information

Move the cursor over a dye color field or over a sample file name field to display information about the samples and dyes.

How to Specify the Information Displayed

The following procedure describes how to specify the information displayed when moving the cursor over a dye color field or a sample file name field:

To specify the information displayed:

Step	Action
1	Select **Project Options** from the **Settings** menu and **Sample Info Display** from the submenu. The **Sample Info Display** dialog box opens. 
2	Select the check boxes in the **Dye/Sample Info & Legend** section to control what appears when you move the cursor over the dye color fields in the **Control** windows. The following table describes the check boxes:

If you select this check box...	This appears...
File Name	Sample file name.
Sample Name	Name of the sample file from the sample file.
Sample Info	Sample information from the sample file.
Comment	Comment from the sample file.

To specify the information displayed: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>In the Sample File Info heading, select the check boxes for the information that you want to display when you move the cursor over the Sample File name field.

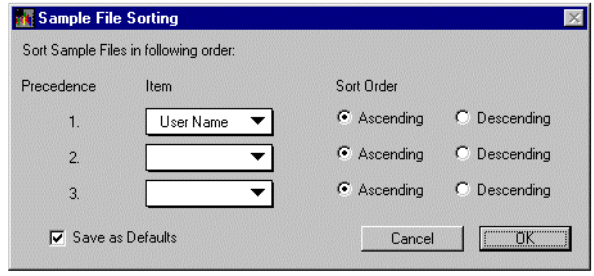
The following table describes the buttons:

<table>
<tr><th>Select this button...</th><th>To display...</th></tr>
<tr><td>Show User Name</td><td>user name from the sample file.</td></tr>
<tr><td>Show Instrument Name</td><td>instrument name from the sample file.</td></tr>
<tr><td>Show Path Name</td><td>path and name of the folder where the file is located.</td></tr>
<tr><td>Show Run Date</td><td>run date and start time from the sample file.</td></tr>
<tr><td>Show Creation Date</td><td>date and time the sample file was created.</td></tr>
</table>
</td></tr>
<tr><td>4</td><td>Select the check box labeled Save as Defaults to have the options you choose saved as the default settings.</td></tr>
<tr><td>5</td><td>Click OK.</td></tr>
</table>

Setting Sample File Sort Order

Use this option to specify how sample files are sorted using three criteria. If no sorting option is specified, then the program sorts by sample number.

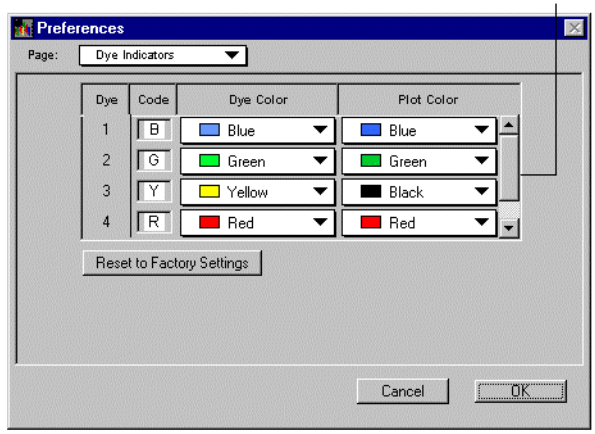
To set sample file sort order:

Step	Action
1	Select **Project Options** from the **Settings** menu and **Sample File Sorting** from the submenu. The **Sample File Sorting** dialog box opens. 
2	From the pop-up menus, select from the following items: ♦ File Name ♦ Directory ♦ Sample Number ♦ User Name ♦ Instrument Name ♦ Run Date ♦ Creation Date ♦ As Added (sorts the files in the order that they were added) The precedence indicates the sorting level.
3	Select a button for each item to indicate whether to sort in ascending or descending order.
4	Select the check box labeled **Save as Defaults** to have the options you choose saved as the default settings.
5	Click **OK**.

Setting Dye Indicator Preferences

The following procedure describes how to change the defaults that determine what dye colors appear on the screen and on printed results. Setting default dye and plot colors sets the colors used for both the Control windows and the Results displays:

To set default dye and plot colors:

Step	Action
1	Select **Preferences** from the **Settings** menu and **Dye Indicators** from the submenu. The **Preferences** window opens. If the **Preferences** window is already displayed, select **Dye Indicators** from the pop-up menu. Vertical scroll bar  **Note** Use the scroll bar to see the orange dye color.
2	The following table describes the **Dye Color** and **Plot Color** columns:
3	Use the vertical scroll bar to change the dye color and plot color for a fifth dye.

Item	Description
Dye Color column	Shows the colors that represent the dyes in the **Control** window list. The dye color is also identified in the left color legend in the **Results** display.
Plot Color column	Shows the colors used for plotting the data in the electropherograms.

To set default dye and plot colors: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>To change a code, type a different character in the appropriate entry field in the Code column.</td></tr>
<tr><td>5</td><td>Take the following action to change a color:

<table><tr><th>To...</th><th>Then...</th></tr><tr><td>change a color</td><td>select a new color from the pop-up menu.</td></tr><tr><td>define a new color</td><td>Select Other from the pop-up menu.

A color picker opens.</td></tr></table></td></tr>
<tr><td>6</td><td>Click OK when finished changing dye indicator preferences.</td></tr>
</table>

Defining Folder Locations

Introduction

The GeneScan Analysis Software looks in the designated folders for the:

- Size Standard file
- Analysis Parameter file
- Matrix file

When saving one of these files for the first time, the default folder locations for saving the files are those same designated folders.

Storing Matrix Files

Store matrix files, that are intended for use by Data Collection software to assign to collection runs, in the AppliedBio folder. The AppliedBio folder is located on the computer on which the Data Collection software is installed.

If Data Collection and Analysis are Performed on Different Computers

Make a copy of a matrix and store it as follows. This is useful when data collection and analysis are performed on different computers.

Store a copy in the...	For use by the...
AppliedBio folder	Data Collection software.
GS Matrix Folder	GeneScan Analysis Software.

Note The ABI PRISM® instrument Data Collection software uses the files installed by the GeneScan Analysis Software in the AppliedBio folder. When you run the analysis software, the program also creates several files (such as a Preference file) and an Analysis Log.

Analyzing Sample Files

4

Overview

In This Chapter Topics in this chapter include the following:

Topics	See Page
About Sample Files	4-2
Converting Macintosh Computer Sample Files	4-3
Converting Sample Files for Use on a Different Platform	4-4
Opening Sample Files	4-7
About the Sample File Window	4-8
Sample Results View	4-9
Sample Info View	4-11
Size Curve View	4-20
Raw Data View	4-22
EPT Data View	4-24
Analyzing a Sample File	4-26

About Sample Files

Sample File Generation on 377

The Gel Processor bundled with 377 Data Collection software creates Sample files after extracting lanes. The information from each lane in the gel file is tracked and extracted, and the resulting Sample files are placed in their respective sample folder. If you change tracking, lane assignment, or Sample Sheet information, you have to regenerate the Sample files.

The software consults Sample Sheet information to determine whether a lane is used (contains sample). The lane tracker uses this information to assign lane numbers to the tracker lines. In addition, the Gel Processor Software only extracts those lanes identified as Used.

What Files Contain

Sample files contain electrophoresis data collected on ABI PRISM® instruments. Unanalyzed data contain raw data.

Sample Files

Refer to the following table on how Sample files are generated:

Instrument	Software That Generates Sample Files	Notes
ABI PRISM 310	310 Data Collection	Sample file is created after each injection. Data Collection software invokes GeneScan software for auto-analysis
ABI PRISM® 377 DNA Sequencer	Gel Processor software	Gel file is created by 377 Data Collection software. Gel Processor tracks gels and extracts data into Sample files. Gel Processor invokes GeneScan for auto-analysis.
ABI PRISM® 3100 Genetic Analyzer ABI PRISM® 3700 DNA Analyzer	Data Extractor	Sample files are extracted by Data Extractor after each run is completed. If set for auto-analysis, the software is invoked to analyze the data.

GeneScan software is used to manually analyze, edit and view sample files generated on any ABI PRISM instrument.

How GeneScan Analyzes Sample Files

The GeneScan® Analysis Software performs the following steps in analyzing sample files:

Step	Action
1	Processes the raw data signals to generate analyzed data signal and then uses the analyzed signals to detect the signal peaks associated with DNA fragments.
2	Performs size calling by identifying the peaks of the in-lane size standard found in each sample.
3	Determines the fragment size of each experimental peak within the sample based on the size calling curve generated using the size standard peaks, the selected size calling method, and by comparing it to the pre-defined size standard file. The algorithmic steps of the process from raw data to analyzed data are as follows: ♦ Baselining ♦ Smoothing, if any ♦ Peak detection

Converting Macintosh Computer Sample Files

About Converting Files

When you insert the GeneScan Analysis Software version 3.7 for Windows NT® platform CD-ROM into a Macintosh® computer's CD-ROM drive, a folder appears that contains two applications, Mac to Win and Win to Mac.

Use these applications to change sample files created on the Macintosh computer to files that can be read by a Windows NT-based computer, and to change files that were created on a Windows NT-based computer so that they can be read by a Macintosh computer.

Converting Sample Files for Use on a Different Platform

Introduction

Use the conversion utilities to change sample files created on the Macintosh® computer to files that can be read by a Windows NT¤-based computer, and to change files that were created on a Windows NT-based computer so that they can be read by a Macintosh computer.

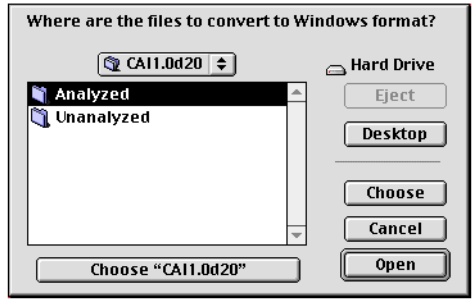
IMPORTANT This utility will run only on a Macintosh computer.

Converting Macintosh Files to Windows NT-Based Computer Files

To convert Macintosh sample files for use on the Windows NT-based computer:

Step	Action
1	Insert the *ABI PRISM® GeneScan Analysis Software v. 3.7 for Windows NT® platform* CD-ROM into the Macintosh computer where your Macintosh sample files are stored.
2	Double-click the CD-ROM icon.
3	Double-click the **Sample File Mac to Win** icon to start the application. The following dialog box is displayed:

To convert Macintosh sample files for use on the Windows NT-based computer: *(continued)*

Step	Action
4	Click **Run**. The following directory dialog box is displayed: 
5	Navigate to the folder that contains the sample files that you want to convert and click **Choose**. The program will perform the task and automatically quit. The converted sample files will have the extension .ab1.

Converting Windows NT-Based Computer Files to Macintosh Files

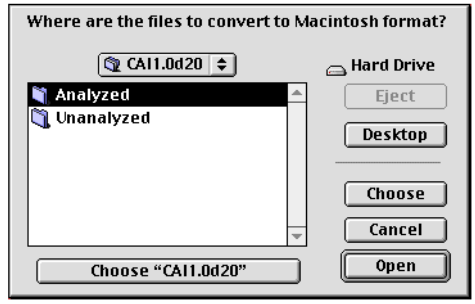
To convert Windows NT-based computer sample files for use on the Macintosh computer:

Step	Action
1	Insert the *GeneScan Analysis Software v. 3.7 for Windows NT platform* CD-ROM into the Macintosh computer where your Windows NT-based sample files are stored.
2	Double-click the CD-ROM icon.

To convert Windows NT-based computer sample files for use on the Macintosh computer: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Click the Sample File Win to Mac icon to start the application.

The following directory dialog box is displayed:

 
</td></tr>
<tr><td>4</td><td>Navigate to the folder that contains the sample files that you want to convert and click the Choose button.

<table><tr><th>If...</th><th>Then...</th></tr><tr><td>there are no problems, the program will perform the task and automatically quit</td><td>When you open the folder, you can double-click the sample files to open them using GeneScan Analysis Software on a Macintosh computer.</td></tr></table></td></tr>
</table>

Opening Sample Files

Introduction

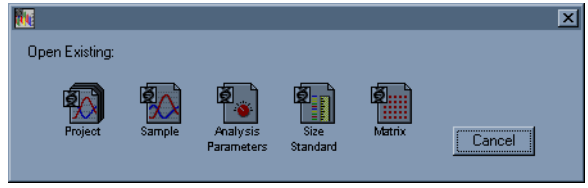
Sample files can be opened as separate files outside of projects, and display related information about each sample file.

To open sample files:

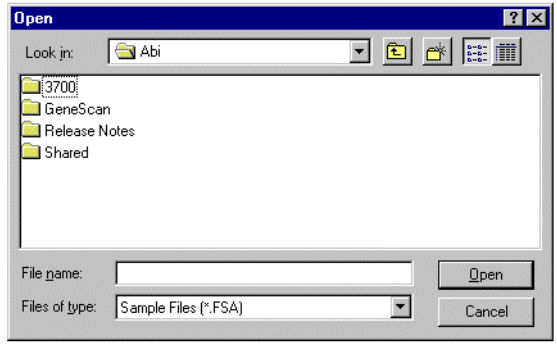
If you are interested in...	Then...
one or two sample files	it is often more convenient to open sample files individually and analyze or view the data without opening an entire project.
multiple sample files	use a project. For information on opening Sample Files from within a Project, see “Accessing Sample Files” on page 3-6

Procedure

To open a sample file as a separate file:

Step	Action
1	Select **Open** (Ctrl+O) from the **File** menu. The **Open Existing** dialog box appears.  **Note** You can also double-click the sample file name in the folder containing the files. If the GeneScan Analysis Software is not running, the software starts and opens the sample file.

To open a sample file as a separate file: *(continued)*

Step	Action
2	Click the Sample icon. An **Open** dialog box appears. 
3	In the dialog box, navigate to the folder and select the sample file that you want to open.
4	Click **Open**. The **Sample File** window appears. For more information on the **Sample File** window, refer to page 4-8.

About the Sample File Window

What It Displays

You can use the display modes in the Sample File window to review the analyzed and raw data, and all pertinent data collection, sizing and sample description information from a single window. The Sample Results view appears as the default.

Five Views

The five views of the Sample File window are:

View	See Page
Sample Results View	4-9
Sample Info View	4-11
Size Curve View	4-20
Raw Data View	4-22
EPT Data View	4-24

Sample Results View

What It Displays

The Sample Results View displays the sample file's analyzed data in both electropherogram and tabular data form.

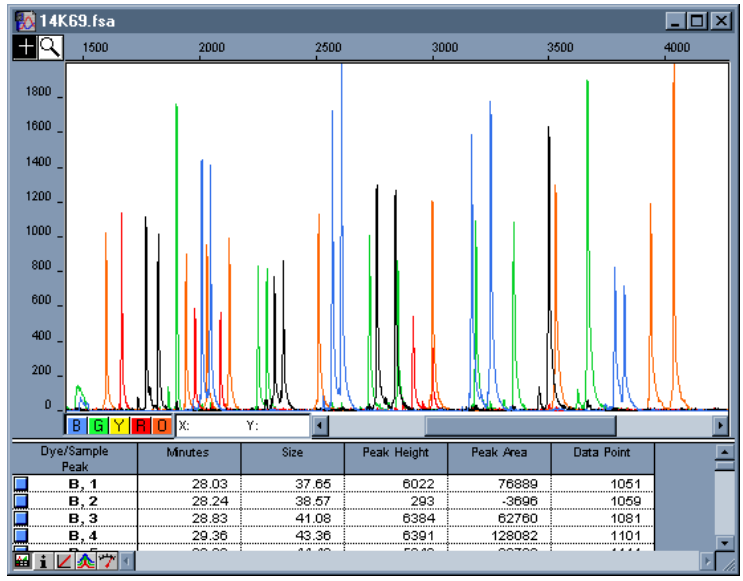
Displaying the View

The following table describes ways to display the Sample Results View:

<table>
<tr><th>To display the view...</th><th>Do this...</th></tr>
<tr><td>from the Sample File window</td><td>
<table>
<tr><th>You can either</th><th>Result</th></tr>
<tr><td>click the button for the Sample Results view at the bottom left of the Sample File window.
 </td><td rowspan="2">The Sample Results View appears.
Refer to “Example of Sample Results View” on page 4-10.</td></tr>
<tr><td>select Sample Results (Ctrl+E) from the Sample menu.</td></tr>
</table>
</td></tr>
<tr><td>from a project window</td><td>a. Select a sample or Ctrl+click to select multiple samples in the Analysis Control (Ctrl+1) or the Results Control (Ctrl+2) window.
b. Select Sample Results (Ctrl+E) from the Sample menu.</td></tr>
</table>

Example of Sample Results View

The following is an example of the Sample Results view:



Description of Columns

The following table describes the columns in the above figure:

This column	Identifies
Dye/Sample Peak	Dye color and Peak number.
Minutes	The time, in minutes, from the start of the run to the time the fragment was detected.
Size	The number of base pairs in the fragment. This value is calculated automatically only if you: ♦ Run the size standard in the same lane or injection as the sample, and ♦ Perform size calling.
Peak Height	Signal size.
Peak Area	Area of the detected peak.
Data point	Data point of the fragment at its maximum peak height.

Differences from the Results Display

The Sample Results view displays the same electropherogram and tabular data as the Results Display, with the following differences:

- One sample file is displayed.
- Show or hide dye/sample data by clicking the buttons below the electropherogram.
- Cannot display legends.
- Cannot use custom plot colors.

Sample Info View

What It Displays

Displays the following sample file information:

Information displayed	See Page
Run Information	4-14
Data Collection Settings	4-14
Sample Information	4-14
Gel Information	4-15
Analysis Records	4-15
Dyes Within Analysis Records	4-16
Data Collection Settings	4-17
Gel Information (Polymer)	4-17
Sample Information	4-18
Analysis Records	4-18
Dyes Within Analysis Records	4-19

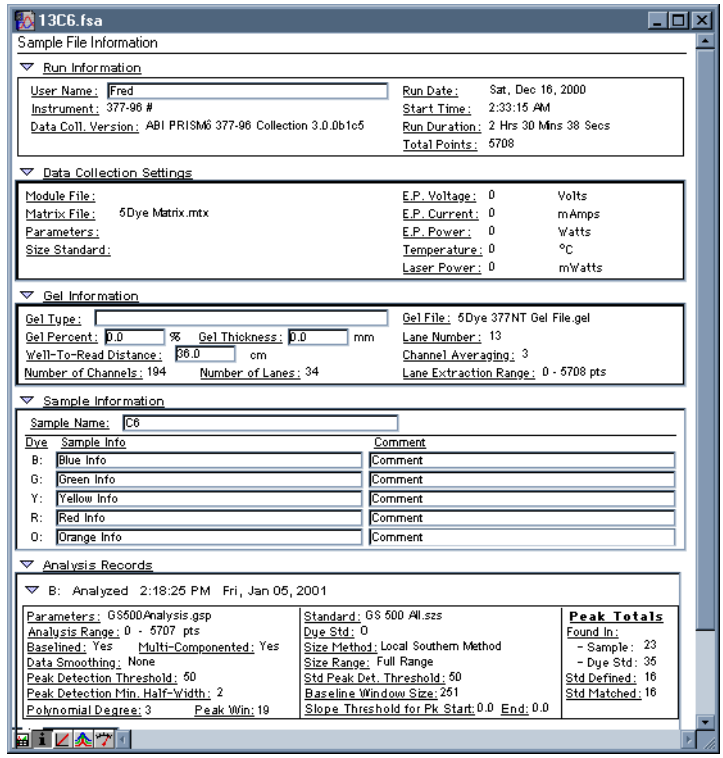
Displaying the View

The following table describes ways to display the Sample Info View:

<table>
<tr><th>To display the view...</th><th>Do this...</th></tr>
<tr><td>from the Sample File window</td><td>
<table>
<tr><th>You can either</th><th>Result</th></tr>
<tr><td>click the button for the Sample Info view at the bottom left of the Sample File window.
 </td><td rowspan="2">The Sample Info view appears.
Refer to “Example of Sample Info View” on page 4-13.</td></tr>
<tr><td>select Sample Info (Ctrl+I) from the Sample menu.</td></tr>
</table>
</td></tr>
<tr><td>from a project window</td><td>a. Select a sample or Ctrl+click to select multiple samples in the Analysis Control (Ctrl+1) or the Results Control (Ctrl+2) window.
b. Select Sample Info (Ctrl+I) from the Sample menu.
The information is organized in four panels.
Click the triangles to expand or collapse the panels to display specific information.</td></tr>
</table>

Example of Sample Info View

The following is an example of the Sample File Information window in Sample Info View:



Description of Information

This information is generally applied to Data Collection software. The following tables list the information in the Sample Info View:

Run Information

Information found under the header	Information entered in
♦ User Name ♦ Instrument ♦ Data Collection software version ♦ Run date and start time ♦ Tube ♦ Run Date ♦ Start Time ♦ Run Duration ♦ Total Points	Data Collection software Run file and run information

Data Collection Settings

Information found under the header	Information is entered in
♦ Module File ♦ Matrix ♦ Analysis Parameters ♦ Size Standard ♦ Run Voltage, Injection Voltage, and Injection Duration ♦ Temperature ♦ Laser power	Data Collection software Run file and run information

Sample Information

Information found under header	Information is entered in
Sample Name	Data Collection software Plate Setup Record
Sample data and comment for each dye color	

Gel Information

This information applies only to samples from the ABI PRISM 377 instrument.

Information found under header	Information is entered in
♦ Gel Type ♦ Gel File name ♦ Gel Percent ♦ Gel Thickness ♦ Well-To-Read Distance ♦ Number of Channels ♦ Lane Number ♦ Channel Averaging ♦ Lane Extraction Range	Data Collection software

Analysis Records

Information found under header	Information is entered in
Date and time each color was analyzed, and more panel-display arrows	Analysis information

Dyes Within Analysis Records

Information found under header	Information is entered
Analysis parameters file and range analyzed	Analysis Settings
Whether baselined or multicomponented	
Data smoothing	
Peak detection threshold and minimum half-width	
Polynomial degree	
Peak window	
Size standard file	Analysis Control window
The dye color used for the standard, Sizing method, and range	
Standard peak detection threshold	Analysis Settings
Baseline window size	
Slope threshold for peak start	
Value for end	
Total number of peaks: found in sample and dye standard, defined in standard matched with standard peaks	Analysis results

Data Collection Settings

Information found under the header	Information is inserted from
♦ Module File ♦ Matrix File ♦ Analysis Parameters ♦ Size Standard ♦ Electrophoresis voltage, current, and power (ABI 373 and ABI PRISM 377 only) ♦ Run Voltage, Injection Voltage, and Injection Duration (ABI PRISM 310 only) ♦ Temperature ♦ Laser power	ABI 373 or ABI PRISM 377 gel file (data collection Run File and run information). This information is embedded in the gel file. ABI PRISM 310 data collection Run file and run information.

Gel Information (Polymer)

Information found under the header	Information is inserted from
ABI PRISM 310	
♦ Gel type	Optional (user entered)
♦ Length to detector	Data Collection information
♦ Lot # and expiration date	
ABI 373 and ABI PRISM 377	
♦ Gel type	Optional (user entered).
♦ Name of gel file	Gel file (data collection Run file) This information is embedded in the gel file.
♦ Gel percentage and thickness	Optional (user entered)
♦ Well-to-read (separation) distance	
♦ Number of channels	Gel file (data collection Run file) This information is embedded in the gel file.
♦ Number of Lanes	

<table>
<tr><th>Information found under the header</th><th>Information is inserted from</th></tr>
<tr><td>♦ Channel Averaging

Note Zero (0) indicates use of pre-averaging offscale data.

The following is an example of how pre-averaging offscale data appears:
<table><tr><th>Extraction method</th><th>Displayed</th></tr><tr><td>3 channel averaging</td><td>3</td></tr><tr><td>3 channel averaging with pre-averaging offscale</td><td>30</td></tr><tr><td>3 channel averaging-weighted</td><td>- 3</td></tr><tr><td>3 channel averaging-weighted, pre-averaging offscale</td><td>- 30</td></tr></table></td><td rowspan="2">Gel processing parameters</td></tr>
<tr><td>♦ Range of data points extracted</td></tr>
</table>

Sample Information

Information found under header	Information is inserted from
Sample data and comment for each dye color	♦ ABI PRISM 310 Sample Sheet ♦ ABI 373 or ABI PRISM 377 gel file (Sample Sheet) This information is embedded in the gel file.

Analysis Records

Information found under header	Information is inserted from
Date and time each color was analyzed, and more panel-display arrows	Analysis information

Dyes Within Analysis Records

Information found under header	Information is inserted from
♦ Analysis parameters file and range analyzed	Analysis Settings
♦ Whether baselined or multicomponented	
♦ Data smoothing	
♦ Peak detection threshold and minimum half-width	
♦ Size standard file	Analysis Control window
♦ The dye color used for the standard, Sizing method, and range	
♦ Standard peak detection threshold	Analysis Settings
♦ Split Peak correction	
♦ Total number of peaks: found in sample and dye standard, defined in standard matched with standard peaks	Analysis results

Size Curve View

What It Displays

Displays sizing curves for sample files. The size curve is a measure of how well the internal size standard matches the standard definition, and whether or not it is linear.

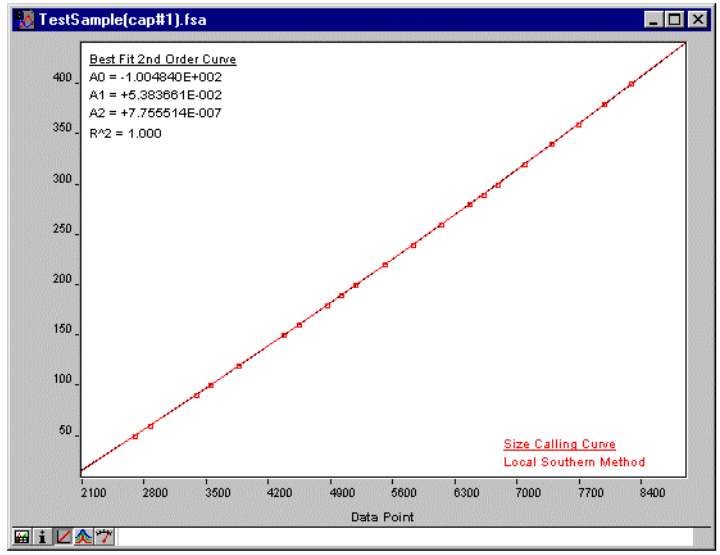
Displaying the View

The following table describes ways to display the Size Curve View:

<table>
<tr><th>To display the view...</th><th>Do this...</th></tr>
<tr><td>from the Sample File window</td><td>
<table>
<tr><th>You can either</th><th>Result</th></tr>
<tr><td>click the button for the Size Curve view at the bottom left of the Sample File window.
 </td><td rowspan="2">The Size Curve view appears.
Refer to “Example of Size Curve View” on page 4-21.</td></tr>
<tr><td>select Size Curve (Ctrl+U) from the Sample menu.</td></tr>
</table>
</td></tr>
<tr><td>from a project window</td><td>a. Select a sample or Ctrl+click to select multiple samples in the Analysis Control (Ctrl+1) or the Results Control (Ctrl+2) window.
b. Select Size Curve (Ctrl+U) from the Sample menu.
For each selected sample, a Sample File window opens and displays its Size Curve view.</td></tr>
</table>

Example of Size Curve View

The Size Curve view displays two curves, as shown in the figure below. For a description of the curves, refer to “Curves Described” below.



Curves Described

The following table describes the curves in the above figure:

Note Sizing errors due to anomalous mobilities may be displayed as nonlinear.

This curve	Represents
Red curve	The sizecalling curve, based on the sizecalling method used to analyze the data.
Black curve	The best-fit least squares curve, which the GeneScan Analysis Software calculates for all samples, regardless of the size calling method. This curve is provided to help evaluate the linearity of the sizing curve. When the sizing curve and best-fit curve match, they overlap so you see only the size curve.

Raw Data View

What It Displays

It displays the raw data collected for a sample. This information is stored in the sample file.

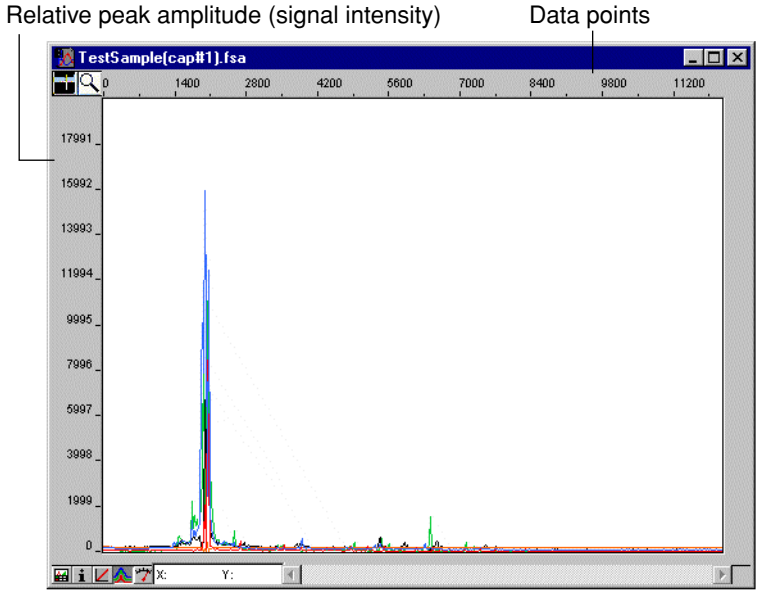
Displaying the View

The following table describes ways to display the Raw Data View:

<table>
<tr><th>To display the view...</th><th>Do this...</th></tr>
<tr><td>from the Sample File window</td><td>
<table>
<tr><th>You can either</th><th>Result</th></tr>
<tr><td>click the button for the Raw Data view at the bottom left of the Sample File window.
 </td><td rowspan="2">The Raw Data View appears.
Refer to “Example of Raw Data View” on page 4-23.</td></tr>
<tr><td>select Raw Data (Ctrl+R) from the Sample menu.</td></tr>
</table>
</td></tr>
<tr><td>from a project window</td><td>a. Select a sample or Ctrl+click to select multiple samples in the Analysis Control (Ctrl+1) or the Results Control (Ctrl+2) window.
b. Select Raw Data (Ctrl+R) from the Sample menu.</td></tr>
</table>

Example of Raw Data View

The following is an example of the Sample File window in Raw Data View:



What to Evaluate

Use the Raw Data view to evaluate:

- Problems or noise in the baseline that could result in poor size calling.
- Start and stop points for analysis.

For information on changing the horizontal (refer to page 9-21) or vertical scale of the data (refer to page 9-22).

EPT Data View

What It Displays

The EPT Data View (electrophoresis power and temperature) displays this information collected for a sample, and it is stored in the sample file.

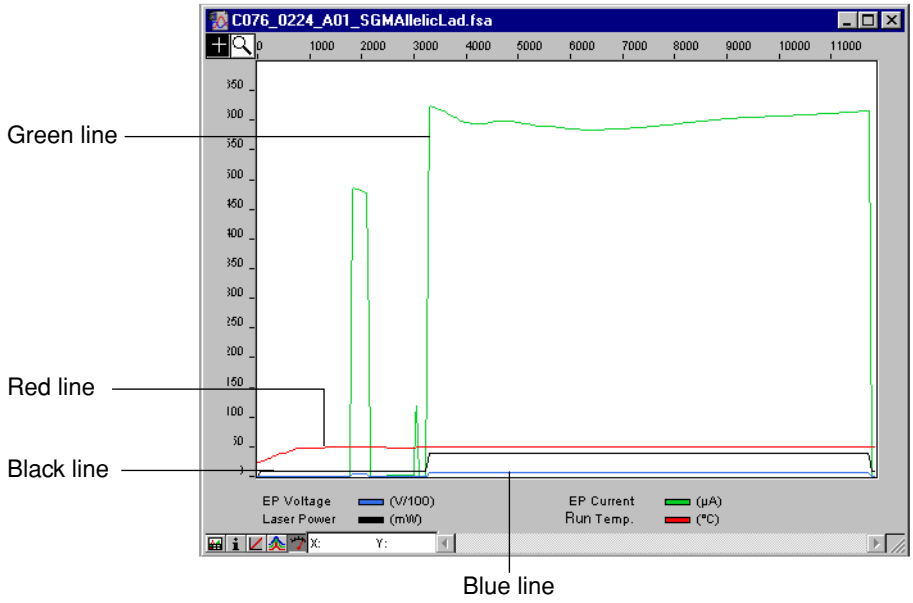
Displaying the View

The following table describes ways to display the EPT Data View:

<table>
<tr><th>To display the view....</th><th>Do this...</th></tr>
<tr><td>from a Sample File window</td><td>
<table>
<tr><th>You can either</th><th>Result</th></tr>
<tr><td>click the button for the EPT Data view at the bottom left of the Sample File window.
 </td><td rowspan="2">The EPT Data view appears.
Refer to “EPT Data View Example” on page 4-25.</td></tr>
<tr><td>select EPT Data (Ctrl+M) from the Sample menu.</td></tr>
</table>
</td></tr>
<tr><td>from a project window</td><td>a. Select a sample or Ctrl+click to select multiple samples in the Analysis Control (Ctrl+1) or the Results Control (Ctrl+2) window.
b. Select EPT Data (Ctrl+M) from the Sample menu, or click the EPT data button.</td></tr>
</table>

EPT Data View Example

The following is an example of the Sample File window in EPT Data View:



Colored Lines Described

The following table describes the lines in the above figure:

Line	Description
Blue	Electric voltage in volts/10
Black	Electric power in watts
Red	Run temperature in °C
Green	Electric current in mA (milliamps)

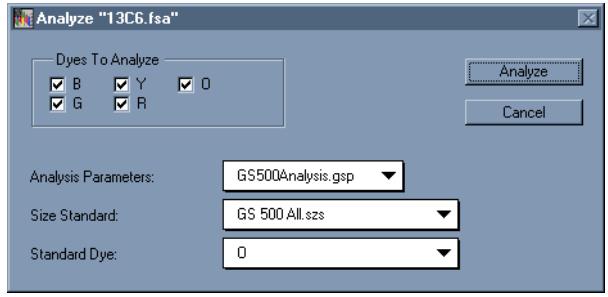
Analyzing a Sample File

Introduction

The GeneScan Analysis Software analyzes raw data stored in sample files according to parameters and standards that you select. You can use the analyzed data to detect peaks associated with DNA fragments and identify those peaks with an established size standard.

Procedure

To analyze a sample file:

Step	Action
1	Select Analyze “Sample File Name” from the **Sample** menu (Ctrl+Y). The Analyze Sample file dialog box appears. 
2	From the Analyze Sample file dialog box, select one of the following options: **Choose...** / **To select...** **Dyes to Analyze** check boxes — any number of dyes to analyze. **Analyze Parameters** pop-up menu — from the default parameters or any parameter files in the folder location specified in the application preferences. **Size Standard** pop-up menu — from the default standard, or any standard files in the folder location that you specify in the application preferences. **Standard Dye** pop-up menu — the inlane standard dye.
3	After analysis, evaluate the results. For more information on evaluating the results, refer to Chapter 8, “Evaluating Analysis Results.”

Installing a New Matrix File

Use the following procedure to install a new matrix file for the Sample file that you want to analyze.

For information on attaching the new matrix to an ABI 373 or ABI PRISM 377 gel file, refer to “Gel Processor User’s Manual.”

To install a new matrix file:

Step	Action
1	Choose **Install New Matrix** from the **Sample** menu. A directory dialog box appears. The Folder Preferences settings determine where the GeneScan Analysis Software looks for the matrix file. For more information, refer to “Defining Folder Locations” on page 3-16.
2	Select the new matrix file in the dialog box and click **Open**. A message appears when the matrix is successfully assigned.
3	Re-Analyze the Sample file. Applying a new matrix file clears previous analysis information, so you must re-analyze the file.

Working with Analysis Parameters 5

Overview

In This Chapter Topics in this chapter include the following:

Topics	See Page
About the Analysis Parameters	5-2
Sizecaller Algorithm Flowchart	5-4
Setting Analysis Parameters	5-5
Using Analysis Parameter Files	5-13

About the Analysis Parameters

What They Are

Analysis parameter files are used by 3100 and 3700 Data Collection software to inform the software of the parameters to use to automatically analyze fragment data. Use the analysis parameters provided, or follow the procedure in “Creating GeneScan Analysis Modules” on page A-1 to create new analysis parameter files. The auto-analysis feature is part of the 3100 and 3700 Data Collection software.

These parameters are also used for analysis using the GeneScan® Analysis Software.

Why They Are Necessary

Analysis parameter files are required because the auto-analysis feature in 3100 and 3700 software has no user interface, so the parameters for analysis cannot be directly configured in the analysis software. The GeneScan Analysis Software creates the analysis parameters, and the software saves the parameters in a folder that can be read by the Data Collection system software that performs auto-analysis.

The analysis parameter file can also be used for manual analysis of fragment data. It is then used by auto-analysis while it is processing run data.

When to Specify a Parameter

Before performing a run, specify the analysis parameter to use for analysis of each sample. Do this when preparing the plate record for the plate that contains the samples on 3100 and 3700 instruments and when preparing the run sheet on 310 and 377 instruments.

Note If there is no analysis parameter specified for a particular sample in the plate record, the sample will not be analyzed by auto-analysis. Use the GeneScan Analysis Software to analyze the data.

Analysis Parameter Files Provided

The following analysis parameter files are provided:

Analysis parameter file	Suggested parameters for using...
GS120Analysis.gsp	GeneScan 120 Size Standard
GS350Analysis.gsp	GeneScan 350 Size Standard
GS400Cubic Analysis.gsp	GeneScan 400 HD Size Standard
GS400HDAnalysis.gsp	GeneScan 400 HD Size Standard
GS400Ord2Analysis.gsp	GeneScan 400 HD Size Standard
GS500Analysis.gsp	GeneScan 500 Size Standard

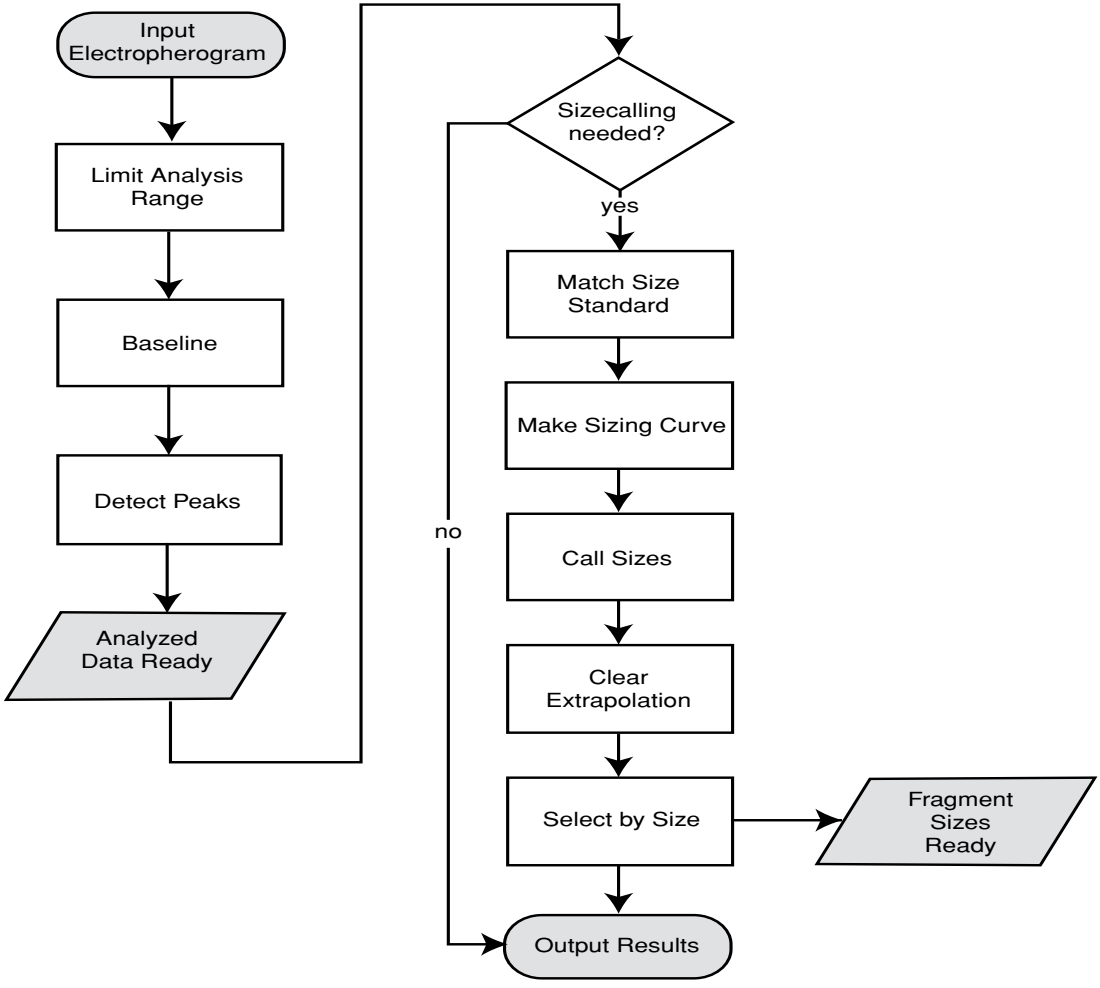
When Analysis Parameters Are Used

Analysis parameter files are opened and read by the Data Extractor program as each sample is extracted.

Note If you make a change to an existing analysis parameter file (without changing its file name), this change will affect any sample extracted after the change is saved.

Sizecaller Algorithm Flowchart

Flowchart The following flowchart shows how the sizecaller algorithm works:



Setting Analysis Parameters

Default Settings

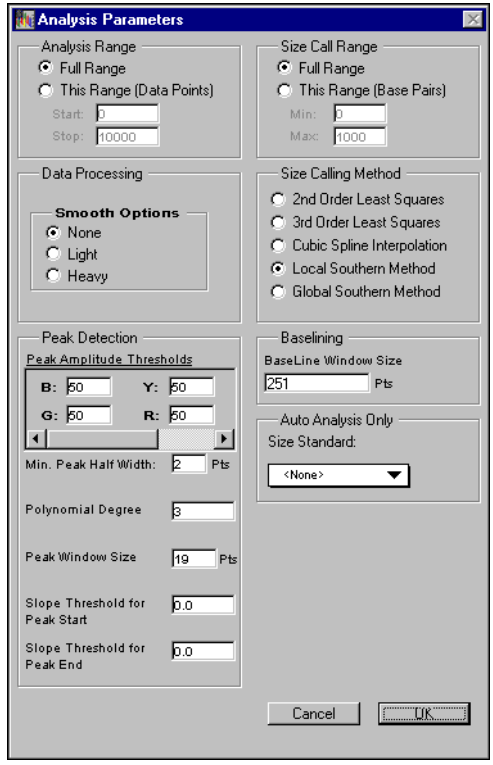
The following table describes the default settings for the analysis parameters. Some of these parameters will be different depending on the analysis setting you select. This is an example of one of the settings.

<table>
<tr><th>Parameter</th><th>Default setting</th></tr>
<tr><td>Analysis Range</td><td>Full Range</td></tr>
<tr><td>Data Processing</td><td>Smooth Options–the Light button is selected</td></tr>
<tr><td>Peak Detection</td><td>Select Analysis Parameters from the Settings menu.The Analysis Parameters dialog box opens.
<table>
<tr><th>Parameter</th><th>Default setting</th></tr>
<tr><td>Peak Amplitude Thresholds</td><td>
<table>
<tr><th>Dye</th><th>Setting</th></tr>
<tr><td>Blue</td><td>50</td></tr>
<tr><td>Green</td><td>50</td></tr>
<tr><td>Yellow</td><td>50</td></tr>
<tr><td>Red</td><td>50</td></tr>
<tr><td>Orange</td><td>50</td></tr>
</table>
</td></tr>
<tr><td>Min. Peak Half Width</td><td>2 points</td></tr>
<tr><td>Degree of Polynomial differentiation</td><td>3</td></tr>
<tr><td>Peak Window Size</td><td>19 points</td></tr>
<tr><td>Slope Threshold for Peak Start</td><td>0.0</td></tr>
<tr><td>Slope Threshold for Peak End</td><td>0.0</td></tr>
</table>
</td></tr>
<tr><td>Full Range</td><td>Full Range</td></tr>
<tr><td>Sizecalling Method</td><td>Local Southern Method</td></tr>
<tr><td>Baselining</td><td>Baseline Window Size 251 points</td></tr>
<tr><td>Auto Analysis</td><td>Size Standard <None></td></tr>
</table>

Displaying Analysis Parameters

Select Analysis Parameters from the Settings menu. The Analysis Parameters dialog box opens.

Note To display the orange Peak Amplitude Threshold, use the scroll bar under the values and scroll to the right.



Analysis Parameters

There are seven analysis parameters:

Analysis parameter	See Page
Analysis Range Options	5-7
Data Processing Options	5-7
Peak Detection Options	5-8
Sizecall Range Options	5-10
Sizecalling Method Options	5-10
Baselining Option	5-12
Auto-Analysis Only Option	5-12

Analysis Range Options

The following are the Analysis Range options:

Item	Description
Full Range button	Use to analyze all the data collected on the genetic analysis instrument for each sample.
This Range (Data Points) button	Enter Start and Stop data point numbers in the entry fields in order to specify only a limited range to be analyzed for each sample. This affects what is displayed in the results display. Normally, set the analysis range to start after the primer peak.

Data Processing Options

The Smooth Options only affect the appearance of the analyzed electropherograms.

Note Since the tabulated peaks are calculated from unsmoothed data, they might not be consistent with the smoothed display. For example, the reported peak heights might not be the same as those visible from the smoothed electropherogram. It is recommended that you select the None button.

Peak Detection Options

About the Peak Detection Options

The Peak Detection options locate peaks at the positive-to-negative zero crossings of the first derivative of the baselined electropherogram. The peak detector computes the first derivative at a data point *i* by fitting a polynomial to a window centered on *i*.

Peak Detection Parameter Options Described

The following table describes the options:

Item	Description	For example
Peak Amplitude Thresholds	The sizecaller reports to the user only those peaks whose heights are at least the Peak Amplitude Threshold for that dye. Set the dye amplitude threshold at a level that allows the software to detect peaks, but eliminate noise. For each dye, the GeneScan Analysis Software detects peaks above the threshold entered in the entry field.	If you leave the default value of 50, peaks with amplitude above 50 are analyzed and appear in the tabular data. Lower amplitude peaks still appear in the electropherogram, but are not analyzed and do not appear in the tabular data.
Minimum Peak Half Width	Defines what constitutes a peak. Use to specify the smallest full width at half maximum for peak detection. The range is 2–99. A typical number might be 3 for microsatellites, or 10 for SSCPs.	If this number is large, the software ignores noise spikes. If the peaks in the data are narrow, set the value to a low number. Experiment with this value to determine the best number for the data.

The following table describes the options: *(continued)*

<table>
<tr><th>Item</th><th>Description</th><th>For example</th></tr>
<tr><td>Polynomial Degree</td><td>Sets the degree of the polynomial.
<table><tr><th>Min. setting</th><th>Max. setting</th></tr><tr><td>2</td><td>5</td></tr></table></td><td rowspan="3">These parameters control the sensitivity of this process. Sensitivity increases with the polynomial degree and decreases with the window size.

Use polynomials of degree 2 or 3 for well-isolated peaks, such as those from a size standard, and a degree 4 for finer control.

For degree 4, the Peak Window Size should be 1 to 2 times the full width at half maximum of the peaks that you wish to detect.

These parameters cannot be set for each color independently.</td></tr>
<tr><td>Peak Window Size</td><td>Sets the width of the window.
<table><tr><th>Min. setting</th><th>Max. setting</th></tr><tr><td>1 above the Degree of Polynomial differentiation setting.</td><td>Number of scans between peaks.</td></tr></table></td></tr>
<tr><td>Slope Threshold for Peak Start

Slope Threshold for Peak End</td><td>Determines where a peak starts and stops.</td><td>For example, a peak ends when the first derivative again exceeds the Slope Threshold for Peak End.

Slope Threshold for peak start must be non-negative and Slope Threshold for peak end must be nonpositive.

Values other than 0 will move the extent of the peak toward its center.</td></tr>
</table>

The following table describes the options: *(continued)*

Item	Description	For example
Baseline Window Size	Controls the slope of the baseliner. The sizecaller determines a baseline value for each scan *i*. Basically, the sizecaller sets the baseline to the lowest electropherogram value that it sees in a window of size ß centered on scan *i*.	To achieve symmetry, you should set ß to an odd value, though even values are acceptable. Small ß will cause the baseline to creep up into peaks. Large ß will create a baseline that does not touch all peaks; that is, the peaks will not be baseline resolved after the sizecaller subtracts the baseline from the electropherogram.

Sizecall Range Options

About the Sizecall Range Options

Use the Sizecall Range parameter options to specify the range of size fragment (in base pairs) to be included in the peak tabular data.

Sizecall Range Parameter Options Described

Item	Description
Full Range button	Select this choice to report all peaks within the range of the matched size standard.
This Range (Base Pairs) button	Select this choice to limit the reported peaks by fragment size.

Sizecalling Method Options

About Sizecalling Method Options

Click a button to select the desired sizecalling method. The GeneScan Analysis Software uses these methods to determine the molecular length of an unknown fragment.

Sizecalling Method Parameter Options Described

Item	Description
2nd Order Least Squares and 3rd Order Least Squares	Both Least Squares Methods use regression analysis to build a best-fit sizecalling curve. For information on the Sizecalling Methods, refer to page B-1.
Cubic Spline Interpolation	Forces the sizing curve through all the known points of the selected GeneScan size standard. For information on the Cubic Spline Interpolation Method, refer to page B-4.
Local Southern Method	Determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility. For information on the Local Southern Method, refer to page B-5.
Global Southern Method	Similar to the Least Squares Method in that it compensates for standard fragments that may run anomalously. For information on the Global Southern Method, refer to page B-7.

Baselining Option

About the Baselining Option

The Baselining option controls the scope of the baseliner. Use this option to set the size *Beta* of the Baseline Window.The sizecaller computes a baseline for the electropherogram of each dye independently.

How the Baselining Option Works

A baseline comprises a value at each data point *i*. Basically, the baseline value at each data point *i*, is the lowest electropherogram value in a window whose width *Beta* is set using the Baselining option, and centered at each data point *i*.

More accurately, the baseline computed in this manner is intermediate. The real baseline value at each data point *i*, is the highest intermediate value, again in a window whose width *Beta* is set using the Baselining options and centered at each data point *i*. The sizecaller baselines an electropherogram by subtracting the baseline from the raw electropherogram.

If the Baseline Window Is Too Small or Too Large

The following table describes what happens if the baseline window is either too small or too large:

Using...	Causes...
a small baseline window size	the baseline to creep into the peaks, resulting in shorter peaks in the analyzed data.
a large baseline window size	the baseline to ride too low, resulting in elevated and possibly not baseline-resolved peaks.

Auto-Analysis Only Option

When performing auto-analysis, select the Size Standard to use from this pop-up menu.

Using Analysis Parameter Files

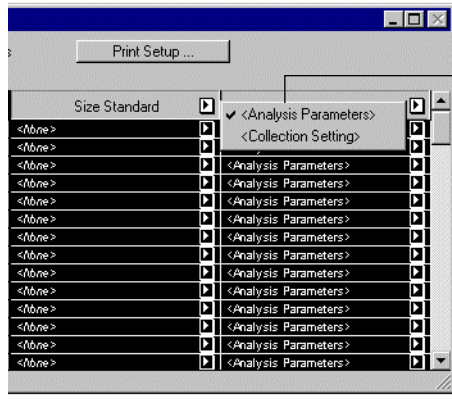
In This Section

This section contains the following topics:

Topic	See Page
Assigning the Same Analysis Parameters to All Files	5-13
Assigning Different Parameters to Single Samples	5-15
Displaying Default Parameters	5-16
Creating Custom Analysis Parameter Files	5-16
Changing an Existing Analysis Parameters File	5-17
Deleting Custom Analysis Parameters	5-17

Assigning the Same Analysis Parameters to All Files

To analyze samples using the same analysis parameters:

Step	Action
1	If the **Analysis Control** window is not displayed, then select **Analysis Control** (Ctrl+1) from the **Windows** menu.
2	Click the arrow in the **Parameters** column heading and choose parameters from the pop-up menu. Your menu choice applies to all fields in the column. **Note** Selecting this pop-up menu automatically selects ALL samples. You cannot assign parameters to only a subset of samples. 

To analyze samples using the same analysis parameters: *(continued)*

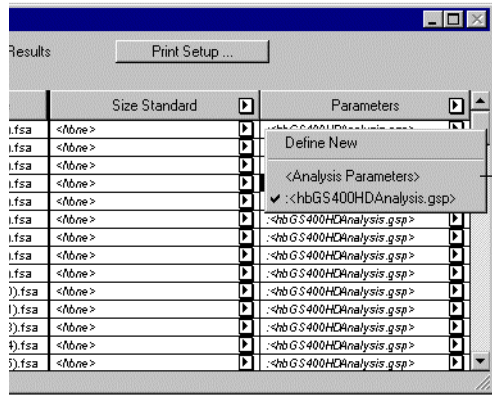
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>The pop-up menu contains the following options:

<table>
<tr><th>Item</th><th>Description</th></tr>
<tr><td>Analysis Parameters</td><td>Applies the parameters that are stored as preferences in the software.</td></tr>
<tr><td>Collection Setting</td><td>Applies the analysis parameters file specified in the Data Collection software, which is embedded in the sample file.</td></tr>
<tr><td>Custom parameters that are listed at the bottom of the menu</td><td>These are files that have been predefined and they are located in the Params folder.

The path is:
D:\AppliedBio\Shared\Analysis\Sizecaller\Params</td></tr>
</table>
</td></tr>
</table>

Assigning Different Parameters to Single Samples

To apply separate analysis parameters to selected samples:

Step	Action
1	If the **Analysis Control** window is not displayed, then select **Analysis Control** (Ctrl+1) from the **Windows** menu.
2	Click the arrow in the **Parameters** column for the sample parameter settings that you want to change. A pop-up menu opens. 
3	The pop-up menu contains the following options: **Choose...** / **To...** **Analysis Parameters** — apply the parameters that are stored as preferences in the software. **Define New** — display the **Analysis Control** dialog box. For information on completing the fields, see “About the Analysis Parameters” on page 5-2.
4	Repeat step 2 and step 3 for each sample. **Note** You can also use the **Cut**, **Copy**, and **Paste** commands from the **Edit** menu.

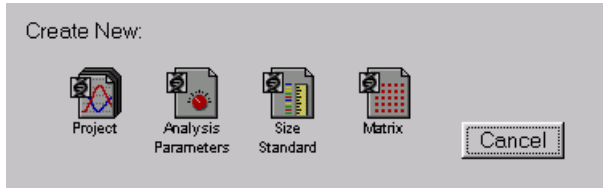
Displaying Default Parameters

There are two ways to display the default parameters:

You can...	Then...
select **Analysis Parameters** from the **Settings** menu.	the **Analysis Parameters** dialog box opens with the default parameters. For information on defining the Analysis Parameters, refer to page 5-2.
double-click **Analysis Parameters** from any entry in the **Parameters** column in the **Analysis Control** window.	

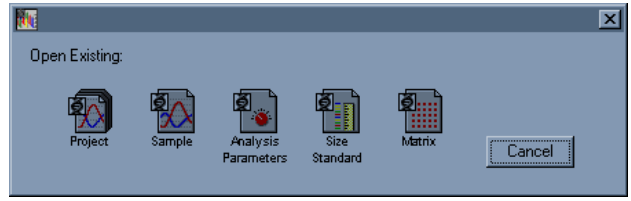
Creating Custom Analysis Parameter Files

To create a custom analysis parameter file:

Step	Action
1	Select **New** from the **File** menu. **Note** You can also select the **Define New** option from the pop-up menu in the **Parameters** column of the **Analysis Control** window. The **Create New** dialog box opens. 
2	Click the **Analysis Parameters** icon. The **Analysis Parameters** dialog box opens.
3	Change the parameters as necessary. For more information on the analysis parameters, refer to page 5-2.
4	Choose **Save** (Ctrl+S) from the **File** menu. A dialog box opens.
5	Enter a descriptive name and click **Save**. The file now opens in the pop-up menu for analysis parameters in the **GeneScan Analysis Control** window. You can also select the file in the Data Collection software for automatic analysis.

Changing an Existing Analysis Parameters File

To change an existing analysis parameter file:

Step	Action
1	Select **Open** (Ctrl+O) from the **File** menu. **Note** You can also double-click the file name in the **Parameters** column of the **Analysis Control** window. The **Open Existing** dialog box opens. 
2	Click the **Analysis Parameters** icon. An **Open** directory dialog box opens. **If...** / **Then...** the Params folder does not appear / navigate to the folder. The path is: D:\AppliedBio\Shared\Analysis\Sizecaller\Params
3	Select a file that you want to change and click **Open**.
4	Make the changes and close the window by clicking **OK**.

Deleting Custom Analysis Parameters

To delete a custom analysis parameters file:

Step	Action
1	Click the **Start** button, and then point to **Programs**.
2	Click **Windows NT® Explorer** and find the file in the folder you specified as the location for the analysis parameters files. **Note** The custom analysis parameters file is in the folder that is normally called the GS Parameters Folder. This folder is inside the ABI PRISM GeneScan folder.
3	Drag the custom analysis parameters file to the Recycle Bin.

6 Making a Matrix File

Overview

This chapter describes the processes of making a matrix file, loading and running samples and evaluating the file. You have the option of selecting four or five dyes depending on the application when creating a new matrix for data collection.

Note This chapter applies only to the 310 and 377 instruments.

In This Chapter

Topics in this chapter include the following:

Topics	See page
About Matrix Files	6-2
Process of Creating a New Matrix File	6-7
Loading and Running Dye Standards for the ABI PRISM 310	6-9
Loading and Running Dye Standards for the ABI PRISM 377	6-12
Generating Matrix Sample Files for the ABI PRISM 377 Instrument	6-15
Choosing a Scan Range for the Matrix Calculation	6-17
Generating a New Matrix File	6-20
Saving and Naming the Matrix File	6-22
Assigning the Matrix File to Sample Files	6-23
Evaluating the Matrix File	6-25
Causes for Bad Matrix Files	6-26

About Matrix Files

Introduction

There are three dye-labeling chemistries currently available to prepare nucleic acid samples to use the GeneScan® Analysis Software on ABI PRISM® instruments:

- Fluorescent NHS-Ester
- Fluorescent dNTP
- Fluorescent Phosphoramidite

Each chemistry has a set of dye labels that fluoresce at different wavelengths when excited by a laser.

During data collection on the...	The wavelengths are separated...
310 or 377, 377XL, or 96-lane upgrade instrument	by a spectrograph into a known spectral pattern across a detection system with the sequencer.

Matrix File Definition

Matrix files are mathematical matrices that correct for spectral overlap of fluorescent emission spectra data collected from ABI PRISM® instruments.

A matrix file allows you to account for spectral overlap when analyzing Sample files.

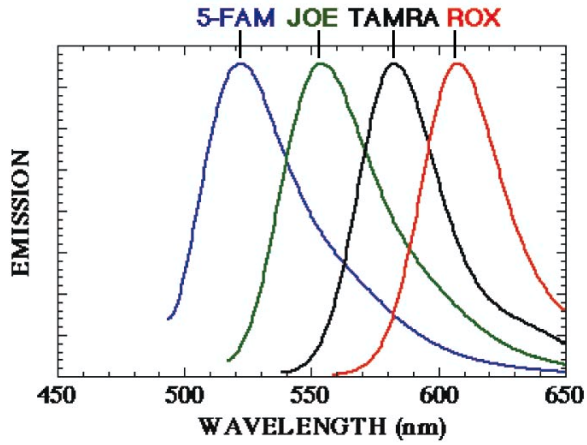
Multicomponent Definition

This process of eliminating the bleed-through caused by spectral overlaps is called multicomponenting.

Applying a matrix file to raw data allows you to generate multicomponented data.

Why Is a Matrix File Necessary

A matrix file is necessary because the four or five dyes used to label the fragments fluoresce at different wavelengths and may have spectral overlaps, as shown below:



When to Create a Matrix File

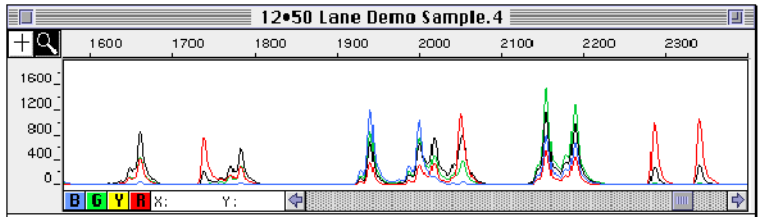
Create a matrix file for each dye set used from that particular instrument before analyzing fragment data.

You may have to create new matrix files for different gel compositions or unusual run conditions.

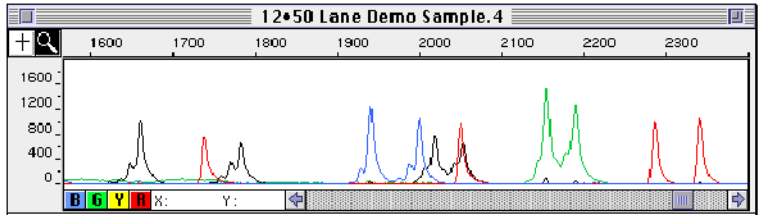
Sample Files Using Matrix File

The figures below show examples of data analyzed with and without a matrix file.

You can see that peak data from a Sample file analyzed without a matrix file displays the expected peak, along with extra peaks in other dye colors, or bleed-through from other dye colors.



Sample file analyzed without a matrix file



Sample file analyzed with a matrix file

Installing a Matrix File

You can install a matrix file into a gel file or into a Sample file.

Normally the matrix from a matrix file is installed within a gel file or a Sample file automatically upon generation during or after a run. Additionally a matrix can be manually installed into a 377/310 Sample file from within the GeneScan Analysis Software.

When to Assign a Matrix File

Before you can successfully analyze Sample files using the GeneScan Analysis Software, you must make a new matrix file or assign an existing one to a set of Sample files.

Limitations to Matrix Files

You can only assign a matrix file to Sample files generated on the same instrument, under the same electrophoresis, gel matrix and buffer conditions, and using the same dye set.

Note If you are using a fifth dye, then you need to create a new matrix file for that dye.

When to Create a New Matrix File

Create a new matrix file in the following conditions:

- For each dye set:
  - NHS-Esters
  - Phosphoramidite set
  - Fluorescent dNTPs
- Whenever you change the dye set you use to label sample fragments, for example, if you are using the fifth dye.
- When you use gel materials or buffers with pH values that differ greatly from the pH value of the gel material or buffer on which the existing matrix files were generated.
- When you use dyes other than those provided by Applied Biosystems.
- When you run the same gel on a different instrument.
- When you see multiple unexpected peaks of different colors under an expected peak.
- When you recalibrate your CCD camera (310 and 377 instruments) and the change is greater than 3 pixels from the original pixel position.
- When you replace the CCD camera (ABI PRISM 310 and ABI PRISM 377).

Considerations Before Making a Matrix File

The following table lists some of the considerations before making a matrix file:

Consideration	Comment
How much dye matrix standard to load?	With the ABI PRISM 377, loading more than 3 µL, produces too much signal. Any amount that results in a signal over 4,000 FUs is too strong.
Which lanes to load with the dye matrix standards?	For gel electrophoresis, load the matrix standards with an empty lane between each sample to avoid contamination of the individual dyes by residual material leaking adjacent samples.
What exact gel data will be used for matrix creation?	After generating a gel image, for ABI PRISM 377 instrument, check that the tracking of the gel file is adequate.

Where to Store Matrix Files

Store matrix files intended for use by Data Collection software in:

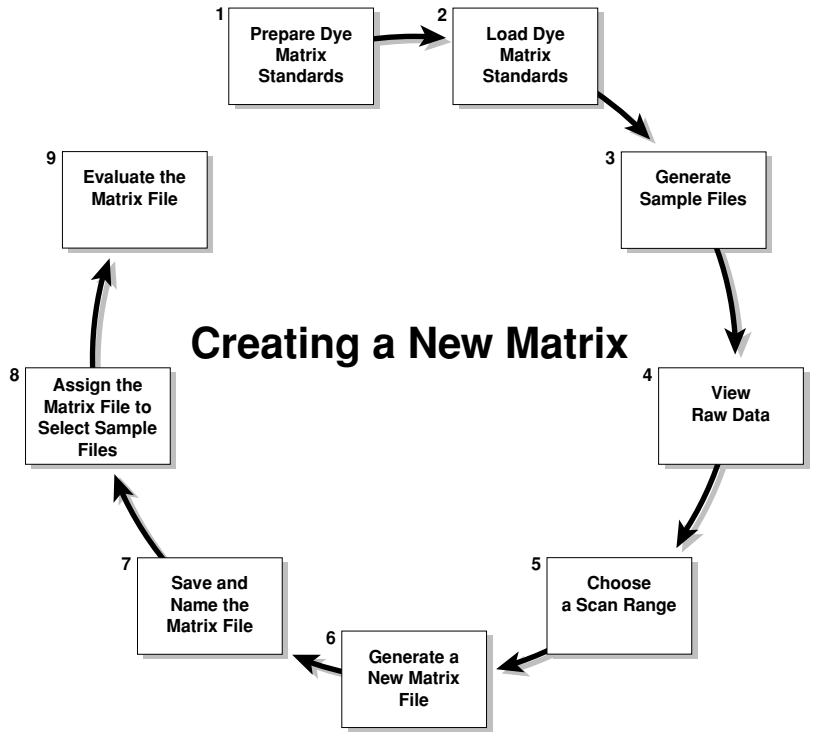
D:\AppliedBio\Shared\Analysis\Sizecaller\Matrix\

If Data Collection and Analysis are installed on different computers, the location is the same. Remember to copy the matrix file from the analysis computer to the Data Collection computer.

Process of Creating a New Matrix File

Process Diagram

The following diagram shows the procedure for making a new matrix file:



For sample preparation and loading information, refer to the appropriate instrument user manual

Steps to Create a New Matrix

The following table lists the steps to create a new matrix file:

Step	Process	See Page
1	Loading and Running Dye Standards for the ABI PRISM 310	6-9
2	Loading and Running Dye Standards for the ABI PRISM 377	6-12
3	Choosing a Scan Range for the Matrix Calculation	6-17
4	Generating a New Matrix File	6-20

The following table lists the steps to create a new matrix file: *(continued)*

Step	Process	See Page
5	Saving and Naming the Matrix File	6-22
6	Assigning the Matrix File to Sample Files	6-23
7	Evaluating the Matrix File	6-25
8	Causes for Bad Matrix Files	6-26

Loading and Running Dye Standards for the ABI PRISM 310

Introduction

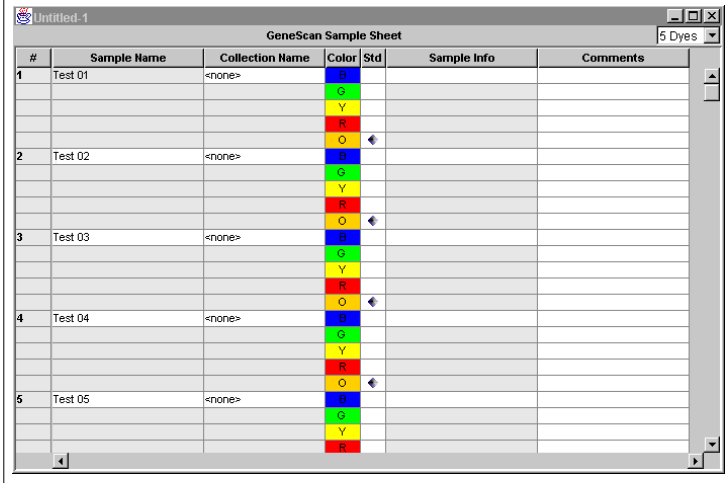
This section describes how to do the following tasks before running matrix standards on the ABI PRISM 310:

Topic	See Page
Creating a GeneScan Sample Sheet	6-9
Creating a GeneScan Injection List	6-10
Starting the Data Collection Software	6-11

Note When loading the matrix standards on an instrument, note which colors you load in which autosampler positions.

Creating a GeneScan Sample Sheet

To create a GeneScan Sample Sheet:

Step	Action
1	Open the ABI PRISM 310 Data Collection software.
2	Choose **New** from the **File** menu.
3	Click the **GeneScan Sample Sheet 48** or **96** tube icon. The **GeneScan Sample Sheet** opens. 
4	Enter the appropriate colors (Blue, Green, Yellow, Red, or Orange) in the positions for each sample name.
5	Enter any additional information about the sample in the **Comments** column.

To create a GeneScan Sample Sheet: *(continued)*

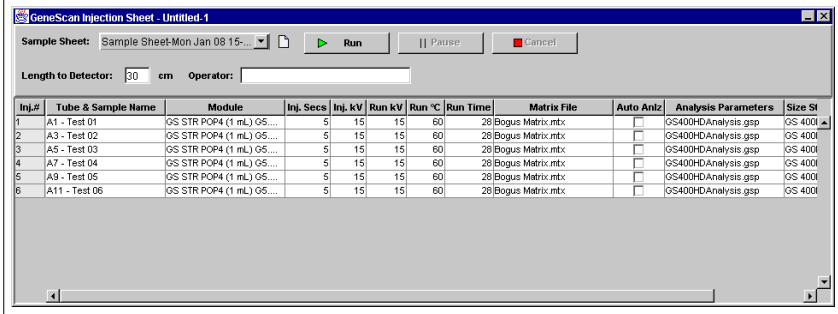
Step	Action
6	Use the **Save As** command and save the Sample Sheet to the Sample Sheet folder.

Creating a GeneScan Injection List

The Sample Sheet is imported into the Injection List, which defines the sample names and the initial injection order.

To create a GeneScan Injection List:

Step	Action
1	Choose **New** from the **File** menu.
2	Click the **GeneScan Injection List** icon. The **GeneScan Injection List** opens.



3	Select a Sample Sheet from the **Sample Sheet** pop-up menu.
4	Select the appropriate module in the **Module** pop-up menu for lines 1 through 4, for example, A1, A3, A5, and A7.
5	Unselect **Auto-Anlz** (analysis).
6	Unselect **Auto-Print**.

Starting the Data Collection Software

To start the Data Collection software:

Step	Action
1	Click the **Run** button. The following windows open:

Window	Description
Raw Data window	Shows the real-time chromatogram of the run.
Log Window	Shows the real-time written record of run events.

Step	Action
2	Choose **Status** from the **Window** menu. The current run is in italics in the Injection List. You can monitor activities such as electrophoresis current, laser power, running time, and gel temperature.

If the Run was Cancelled

If the run was cancelled and you are using Data Collection Software Version 1.0.4 or later (Macintosh or Windows version), the sample file is saved if you skip to the next sample or cancel a run.

Run Time

Run time is approximately 30 minutes for the GS STR POP-4 module, so the total run time will be about 120 minutes.

Assigning Matrix File

For information on “Assigning the Matrix File to Sample Files,” refer to page 6-23.

Loading and Running Dye Standards for the ABI PRISM 377

Introduction

This section describes how to do the following tasks using the 377 and the 377 with XL Upgrade instruments:

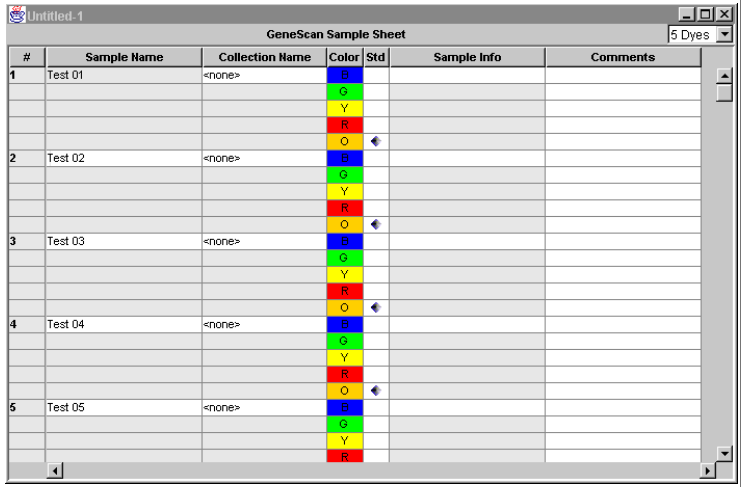
Topic	See page
Creating a GeneScan Sample Sheet	6-12
Loading Matrix Standards	6-13
Running the Matrix Standards	6-14

Note When loading the matrix standards on an instrument, note which colors you load in which lanes for gel-based systems.

Creating a GeneScan Sample Sheet

The GeneScan Sample Sheet assigns sample and dye information to the appropriate lane.

To create a GeneScan Sample Sheet:

Step	Action
1	Open the ABI PRISM 377 Data Collection software. Define or verify the data collection preferences.
2	Choose **New** from the **File** menu.
3	Click the **GeneScan Sample Sheet** icon. The GeneScan Sample Sheet opens. 

To create a GeneScan Sample Sheet: *(continued)*

Step	Action
4	Enter the individual colors in the appropriate lanes where the matrix standards are loaded. **Note** It is important to fill out the Sample Sheet completely.
5	Enter any additional information about the sample in the **Comments** column.
6	Use the **Save As** command and save the Sample Sheet to the **Sample Sheet** folder.

Loading Matrix Standards

To load matrix standards:

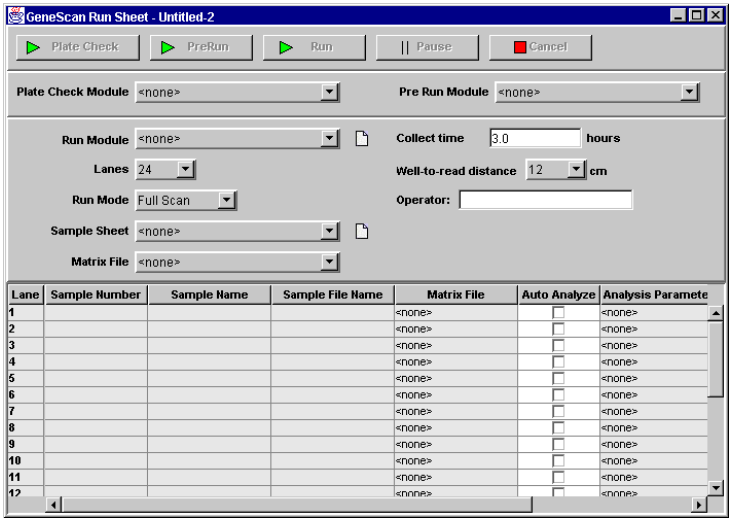
Step	Action
1	For denaturing gels load: ♦ 0.5–2 µL of matrix standard per lane. ♦ 8 lanes with different colors, leaving an empty lane between each lane of matrix standard.
2	Complete the information in the data collection Run sheet, making sure to choose the appropriate PreRun and Run modules. Take the following action:
3	Electrophorese samples according to conditions specified in your instrument manual.

For this matrix...	Choose modules that use...	Module file
Dye Primer matrix	Virtual Filter A	GS 36A...
Fluorescent Amidite	Virtual Filter C	GS36C...

Running the Matrix Standards

Run the matrix standards under the precise conditions you want to generate a matrix file.

To run the matrix standards:

Step	Action
1	Complete the information in the data collection Run sheet. Refer to the figure below to select the appropriate **PreRun** and **Run Modules**. 
2	Take the following action: **For this matrix...** / **Choose modules that use...** / **Module file** Dye Primer or dNTP matrix / Virtual Filter A / GS 36A... GS Amidite matrix / Virtual Filter C / GS36C...
3	Start the electrophoresis run according to the conditions specified in your instruction manual.
4	Go to “Generating Matrix Sample Files for the ABI PRISM 377 Instrument” on page 6-15.

Generating Matrix Sample Files for the ABI PRISM 377 Instrument

Who Should Use This Step

Follow these guidelines to use this step:

Who should use this step	Who should not use this step
This step is only necessary if you are using an: ♦ ABI PRISM 377 instrument ♦ XL-upgraded instrument ♦ 96-lane upgrade	The ABI PRISM 310 Genetic Analyzer automatically processes collection data and generates Sample files when the run completes. If you ran your Dye Matrix Standards on the ABI PRISM 310, go to "Choosing a Scan Range for the Matrix Calculation" on page 6-17.

Gel Handling on Windows

The 377 Data Collection for Windows generates gel files. There is a new program on Windows called Gel Processor. This program handles all gel file-related tasks, including tracking and sample file generation. For more information on the Gel Processor, refer to the Gel Processor user's manual.

Verify Tracking

Before generating Sample files, verify that the lanes were set and tracked correctly. Once you have successfully completed your run of Dye Matrix Standards, open the gel file in the Gel Processor and visually verify the positions of the tracker lines.

Generating Sample Files

To generate Sample files from within Gel Processor:

Step	Action
1	Click the appropriate **Lane Indicators** at the top of the **Gel** window to make sure the tracker line is optimally aligned over each band in all lanes. If any of the lanes are not properly tracked, use the tracker line editing tools to align the tracker lines in each lane. Refer to the *Gel Processor User's Manual*.
2	If you change any of the lane assignments in any way, save the changes.

To generate Sample files from within Gel Processor: *(continued)*

Step	Action
3	From the **Gel** menu, choose **Track & Extract Lanes**. **Note** If the gel is already tracked, choose **Extract Lanes** from the **Gel** menu. The project Analysis Control window opens containing each of the Sample files.

Choosing a Scan Range for the Matrix Calculation

Introduction

Depending on how well your Matrix Standards run, it may be necessary for you to choose a specific range of data points to be considered for your matrix calculation.

In order to choose appropriate values for the Scan range, you must first view the Sample file raw data from each of the matrix standard files, so you can decide where to choose the start and stop points for the scan range.

Viewing the Raw Data

When you have multiple Sample files, raw data can be accessed more easily through a project's Analysis Control window. Raw data can provide useful information about the Sample files you have created.

Note You can view Sample files without opening a project. However, this procedure is easier if you use a project to organize the Sample files.

To view raw data:

Step	Action
1	Use the following steps to create a project for the Dye Matrix Standards: a. Choose **New** from the **File** menu.The Create New dialog box opens. b. Click the **Project** icon. An untitled Analysis Control window opens. c. Choose **Add Sample Files** from the **Project** menu. d. Find and open your matrix run folder. e. Select the five Sample files representing the blue, green, yellow, red, and orange dye-labeled runs, and click **Add**. f. Click **Done** after the Sample files are transferred. For more information, refer to "Creating a New Project" on page 2-9.
2	From the **Analysis Control** window, select the matrix standard Sample files by clicking on the first Sample file, holding down the mouse button, and releasing on the last Sample file.

To view raw data: *(continued)*

Step	Action
3	From the **Project** menu, choose **Raw Data** (Ctrl+R). Electropherograms displaying raw data from the matrix standard Sample files appear. **Note** For the ABI PRISM 377 instrument, you can also view raw data from the gel display by selecting one of the lanes containing Dye Matrix Standard and looking at the Slice View to the left of the gel image. For more information about viewing the gel image, refer to the *Gel Processor User's Manual*.

What to Look For in the Raw Data Display

In the raw data display of the Sample files verify the following:

- Data peaks are present in all four of the matrix standards.
- There are no anomalies.
- The baseline is stable.
- Peaks should be on-scale—no more than 4000 relative fluorescent units—and the peaks of the dye of interest should have a value of at least 200.

If peak data does not show these characteristics, refer to "Causes for Bad Matrix Files" on page 6-26, for possible interpretations of your peak data.

Choosing a Scan Range

To choose a scan range:

Step	Action
1	Move the cursor well away from the primer peak, in a region at the beginning of the run and in a flat part of the baseline, and record the scan numbers. **Note** When choosing the start point, do not include primer peaks in the scan range (refer to "Eliminating Primer Peaks"). Also, the region for both the start and stop points should be flat points at the baseline.
2	Record the data point values for both the start and stop points of the scan range. You will need to enter these values in the next step when generating the new matrix file (refer to page 6-20).

To choose a scan range: *(continued)*

Step	Action
3	Close the raw data boxes and the project by clicking in the upper left-hand corner of the window. **Note** Holding down the Alt key while clicking in the upper left-hand corner of the window will close the windows simultaneously.

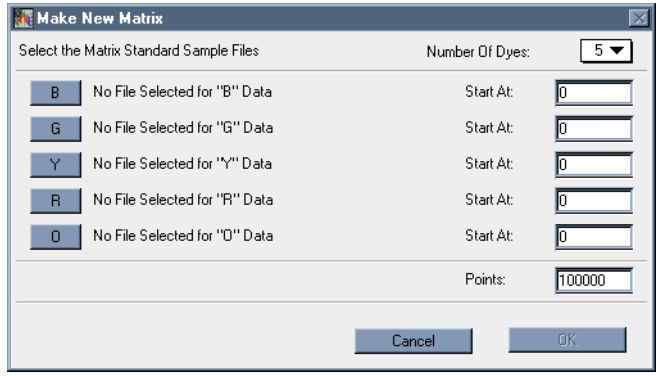
Eliminating Primer Peaks

Both the primer peaks and the data peaks are displayed when viewing the raw data of your matrix standards. Any time you run dye-labeled samples on a gel (377 instrument), or capillary (310 instrument), you always have excess dye-labeled primer in the reaction. The primer peak displays as the first peak, usually off-scale because it is in molar excess.

Eliminate the primer peak when making a matrix, by choosing the start point after the primer peak in a flat area with a stable baseline.

Generating a New Matrix File

Procedure To generate a new matrix file:

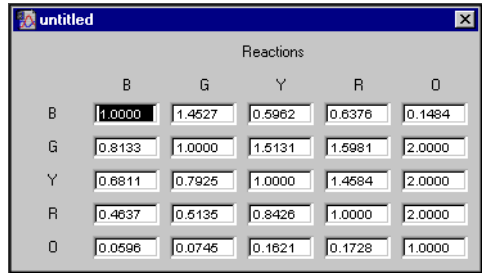
Step	Action
1	Choose **New** from the **File** menu. The Create New dialog box opens.
2	Click the **Matrix** icon. The **Make New Matrix** dialog box opens. 
3	Choose the number of dyes from the **Number of Dyes** pop-up menu. If 5 dyes are selected, a button is added to the bottom of the list.
4	The B, G, Y, R, and O buttons represent dye colors. a. Click a button to display a pop-up menu. b. Use the pop-up menu to access a Sample file to link to each of the dye-labeled primers. c. Choose the Sample file that represents the dye color for that button.
5	Enter the start point that you determined when choosing a scan range in the Start at field. Refer to “Choosing a Scan Range” on page 6-18.

To generate a new matrix file: *(continued)*

Step	Action
6	Enter the total number of data points to include to calculate the matrix in the Points field. **Note** You must have at least five peaks to make a matrix. In most cases, leave the default value, unless you must exclude a portion of your data because of artifacts or bleed-through.
7	Click **OK**. This generates a new matrix file.

Matrix File Example

The following is an example of the Matrix Values window that opens showing the values used to calculate the overlap correction.



For each dye, the value where the dye fluorescence is read by the appropriate filter is 1.000. The adjacent colors show the amount of overlap for which the system must compensate. The adjacent values, in most cases, should be less than 1.000, but equal to or greater than 0.0000.

Saving and Naming the Matrix File

Introduction

The matrix file is instrument-specific. You cannot apply a matrix file you made on the ABI PRISM 377 to data you collected on an ABI PRISM 310, nor can you apply a matrix file made on an ABI PRISM 377 to a Sample or gel file made on another ABI PRISM 377. In other words, you cannot apply matrix files created on one instrument to other instruments of the same model.

Naming Considerations

When naming a matrix file, consider including the following information in the name:

Item	For example
Instrument type	ABI PRISM 377, or ABI PRISM 310.
Filter set used	D, C, F, G5 and E5
Gel conditions	native or denaturing.

Saving the Matrix File

To save the matrix file:

Step	Action
1	Select **Save** from the **File** menu. A dialog box opens.
2	Enter a descriptive name for the new matrix file and click **Save**.

Where to Store the Matrix File

Store matrix files that are intended for use by data collection to assign to collection runs in:

D:\AppliedBio\Shared\Analysis\Sizecaller\Matrix\

When Data Collection and Analysis Are Performed on Different Computers

Be sure to copy matrix files generated on the analysis computer (the computer running GeneScan analysis) to the Data Collection computer, if different. This will ensure that the correct matrix is stored in the GeneScan sample file. The proper matrix is required for accurate analysis of 310 and 377 sample files.

Assigning the Matrix File to Sample Files

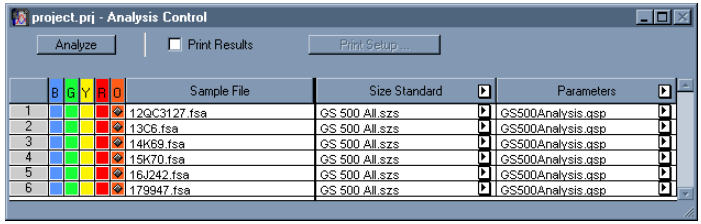
Introduction

After generating the new matrix file, assign it to all the Sample files that you want to analyze.

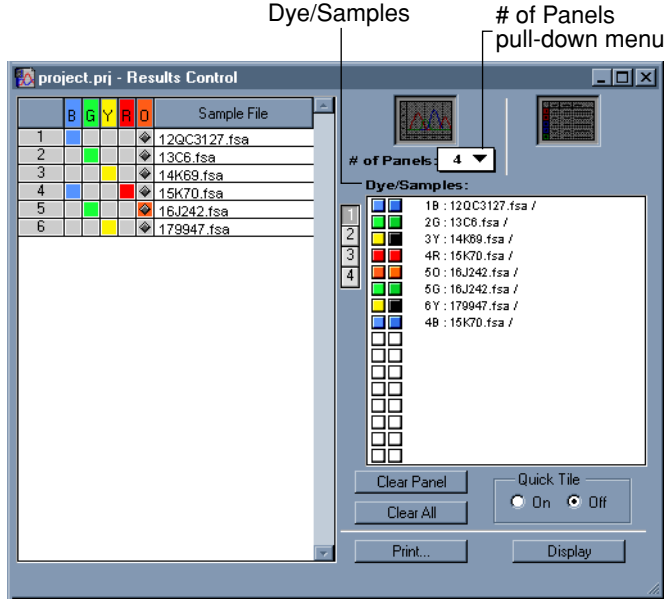
IMPORTANT After assigning your matrix file to Sample files, refer to "Evaluating the Matrix File" on page 6-25.

Procedure

To assign a matrix file to Sample files and review them:

Step	Action
Assigning a matrix file to Sample files:	
1	From the project that contains your matrix standard Sample files, open the **Analysis Control** window. 
2	Select the matrix standard Sample files.
3	Select **Assign New Matrix** from the **Project** menu.
4	Select the matrix file you just created.
5	Select numbers **1**, **2**, **3**, and **4** on the left side of the window to highlight the colors for each row.
6	Select **Set Analysis Parameters** from the **Settings** menu.
7	Enter the appropriate range for the Analysis Range and click **OK** to return to the Analysis Control window.
8	Click **Analyze**.

To assign a matrix file to Sample files and review them: *(continued)*

Step	Action
Reviewing the results:	
1	Choose **Results Control** from the **Windows** menu. The Results Control window opens as shown below. 
2	Select **4** from the **# of Panels** pull-down menu (see above).
3	Click **1** under **Dye/Samples** (see above).
4	Click **1** on the **Sample Files** side of the **Results Control** window.
5	For the rest of the matrices, click **Analyze** and repeat steps 1 and 2.

Evaluating the Matrix File

Introduction

After creating a new matrix file and assigning it to select Sample files, the next step is evaluating the quality of the matrix file. The quality of the matrix file has a direct impact on the quality of the results data.

Procedure

To evaluate the matrix file:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Analyze the Sample files used to make the matrix.</td></tr>
<tr><td>2</td><td>Display Results data for all the Dye Matrix Standard Sample files on one screen, showing only electropherogram data.</td></tr>
<tr><td>3</td><td>For each displayed Sample file:
<table>
<tr><th>You should see...</th><th>If not...</th></tr>
<tr><td>that the only visible peaks represent the color of the Dye Matrix Standard run in that lane, or for that injection (ABI PRISM 310).</td><td rowspan="3">you probably have a bad matrix file.

For instructions on how to identify and correct problems with bad matrix files see, “Causes for Bad Matrix Files” on page 6-26.</td></tr>
<tr><td>all other lines should be relatively flat.

This indicates that the matrix properly compensated for the spectral overlap.

For example, for the blue matrix standard Sample file, you should only see blue.</td></tr>
<tr><td>sharp, well-defined, singularly colored peak data.</td></tr>
</table></td></tr>
</table>

Causes for Bad Matrix Files

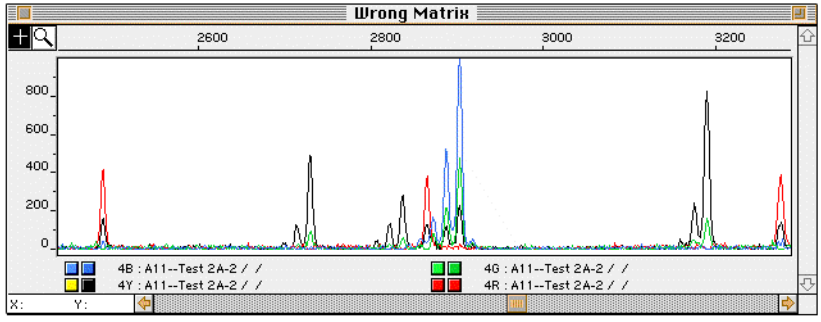
If an Error Message Appears

There are two possible causes for the error messages shown in the following table:

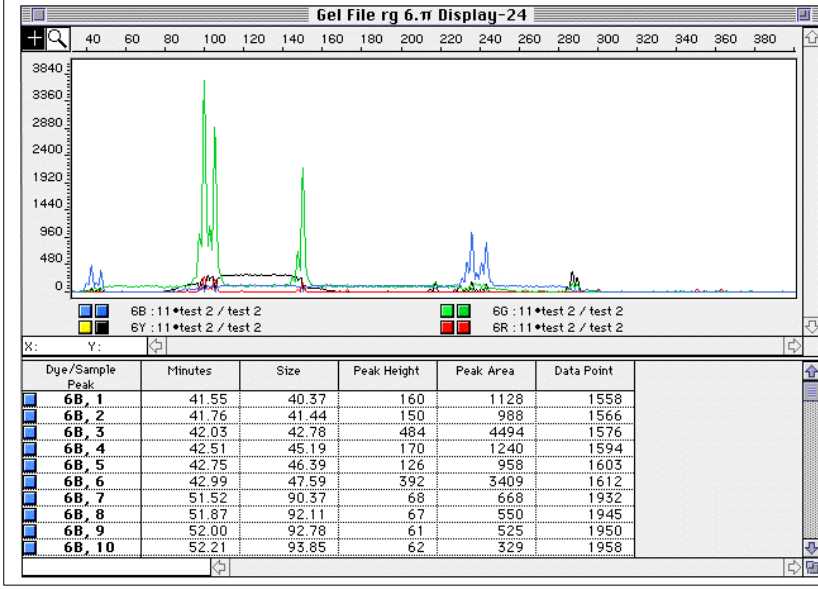
For this cause...	Take this action...
designated the wrong files.	reassign the matrix files. Refer to “Assigning the Matrix File to Sample Files” on page 6-23.
signal is too weak to make a matrix.	rerun the matrix standards.

Two Causes of Bad Matrix Files

The following table lists two common causes of bad matrix files:

Problem	Cause	What to do
Artifact peaks of different colors under the true peaks. Refer to the figure below.	Loading too much dye when running matrix standards, resulting in dye bleed-through.	Complete another run and recreate the matrix.
		
Noisy baseline.	If the matrix subtracts too much of a particular color from the sample, then the baseline may become too elevated, resulting in false peaks. Refer to the figure below.	Complete another matrix run and make sure you do not have any off-scale data.

The following table lists two common causes of bad matrix files: *(continued)*

Problem	Cause	What to do
		

Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
6B, 1	41.55	40.37	160	1128	1558
6B, 2	41.76	41.44	150	988	1566
6B, 3	42.03	42.78	484	4494	1576
6B, 4	42.51	45.19	170	1240	1594
6B, 5	42.75	46.39	126	958	1603
6B, 6	42.99	47.59	392	3409	1612
6B, 7	51.52	90.37	68	668	1932
6B, 8	51.87	92.11	67	550	1945
6B, 9	52.00	92.78	61	525	1950
6B, 10	52.21	93.85	62	329	1958

Working with Size Standards 7

Overview

In This Chapter Topics in this chapter include the following:

Topics	See Page
About Size Standards	7-2
Defining the Size Standard	7-3
Using Size Standards	7-9

About Size Standards

What Are Size Standards

Size standards are specific DNA fragments of known sizes. After defining the peaks of a size standard, the GeneScan® Analysis Software matches this definition to the internal size standard included with the run. The software assigns the defined size values to the appropriate peaks of the internal size standard, and uses this information with the selected sizecalling method to size all unknown fragments.

Advantages of Using Size Standard

Running an internal size standard results in determining accurate and precise molecular length. This is because the internal size standard and the unknown fragments undergo exactly the same electrophoretic forces. The GeneScan Analysis Software can then compensate for band-shift artifacts caused by variations in the gel and in the sample from lane to lane or injection to injection.

Size Standards Provided

Applied Biosystems provides several fluorescently labeled size standards, which are described in Appendix C.

You can also label and use other fragments if they better suit the fragment sizes with which you are working.

When to Define Size Standards

Normally, a size standard is defined using the GeneScan Analysis Software after running the size standard with samples on the instrument. The software detects peaks for a selected dye color in a selected sample file and allows you to define the peak sizes. You can save the defined size standard in a file and use it to automatically analyze other samples run with the same size standard and under the same conditions.

For a list of the size standards that are supplied with the GeneScan Analysis Software, refer to Appendix C, “GeneScan Size Standards.”

If Split Peaks Appear

Split peaks might appear in size standards in which both DNA strands are labeled. For some peaks, the two strands migrate at different rates when running under denaturing conditions, and they appear as two peaks approximately half the height of normal, non-split, peaks. One peak of the two runs is true to size. Assign a size to that peak for the size standard definition, and assign zero to the other peak.

Defining the Size Standard

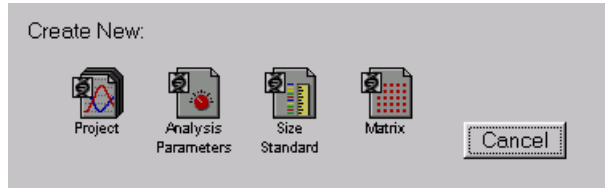
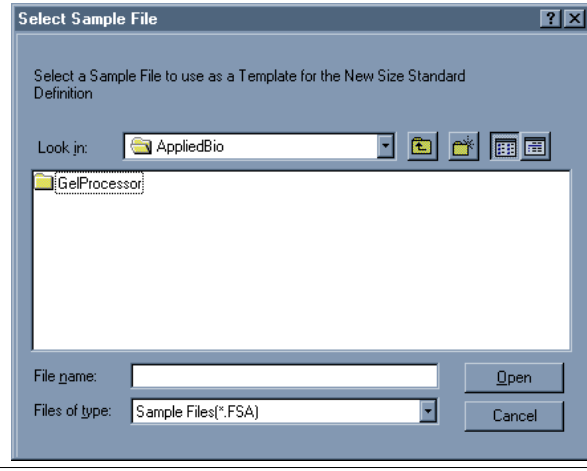
Two Ways to Define the Size Standard

There are two ways to define a new size standard:

Topic	See Page
Using the New Command	7-3
Using the Analysis Control Window	7-7

Using the New Command

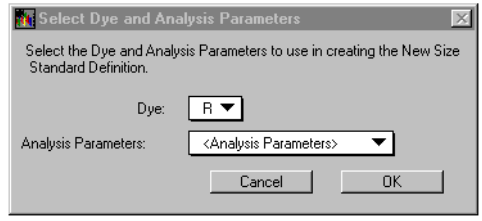
To use the New command to define a new size standard:

Step	Action
1	Select **New** from the **File** menu. The **Create New** dialog box opens. 
2	Click the **Size Standard** icon. The following dialog box opens: 

To use the New command to define a new size standard: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Select the sample file that contains the dye standard you want to use as a template for the new size standard and click Open.

The Select Dye and Analysis Parameters dialog box opens.

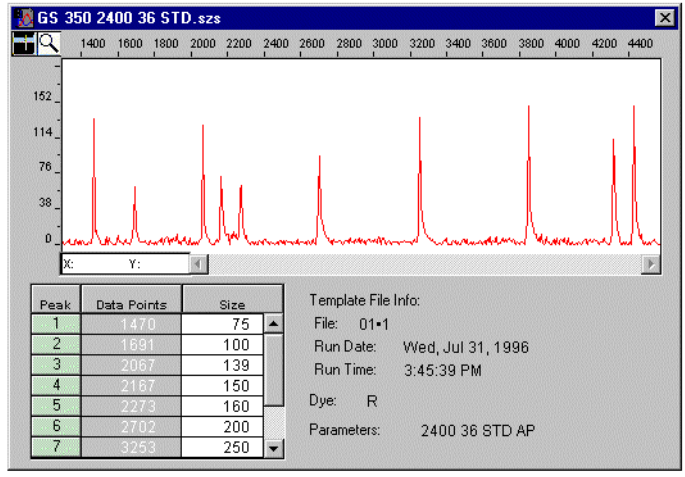
 
</td></tr>
<tr><td>4</td><td>From the Dye pop-up menu, select the code that represents the dye label of the size standard in the selected sample File.</td></tr>
<tr><td>5</td><td>From the Analysis Parameters pop-up menu, select the analysis parameters to use.

The pop-up menu contains the following options:

<table>
<tr><th>Item</th><th>Description</th></tr>
<tr><td>Analysis Parameters</td><td>Applies the parameters that are stored as preferences in the software.</td></tr>
<tr><td>custom parameters that are listed at the bottom of the menu</td><td>These are files that you defined and they are located in the Params folder.

The path is: D:\AppliedBio\Shared \Analysis\Sizecaller\Params.</td></tr>
</table></td></tr>
</table>

To use the New command to define a new size standard: *(continued)*

Step	Action
6	Click **OK**. A window opens (see below) showing the electropherogram and a table of peaks for the dye color and sample selected. You should be able to recognize the peak pattern of the size standard in the electropherogram. **Note** You can only change the peak size value in the right column of the table. You cannot change or rearrange the peak numbers. **If...** / **Then...** too many peaks appear in the electropherogram or the baseline is too high → you may need to adjust the analysis parameters. See "Using Analysis Parameter Files" on page 5-13. The software assigns a number to each peak found in the electropherogram in order, from left to right. 

To use the New command to define a new size standard: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>7</td><td>Specify the peaks of the size standards and their sizes as follows:
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a.</td><td>Click the peak you want to define either in the electropherogram or in the table.

Use the Zoom In (Ctrl+ Plus sign) and Zoom Out (Ctrl+ Minus sign) commands from the View menu to zoom the electropherogram for easier viewing.

– If you click a peak in the electropherogram, the corresponding row in the table is highlighted.

– If you click a peak number in the table, the corresponding peak in the electropherogram is highlighted.</td></tr>
<tr><td>b.</td><td>Type the value for the selected peak in the corresponding Size field in the table.

Refer to Appendix C, “GeneScan Size Standards,” for values and peaks patterns.

Note Leave a zero in the Size field to ignore a peak for the size standard definition.</td></tr>
<tr><td>c.</td><td>Press Enter to automatically move to the next size standard peak.</td></tr>
</table></td></tr>
<tr><td>8</td><td>When you finish defining the peaks, save the size standard by selecting Save As from the File menu.

Note You can also click the Close button.</td></tr>
<tr><td>9</td><td>Enter a descriptive name for the size standard and click Save.

Note Run conditions are not stored in the Size Standard file. Use a name that clearly defines the size standard for future use.

This file is automatically saved in the SizeStandards folder.

The path is:
D:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards</td></tr>
</table>

Using the Analysis Control Window

To define a new size standard:

Step	Action
1	Open an existing project or create a new project. **For information on...** / **See Page** Opening an Existing Project — 2-8 Creating a New Project — 2-9 The Analysis Control window should open. If it does not, select **Analysis Control** (Ctrl+1) from the **Windows** menu.
2	In the **Analysis Control** window, find the row that contains the sample for which you want to define the size standard.
3	In that row, Ctrl+click the dye color cell that represents your size standard. A diamond symbol (◆) appears in the cell, identifying it as the size standard.
4	Click the arrow in the **Parameters** field of the same row, and select an option from the pop-up menu that opens. The pop-up menu contains the following options: **Item** / **Description** Analysis Parameters — Applies the parameters that are stored as preferences in the software. filename — Applies the settings specified in the data collection run file. Custom parameters that are listed at the bottom of the menu — These are files that you defined and they are located in the Params folder. The path is: D:\AppliedBio\Shared\Analysis\Sizecaller\Params
5	In the same row, click the arrow in the **Size Standard** field, and select **Define New** from the pop-up menu. A window opens showing the electropherogram and a table of peaks for the dye color and sample you selected. You should be able to recognize the peak pattern of the size standard in the electropherogram.

To define a new size standard: *(continued)*

Step	Action
6	Follow step 7 to step 9 on page 7-6. The name of the size standard appears in the **Size Standard** pop-up menu in the **Analysis Control** window.

Using Size Standards

In This Section

This section contains the following topics:

Topic	See Page
Changing the Number of Peaks Detected	7-9
Editing the Size Standard Definition	7-9
Using the Open Command to Edit an Existing Size Standard	7-10
Using the Analysis Control Window to Edit an Existing Size Standard	7-11
Deleting an Existing Size Standard	7-12
Analyzing Samples Using the Same Size Standard	7-13
Selecting Separate Size Standards for Samples	7-14

Verifying Size Calculations

For information, see “Verifying Size Calculations” on page 9-33.

Changing the Number of Peaks Detected

Use the Analysis Parameters dialog box to change the number of peaks detected in the Define New Standard window.

For information, refer to “Peak Detection Options” on page 5-8.

Editing the Size Standard Definition

The following procedure describes how to edit the size standard definition (Collection Setting standard) that is embedded in the sample file. Double-clicking the size standard definition will not open the file.

To edit the size standard definition:

Step	Action
1	Choose **Open** from the **File** menu. The **Open Existing** dialog box opens. The Collection Setting does not change.
2	Click the **Size Standard** icon. An **Open** dialog box opens.

To edit the size standard definition: *(continued)*

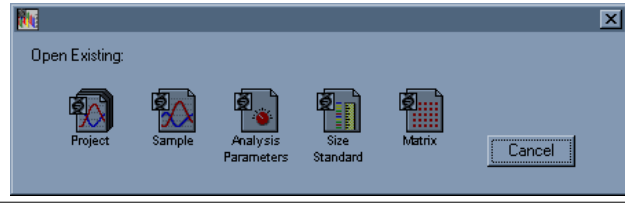
Step	Action
3	Navigate to the SizeStandards folder, select the file that you want to edit, and click **Open**. The path is: D:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards
4	Make any changes to the file and click the **Close** button.
5	Choose the edited file from the **Size Standard** pop-up menu to apply the changes.

Editing an Existing Size Standard

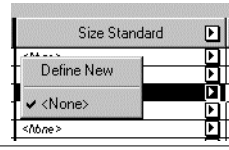
The two ways to edit a previously defined size standard are by:

- Using the Open Command to Edit an Existing Size Standard
- Using the Analysis Control Window to Edit an Existing Size Standard

Using the Open Command to Edit an Existing Size Standard

Step	Action
1	Choose **Open** (Ctrl+O) from the **File** menu. The **Open Existing** dialog box opens. 
2	Click the **Size Standard** icon. An **Open** dialog box opens.
3	Navigate to the SizeStandards folder, select the size standard file that you want to modify, and click **Open**. The path is: D:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards
4	Edit the peak size values by following steps 7 to 9 on page 7-6.
5	Select **Save** (Ctrl+S) from the **File** menu to update an existing file, or **Save As** to specify a new name.

Using the Analysis Control Window to Edit an Existing Size Standard

Step	Action
1	If the **Analysis Control** window is not open, select **Analysis Control** (Ctrl+1) from the **Windows** menu.
2	Click the arrow in the **Size Standard** column for the sample that you want to change. 
3	Select a size standard from the pop-up menu.
4	Edit the peak size values by following steps 7 to 9 on page 7-6
5	Select **Save** (Ctrl+S), or **Save As** from the **File** menu to save changes and specify a new file name.

Deleting an Existing Size Standard

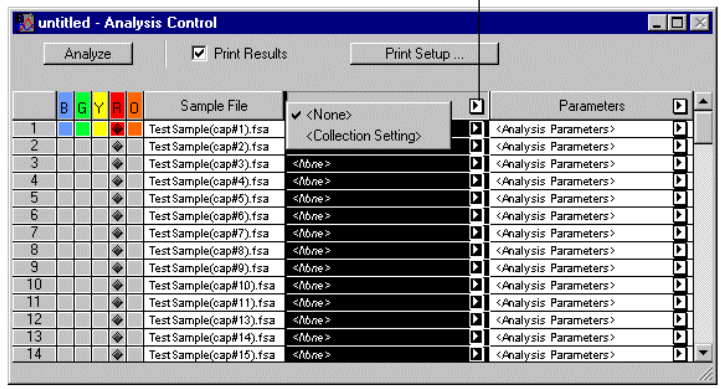
The following procedure describes how to delete a user-defined size standard from the Params Folder so that it no longer appears in the Size Standard pop-up menus. The size standard is permanently removed, and you must redefine it to use it again.

To delete an existing size standard:

Step	Action
1	Click the **Start** button, and then point to **Programs**.
2	Click **Windows NT® Explorer** to find and open the SizeStandards folder. The path is: D:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards
3	Select the size standard file that you want to delete.
4	Drag the file to the **Recycle Bin**. **Note** You can also drag the size standard to another folder for storage.

Analyzing Samples Using the Same Size Standard

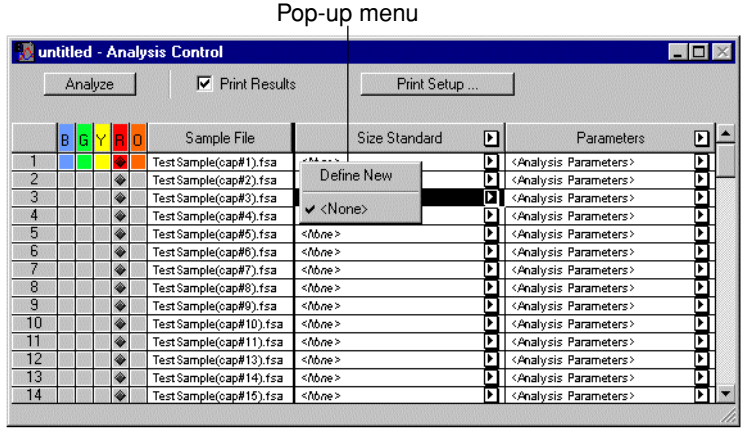
To select the same size standard to analyze all samples:

Step	Action
1	If the **Analysis Control** window is not displayed, select **Analysis Control** (Ctrl+1) from the **Windows** menu.
2	Click the arrow in the **Size Standard** column heading and choose a size standard file from the pop-up menu. Your menu choice applies to all fields in the column. **Note** Alternatively, you can choose a value from the pop-up menu, click the header to select the entire column, and select **Fill Down** (Ctrl+D) from the **Edit** menu. Pop-up menu 
3	The pop-up menu contains the following options: **Item** / **Description** None — Apply no standard definition. Collection Setting — Apply the size standard specified in the Data Collection software, which is embedded in the sample file. For information on editing this file, see “Editing the Size Standard Definition” on page 7-9. Custom standards that are listed at the bottom of the menu — These are files that you defined and they are located in the SizeStandards folder. The path is: D:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards

Selecting Separate Size Standards for Samples

To apply separate size standards to selected samples, click the arrow in the Size Standard column for the sample that you want to change (refer to the figure below) to open the pop-up menu.

For information on using the pop-up menu, see step 3 above.



Evaluating Analysis Results

8

Overview

In This Chapter Topics in this chapter include the following:

Topic	See Page
Process of Evaluating Analysis Results	8-2
Ways to Display Analysis Results	8-3
About the Results Display Window	8-4
Using the Results Control Window	8-7
Changing How the Results Are Displayed and Printed	8-13
About the Sample Results View	8-15
Updating the Results	8-16
Saving and Renaming the Results Control Format	8-17

Process of Evaluating Analysis Results

Evaluating Analysis Results

The following table describes the steps to evaluate the analysis results:

Step	Action	For information, see...
1	Displaying analysis results	"Ways to Display Analysis Results" on page 8-3
2	Using electropherogram and tabular data displays	"About Electropherogram and Tabular Data Displays" on page 9-2
3	Viewing electropherograms	"Displaying Electropherogram Data" on page 9-9
4	Verifying analysis results	"Process of Verifying Results" on page 9-31
5	Displaying other sample file data	♦ "Sample Info View" on page 4-11 ♦ "Size Curve View" on page 4-20 ♦ "Raw Data View" on page 4-22 ♦ "EPT Data View" on page 4-24
6	Using the analysis log	"Using the Analysis Log" on page 9-36
7	Remembering and renaming the results display	"Saving and Renaming the Results Control Format" on page 8-17

Ways to Display Analysis Results

Two Ways to Display Analysis Results

The following table describes two ways to display analysis results:

You can use...	Description	See Page
About the Results Display Window	This window is created from the **Results Control** window of a project. Use to group and view multiple sample files as electropherogram and tabular data.	8-4
About the Sample Results View	This view displays one sample file at a time, like the **Results Display** window, but is more convenient to view analysis results from a single sample file. It also allows quick access to supporting information views.	8-15

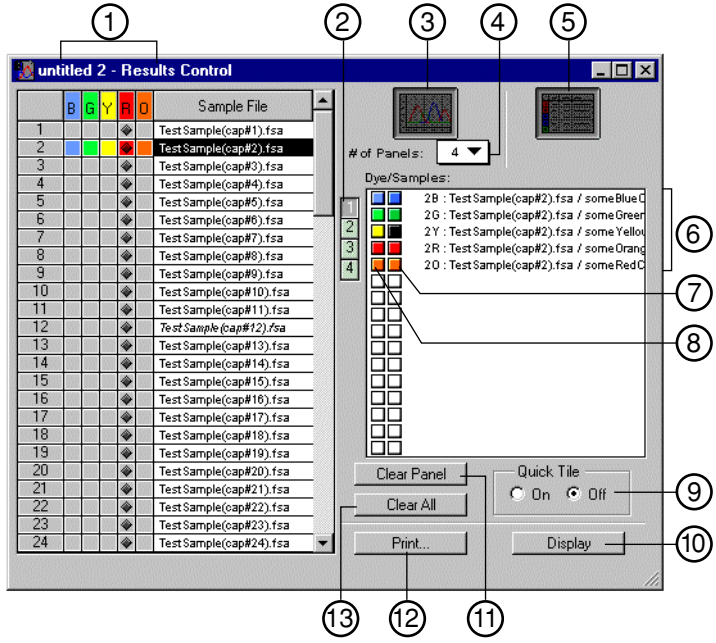
About the Results Display Window

Introduction

The Results Display window is created from the Results Control window of a project. It allows you to group and view multiple sample files as electropherogram or tabular data. You can use the window to show up to eight panels, with multiple dye/samples per panel.

Displaying the Window

When you select Results Control (Ctrl+2) from the Windows menu, a window opens like the example shown below. The callouts are described in the table that follows this example.



Results Control Window Callouts Described

The following table describes the callouts in the previous figure of the Results Control window:

<table>
<tr><th>Callout</th><th>Description</th></tr>
<tr><td>1</td><td>Click one of the dye color fields (B, G, Y, R, O) to select dye/sample information.</td></tr>
<tr><td>2</td><td>Click to display electropherograms for the selected samples.</td></tr>
<tr><td>3</td><td>Click to show which sample is selected for this panel.</td></tr>
<tr><td>4</td><td>Choose the number of electropherogram panels available for display from the pop-up menu.</td></tr>
<tr><td>5</td><td>Click to display tabular data for the selected samples.</td></tr>
<tr><td>6</td><td>Identifies the sample by row number and dye code.

Sample information is displayed as specified in the project options.</td></tr>
<tr><td>7</td><td>Plot color indicator

<table>
<tr><th>If you...</th><th>Then...</th></tr>
<tr><td>double-click the plot color indicator</td><td>the Choose a Plot Color dialog box opens.

For more information, refer to “Defining Individual Plot Colors” on page 9-26.</td></tr>
<tr><td>Ctrl+double-click the dye color indicator</td><td>the plot color indicator returns to the default color.

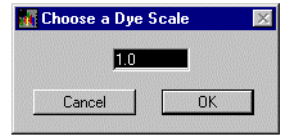
For more information, refer to “Setting Dye Indicator Preferences” on page 3-14.</td></tr>
</table>

Note If you change the plot color, a vertical line appears beside the indicator, showing that it has been modified.</td></tr>
</table>

The following table describes the callouts in the previous figure of the Results Control window: *(continued)*

<table>
<tr><th>Callout</th><th>Description</th></tr>
<tr><td>8</td><td>Dye color indicator

<table>
<tr><th>If you...</th><th>Then...</th></tr>
<tr><td>double-click the dye color indicator</td><td>the Choose a Dye Scale dialog box opens.

For more information, see “Changing the Dye Scale in Electropherograms” on page 9-28.</td></tr>
<tr><td>Ctrl+double-click the dye color indicator</td><td>the dye color indicator returns to the default scale.

For more information, see “Changing the Dye Scale Preferences” on page 9-30.</td></tr>
</table>

Note If you change the scale, a vertical line appears beside the indicator, showing that it has been modified.</td></tr>
<tr><td>9</td><td>Quick Tile buttons.

See “Creating Tiled Electropherogram Displays” on page 8-10.</td></tr>
<tr><td>10</td><td>Display button.

See “Displaying the Results” on page 8-12.</td></tr>
<tr><td>11</td><td>Clear Panel button.

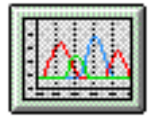
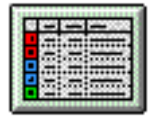
See “Removing Samples” on page 8-12.</td></tr>
<tr><td>12</td><td>Print button.

See “Printing the Results” on page 8-12.</td></tr>
<tr><td>13</td><td>Clear All button.

See “Removing Samples” on page 8-12.</td></tr>
</table>

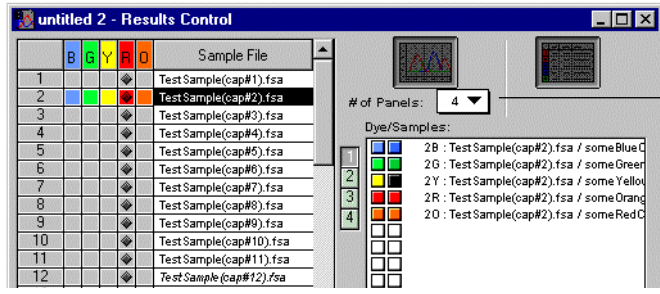
Selecting Display Format

Choose the results display format by clicking the electropherogram or tabular icons, or both. You must select one of the icons to display or print data.

Click this icon...	To display...	And the...
	electropherogram data	panel information below the icon is enabled. Refer to the figure below.
	tabular data	Tabular data appears in a single table, so electropherogram panel configuration is not relevant.

Selecting Electropherogram Panels

In electropherograms, the data appears in panels. You can overlay up to 16 samples within up to eight panels. Select the number of available panels (up to eight) from the pop-up menu labeled # of Panels.



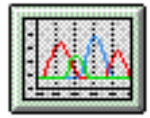
Note Set the number of panels when using the Quick Tile option. Using this option causes the GeneScan® Analysis Software to automatically change panels as you select samples to display (see “Creating Tiled Electropherogram Displays” on page 8-10).

Selecting Samples to Display

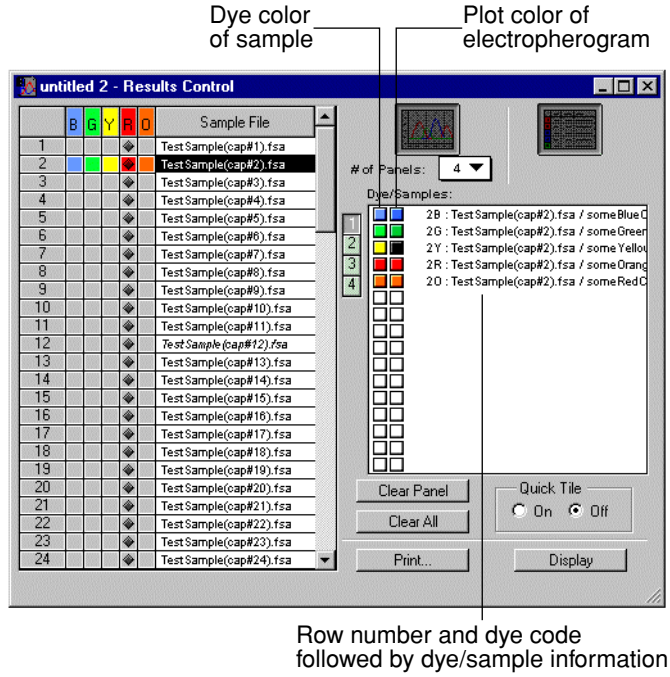
Use the dye color field on the left side of the Results Control window to select the dye to display.

You can select any or all dye colors, including the standard, for each sample file.

To select samples to display:

Step	Action
1	Click the Electropherogram icon.  The panel below the button is enabled.
2	Click a panel number in the list to the left of the **Dye/Samples** list. If fewer than eight panels are available and you want to use more, choose a larger number (up to eight) from the **# of Panels** pop-up menu.

To select samples to display: *(continued)*

Step	Action
3	Click the dye color fields to select or unselect the corresponding samples. Take the following action: **To...** / **Click the...** select the entire column — header of the column. unselect the entire column — **Clear All** button, or click the header if it is already selected. select all colors for a sample file — index number to the left of the color columns. The samples corresponding to the selected dyes appear in the **Dye/Samples** list to the right of the **Sample File** list, as shown in the figure below. 

To select samples to display: *(continued)*

Step	Action
4	When you change to a new panel, the dye colors of the samples you selected in other panels appear dark gray to indicate that they have been selected. You can select them again in the current panel.

Creating Tiled Electropherogram Displays

Procedure

To create tiled electropherogram displays:

Step	Action
1	Choose the number of panels you want to display from **# of Panels** pop-up menu.
2	Click the **On** button under **Quick Tile**.
3	Select samples by clicking color fields. For information on: **Topic** — **See Page** Selecting Samples to Display — 8-8 Setting the tiled electropherogram preferences — 8-13 Each time a sample is selected, the program automatically changes to the next panel, so each selection is placed after the one containing your previous selection. After you select a sample for the last panel, the panel displays the first panel again.

Example

The following table describes two examples of how to use the Quick Tile feature:

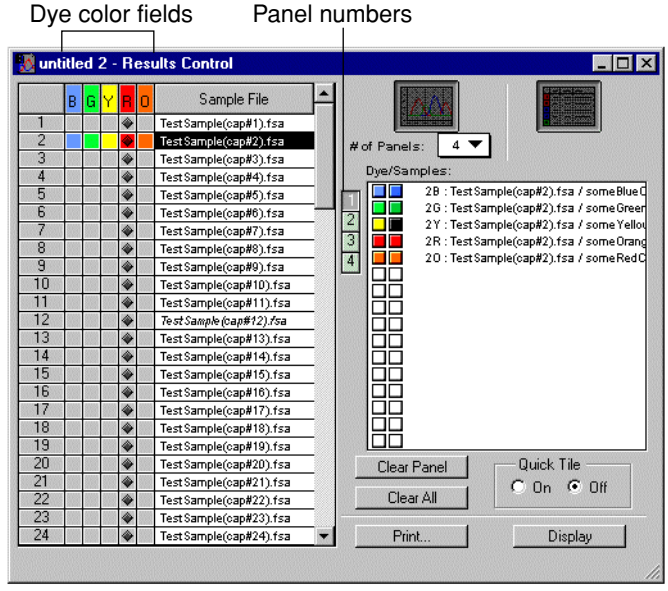
If you...	Then...
have four samples and choose four panels for display	click the column heading for the blue dye to select all four blue dye-labeled samples. The blue dye for each sample file appears in a separate panel.

The following table describes two examples of how to use the Quick Tile feature: *(continued)*

If you...	Then...
click the row index number for the first sample file to select all dye colors for one sample file	each dye for that sample file appears in a separate panel.

Unselecting Samples

To unselect the samples that you have selected for display:

Step	Action
1	If you specified the Electropherogram display, click the panel number to the left of the **Dye/Samples** list to display the panel containing the samples you want to unselect. 
2	On the left side of the **Results Control** window, unselect the dye color fields corresponding to the samples you want to remove. The dye/sample identifiers are removed from the **Dye/Samples** list as you unselect samples.

Removing Samples

To remove samples:

Step	Action
1	Select the panel number on the buttons to the left of the **Dye**/**Samples** list.
2	You can take the following action: **To remove…** — **Click…** all the samples you selected to display in a panel — **Clear Panel**. the samples you have selected to display in all panels — **Clear All**.

Displaying the Results

To display the results on the screen, you can either:

- Click the Display button.
- Press the Return or Enter key.

Printing the Results

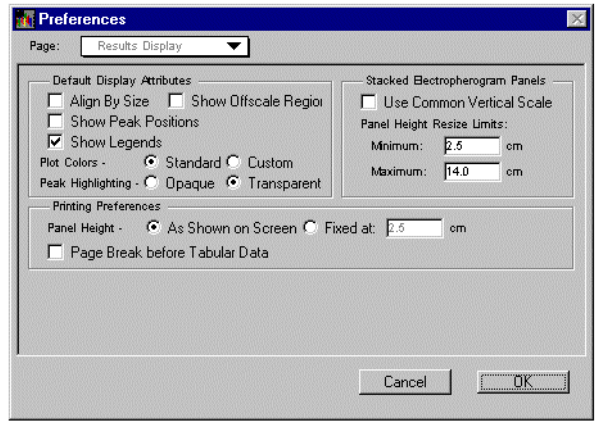
To print the results:

Step	Action
1	You can either: ♦ Click the **Print** button, or ♦ Select **Print** (Ctrl+ P) from the **File** menu.
2	Click **OK** in the dialog box that opens.

Changing How the Results Are Displayed and Printed

Procedure You can set certain display preferences that remain in effect each time you display or print results data.

To change how results are displayed and printed:

Step	Action
1	Select **Preferences** from the **Settings** menu and **Results Display** from the submenu. The following dialog box opens:  There are three **Results Display** preference categories: ♦ Default Display Attributes ♦ Stacked Electropherogram Panels ♦ Printing Preferences

To change how results are displayed and printed: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Set the Default Display Attributes to control the display attributes of new results displays, as follows:
<table>
<tr><th>You can select the...</th><th>For more information...</th></tr>
<tr><td>Align By Size check box</td><td>“Showing Data by Fragment Size” on page 9-20.</td></tr>
<tr><td>Show Peak Positions check box</td><td>“Displaying Peak Positions” on page 9-14.</td></tr>
<tr><td>Show Legends check box</td><td>“Using Legends to Change the Display” on page 9-15.</td></tr>
<tr><td>Show Offscale Region check box</td><td>“Showing Off-Scale Data” on page 9-18.</td></tr>
<tr><td>Standard
or
Custom Plot Colors buttons</td><td>“Defining Custom Colors” on page 9-25.</td></tr>
<tr><td>Opaque
or
Transparent Peak Highlighting buttons</td><td>“Highlighting Peaks” on page 9-15.</td></tr>
</table></td></tr>
<tr><td>3</td><td>Set the Stacked Electropherogram Panels, as follows:
<table>
<tr><th>Choose...</th><th>To set...</th></tr>
<tr><td>Use Common Vertical Scale check box</td><td>all panels in a display so they have the same vertical scale.
The common scale is based on the electropherogram with the largest vertical scale.</td></tr>
<tr><td>Panel Height Resize Limits</td><td>minimum and maximum values for electropherogram panel height in the results display.
Use to limit how much the electropherogram panels stretch or shrink to fit the size of the window.</td></tr>
</table></td></tr>
</table>

To change how results are displayed and printed: *(continued)*

Step	Action
4	Set the **Printing Preferences**, as follows: **If...** / **Then...** the height of the panels that appears on screen is acceptable → select the **As Shown on Screen** button. you want to print the electropherogram at a specified height → click the **Fixed at** button and enter a value in the field. you want to force a page break after the electropherograms have been printed → select the **Page Break before Tabular Data** check box.
5	Click **OK**.

About the Sample Results View

About the View

The Sample Results view is displayed within a Sample File window. You can access the window through a sample file or through a project's Analysis Control or Results Control window.

If...	Then...
you are opening a sample file as a stand-alone file	the **Sample Results** view is the default display within the **Sample File** window.
For more information on...	**See Page**
the **Sample Results** view	4-9

Updating the Results

Re-analyzing the Data

The Results Control and the Sample Results windows are dynamic.

If you re-analyze your data with either window active, then the software updates this window.

Saving and Renaming the Results Control Format

Introduction

You can use the Results Control window to view multiple sample files in electropherogram and tabular format. The GeneScan Analysis Software allows you to save formats for future use. You can then redisplay or print these formats without having to redefine them again.

Important Considerations

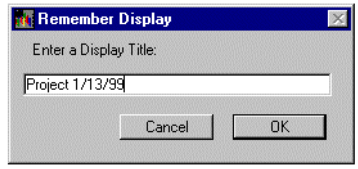
The following are important considerations for saving a Results Control format:

- You must save the project for the display to be available when you open the project again.
- Remembering a display preserves the combination of windows/panels/data and customized color settings.

  It does not preserve any zooming you have performed.

Saving the Display Format

To save a display format for future viewing:

Step	Action
1	With the **Results Control** window set for the display, either: ♦ Click the **Display** button, or ♦ Press the **Enter** key.
2	With the display on the screen, select **Remember Display** from the **Project** menu. The **Remember Display** dialog box opens. 
3	Enter a name for the display and click **OK**.

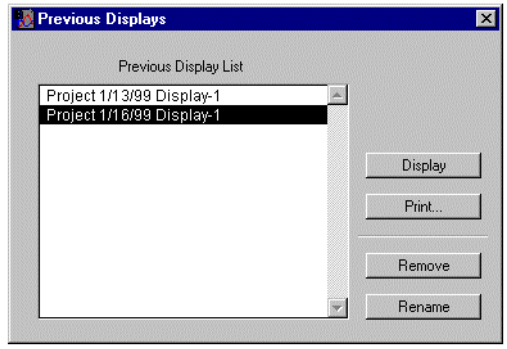
Working with a Previously Saved Display

Use the Previous Displays dialog box to display, print, remove or rename a saved display.

To work with a previously saved displays:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Select Previous Displays from the Project menu.

The following is an example of the Previous Displays dialog box:

 
</td></tr>
<tr><td>2</td><td>Select a display or multiple displays and take the following action:

<table>
<tr><th>To...</th><th>Click an item in the list and click...</th></tr>
<tr><td>display the saved formats</td><td>Display.</td></tr>
<tr><td>print the saved formats</td><td>Print.

The standard print dialog box opens.</td></tr>
<tr><td>remove the saved formats</td><td>Remove.

An alert opens.</td></tr>
<tr><td>rename a currently saved format

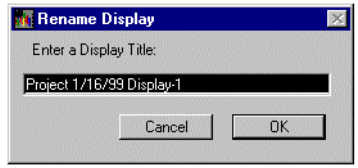
Note You can only rename one display at a time.</td><td>Rename.

The Rename dialog box opens.

See “Renaming the Current Results Display” on page 8-19.</td></tr>
</table>
</td></tr>
</table>

Renaming the Current Results Display

To rename the Results display that is currently on the screen and to save the display under a different name:

Step	Action
1	Ensure that the display is the active window.
2	Select **Rename Display** from the **Project** menu. The following is an example of the **Rename Display** dialog box: 
3	Enter a new name for the display and click **OK**. The new name opens in the **Previous Displays** dialog box.

Evaluating Electropherograms

9

Overview

In This Chapter Topics in this chapter include the following:

Topic	See Page
About Electropherogram and Tabular Data Displays	9-2
Displaying Electropherogram and Tabular Data	9-4
Displaying Electropherogram Data	9-9
Working with Electropherogram Data	9-12
Defining Custom Colors in Electropherograms	9-24
Changing the Dye Scale in Electropherograms	9-28
Process of Verifying Results	9-31
Verifying Size Calculations	9-33
Using the Analysis Log	9-36
Verifying Peak Detection	9-38

About Electropherogram and Tabular Data Displays

Introduction

After analyzing the data, you can display the results for each sample in electropherogram and tabular data. You can also customize the electropherogram and tabular data display.

Note Altering the appearance of the electropherograms and the tabular data displays does not change the analyzed data contained in the sample file on which they are based.

How the Window Is Divided

When electropherogram and tabular data are displayed together, the window is divided into upper and lower windows.

Window	Contains
Upper window	electropherogram data
Lower window	tabular data
For more information on...	**See**
customizing the window's appearance by adjusting the relative size of each window	"Adjusting Window Size" on page 9-7.

What Tabular Data Contains

If you analyze samples and perform sizecalling, the tabular data contains the estimated sizes (in base pairs) of all detected fragments. Use this information for detailed data analysis and further calculations. The peaks matched to the defined size standard are identified by dots next to the Dye/Sample Peak field.

Sample peaks that are larger (in base pairs) than the largest defined peak in the selected standard are not sized. The corresponding size fields are blank.

Note Tabular data displays only peaks that are detected based on the Dye Amplitude Thresholds and Minimum Peak Half Width setting of the analysis parameters.

How Electropherogram Panels Are Sized

In the Results Display window, the GeneScan® Analysis Software sizes all electropherogram panels to fit within the electropherogram portion of the window by using the largest size that fits them into the visible area. You can scroll to see the portion of the display that is not visible.

For More Information

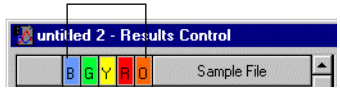
For more information, see the following topics:

Topic	See Page
Displaying Electropherogram and Tabular Data	9-4
Displaying Electropherogram Data	9-9
Working with Electropherogram Data	9-12

Displaying Electropherogram and Tabular Data

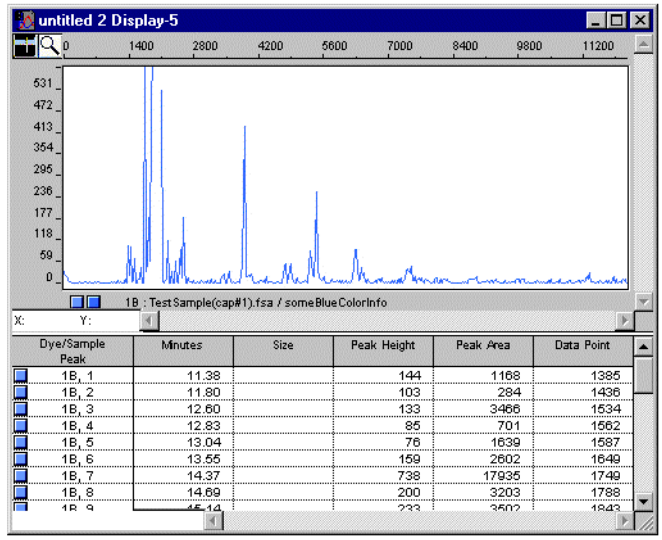
Procedure

To display electropherogram and tabular data:

Step	Action
1	Select **Results Control** (Ctrl+2) from the **Windows** menu to open the **Results Control** window.
2	Click the Electropherogram button and the Tabular button .
3	If applicable, select the number of electropherogram panels from the **# of Panels** list.
4	Click the dye color fields to select or unselect the corresponding samples. Dye color fields  Take the following action: **To...** / **Click the...** select the entire column / header. unselect the entire column / **Clear All** button. select all colors for a sample file / index number to the left of the color columns. The samples corresponding to the selected dyes appear in the **Dye/Samples** list to the right of the **Sample File** list.
5	Click **Display**. The electropherogram and tabular data are displayed in the **Results Display** window. See also “Working with Electropherogram Data” on page 9-12.

Example of Tabular Data and Electropherogram

The following is an example of tabular data with a corresponding electropherogram:



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
1B, 1	11.38		144	1168	1385
1B, 2	11.80		103	284	1436
1B, 3	12.60		133	3466	1534
1B, 4	12.83		85	701	1562
1B, 5	13.04		76	1639	1587
1B, 6	13.55		159	2602	1649
1B, 7	14.37		738	17935	1749
1B, 8	14.69		200	3203	1788

Table Describing Columns

The following table describes the columns in the “Example of Tabular Data and Electropherogram” above:

Column heading	Identifies...
Dye/Sample Peak	♦ Sample index number ♦ Dye color ♦ Peak number
Minutes	The time, in minutes, from the start of the run to the time the fragment was detected
Size	The differences in fragment mobility This value is calculated automatically only if you: ♦ Run the size standard in the same lane or injection as the sample, and ♦ Perform sizecalling
Peak Height	Signal size (RFU)

The following table describes the columns in the “Example of Tabular Data and Electropherogram” above: *(continued)*

Column heading	Identifies...
Peak Area	Area of the detected peak
Data point	Data point of the fragment at its maximum peak height

Why Some Peaks May Be Visible Only in an Electropherogram

Peaks may be visible in the electropherogram and not listed in the tabular data because:

Reason	For more information, see...
The software detects the peaks based on the Peak Amplitude Thresholds and Min Peak Half Width.	“Peak Detection Options” on page 5-8
Electropherograms display the peaks that fall within the range specified by the Sizecall Range parameters that are defined in the Analysis Parameters dialog box.	“Sizecall Range Options” on page 5-10

Why Some Peak Areas May Have a Negative Number

If negative values appear in peak areas in the electropherogram, it is because a portion of the peak is below the baseline. The GeneScan Analysis Software display does not show the part of the electropherogram that is below the baseline.

Highlighting Information

To highlight information for one peak in the electropherogram and tabular data:

Click...	Then...
the peak in the electropherogram	the peak fills with color and the corresponding row in the tabular data window is highlighted.
the **Dye/Sample Peak** number in the tabular data window	highlights the corresponding peak in the electropherogram.

Changing the Highlight Color

To change the highlight transparency:

Step	Action
1	Select **Peak Highlighting** from the **View** menu.
2	Then select either **Opaque** or **Transparent** from the submenu.

Note For more information on highlighting peaks, see page 9-15.

Adjusting Window Size

To adjust the relative size of the electropherogram and tabular windows:

Step	Action
1	Move the cursor to the window divider (the double line between the two windows).
2	When the cursor changes to a bidirectional arrow ( ), click the window divider line and drag it up or down.

Hiding Selected Rows of Data

To hide selected rows of data:

Step	Action
1	Take the following action:

If you want to...	Then...
select a row	either: ♦ Click the first field in the row, or ♦ Click the corresponding peak in the electropherogram.
select several rows that are not next to each other	Ctrl+click the rows.

Step	Action
2	Select **Hide Selected Rows** (Ctrl+H) from the **View** menu.

Limiting the Rows to Display

To limit the display to the selected rows of data:

Step	Action
1	Select the rows you want to display.
2	Select **Show ONLY Selected Rows** (Ctrl+G) from the **View** menu. **Note** Select **Show All Rows** (Ctrl+G) from the **View** menu to display all of the tabular data after limiting the display.

Displaying Electropherogram Data

Definition

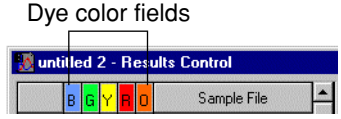
Each electropherogram provides a profile of the selected dye samples it represents. The y-axis represents the relative fluorescence of the detected fragments as they occurred over time. The x-axis represents time and can be displayed by data points or base pairs.

Base Pairs Versus Data Points

The tick marks on the x-axis can represent size in base pairs instead of data points. This option is only available for runs that include an internal size standard with the sample (see “Showing Data by Fragment Size” on page 9-20).

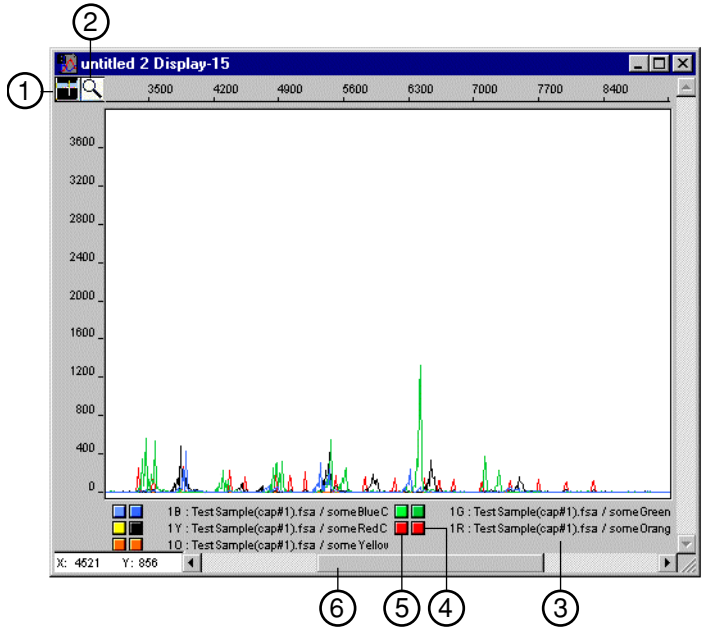
Procedure to Display Data

To display electropherogram data:

Step	Action
1	Select **Results Control** (Ctrl+2) from the **Windows** menu to open the **Results Control** window.
2	Click the **Electropherogram** button.
3	If applicable, select the number of electropherogram panels from the **# of Panels** pop-up menu.
4	Click the dye color fields to select or unselect the corresponding samples. Dye color fields  Take the following action: **To...** / **Click the...** select the entire column — header. unselect the entire column — **Clear All** button. select all colors for a sample file — index number to the left of the color columns. The samples corresponding to the selected dyes appear in the **Dye/Samples** list to the right of the **Sample File** list.
5	Click **Display**. The electropherogram is displayed in the **Results Display** window as shown in the example below.

Electropherogram Example

The following is an example of an electropherogram:



Electropherogram Callouts Described

The following table describes the callouts in the figure above:

Call out	Description	See...
1	Cross hairs	"Displaying X- and Y-Axis Positions" on page 9-13
2	Magnifying glass Use to zoom in a specific area or hold down while pressing the **Alt** key to zoom out to a smaller scale. Or, click and drag a marque around an area to zoom in to that area.	"Zooming In and Out" on page 9-17
3	Legend Text from the sample file that appear beneath electropherogram panels in the **Results Display** window.	"Using Legends to Change the Display" on page 9-15

The following table describes the callouts in the figure above: *(continued)*

Call out	Description	See...
4	Dye color indicator	“Dye color indicator” on page 8-6
5	Plot color indicator	“Plot color indicator” on page 8-5
6	Scroll bar Use the scroll bar to scroll horizontally.	“Scrolling the Display” on page 9-16

Working with Electropherogram Data

In This Section This section describes how to perform the following tasks:

Task	See Page
Displaying X- and Y-Axis Positions	9-13
Moving the Electropherogram	9-13
Changing the Dye Color	9-14
Displaying Peak Positions	9-14
Highlighting Peaks	9-15
Using Legends to Change the Display	9-15
Scrolling the Display	9-16
Zooming In and Out	9-17
Showing Off-Scale Data	9-18
Electropherogram Displaying Off-Scale Data	9-19
Electropherogram Displaying the Flat-Topped Effect	9-19
Showing Data by Fragment Size	9-20
Changing the Horizontal Scale	9-21
Changing the Vertical Scale	9-22
Assigning Standard or Custom Colors	9-23

Displaying X- and Y-Axis Positions

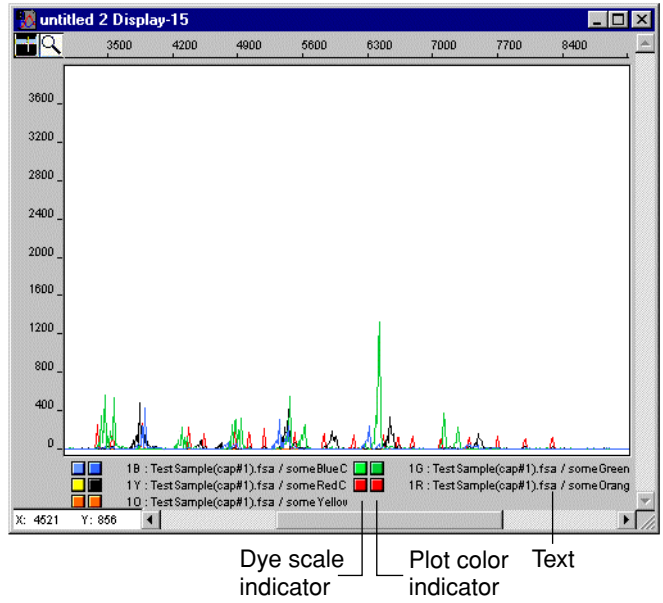
The following table describes how to display the x- and y-axis positions:

To...	Then...
display the x- and y-axis positions.	click the cross hairs and select an area in the Electropherogram. The x- and y-axis values appear in the box in the lower left corner of the electropherogram. If tabular data is also displayed, the row in the table is highlighted.

Moving the Electropherogram

Move the associated electropherogram to the front by clicking one of the following in the legend:

- Dye scale indicator
- Plot color indicator, or
- Text



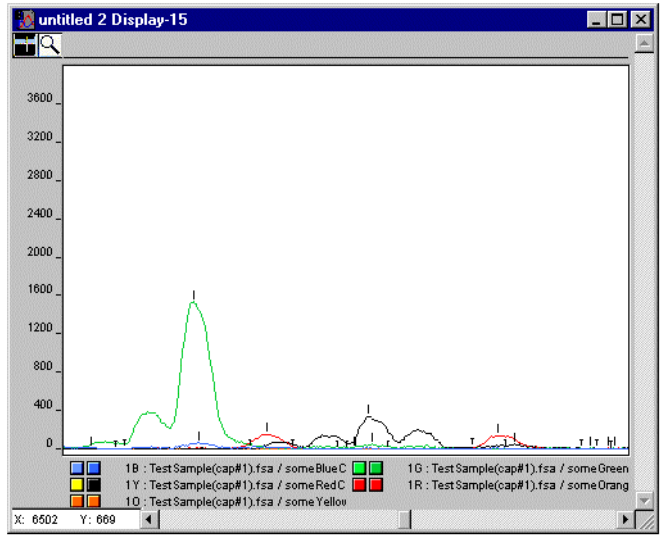
Changing the Dye Color

The following table describes how to change the dye color and how to return to the default dye color:

To...	Then...
change the dye color	double-click the dye color indicator.
return to the default dye color	Ctrl+double-click the dye color indicator.
If...	**Then...**
you change the dye color or scale	a vertical line appears beside the indicator, showing that it has been modified.

Displaying Peak Positions

Use Show Peak Positions from the View menu to examine how the GeneScan Analysis Software defines peaks by displaying markers that identify the beginning, center, and end of each peak.



Highlighting Peaks

Use the Peak Highlighting command to highlight a selected peak with the dye/sample's plot color.

To highlight selected peaks:

Step	Action
1	Select **Peak Highlighting** from the **Views** menus and either **Opaque** or **Transparent** from the submenu.
2	Click a detected peak in an electropherogram. The peak is highlighted.

Use this option...	To...
Opaque	fill the peak with a solid color that can obscure peaks behind the selected peak.
Transparent	use a slightly diffused plot color that allows you to view overlapping peaks.

Using Legends to Change the Display

The following table shows how to use legends to change how electropherograms are displayed:

If you want to...	Then...
show or hide legends	select **Show Legends** from the **View** menu.
open sample file windows	double-click the corresponding legend text.
reorganize overlaid electropherograms	a. Display the electropherograms with legends. b. Click either the dye scale indicator, plot color indicator, or the text for the sample you want to move to the front.

Scrolling the Display

The following table describes ways to scroll the display:

<table>
<tr><th>Use the</th><th>Description</th></tr>
<tr><td>scroll bar</td><td>
<table>
<tr><th>If you want to...</th><th>Then...</th></tr>
<tr><td>shift the electropherogram to the right or the left</td><td>click in the gray region of the scroll bar to the right or left of the scroll box.</td></tr>
<tr><td>scroll across the electropherogram</td><td>click an arrow at the end of the scroll bar.</td></tr>
<tr><td>control the amount of scroll</td><td>drag the scroll bar to the right or the left.</td></tr>
</table>
</td></tr>
<tr><td>scroller symbols</td><td>a. Hold the mouse cursor over either the vertical or horizontal scale of the electropherogram.
Either a vertical scroller symbol ( ) or a horizontal scroller symbol ( ) appears.
b. Hold down the mouse button and move the mouse in the direction of the information you want to view.</td></tr>
</table>

Zooming In and Out

About Zooming In and Out

By default, the GeneScan Analysis Software scales each electropherogram horizontally to show all peaks detected during the run. While this provides a good overview of the run, some peaks may be quite compressed.

Improving Visibility

To improve visibility, you can change the horizontal scale of the electropherograms by zooming.

Zooming affects:

- Only the horizontal scale, and zooms the middle portion of the window
- All displayed electropherogram panels

How to Change the View Scale

If you want to...	Then...
see views with greater detail	♦ Select **Zoom In** (Ctrl++) from the **View** menu, or ♦ Click the magnifying glass in the upper-left corner of the window, and drag around a specific area to zoom in on the electropherogram.
see a smaller scale view of the data after zooming in	♦ Select **Zoom Out** (Ctrl+ -) from the **View** menu, or ♦ Click the magnifying glass cursor, hold down the **Alt** key, and click the electropherogram. The data appears in successively smaller scale views.
quickly scale the data so that the entire length fits within the window, again	Select **Zoom Out** (Full Range) from the **View** menu.

Showing Off-Scale Data

This section contains the following information:

Topic	See Page
Procedure	9-18
About Flat-Topped Peaks	9-18
Electropherogram Displaying Off-Scale Data	9-19
Electropherogram Displaying the Flat-Topped Effect	9-19

Procedure

To show off-scale data:

Step	Action
1	Select **Preferences** from the **Settings** menu and **Results Display** from the submenu. The **Results Display Preferences** dialog box opens.
2	Select the **Show Offscale Regions** check box to highlight with a red bar regions in the electropherogram that contain off-scale data (see "Electropherogram Displaying Off-Scale Data" on page 9-19). **Note** Select the **Zoom In** (Ctrl++) command from the **View** menu to more clearly show the areas of off-scale data. **If...** / **Then...** the sample was sized / the **Analysis Log** lists the numbers of off-scale regions in the analysis range for each sample file. **Note** You can toggle this command for individual electropherograms by selecting **Hide/Show Offscale Regions** (Ctrl+`) from the **View** menu.

About Flat-Topped Peaks

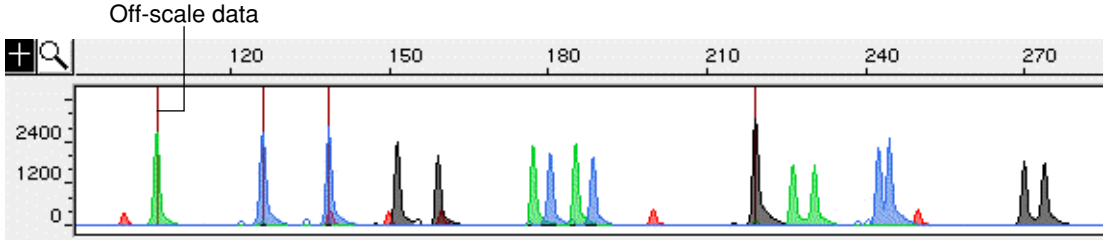
An additional feature is that peaks that contain off-scale data points are drawn in the electropherograms as "flat topped;" that is, the top section of the peak is flat rather than pointed (see "Electropherogram Displaying the Flat-Topped Effect" on page 9-19).

This feature can be seen when the data is analyzed with no or light smoothing; the flat-topped peaks may not be apparent with heavy smoothing.

Select Analysis Parameters from the Settings Menu (see “Data Processing Options” on page 5-7).

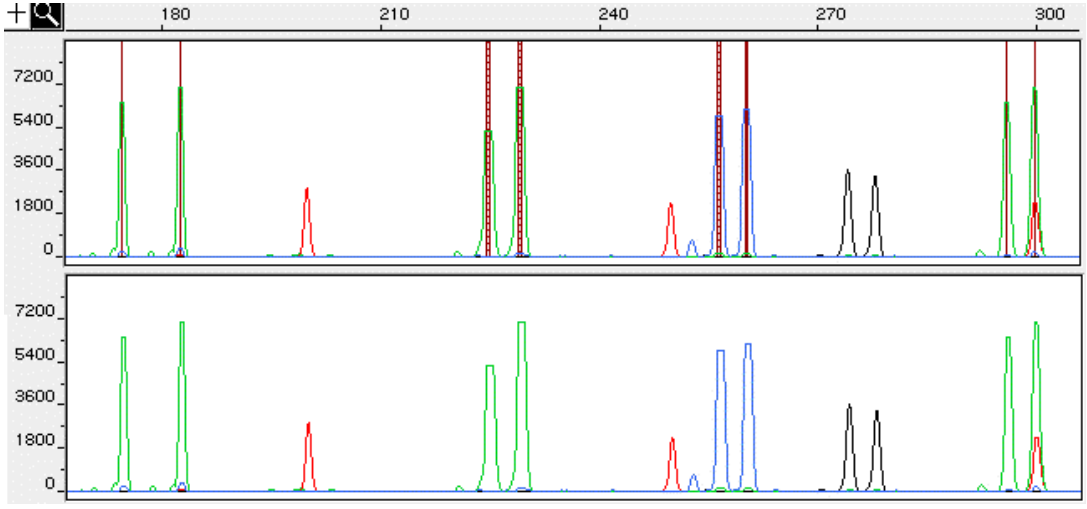
Electropherogram Displaying Off-Scale Data

The following is an example of an electropherogram displaying off-scale data:



Electropherogram Displaying the Flat-Topped Effect

The off-scale peaks in the electropherogram in the expanded view below illustrate the flat-topped effect.



Showing Data by Fragment Size

The following table explains how to show data by fragment size:

If...	Then...
you analyze your samples with an internal size standard	use the **Align by Size** (Ctrl+T) command from the **View** menu to align the horizontal scale of the electropherograms by fragment size instead of by data point. **Note** You can display data by size only if you analyzed and performed sizecalling of your samples using a size standard.
For example, if	**Then...**
you run two identical samples in different runs	the **Align by Size** command adjusts for run-to-run variations by aligning peaks by size value. This eliminates any apparent differences that were caused by run discrepancies. **Note** You can display overlaid samples in the same dye in different colors. See “Defining Custom Colors in Electropherograms” on page 9-24.

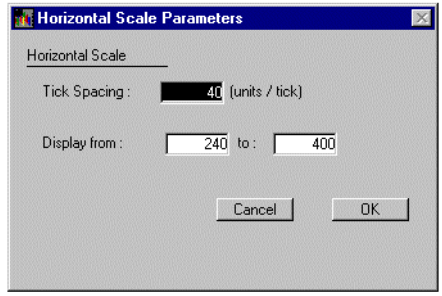
How to Switch Between Size and Data Point Display

If you want to...	Then...
show data by size	select **Align by Size** (Ctrl+ T) from the **View** menu. When the data is aligned by size, the menu command changes to **Align by Data Point**. Select the command again, to show the data aligned by data point.
set the default peak alignment	select **Preferences** from the **Settings** menu, and select **Results Display** from the submenu. You can use the **Results Display Preferences** dialog box to set certain preferences that remain in effect each time you display or print results data. For more information, see “Changing How the Results Are Displayed and Printed” on page 8-13.

Changing the Horizontal Scale

Changing the Horizontal Scale for All Electropherograms

To change the scale of the horizontal axis for all electropherograms:

1	Display the electropherogram panels you want to change.
2	Select **Horizontal Scale** from the **View** menu. The **Horizontal Scale Parameters** dialog box opens. You can also move the cursor over the horizontal axis of a displayed electropherogram and double-click. 
3	Enter the increments represented by the tick marks for the horizontal axis in the **Tick Spacing** box.
4	Enter a range in the entry fields labeled **Display from** and **Display to**.
5	Click **OK**.

Changing the Horizontal Scale for Individual Electropherograms

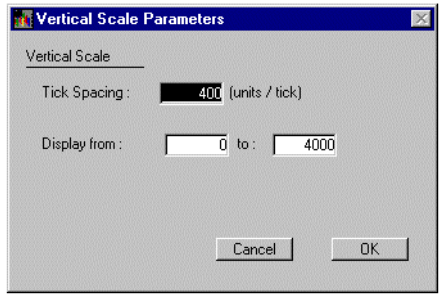
To change the horizontal scale for individual electropherograms:

Step	Action
1	Display the electropherograms.
2	Move the cursor over the horizontal axis of the panel that you want to change and double-click. The **Horizontal Scale Parameters** dialog box opens.
3	Enter the increments represented by the tick marks for the horizontal axis in the **Tick Spacing** box.
4	Enter a range in the entry fields labeled **Display from** and **Display to**.
5	Click **OK**. The horizontal scale changes.

Changing the Vertical Scale

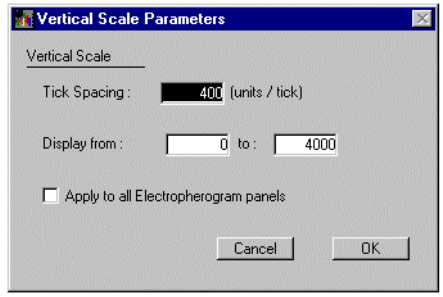
Changing the Vertical Scale for All Electropherograms

To change the vertical scale for all electropherograms:

Step	Action
1	Display the electropherogram panel that you want to change.
2	Select **Vertical Scale** from the **View** menu. The **Vertical Scale** dialog box opens. 
3	Enter the increments represented by the tick marks for the vertical axis in the **Tick Spacing** box.
4	Enter a range in the entry fields labeled **Display from** and **Display to**.
5	Click **OK**.

Changing the Vertical Scale for Individual Electropherograms

To change the vertical scale for individual electropherograms:

Step	Action
1	Display the electropherograms.
2	Move the cursor over the vertical axis of the panel that you want to change, and double-click. The following dialog box opens: 
3	Enter tick mark increments and a range.
4	Ensure that the **Apply to all Electropherogram panels** check box is not selected. Select the check box only to apply the changes to all displayed electropherogram panels.
5	Click **OK**. The vertical scale changes only for the electropherogram panel that you selected.

Assigning Standard or Custom Colors

The following table describes how to assign standard or custom colors and where to look for more information:

To...	Then...
assign standard or custom colors	select the **Plot Colors** command from the **View** menu and either **Standard** or **Custom** from the submenu. For information on defining custom colors, see “Defining Custom Colors in Electropherograms” on page 9-24.

Defining Custom Colors in Electropherograms

Introduction

The GeneScan Analysis Software assigns a plot color to each dye/sample added to an electropherogram. Normally, it is the color associated with the individual dye/sample by the Dye Indicators Preferences.

Note To change the default dye colors in the Analysis Control window and the Results displays, see “Setting Dye Indicator Preferences” on page 3-14.

Note Custom plot colors are not available in the Sample Results view.

Why Change Colors in the Electropherogram

Change the colors in the electropherogram to:

- Differentiate between different samples labeled with the same color dye
- Improve contrast between different dye colors
- Show data in a special color for a presentation
- Optimize plot colors for a particular printer

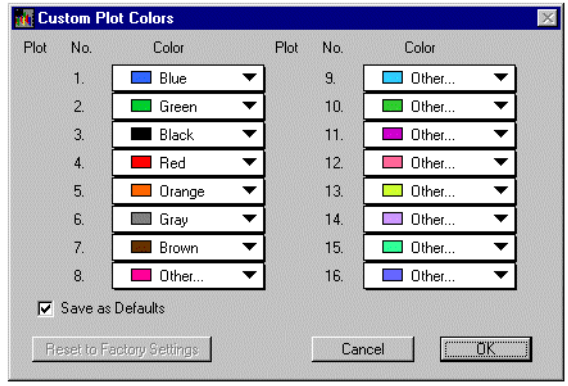
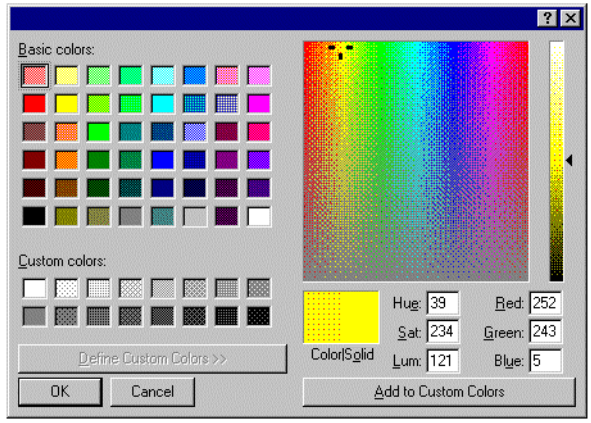
Saving the Display Format

The following table describes the options to save the display format after customizing the display colors:

If you...	And then...
specify saving the display format of a **Results Display** window after customizing the display	open it at a later time, the custom colors still appear. For more information, see “Saving and Renaming the Results Control Format” on page 8-17.
do not save the display format after manually customizing the colors	display the same results again. The electropherograms are redrawn using default colors.

Defining Custom Colors

To define custom colors for all electropherograms:

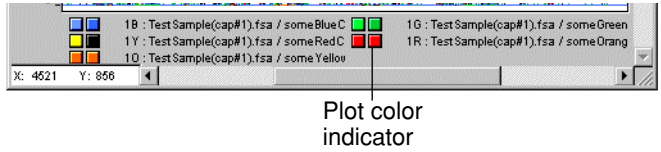
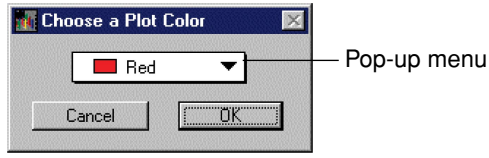
Step	Action
1	Select **Project Options** from the **Settings** menus and **Choose Custom Plot Colors** from the submenu. The **Custom Plot Colors** dialog box opens. 
2	Select new colors from the pop-up menus beside the 16 plot numbers. **Note** The plot numbers indicate the order of the samples in the electropherogram legend.
3	Select **Other** from the pop-up menu to specify a color that does not appear in the pop-up menu. The following is an example of a color picker that opens: 

To define custom colors for all electropherograms: *(continued)*

Step	Action
4	Position the cross hairs pointer on the color you want, and click. The color appears in the **Custom Color** box.
5	To save the custom color, click **Add to Custom Colors**.
6	Click **OK**. The **Color Picker** window closes.
7	In the **Custom Plot Colors** dialog box, select the check box labeled **Save As Defaults** to save the customized colors.
8	Click **OK**.

Defining Individual Plot Colors

To define the individual plot colors:

Step	Action
1	Display the electropherograms with legends.
2	Double-click the plot color indicator next to the sample you want to change.  Plot color indicator The **Choose a Plot Color** dialog box opens:  Pop-up menu
3	Select a **new color** from the pop-up menu. If you select **Other**, then a color picker opens.

To define the individual plot colors: *(continued)*

Step	Action
4	Click **OK**. The color of the electropherogram for the individual sample changes, and a vertical line appears beside the plot color indicator to signify that it has been modified. **Note** You can change the plot color in the same way from the **Results Control** window. When you do so, the dye/sample is plotted with the set color each time you open the applicable **Results Display** window. **Note** Press Ctrl and double-click the plot color indicator to reset it to the original color.

Changing the Dye Scale in Electropherograms

What the Dye Scale Defines

The dye scale defines how dyes in an electropherogram appear relative to each other. You can compensate for peaks with different intensities by redefining the dye scale.

Note Changing the dye scale affects only the display, not the underlying data.

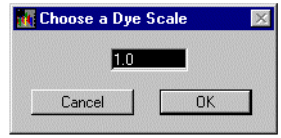
Increasing the Dye Scale Example

The following table describes one way to increase the dye scale:

If...	Then...	Action...
you loaded a smaller amount of green sample in relation to the red sample	the peaks for the green sample might appear half as tall as those of the red sample.	To make it easier to view both samples on the same scale, increase the dye scale value of the green sample to make the peaks appear similar.

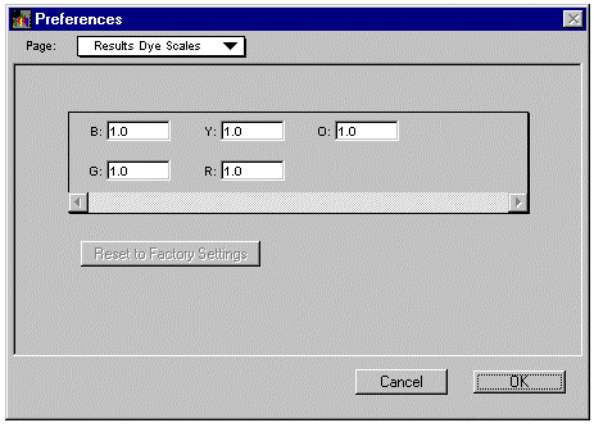
Changing the Dye Scale of an Electropherogram

To change the dye scale of an individual electropherogram:

Step	Action
1	Display the electropherograms with legends.
2	Double-click the dye color indicator next to the sample you want to change. Plot color indicator The **Choose a Dye Scale** dialog box opens. 
3	Enter a new scale in the dialog box and click **OK**. The dye scale for the individual sample changes, and a vertical line appears beside the dye color indicator to signify that it is modified. **Note** You can change the dye color in the same way from the **Results Control** window. The dye is scaled each time you open the **Results Display** window.

Changing the Dye Scale Preferences

To change the dye scale preferences:

Step	Action
1	Select **Preferences** from the **Settings** menu and select **Results Dye Scales** from the submenu. The **Preferences** dialog box opens with the **Results Dye Scales** pop-up menu displayed. 
2	Enter a positive number between 0.1 and 100 for each sample relative to any other sample, and click **OK**. **Note** Dye scale values do not automatically revert to default values. Change them back to the defaults before examining results of another run.

Process of Verifying Results

Introduction

You can use the electropherogram and tabular displays to verify the results of analysis by checking the GeneScan Analysis Software calculated sizes and peaks.

Note The sizecalling of the standard and of sample fragments varies according to the sizecalling method you defined in the Analysis Parameters and the accuracy of the defined standard.

Steps to Verify Size Calculation

To verify size calculations:

Step	Action	See
1	Compare how well multiple size standard electropherograms line up within a **Results Display** window when aligned by size.	"Verifying Size Calculations" on page 9-33.
2	View the sizing curve calculated by the GeneScan Analysis Software.	"Example of Size Curve View" on page 4-21.
3	Determine how well the defined size standard matches the size standard run with your sample. Use the **Peak Total** information in the **Sample Info** view of the **Sample File** window.	"Sample Info View" on page 4-11.
4	View the **Analysis Log**, which provides messages for each analyzed sample file. If there is a problem or a questionable condition during sizecalling, a warning message is displayed in the **Analysis Log**.	"Using the Analysis Log" on page 9-36.
5	Use the **Raw Data** view to display information about the raw data for a sample. Analyzed sample files contain raw and analyzed data.	"Example of Raw Data View" on page 4-23.

To verify size calculations: *(continued)*

Step	Action	See
6	Use EPT Data to troubleshoot problems caused by poor run conditions, such as: ♦ EP voltage ♦ EP current ♦ Laser power ♦ Run temperature versus time EPT data can be displayed for each sample file.	"EPT Data View Example" on page 4-25.

Verifying Size Calculations

Introduction

This section describes the following topics:

- Verifying for the GeneScan-350 Standard
- Evaluating for Multiple Size Standards

Verifying for the GeneScan-350 Standard

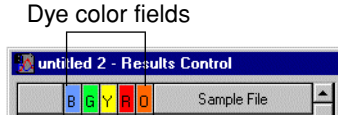
About the Standard

In the GeneScan-350 standard, the size of the first peak should be approximately 50 bp, the second 75 bp, and so on, assuming that processing started after the 35-bp fragment passed the scan region. If peaks appear to be correctly measured for your run, measurement of the sample fragments that ran with the standard should also be correct.

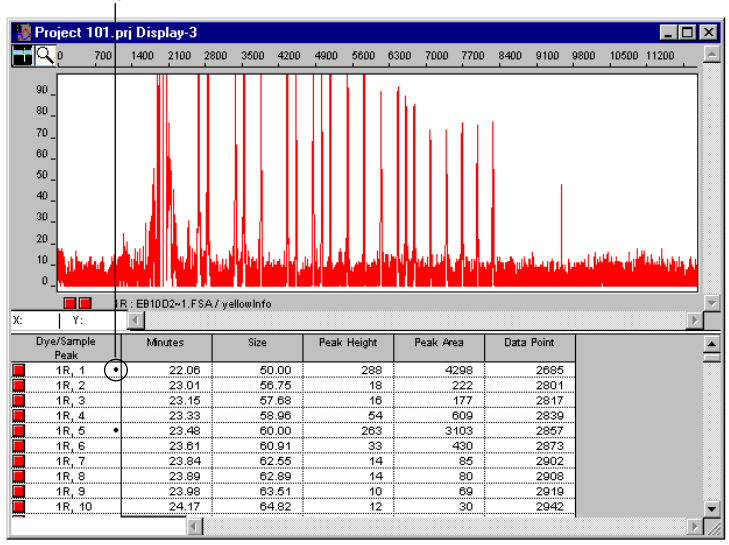
Note For a complete list of fragment sizes, refer to Appendix C, “GeneScan Size Standards.”

Procedure

To verify the size calculation for the GeneScan-350 standard:

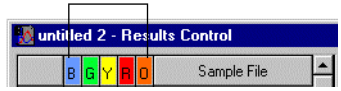
Step	Action
1	In the **Results Control** window, select the **Electropherogram** button and the **Tabular** button.
2	Click the **Clear All** button to clear the panels.
3	Click the dye color for the size standard you want to view. Dye color fields 
4	Click **Display**. **Note** You can also open the **Sample File** window for the sample file of interest to verify sizecalling.

To verify the size calculation for the GeneScan-350 standard: *(continued)*

Step	Action
5	Click each peak in the electropherogram and check the tabular data to ensure it is the correct size (see figure below). **Note** The peaks matched to the defined size standard are identified by dots next to the **Dye/Sample Peak** field. Indicates peak matched to defined size standard  Dye/Sample Peak | Minutes | Size | Peak Height | Peak Area | Data Point 1R, 1 | 22.06 | 50.00 | 288 | 4298 | 2685 1R, 2 | 23.01 | 56.75 | 18 | 222 | 2801 1R, 3 | 23.15 | 57.68 | 16 | 177 | 2817 1R, 4 | 23.33 | 58.96 | 54 | 609 | 2839 1R, 5 | 23.48 | 60.00 | 263 | 3103 | 2857 1R, 6 | 23.61 | 60.91 | 33 | 430 | 2873 1R, 7 | 23.84 | 62.55 | 14 | 85 | 2902 1R, 8 | 23.89 | 62.89 | 14 | 80 | 2908 1R, 9 | 23.98 | 63.51 | 10 | 69 | 2919 1R, 10 | 24.17 | 64.82 | 12 | 30 | 2942
6	If you find that the sizes were not calculated correctly, you can: ♦ Redefine the size standard, or ♦ Change the analysis parameters and re-analyze the affected samples (see “Sizecaller Algorithm Flowchart” on page 5-4)

Evaluating for Multiple Size Standards

To evaluate size calculations for multiple size standards:

Step	Action
1	In the **Results Control** window, select the **Electropherogram** button.
2	Click the **Clear All** button to clear the panels.
3	Click the **On** button to turn on the **Quick Tile** option.
4	Click the dye colors for the size standards you want to view. Dye color fields  When the **Quick Tile** option is on, the GeneScan Analysis Software inserts each in a separate panel. **Note** Click the header of the appropriate dye/sample column to display all standards in the project that are the same color dye.
5	Click **Display**. The standards appear in tiled electropherogram displays. For information on setting the tiled electropherogram displays, see “Creating Tiled Electropherogram Displays” on page 8-10.
6	Select **Align by Size** (Ctrl+T) from the **View** menu if the electropherograms are not already aligned by size. The size standards should line up when aligned by size. **Note** You can set preferences so that all new displays show data aligned by size by selecting **Preferences** from the **Settings** menu and **Results Display** from the submenu. Click the **Align By Size** check box. For more information, see “Saving and Renaming the Results Control Format” on page 8-17.

Using the Analysis Log

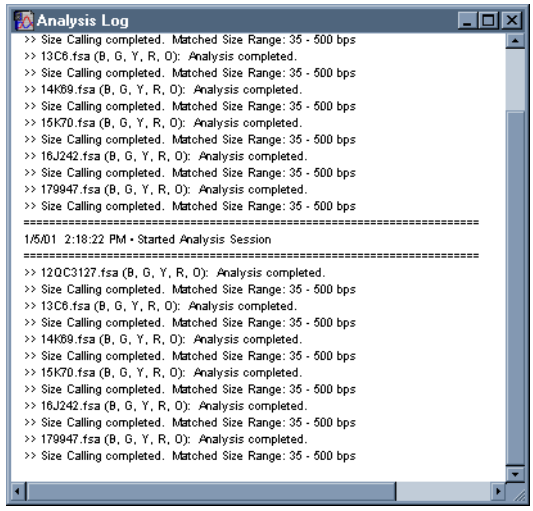
What Is the Analysis Log

The Analysis Log maintains a running record of analysis performed by the GeneScan Analysis Software. If a problem occurs during analysis of a sample file, the Analysis Log automatically opens in the foreground as an alert.

Displaying the Analysis Log

Select Analysis Log (Ctrl+0) from the Windows menu.

The following is an example of the Analysis Log:



What to Evaluate

Evaluate the following:

What to evaluate	What not to evaluate
Potential problems that the GeneScan Analysis Software might have had during sizecalling. **Analysis Log** will alert you if more than two defined size standard peaks were not matched.	The GeneScan Analysis Software will not alert you to any consecutive peaks at the end of the definition. This is to avoid logging warnings when your sample was not run long enough to include all the defined size standard peaks. This prevents you from having to create a new standard for shorter runs. The **Analysis Log** will, however, alert you if less than 50% of the defined size standard peaks were not matched, regardless of the peak locations in the definition.

Removing Information from the Analysis Log

To remove information from the Analysis Log:

Step	Action
1	Select the information you want to remove. **Note** Choose **Select All** (Ctrl+A) from the **Edit** menu to select all the information.
2	Select **Clear** from the **Edit** menu.

Closing the Analysis Log

You can either:

- Click the **Close** button in the upper-left corner, or
- Select **Close** (Ctrl+W) from the **File** menu

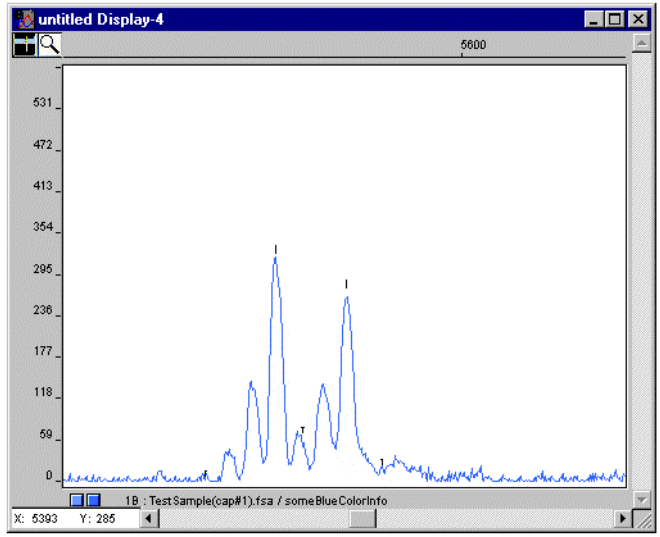
Verifying Peak Detection

Introduction

Use the Show Peak Positions command from the View menu, while the electropherogram and associated tabular data are displayed, to verify results by examining how the GeneScan Analysis Software defined the total area that comprises each peak and the center of the peak.

Verifying Peak Detection

To verify peak detection:

Step	Action
1	Select **Show Peak Positions** from the **View** menu. Markers appear that identify the beginning, center, and end of each peak. **Note** For a better view, select **Zoom In** from the **View** menu, or use the Zoom tool (see “Zooming In and Out” on page 9-17). 
2	Examine the display to ensure that each peak’s center, beginning, and end points are correct.

To verify peak detection: *(continued)*

Step	Action
3	Select **Hide Peak Positions** from the **View** menu to suppress the display of the peak markers. **Note** You can also use the **Sample Info** view to display information about the peaks detected and matched. For more information, see “Description of Information” on page 4-14.

Saving, Archiving, and Copying Files 10

Overview

In This Section Topics in this chapter include the following:

Topics	See Page
Why Save GeneScan Files	10-2
Saving GeneScan Files	10-3
Archiving Sample Files	10-4
Transferring Data to Other Applications	10-5

Why Save GeneScan Files

Reasons for Saving Files

The following table explains why you save projects, sample files, and Results Displays. For information on archiving files, see “Archiving Sample Files” on page 10-4.

Save	Because	See
GeneScan® Analysis Software projects	It protects the links to sample files and their preferences. Projects contain links to sample files and preferences regarding display and analysis.	“Saving Projects” on page 10-3.
Sample files	It protects the links to projects and their preferences. Sample files also contain raw data and critical information about the run, settings, and analysis control.	“Saving Sample Files” on page 10-3.
Results Displays	It saves the Results Display settings in projects when you have a display format that suits your needs.	“Saving Results Displays” on page 10-3.

Saving GeneScan Files

Introduction

This section describes how to save projects, sample files, and results displays.

Saving Projects

Note You do not need to save a sample file after analysis. The analyzed data is written directly to the sample file during analysis.

The following table describes the options to save a project:

<table>
<tr><th>If you choose...</th><th>Then...</th></tr>
<tr><td>Save Project
(Ctrl+S)</td><td>you can take the following action:<table><tr><th>If you...</th><th>Then...</th></tr><tr><td>previously saved the project</td><td>it is automatically saved using the same name.</td></tr><tr><td>had not saved the project</td><td>the Save this document as dialog box opens.

Select a location for the file, enter a name, and click Save.</td></tr></table></td></tr>
<tr><td>Save Project As</td><td>the Save this document as dialog box opens.

Select a location for the file, enter a name, and click Save.</td></tr>
</table>

Saving Sample Files

To save sample files, select **Save** (Ctrl+S) from the **File** menu. If you select **Close** from the **File** menu or click the **Close** button when you have not saved the changes, a dialog box opens with a message asking if you want to save them.

Saving Results Displays

You can combine electropherograms and tabular data in many ways for display, and the GeneScan Analysis Software allows you to save display combinations and formats for future viewing.

For more information on saving a display for future viewing, refer to “Saving and Renaming the Results Control Format” on page 8-17.

Archiving Sample Files

When to Archive Sample Files

Archive sample files when you feel confident that the channel selections (tracking) used to generate them were correct.

Procedure

A sample file is 60 KB to 150 KB in size, depending on the length of the run.

Step	Action
1	To archive sample files, drag the file icon or the run folder containing the files to the floppy disk icon or to an alternative storage device.
2	A 1.4 MB high-density disk holds about 12 files.

Transferring Data to Other Applications

Genotyper Software

GeneScan Analysis Software files can be read by Genotyper® software for Windows NT® platform.

Cutting and Pasting Tabular Data

To cut and paste tabular data:

Step	Action
1	Display the tabular data you want to copy.
2	Select the rows you want to copy by taking the following action: **If you want to select...** / **Then...** all tabular data — Choose **Select All** (Ctrl+A) from the **File** menu. several consecutive rows — Shift-click the first and last row in the group you want to select. several rows that are not listed next to each other — Ctrl+click the rows.
3	Select **Copy** (Ctrl+C) from the **Edit** menu.
4	Optional: To view the contents of the clipboard before pasting, from the **Edit** menu, select **Show Clipboard**.
5	Open the new application and click where you want to place the information.
6	Select **Paste** (Ctrl+V) from the **Edit** menu.

Creating a Text File

To create a text file from tabular data:

Step	Action
1	Display the tabular data.
2	Select **Export Table** from the **File** menu. The **Save As** dialog box opens.
3	Choose a name and file location in the dialog box and click **Save**.

11 Printing Results

Overview

In This Chapter

Topics in this chapter include the following:

Topics	See Page
About Printing	11-1
Printing Run Results Automatically	11-2
Printing Selected Sample Files	11-3

About Printing

Ways You Can Print

You can print the results of the run automatically at the end of the run or interactively, as selected sample files or display combinations.

If You Get Unexpected Results

You might initially get unexpected results from autoprinting or the Print One command if you switch printers.

Step	Action
1	The first time you print after changing the printer configuration, select **Print Setup** (Ctrl+J) from the **File** menu and click **OK**.
2	Select **Print** (Ctrl+P) from the **File** menu and use the **Print** dialog box.

Printing Run Results Automatically

Introduction

You can specify that the results are printed in the 310 and 377 Data Collection software or in the GeneScan® Analysis Software.

From the Data Collection Software

When you choose automatic printing from the 310 and 377 Data Collection software, the GeneScan Analysis Software prints a separate page for each designated Sample file, showing electropherograms and tabular data as specified in the Auto-Analysis Defaults (see step 2 on page 2-6).

Choose automatic printing in the 310 and 377 Data Collection software as follows:

On this instrument...	Choose...
ABI PRISM® 310	Auto-Print in the Injection list.
ABI PRISM® 377	Auto-Print in the Run Sheet.

For information about setting up your run, refer to the instrument user manual.

Procedure

To print the results automatically as the samples are analyzed:

Step	Action
1	In the **Analysis Control** window, select the check box labeled **Print Results**.
2	Select the samples you want to analyze. For more information, see “Analyzing a Sample File” on page 4-26.
3	Click the **Print Setup** button to specify the samples and the format. For more information, see “Specifying the Format for Printed Results” on page 3-8.
4	Click **OK**. The results are printed after the results are analyzed.

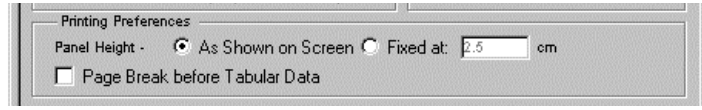
Printing Selected Sample Files

Introduction

Print selected sample files by using the Results Control window or by choosing the sample file.

Setting Printing Options

To set the printing options:

Step	Action
1	Select **Preferences** from the **Settings** menu and **Results Display** from the submenu. The **Results Display** Preferences dialog box opens.
2	Use the **Printing Preferences** section, change the electropherogram height and page breaks.  Depending on how you set these options, the format that prints may be different from what is on the screen. For information on printing saved Results Control formats, see “Working with a Previously Saved Display” on page 8-18.

Printing from the Results Control Window

Print selected samples after analysis, regardless of whether you choose automatic printing.

To print results for selected sample files after analysis:

Step	Action
1	In the **Results Control** window, select the dye/samples and format you want to print. Use the same technique as you did to select the format and the dye/samples to display the data. For more information, see “Using the Results Control Window” on page 8-7.

To print results for selected sample files after analysis: *(continued)*

Step	Action
2	You can take the following action:

You can either...	Then...
Click the **Print** button.	The **Print** dialog box opens.
Select **Print** (Ctrl+P) from the **File** menu.	Make any changes to the settings and click **OK**.
Select **Print One** from the **File** menu.	The sample files are printed. **Note** The **Print** dialog box does not appear.

Printing from the File Menu

To print a sample file from the File menu:

Step	Action
1	Select **Open** from the **File** menu. The **Open Existing** dialog box opens. **Note** You can also double-click the sample file name in the folder containing the files. If the GeneScan® Analysis Software is not running, the software starts and opens the sample file.
2	Click the **Sample** icon. An **Open** dialog box opens.
3	In the dialog box, navigate to the folder and select the sample file that you want to open.
4	Click **Open**. The **Sample File** window opens. For more information about the **Sample File** window, see page 4-8.
5	Select one of the five views of the **Sample File** window, and select **Print** or **Print One** from the **File** menu. **Note** If you select **Print One**, then the **Print** dialog box does not appear.

Creating GeneScan Analysis Modules

A

Overview

In This Chapter

Analysis modules provide the auto-analysis feature with the parameters to use for the GeneScan® Analysis Software. For more information about analysis parameters, refer to Chapter 5, “Working with Analysis Parameters.”

This appendix includes the following topics:

Topic	See Page
About GeneScan Analysis Modules	A-2
Creating GeneScan Analysis Modules	A-5

About GeneScan Analysis Modules

Introduction

The GeneScan Analysis Software modules contain the following analysis options:

- Analysis range to use
- Statistical method used to fit the standard curve to the size standards data

Analysis Module Format

GeneScan analysis modules have the file name format *filename*.gsp and are stored in the Params folder.

The path is: D:\AppliedBio\Shared\Analysis\Sizecaller\Params

Files Referenced by the Module

GeneScan analysis modules reference two types of companion files that contain other analysis parameter information.

Default Settings

The following table describes the default settings for the analysis parameters:

File type	Description
Sizecaller standard file	Size standards are specific DNA fragments of known sizes. After defining the peaks of a size standard, the GeneScan® Analysis Software matches this definition to the internal size standard included with the run. The software assigns the defined size values to the appropriate peaks of the internal size standard, and uses this information with the selected sizecalling method to size all unknown fragments. Size standard setting is used only during auto-analysis by 3100 and 3700 Data Collection software. For more information, refer to “What Are Size Standards” on page 7-2.

The following table describes the default settings for the analysis parameters: *(continued)*

<table>
<tr><th>File type</th><th>Description</th></tr>
<tr><td>Sizecaller parameter file</td><td>There are two sizecaller parameter files. The format is filename.scp, and they are stored in the SizeStandards folder at the following directory location:

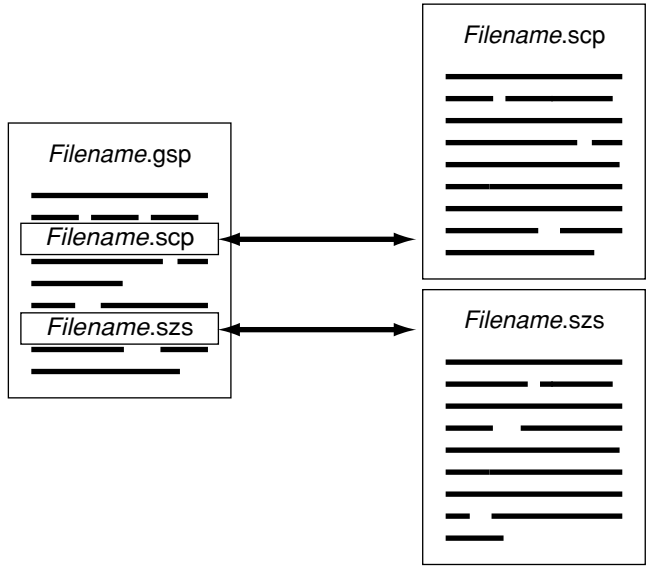
D:\AppliedBio\Shared\Analysis\Sizecaller\Params

<table>
<tr><th>File</th><th>Use this file...</th></tr>
<tr><td>ABISizecallerAutoAnalysis.scp</td><td>when the run data is being analyzed automatically (the first time it is analyzed).</td></tr>
<tr><td>ABISizecallerGSAnalysis.scp</td><td>if the data is re-analyzed.</td></tr>
</table>

Note Do not move or delete these files.</td></tr>
</table>

Relationship Between a Module and the Files

The relationship between an analysis module and the two companion files is illustrated below.



Written in ABI Format, a GeneScan Analysis Software module contains the name of the basecaller settings file to be referenced.

The sizecaller standard fie and sizecaller parameter file are referenced while the analysis module is being read automatically.

Never Modify the Files

Never modify the sizecaller standard file or the sizecaller parameter file. If you modify one of these files, it will no longer work.

Creating GeneScan Analysis Modules

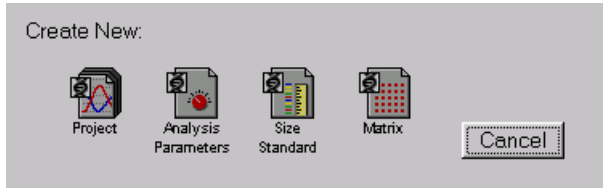
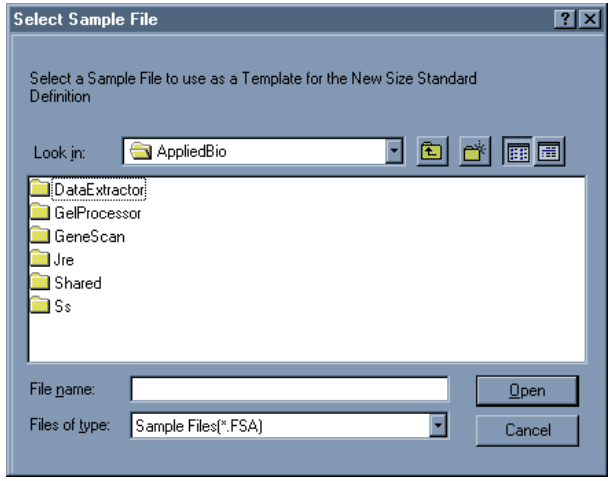
Summary of the Procedure

Follow these steps to create an analysis module:

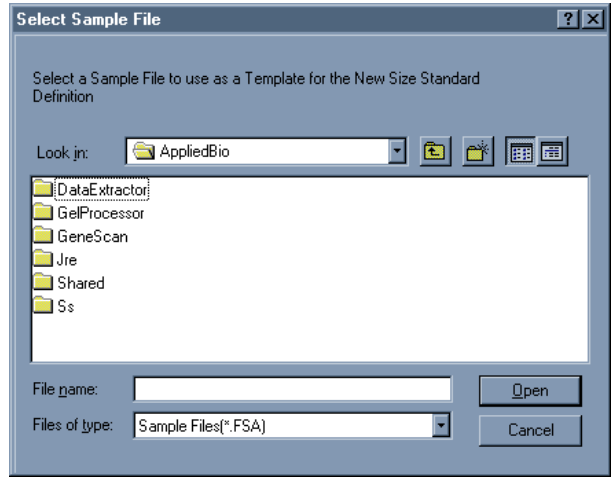
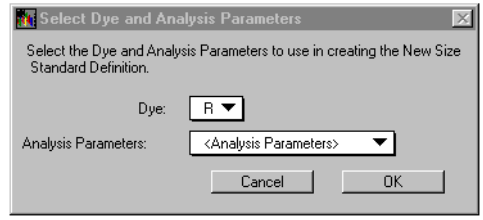
Step	Action
1	Review the size standards data and select the analysis parameters that remove unwanted noise and peaks outside of the size range of the standards. See step 1 in the “Creating a Size Standard File” procedure below.
2	Create a size standard file (*filename*.szs) for the reviewed standards. See step 2 on page A-6 to step 10 on page A-9.
3	Review the sample file of a sample to be sized, selecting the analysis parameters that optimize the appearance of the data. See step 1 on page A-9 to step 4 on page A-11.
4	Save the analysis parameters as a new .gsp file, referencing the size standard file just created. See step 5 on page A-11 to step 8 on page A-12.

Creating a Size Standard File

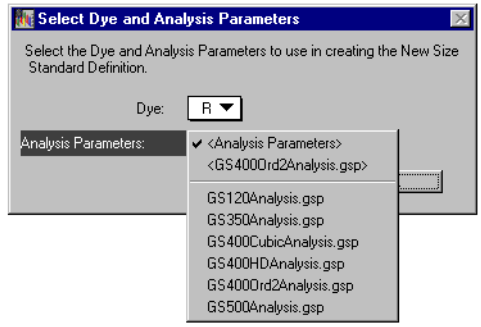
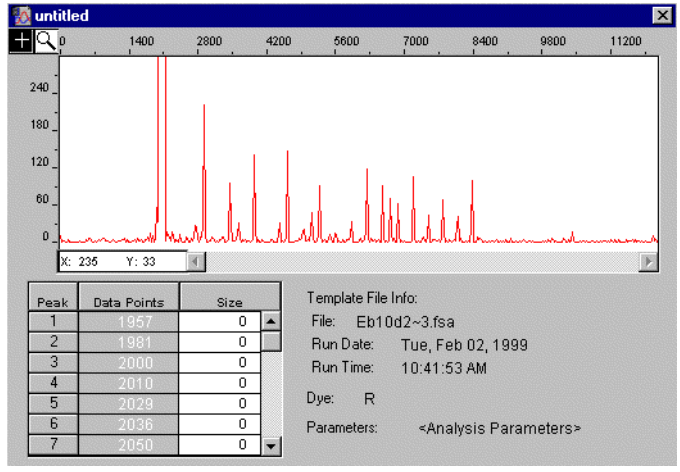
To create a size standard file:

Step	Action
Reviewing and Choosing a Size Standard as a Template	
1	Review the size standard data and optimize the analysis parameters.
2	Select **New** (Ctrl+N) from the **File** menu. The **Create New** dialog box opens. 
3	Click the **Size Standard** icon. The **Select Sample File** browser box opens. 
4	Navigate to the Completed folder at the following directory location: D:\AppliedBio\DataExtractor\Completed

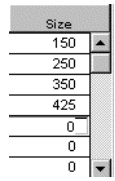
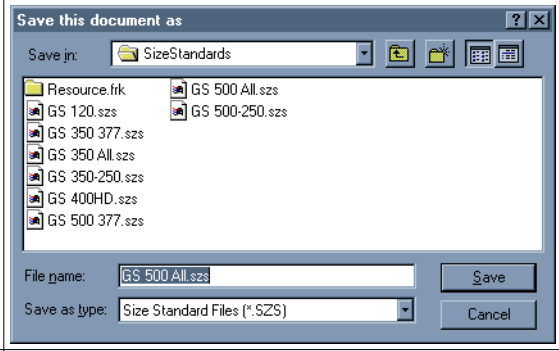
To create a size standard file: *(continued)*

Step	Action
5	Select the GeneScan Analysis Software sample file, with the extension .fsa, that you want to use as a template. 
6	Click **Open**. The **Select Dye and Analysis Parameters** dialog box opens. 
7	From the **Dye** pop-up menu, select the dye that was used to label the size standard DNA fragments.

To create a size standard file: *(continued)*

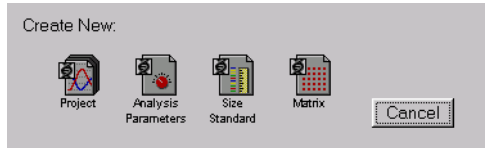
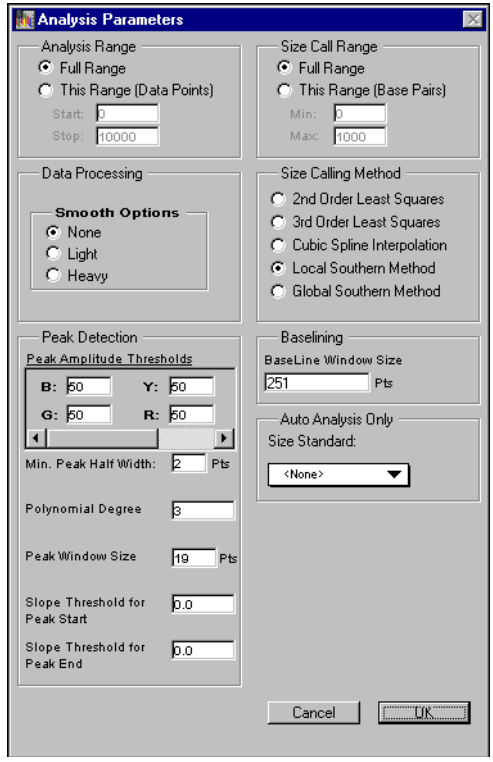
Step	Action
8	From the **Analysis Parameters** pop-up menu, select **Analysis Parameters**.  This references the current analysis parameter setting rather than a specific analysis parameter file.
9	Click **OK**. The following is an example of the dialog box that opens: 

To create a size standard file: *(continued)*

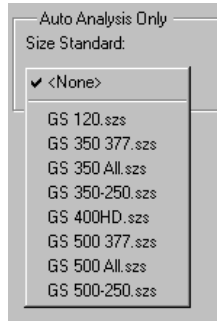
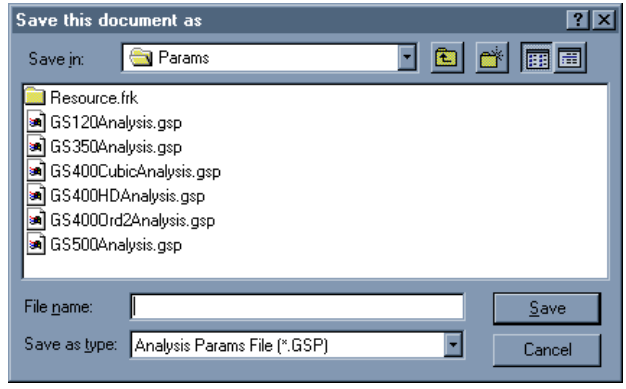
Step	Action
10	In the **Size** column, enter the known sizes of the standard's peaks. 
Saving the Size Standard	
1	Select **Save** from the **File** menu. The **Save this document as** dialog box opens.
2	Navigate to and open the **Size Standards** folder (see below). The folder contains size standards (.szs) files. The path is D:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards 
3	Enter a name in the **File name** text box for the size standards files, and click **Save**. The dialog box closes and the file is saved to the correct location for auto-analysis to read.
4	Click the **Close** button ( ) in the newly created *Filename*.szs dialog box. The dialog box closes.

Creating an Analysis Parameter File

To create an analysis parameter file:

Step	Action
1	In the GeneScan Analysis Software, select **New** from the **File** menu. The **Create New** dialog box opens. 
2	Click the **Analysis Parameters** icon. An untitled **Analysis Parameters** dialog box opens. **Note** To display the orange Peak Amplitude Threshold use the scroll bar under the values and scroll to the right. **Note** The Size Standard option is only specific to the 3100 and 3700 instruments. 

To create an analysis parameter file: *(continued)*

Step	Action
3	Complete the dialog box using the definitions in the “Sizecaller Algorithm Flowchart” on page 5-4.
4	In the **AutoAnalysis Only** group box, select from the pop-up menu the size standard that you just created. 
5	Select **Save** from the **File** menu. The **Save this document as** dialog box opens.
6	Navigate to and open the Params folder. The path is: D:\AppliedBio\Shared\Analysis\Sizecaller\Params 

To create an analysis parameter file: *(continued)*

Step	Action
7	Enter a file name for the analysis parameter file in the **File name** text box, and click **Save**. This saves the file to the correct location for auto-analysis to read.
8	Click the **Close** button in the newly created *Filename*.gsp dialog box.

Sizecalling Methods B

Overview

In This Appendix Topics in this appendix includes the following:

Topic	See Page
Least Squares Method	B-2
Cubic Spline Interpolation Method	B-4
Local Southern Method	B-5
Global Southern Method	B-7

Least Squares Method

About This Method

Both Least Squares Methods (2nd Order and 3rd Order) use regression analysis to build a best-fit sizecalling curve. This curve compensates for any fragments that may run anomalously. As a result, this method normally results in the least amount of error for all the fragments, including the size standards and the samples.

Depending on whether you choose the 2nd or 3rd Order Least Squares Method in the Analysis Parameters dialog box, the resulting size curve is either a quadratic function or a cubic function. The software uses the known standard fragments and the associated scan number positions to produce a sizing curve based on multiple linear regression.

Least Squares Sizecalling Examples

The first figure below shows the 2nd Order Least Squares sizecalling curve, and the second figure shows the 3rd Order Least Squares sizecalling curve.

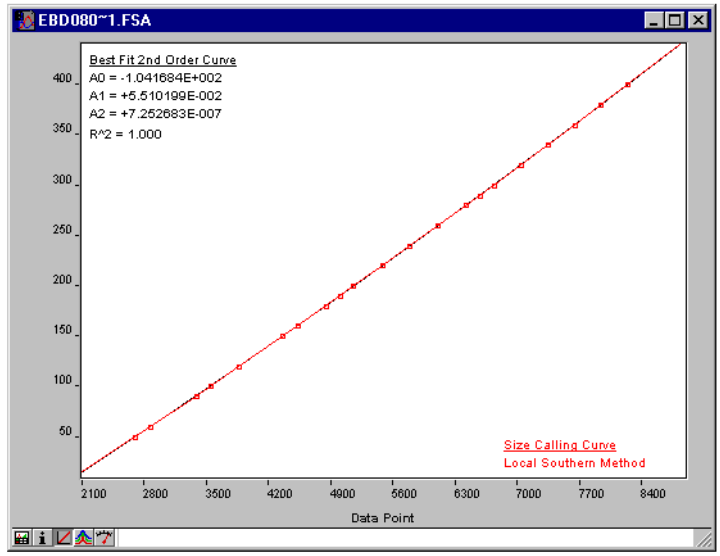


Figure B-1 2nd Order Least Squares sizecalling curve

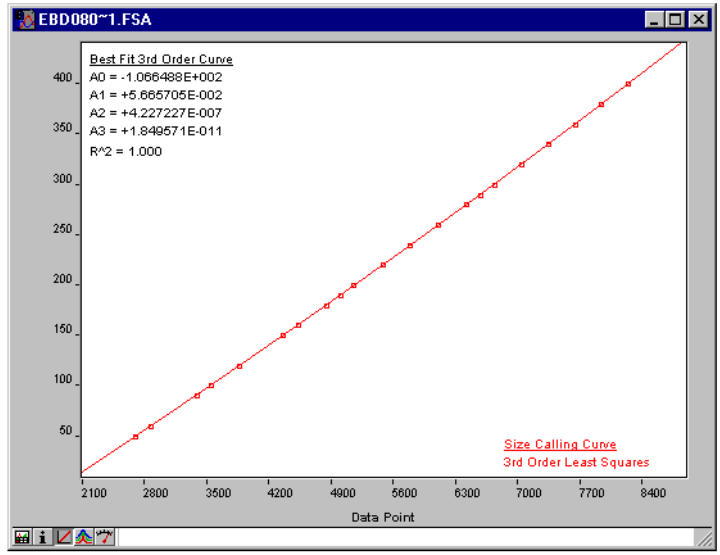


Figure B-2 3rd Order Least Squares sizecalling curve

Advantages

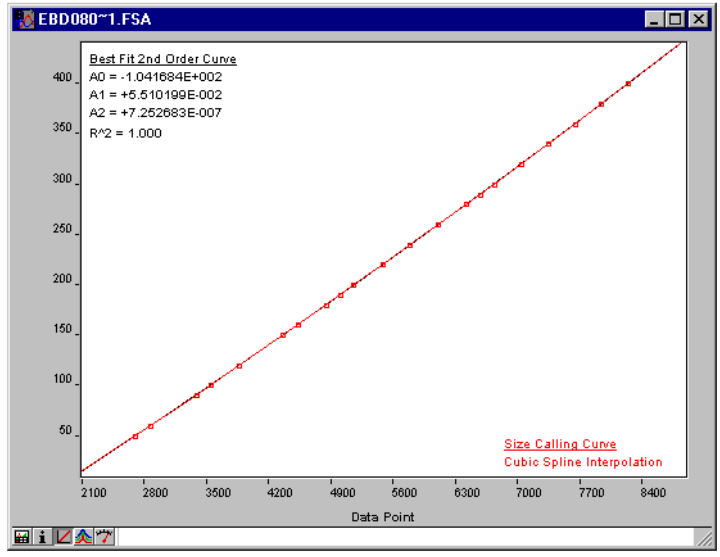
In nearly all instances in the “Least Squares Sizecalling Examples” above, the mobility of an individual DNA fragment is coincident with the best curve fit of the entire data set. Stated differently, the mobility of most DNA fragments is strictly length dependent. This method automatically compensates for fragments that run anomalously.

GeneScan® Analysis Software calculates a best-fit least squares curve for all samples, regardless of the sizecalling method you choose. The curve is black in the Standard Sizing Curve window.

Cubic Spline Interpolation Method

About This Method

By definition, the Cubic Spline Method forces the sizing curve through all the known points of the selected GeneScan size standard. Although this produces exact results for the values of the standards themselves, it does not compensate for standard fragments that may run anomalously.



Possible Local Sizing Inaccuracy

Mobility of any DNA fragment can be affected by its sequence and by secondary and tertiary structure formation. If any internal size standard fragment has anomalous mobility, the Cubic Spline Method may exhibit local sizing inaccuracy.

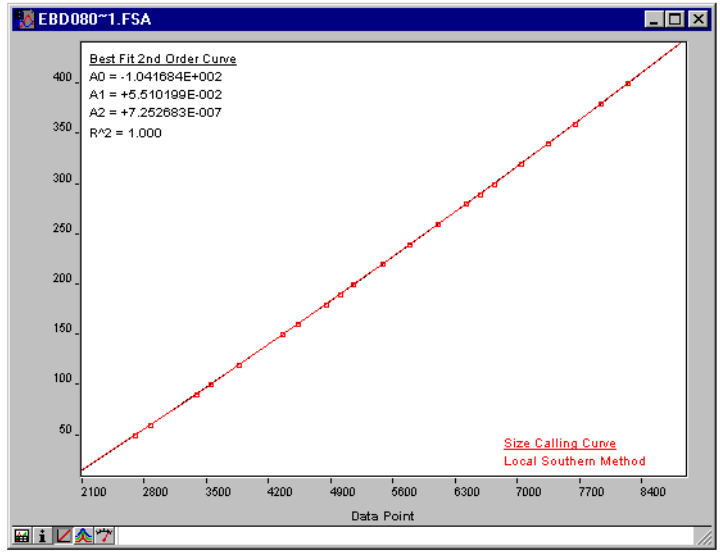
For example: Assume that a standard fragment is close in molecular length to an unknown sample fragment. Assume further that the standard fragment runs anomalously. The Cubic Spline Method assigns the official value to this standard fragment, even though it may be slightly incorrect. The size of the unknown fragment is then likely to be calculated incorrectly as well.

Note This method does not determine the amount of sizing accuracy error.

Local Southern Method

About This Method

The Local Southern Method determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility, as described by E. M. Southern (1979).



The Equation

The following equation attempts to describe the reciprocal relationship between the mobility, m, and the length, L0, of the standard fragments:

$$L = [c/(m - m0)] + L0$$

How This Method Works

This method, which is similar to the Cubic Spline Method, uses the four fragments closest in size to the unknown fragment to determine a best-fit line value. Using this method, only the region of the size ladder near the fragment of unknown length is analyzed.

Note Size estimates may be off if any of the standard fragments run anomalously.

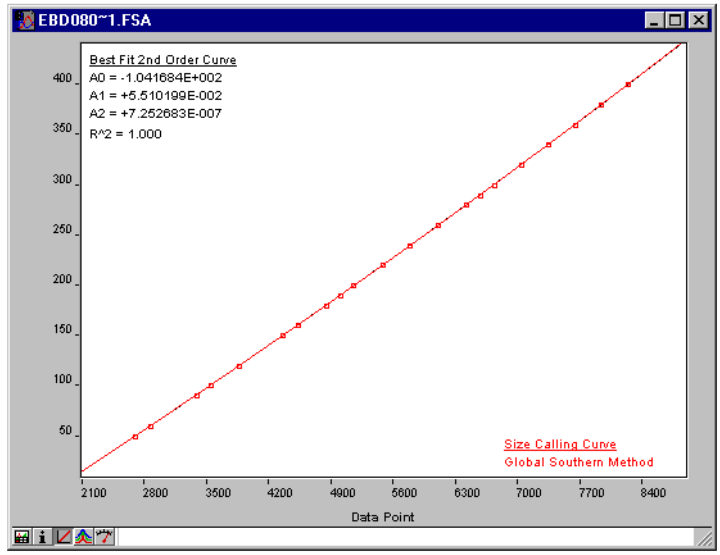
The following table summarizes how the Local Southern Method works:

Step	Action
1	The fitting constants of the curve are calculated for each group of three neighboring points on the standard. A separate curve is created for each set of three points.
2	A curve is then created by using three standard points (two points below and one point above the fragment), and a fragment size is determined.
3	Another curve is created by looking at an additional set of three points (one point below and two points above the fragment) and another value is assigned.
4	The two size values are averaged to determine the unknown fragment length.

Global Southern Method

About This Method

This method is similar to the Least Squares Method in that it compensates for standard fragments that may run anomalously. The method creates a best-fit line through all the available points, and then uses values found on that line to calculate the fragment values.



The Equations

The following table describes how the equations work:

Equation	Description
$L = [c/(m - m0)] + L0$	Attempts to describe the reciprocal relationship between the mobility, m, and the length, L0, of the standard fragments.
$\sum i(Li - (c/(mi - m0) + L0))2$	The fitting constants L0, m0, and c are calculated by a least squares fit to minimize the following quantity.

How This Method Works

All points in the standard are weighted equally, and the curve is not constrained to go through any specific point. The software can analyze a large range of fragment sizes with this method.

DNA fragments that are...	Are sized using...
not bracketed within the size standard curve	a second-order least squares curve extrapolation.
bracketed within the size standard curve	the method that was chosen.

For best results, use a standard that brackets all the fragments of interest.

GeneScan Size Standards

C

Overview

About the Size Standards

The GeneScan Analysis Software comes with several ready-to-use size standard definition files that you can choose from to analyze fragments run on the ABI PRISM® 3700 DNA Analyzer. The size standards are stored in the SizeStandards folder.

The path is:
D:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards

See also “About Size Standards” on page 7-2.

Size Standards Included

The following table lists the ready-to-use size standards:

Size Standard	See Page
GS 120.szs	C-2
GS 350 All.szs	C-4
GS 400HD.szs	C-9
GS 500 All.szs	C-11

The table below lists size standards in which some of the fragments have been set to 0.

Note You can easily remove any of these sizes by opening the definition and setting any unwanted sizes to 0.

Size Standard	See Page
GS 350 377.szs	C-7
GS 350-250.szs	C-8
GS 500 377.szs	C-13
GS 500-250.szs	C-14

GeneScan 120 Size Standard

About This Size Standard

You can use the GeneScan-120 LIZ™ size standard to determine fragment lengths between 15 and 120 base-pairs.

Special Uses

This size standard was designed to provide accurate sizing of short DNA fragments. Therefore, it is particularly useful for SNP analysis. All fragments have been checked for migration that is true to size under a wide variety of run conditions. There are no anomalous fragments.

How It Is Prepared

All aspects of the preparation of the GeneScan 120 LIZ size standard are proprietary. Each fragment contains a single LIZ fluorophore.

Fragment Lengths

The following table lists the lengths of the nine fragments that comprise the GeneScan 120 LIZ size standard:

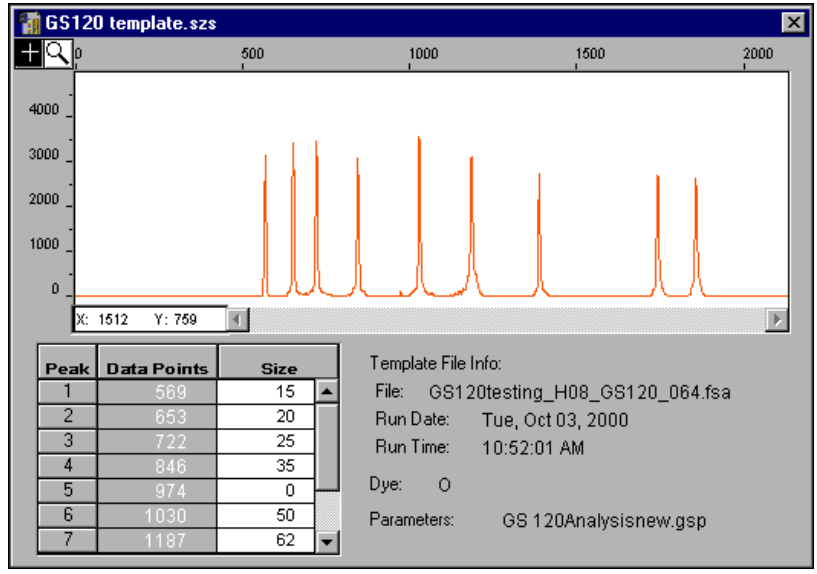
15
20
25
35
50
62
80
110
120

Denaturing Electropherogram

The GeneScan 120 LIZ size standard is made of single-stranded DNA fragments. The following figure shows the peak patterns of GeneScan 120 fragments run under denaturing conditions. Fragments were run using the 3700 POP-5 polymer at 60 °C.

Electropherogram of GeneScan 120 LIZ

The following is an electropherogram of GeneScan 120 LIZ:



GeneScan 350 All Size Standard

About This Size Standard

The GeneScan 350 All size standard contains sizes for all fragments in the GS 350 size standard. This size standard is useful for sizing fragments between 35 and 350 base-pairs. The native fragments are uniformly spaced to provide accurate sizecalling.

How It Is Prepared

The GeneScan 350 All size standard is prepared by Pst 1 digestion of plasmid DNA, followed by ligation of a TAMRA or ROX-labeled 22-mer oligodeoxynucleotide to the cut ends. A subsequent enzymatic digestion with BstU 1 yields DNA fragments containing a single TAMRA or ROX dye (see “GeneScan 350 Molecular Lengths” below).

GeneScan 350 Molecular Lengths

The following table lists the GeneScan 350 Denatured Fragment Molecular Lengths (Nucleotides):

35	160
50	200
75	250
100	300
139	340
150	350

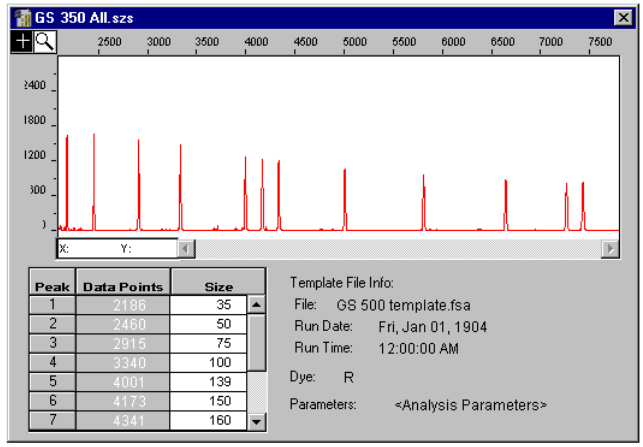
Running Under Denaturing Conditions

The following table describes running the GeneScan 350 All standard under denaturing conditions:

Like the GeneScan 2500 and GeneScan 1000 standard...	However, like the GeneScan 250 standard...	Consequently
the GeneScan 350 standard is made of double-stranded DNA fragments.	the GeneScan 350 standard has only one labeled strand.	under denaturing conditions, even if the two strands migrate at different rates, only the labeled strand is detected. Refer to “Electropherogram of GeneScan 350” below. Because of this, split peaks are avoided that result when two strands move through a denaturing polymer at different rates.

Electropherogram of GeneScan 350

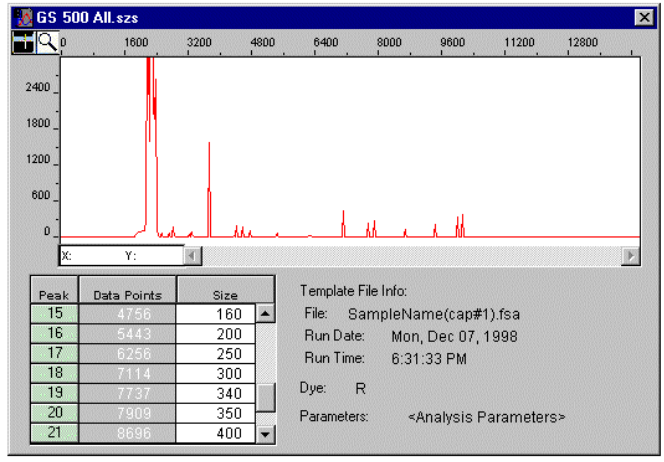
The following is an electropherogram of GeneScan 350 run under denaturing conditions:



Double-Stranded GeneScan 500 Fragments

The following figure shows the sizes of double-stranded GeneScan 500 fragments. Use these values to size fragments run under native conditions.

IMPORTANT An asterisk (*) for the 250 and 340 base-pair peaks denotes peaks resulting from abnormal migration of double strands that did not completely separate under denaturing conditions when analyzed on the 3100 and 3700 instruments. Do not use these peaks to size samples. The peaks show smaller values than the actual size of the fragments.



GeneScan 350 377 Size Standard

About This Size Standard

The GeneScan 350 377 size standard contains all GS 350 fragment sizes except the 35 and 50 base-pair sizes. The two smallest fragments (35 and 50 base-pairs) are often lost in the primer peak on gel instruments. The construction of this size standard differs from the GS 500 377.szs. This is because its electropherogram begins only after the 50 base-pair peak (depending on the run conditions), so the two smallest sizes are not present and do not need to be set to 0 (as in the GS 500 377 size standard).

This size standard can be used on any instrument.

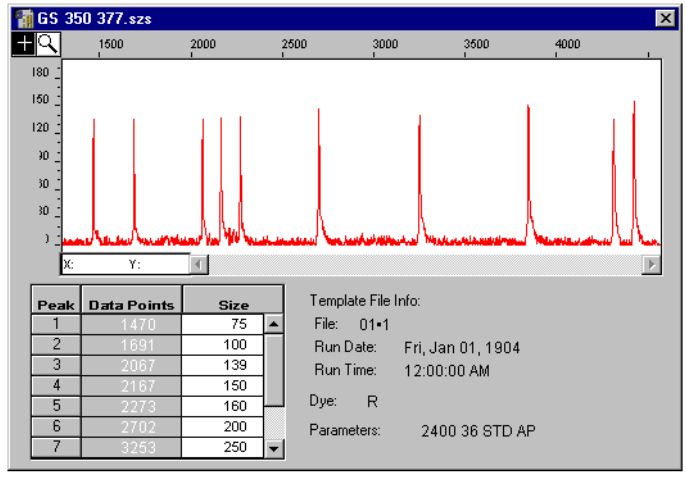
GeneScan 350 377 Molecular Lengths

The following table lists the GeneScan 350 denatured fragment molecular lengths (nucleotides):

75	200
100	250
139	300
150	340
160	350

Electropherogram of GeneScan 350 377

The following is an electropherogram of GeneScan 350 377 run under denaturing conditions:



GeneScan 350-250 Size Standard

About This Size Standard

The GeneScan 350-250 size standard contains all GS 350 fragment sizes except the 250 base-pair size, which has been set to 0.

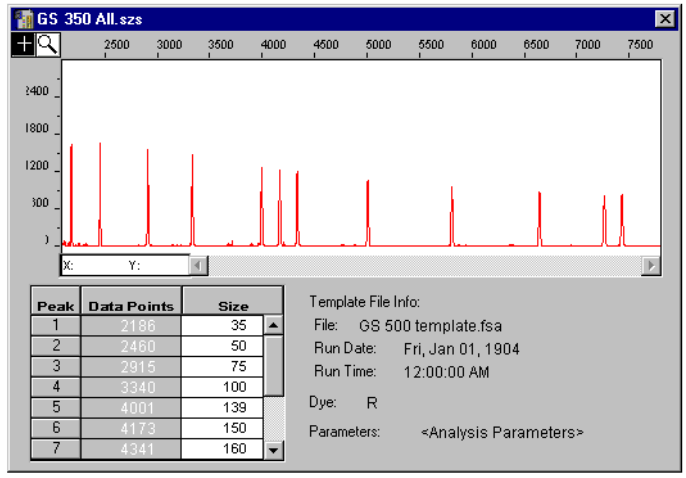
GeneScan 350-250 Molecular Lengths

The following table lists the GeneScan Denatured Fragment Molecular Lengths (Nucleotides):

35	160
50	200
75	0
100	300
139	340
150	350

Electropherogram of GeneScan 350-250

The following is an electropherogram of GeneScan 350-250 run under denaturing conditions:



GeneScan-400HD Size Standard

About This Size Standard

Use the GeneScan-400HD (High Density) size standard to determine fragment lengths between 50 and 400 base-pairs.

Special Uses

The high density of marker bands in this standard makes it particularly useful for microsatellite analysis. All fragments have been checked for migration that is true to size under a wide variety of run conditions on all ABI PRISM® instruments. There are no anomalous fragments (compared with the 250-bp fragment in GeneScan 350 or 500 on the 3700 Analyzer).

GeneScan-400HD is the recommended size standard for use with the ABI PRISM Linkage Mapping Sets.

How It Is Prepared

All aspects of the preparation of the GeneScan-400HD size standard are proprietary. Each fragment contains a single ROX fluorophore.

Fragment Lengths

The following table lists the lengths of the 21 fragments that make up the GeneScan-400HD size standard:

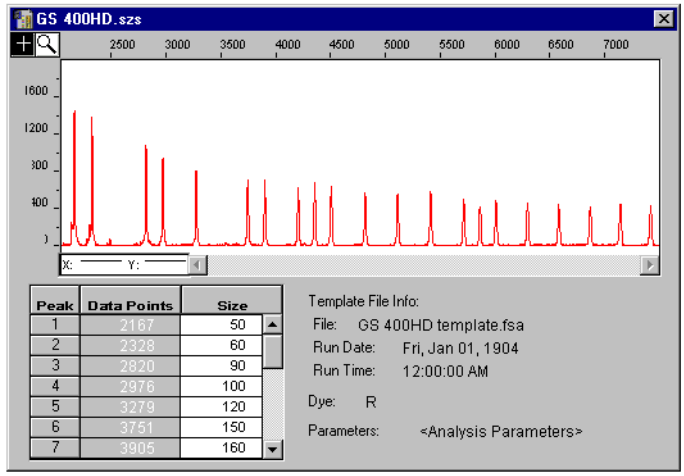
50	160	260	360
60	180	280	380
90	190	290	400
100	200	300	
120	220	320	
150	240	340	

Denaturing Electropherogram

Although the GeneScan-400HD size standard is made of double-stranded DNA fragments, only one of the strands is labeled. Consequently, even if the two strands migrate at different rates under denaturing conditions you will not need to worry about peak splitting. The following figure shows the peak patterns of GeneScan-400HD fragments run under denaturing conditions. Fragments were run using the 3700 POP-6™ polymer at 50 °C.

Electropherogram of GeneScan-400HD

The following is an electropherogram of GeneScan-400HD:



GeneScan 500 All Size Standard

About This Size Standard

The GeneScan 500 All size standard contains all fragments in the GS 500 size standard. This size standard is useful for sizing fragments between 35 and 500 base-pairs. The native fragments are uniformly spaced to provide accurate base calling.

How It Is Prepared

The GeneScan 500 All size standard is prepared by Pst 1 digestion of plasmid DNA, followed by ligation of a TAMRA or ROX-labeled 22-mer oligodeoxynucleotide to the cut ends. A subsequent enzymatic digestion with BstU 1 yields DNA fragments containing a single TAMRA or ROX dye (see “GeneScan 350 Molecular Lengths” below).

GeneScan 500 Molecular Lengths

The following table lists the GeneScan 500 denatured fragment molecular lengths (nucleotides):

35	250
50	300
75	340
100	350
139	400
150	450
160	490
200	500

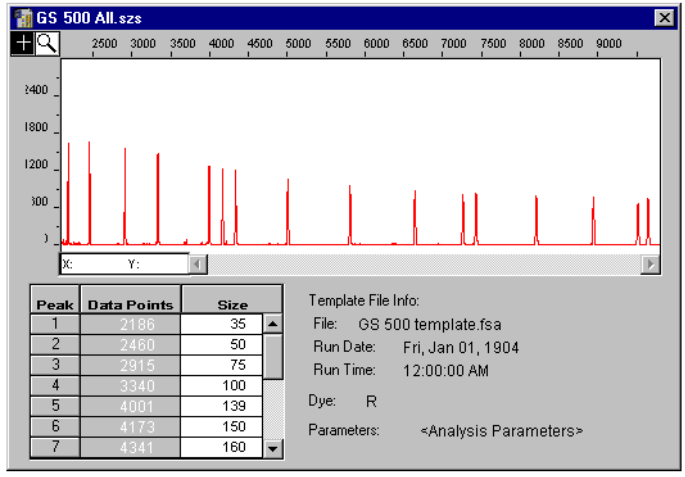
Running Under Denaturing Conditions

Like the GeneScan 2500 and GeneScan 1000 standard, the GeneScan 500 standard is made of double-stranded DNA fragments. However, with the GeneScan 500, only one strand of the double-stranded DNA is labeled, whereas the other two standards have labels on both strands. Consequently, under denaturing conditions, even if the two strands migrate at different rates, only the one labeled strand is detected. Because of this, split peaks are avoided that result when two strands move through a denaturing polymer at different rates.

Refer to “Electropherogram of GeneScan 500” below.

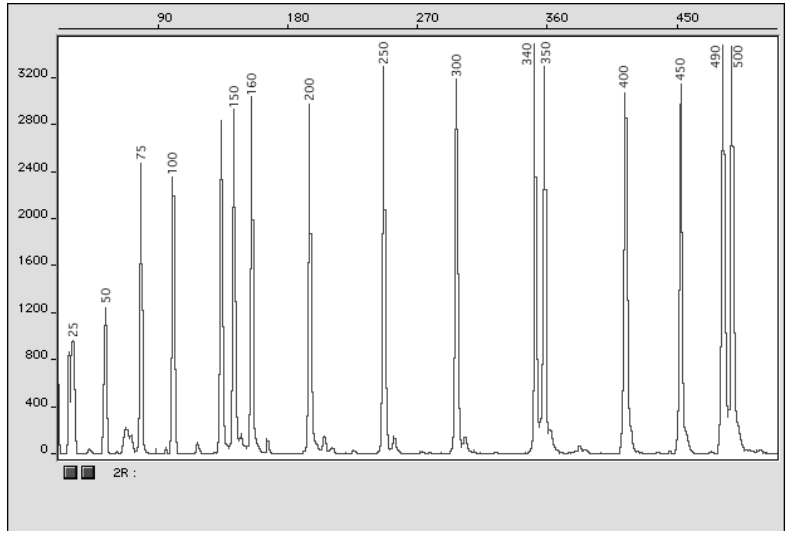
Electropherogram of GeneScan 500

The following is an electropherogram of GeneScan 500 run under denaturing conditions:



Double-Stranded GeneScan 500 Fragments

The following figure shows the sizes of double-stranded GeneScan 500 fragments. Use these values to size fragments run under native conditions.



GeneScan 500 377 Size Standard

About This Size Standard

The GeneScan 500 377 size standard includes all GS 500 fragment sizes except the 35 and 50 base-pairs, which have been set to 0. The two smallest fragments (35 and 50 base-pairs) are often lost in the primer peak on gel instruments.

This size standard can be used on any instrument.

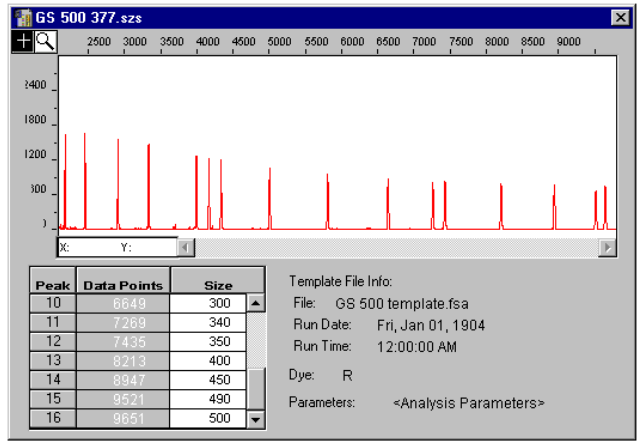
GeneScan 500 377 Molecular Lengths

The following table lists the GeneScan 500 377 denatured fragment molecular lengths (nucleotides):

0	250
0	300
75	340
100	350
139	400
150	450
160	490
200	500

Electropherogram of GeneScan 500 377

The following is an electropherogram of GeneScan 500 377 run under denaturing conditions:



GeneScan 500-250 Size Standard

About This Size Standard

The GeneScan 500-250 size standard has the 250 base-pair peak set to 0, since this peak does not migrate as it should on capillary instruments.

Note The 340 base-pair peak is still present in this file and may not migrate properly. You can check this for the run conditions by changing the 340 base-pair definition to 0, and then use the GeneScan Analysis Software to size the size standard. If you are not happy with GeneScan's size for the 340 base-pair fragment, then it is not migrating properly and you should not use it for your definition.

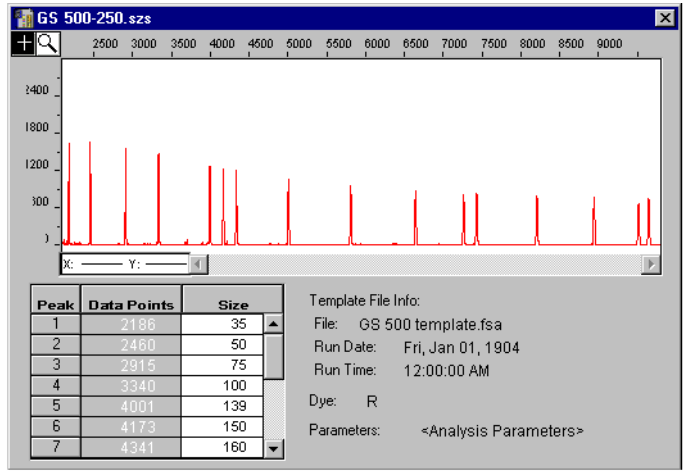
GeneScan 500-250 Molecular Lengths

The following table lists the GeneScan 500-250 denatured fragment molecular lengths (nucleotides):

35	0
50	300
75	340
100	350
139	400
150	450
160	490
200	500

Electropherogram of GeneScan 500-250

The following is an electropherogram of GeneScan 500-250 run under denaturing conditions:



Troubleshooting the GeneScan Software

D

Introduction

In This Appendix

The tables in this section present information about problems you might experience with your GeneScan® Analysis Software runs and suggest possible causes and corrections.

Topics in this appendix include the following:

Topics	See Page
Troubleshooting Projects and Results	D-2
Troubleshooting Gel Data	D-4
Troubleshooting Genotyping Results	D-7
GeneScan Analysis Software Error Messages	D-8

Troubleshooting Projects and Results

Table Description

The following table describes the problem, probable cause, and correction for troubleshooting projects and results:

Problem	Probable Cause	Correction
File name is dimmed in **Size Standard** or **Parameters** column in **Analysis Control** window.	The file has been moved from the folder of its original location.	a. Move the file back to its original location. b. Reset preferences to specify the new folder location. c. Create or select a new file.
Peaks appear on display but the GeneScan Analysis Software does not detect them (cannot select them in electropherogram display).	♦ **Peak Amplitude Threshold** set too high. ♦ **Minimum Peak Half Width** set too high. ♦ Electrophoresis run too quickly resulting in poor resolution.	a. Adjust minimum peak height to include smallest peaks desired and re-analyze. b. Reduce minimum peak half-width setting and re-analyze. c. Repeat electrophoresis at reduced power. For more information, refer to “Peak Detection Options” on page 5-8.
At the position of one strong peak additional colors appear underneath the peak.	Off-scale data not multicomponented correctly.	a. Repeat electrophoresis; load less sample. b. Regenerate sample files.
Peaks appearing in a dye color that should not be present.	Bleed-through from other colors because of off-scale data.	Repeat electrophoresis; load less sample.

The following table describes the problem, probable cause, and correction for troubleshooting projects and results: *(continued)*

Problem	Probable Cause	Correction
Peak centers seem to be incorrect in electropherogram.	♦ Resolution of the gel might be inadequate (ABI PRISM® 377). ♦ Signal-to-noise ratio might be too low.	Repeat electrophoresis at lower power.
Software cannot display the sizing curve for a sample.	♦ Sample's in-lane size standard does not match defined size standard. ♦ Sample file was not sizecalled.	♦ Re-analyze the sample file with a different size standard or create a new one. In the **Analysis Control** window: a. Ctrl+click dye/sample that represents Size Standard for the sample file. b. Select a size standard definition file and re-analyze.
Peaks disappear in the electropherogram.	Included the primer peak in the analysis.	Re-analyze sample file without primer peak.

Troubleshooting Gel Data

Table Description

The following table lists the problem, probable cause, and correction for troubleshooting of gel data:

Problem	Probable Cause	Correction
At the position of one strong peak, additional colors appear underneath the peak.	♦ Off-scale data not multicomponented correctly. ♦ Poor / incorrect matrix. ♦ Gel Image not multicompo-nented.	a. Repeat electrophoresis; load less sample. b. Attach a new gel matrix, regenerate the gel image. c. Regenerate the gel image with multicomponenting selected.
Peaks appearing in a dye color that should not be present.	Bleed-through from other colors because of off-scale data.	Repeat electrophoresis; load less sample.
TAMRA-labeled size standard appears yellow on the gel display.	Collected using: ♦ Filter set A (ABI 373), or ♦ Virtual filter A (ABI PRISM 377).	Repeat electrophoresis with: ♦ Filter set B (ABI 373), or ♦ Virtual filter C (ABI PRISM 377).
TET-labeled products not seen on gel display.	Collected using: ♦ Filter set A (ABI 373), or ♦ Virtual filter A (ABI PRISM 377).	Repeat electrophoresis with: ♦ Filter set B (ABI 373), or ♦ Virtual filter C (ABI PRISM 377).

The following table lists the problem, probable cause, and correction for troubleshooting of gel data: *(continued)*

Problem	Probable Cause	Correction
Signal showing up in neighboring lanes.	Leaking wells of gel.	♦ Consider using a square-tooth comb instead of a shark-tooth comb. ♦ If using 96 lanes, then rerun gel using protocol in the *ABI PRISM 377 DNA Sequencer 96-Lane Upgrade User's Manual* (P/N 4305423).
	Signal intensity very high and signal is being detected in neighboring lanes due to closeness of spacing.	Move tracker lane position from center of band to edge of the band away from strong signal and extract as usual.
		Use 1- or 2-lane averaging to extract lanes.
HEX-labeled products appear green on gel display.	Collected using: ♦ Filter set A (ABI 373), or ♦ Virtual filter A (ABI PRISM 377).	Repeat electrophoresis with: ♦ Filter set B (ABI 373), or ♦ Virtual filter C (ABI PRISM 377).
Collection time was sufficient, but only a small portion of gel displayed.	♦ Gel Image Processing preferences did not include enough scans to display entire gel. ♦ Electrophoresis power too low.	a. Regenerate gel image with new scan range. b. Adjust to correct settings; repeat electrophoresis.

The following table lists the problem, probable cause, and correction for troubleshooting of gel data: *(continued)*

Problem	Probable Cause	Correction
Improper tracking results.	Bad matrix.	Attach new matrix.
	Sample Sheet not filled out properly.	Fill out Sample Sheet properly.
	Comb types set improperly.	a. Fix and type in gel preferences. b. Retrack gel.
	Peak height or red signal too low.	Rerun gel with more size standard.

Troubleshooting Genotyping Results

Table Description

The following table describes the problem, probable cause, and correction for troubleshooting the Genotyper® software results:

Problem	Probable Cause	Correction
Allele peaks seen in correct molecular weight range, with additional peaks seen outside this range.	♦ Bleed-through from other colors because of off-scale data. ♦ Primers not fully optimized.	a. Repeat electrophoresis; load/inject less sample. b. Check optimization.
With allele peaks of high intensity, the GeneScan Analysis Software calls many small peaks.	♦ Background above minimum peak height. ♦ Too much PCR product loaded.	a. Adjust minimum peak height threshold; re-analyze. b. Repeat electrophoresis; load/inject less sample. For more information, refer to “Peak Detection Options” on page 5-8.
A homozygous individual shows a dip at the top of an allele peak which may be called as two separate peaks.	Truncated single peak because of off-scale data can appear as two peaks.	Repeat electrophoresis; load/inject less sample.
Warning message: “Could not complete ‘Run Macro’ command because the labeled peak could not be found.”	The first allele peak for one or more loci in the allelic ladder is lower than the preset minimum peak height specification in the categories list.	a. Adjust minimum peak height; re-analyze. b. Repeat electrophoresis; load/inject less sample. For more information, refer to “Peak Detection Options” on page 5-8.

GeneScan Analysis Software Error Messages

Introduction

This section includes two tables:

- Analysis Log Error Messages
- Error Messages When Defining Size Standards

Analysis Log Error Messages

The following table describes the error messages you might encounter in the GeneScan Analysis Software Log:

Analysis Log Error Message	Comment/Correction	Refer To
The **Analysis Range** parameter does not include enough data points. Check your analysis parameters.	Make sure the **Analysis Range** in your analysis parameters contains at least 250 data points.	"Setting Analysis Parameters" on page 5-5.
The **Range of Data Points** parameter to analyze is too large. Check your analysis parameters.	Specify a smaller range in the analysis parameters.	"Setting Analysis Parameters" on page 5-5.
The analysis Parameters could not be accessed. Check your Analysis Parameters Setting.	Make sure the Analysis Parameters file specified in the **Analysis Control** window is valid and accessible.	"Setting Analysis Parameters" on page 5-5.

Error Messages When Defining Size Standards

The following table describes the error messages you might encounter while defining size standards:

Error Message	Comment/Correction	Refer To
The affected sample file is not available. Locate the sample file and try again.	If the sample file name is dim in the **Analysis Control** window, the GeneScan Analysis Software has not located the sample file. You can instruct the program to search for the sample file.	"Finding Missing Sample Files" on page 2-14.
A Dye Standard is not selected for the affected sample file. Select a Dye Standard and try again.	Select the dye/sample that represents the standard by Ctrl+clicking the appropriate dye/sample field.	
The affected sample file does not have a valid Analysis Parameters Selection. Select new analysis parameters and try again.	Select either: ♦ The default program parameters (<Analysis Parameter >), or ♦ A valid analysis parameters file in the **Analysis Control** window.	"Using Analysis Parameter Files" on page 5-13.
No peaks were found within the Analysis Range. Check your analysis parameters.	♦ Make sure the **Peak AmplitudeThreshold** setting allows for detection of the peaks in your sample. ♦ If peaks in your data are narrow, make sure the **Minimum Peak Half Width** is a small number.	"Sizecaller Algorithm Flowchart" on page 5-4.

GeneScan Analysis Software Files

E

Table of Files The following table lists the files that the GeneScan Analysis Software reads, writes, and, in most cases, creates. The software does not create gel files, and creates Sample files only through lane extraction from a gel.

Table E-1 GeneScan Analysis Software files

File Type	Created by	Location	Where is it used
.fsa	3700 Data Extractor 3100 Data Extractor Gel Processor (377) 310 Data Collection	In a folder often with the title "Run Folder"	GeneScan software can be used to display, view and edit any .fsa file.
.gel	377 Data Collection	In a folder often with the title "Run Folder"	The gel file contains the raw data collected during the instrument run. The gel file is tracked and data is extracted into sample files by Gel Processor.
filename.gsp	Shipped with GeneScan software. Users can create custom files.	D:\AppliedBio\Shared\Analysis \Sizecaller\Params	These files specify certain ranges and methods used during data analysis. Users can create custom .gsp files.
filename.szs	Shipped with GeneScan software. Users can create custom files.	D:\AppliedBio\Shared\Analysis \Sizecaller\SizeStandards	These files are used to identify peak sizes for specified size standards run under certain conditions. Users can define the standards after running them on the instruments.

Table E-1 GeneScan Analysis Software files *(continued)*

File Type	Created by	Location	Where is it used
filename.prj	GeneScan Software	User can specify location. This file is often stored in a folder with the sample files in of the corresponding project	Projects contain references to Sample files. Sample files of a single project can be from one or multiple runs. Projects allow a group of data to be organized, displayed and analyzed together.
filename.mxt	GeneScan Software, using the Matrix making feature		Matrix files must be created for each instrument. The matrix standards are collected on 310 and 377 instruments. These files are used to create .mxt files using GeneScan Analysis software.
Sample log.log	GeneScan Software	D:\AppliedBio\GeneScan\Bin	Contains a running record of analysis performed by the software
Analysis log.log	GeneScan Software	D:\AppliedBio\GeneScan\Bin	Contains a running record of analysis performed by the software

Technical Support

F

Contacting Technical Support

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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
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FMAT™ 8100 HTS System and CytoFluor® 4000 Fluorescence Plate Reader	**1-800-899-5858**, then press **16**	**1-508-383-7855**
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G

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Glossary

This glossary defines special terminology used in the *GeneScan Analysis Software User's Manual*. The terms are listed in alphabetical order. Many terms are defined in the text of the manual, so if you do not find a term here, check the index to see if you can locate it in the manual.

align-by-size curve	Curve created by the GeneScan® Analysis Software for aligning data by size. The software calculates a best-fit, least-squares curve for all samples. This is a third-order curve when you use the Third Order Least Squares sizecalling method; for all other sizecalling methods it is a second-order curve. This curve is black in the Standard Sizing Curve window, although when the sizing curve and this curve match, they overlap so you see only the sizing curve. See also sizecalling curve and size standard spline interpolation curve.
Align By Size/Data Point	Shows the horizontal scale of the electropherograms by fragment size or by data point. You can display data by size only if you ran an internal size standard with your samples and sizecalled the data.
analysis parameters	Options that specify certain ranges and methods used during analysis using the GeneScan Analysis Software. The software has default analysis parameters that are stored in the project itself. These parameters apply globally, unless you create your own parameters files for use with specific protocols.
baselining	Adjusting the baselines of detected dye colors to the same level for a better comparison of relative signal intensity.

Sequence Collector	Sequence Collector (formerly BioLIMS™) is a genetic information management system that provides a relational database for storage and retrieval of DNA sequence and fragment data. In addition to the database itself, Sequence Collector contains a set of software applications and tools for maintaining and interacting with the database. The Sequence Collector database resides on a UNIX workstation and uses a Sybase® or an Oracle® database server. The client applications run on Windows NT®-based computers and/or on UNIX workstations.
data point	The 3700 Data Collection program samples data as it passes by the detector. Each “sampling” is stored as a data point.
dye color indicator	Left color box in the Results Control window and the legend of the Results Display. In the Results Display, click this box to move the associated electropherogram to the front. In the Results Control and the Results Display windows, double-click this box to change the dye scale, or Ctrl+double-click it to reset the dye scale to the default.
dye/sample	Individual sample labeled with a single dye within a sample file. Sample files normally contain up to four dye/samples, depending on how many labeled samples you included in each lane or injection of your 3700 Data Collection program run.
electropherogram	Four-color picture of a sequence, showing peaks that represent the bases. The term is used interchangeably with chromatogram in this manual.
grid	Spreadsheet-like display used for entering data in tabular format. The Analysis Control and Results Control windows display grids for entering sample information.

internal size standard	Also called internal lane or injection size standard, DNA fragment of known size that you include with your run. On the ABI PRISM 3700 DNA Analyzer you include the size standard with each injection. Running an internal lane standard results in particularly accurate and precise molecular length determination because the internal lane standard and the unknown fragments undergo exactly the same electrophoretic forces. The software can then compensate for band-shift artifacts caused by variations in the run.
legend	Informational text that appears beneath electropherogram panels in the results displays. You can show or hide legends, and use the color boxes displayed in them to bring specified electropherograms to the front of the panel, or customize the colors.
matrix file/multicomponent matrix	File used to adjust for the spectral overlap between the fluorescent dyes used on the ABI PRISM® instruments. A mathematical matrix of the spectral overlaps is created and the inverse matrix is used to correct the data during analysis. Matrix files are stored in the AppliedBio folder inside the Macintosh® computer System folder, or in the matrix folder in the GeneScan Analysis Software folder. The values of the matrix are stored in the gel file (ABI 373 and ABI PRISM 377) and in the Sample files. For more information, see Chapter 6, “Making a Matrix File.”
overlaid	Displayed together so they overlap. In the GeneScan Analysis Software Results Display window, all electropherograms in a single panel are overlaid. You can bring a specific one to the front by clicking the color box that represents it in the legend.
plot color indicator	Right color box in the Results Control window and the legend of the Results Display. In the Results Display window, click this box to move the associated electropherogram to the front. In the Results Control and the Results Display windows, double-click this box to change the plot color, or Ctrl+double-click it to reset the plot color to the default.

preferences	Defaults you can set so that certain parameters are automatically applied when you are working with a project. The GeneScan Analysis Software remembers preferences and applies them globally to all new projects.
project	File containing links to a set of sample files that you want to analyze and display together. A project can contain sample files from multiple runs. Adding a sample file to a project creates a reference to the file. It does not copy the file into the project.
project options	Formatting information you can set for the current project. Project options are remembered by the project when you open it again.
sample files	Computer files that contain raw and analyzed data. Sample files are created directly by the 3700 DNA Analyzer. Sample files contain data such as peak locations, sizecalling values, and a record of analysis settings.
sizecalling curve	Curve created by the GeneScan Analysis Software for sizecalling. The software calculates this curve based on the sizecalling method you specify for data analysis. This curve is read in the Standard Sizing Curve window. When it matches the align-by-size curve, the two overlap so you see only this curve. See also “align-by-size curve” and “size standard spline interpolation curve.”
size standard	Specific DNA fragments of known sizes. After you define the peaks of a size standard, the GeneScan Analysis Software matches this definition to the internal lane or injection standard that you include with your run. The software assigns the defined size values to the appropriate peaks of the internal lane or injection standard, and uses this information with the selected sizecalling method to size all unknown fragments.

size standard spline interpolation curve	Curve created by the GeneScan Analysis Software for aligning data by size. The software creates this curve if you use the Local Southern or Cubic Spline Interpolation sizecalling method and the size standard data does not match the best-fit curve, which is normally used for aligning the data by size. This curve is blue in the Sizing Curve window. See also align-by-size curve and sizecalling curve.
tiled	Displayed so they do not overlap. The GeneScan Analysis Software displays tiled electropherogram panels in the Results Display. If you display more than one electropherogram in each panel, all electropherograms in the panel are overlaid.

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