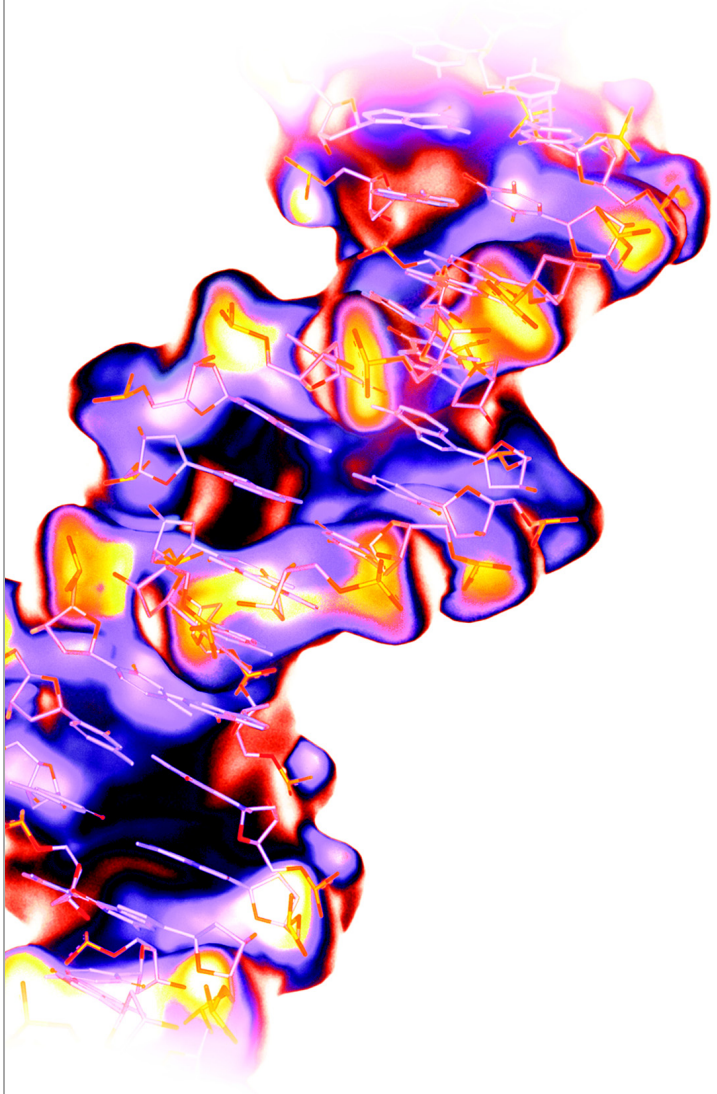
Applied Biosystems

Reference and Troubleshooting Guide



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Reference and Troubleshooting Guide

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Part Number 4366831 Rev. A
06/2005

Contents

Preface

How to Use This Guide

Purpose of This Guide

This guide describes the function of the Process Quality Values (PQV) for the supported analyses of the GeneMapper® Software, explains the fundamental algorithms used by the software, and provides basic troubleshooting techniques.

Audience

This guide is intended for trained laboratory personnel. Applied Biosystems is not liable for damage or injury that results from use of this guide by unauthorized or untrained parties.

Assumptions

This guide assumes that:

- You have installed GeneMapper® Software Version 4.0 as described in the *GeneMapper® Software Version 4.0 Installation and Administration Guide* (PN 4363080).
- You have a working knowledge of the Microsoft® Windows® operating system.

Text Conventions

This guide uses the following conventions:

- **Bold** indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The size of the column affects the run time.

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid Oracle user ID and password.

IMPORTANT! You must create a separate Sample Entry Spreadsheet for each 96-well plate.

How to Obtain More Information

Safety Information

See the *GeneMapper® Software Version 4.0 Installation and Administration Guide* (PN 4363080) for safety information.

Software Warranty and License

See the *GeneMapper® Software Version 4.0 Installation and Administration Guide* (PN 4363080) for warranty and licensing information.

Related Documentation

The following related documents are shipped with the software:

- ***GeneMapper® Software Version 4.0 Installation and Administration Guide*** – Provides procedures for installing, securing, and maintaining version 4.0 of the GeneMapper Software.
- ***GeneMapper® Software Version 4.0 Getting Started Guides*** – Five guides that explain how to analyze the application-specific example data provided with the GeneMapper Software. The guides provide brief, step-by-step procedures for the analysis of microsatellite, LOH, AFLP® system, SNaPshot® kit, and SNPlex™ system data generated by compatible Applied Biosystems electrophoresis instruments and Data Collection Software. The guides are designed to help you quickly learn to use basic functions of the GeneMapper Software.
- ***GeneMapper® Software Version 4.0 Online Help*** – Describes the GeneMapper Software and provides procedures for common tasks. Access online help by pressing **F1**, selecting **Help ▸ Contents and Index**, or clicking in the toolbar of the GeneMapper window.
- ***GeneMapper® Software Version 4.0 Quick Reference Card*** – Provides workflows for specific analysis types and lists instruments, software, and analysis applications compatible with the GeneMapper Software.
- ***GeneMapper® Software Version 4.0 Reference and Troubleshooting Guide*** – Provides reference information such as theory of operation and includes troubleshooting information.

Portable document format (PDF) versions of this guide and the other documents listed above are available on the *GeneMapper® Software Version 4.0 Documentation CD*.

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

Send Us Your Comments

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

techpubs@appliedbiosystems.com

Obtaining Information from the Online Help

The GeneMapper® Software features an online help system that describes how to use each feature of the user interface. To access the online help, click in any window or dialog box (**Help ▸ Contents and Index** if available) for more information.

How to Obtain Support

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

At the Support page, you can:

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

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

1 Process Quality Values and Basic Troubleshooting

Chapter 1
Process Quality Values and Basic Troubleshooting

Chapter 2
SNPlex™ System Troubleshooting

Chapter 3
Algorithms

In this chapter:

Diagnosing and Resolving Basic Problems

PQVs and the Troubleshooting Process

The GeneMapper® Software has a system of Process Quality Values (PQVs) that provide the basis for troubleshooting fragment analysis data using the software. PQVs are application-specific metrics, where each evaluates the data for a specific quality that is consistent with a problem associated with the type of analysis. In this way, the PQV system can alert you to potential problems and provide you with a starting point for investigation.

About Process Quality Values (PQVs)

Each individual PQV displays the result of a unique algorithmic test that evaluates a specific property of the fragment analysis data. The software performs the PQV tests in a specific sequence during the analysis. With the exception of the Sizing Quality (SQ) PQV, the software completes the analysis of each sample in a project even if a sample fails one or more PQV tests.

The majority of PQV metrics yield numeric values between 0 and 1, where 1 indicates that the related sample data or genotype completely passed the associated test. Following the analysis, the software uses the upper and lower thresholds for each PQV to translate the numeric score into one of three symbols, displayed in the Samples or Genotypes tab of the GeneMapper window.

Note: If the thresholds of a PQV can be customized, the software displays the parameters in the Quality Flags tab of the analysis method.

Symbol	Definition	Default Range
	Pass: The sample or genotype passed the PQV test.	0.75 to 1.0
	Check: A possible problem exists for the sample or genotype.	0.25 to 0.75
	Low Quality/Fail: There is a strong possibility that a problem exists for the sample or genotype.	0.0 to 0.25

Note: Applied Biosystems recommends examining all samples that produce  (Check) or  (Low Quality).

Note: The Allele Display Overflow (ADO) and Allele Edit (AE) PQVs of the Genotypes tab report their results as ✕ instead of the colored icons.

Note: The GeneMapper Software does not complete the analysis of samples that display (Low Quality) for the Sizing Quality (SQ) PQV test.

Adjusting the Threshold Settings of PQV Tests

Some PQV metrics include components that can be customized. In those cases, the user-defined parameters for the PQV appear in the Peak Quality tab of analysis methods for the applicable analysis type.

Adjusting the Weights of PQV

The majority of PQV contribute to the Genotype Quality (GQ) PQV, a metric used to gauge the confidence of each genotype call. In those cases, some PQV contain user-defined weights that determine how significantly the associated PQV affect the GQ PQV calculation. For those PQV, the user-defined weights appear in the Quality Flag tab of analysis methods for the applicable analysis type. For more information on the calculation of the GQ PQV, see "GQ (Genotype Quality)" on page 13.

Note: You can configure a PQV so that it does not contribute to the GQ (by setting the weight to 0). However, the PQV remains active.

Rules for PQV Columns

- Quality metrics with Pass/Check values and no Low Quality values are warning flags. Analysis does not stop if problems are detected with these properties, but Applied Biosystems recommends examining results flagged as (Check).
- Holding the cursor over a column header displays a tooltip that lists the full name of the column (the default names are often acronyms).

Table 1-1 PQV values by application

PQVs		Analysis					See Page
			Microsatelite				
Abb.	Name	AFLP™ System	2n	Other	SNaPshot® Kit	SNPlex™ System	
ADO	Allele Display Overflow	✔	✔	✔	✔	✔	8
AE	Allele Edit	✔	✔	✔	✔	✔	8
AN	Allele Number		✔	✔	✔	✔	9
BD	Broad Peak	✔	✔	✔	✔	✔	9
BIN	Out of Bin Allele		✔	✔		✔	10
CC	Control Concordance		✔	✔	✔	✔	11
DP	Double Peak				✔		12
GQ	Genotype Quality	✔	✔	✔	✔	✔	13
LPH	Low Peak Height		✔	✔	✔	✔	15
MNF	Matrix Not Found	✔	✔	✔	✔	✔	16
NB	Narrow Bin				✔		18
OBA	One Basepair Allele		✔				18
OS	Off-Scale	✔	✔	✔	✔	✔	19
PHR	Peak Height Ratio		✔	✔	✔	✔	21
SFNF	Sample File Not Found	✔	✔	✔	✔	✔	22
SHP	Sharp Peak		✔				23
SNF	Size Standard Not Found	✔	✔	✔	✔	✔	23
SP	Split Peak		✔				24
SPA	Single Peak Artifact		✔				24
SPU	Spectral Pull-Up	✔	✔	✔	✔	✔	25
SQ	Sizing Quality	✔	✔	✔	✔	✔	25
XTLK	Cross Talk		✔	✔		✔	8

Common Troubleshooting Procedures

About the Procedures

Procedures commonly used to troubleshoot errors and irregularities in fragment analysis data:

Displaying Sample Information/Raw and EPT Data

1. In the GeneMapper window, select the **Samples** tab.
2. In the Navigation Pane of the GeneMapper window:
   a. Click ⊞ to expand the contents of the project folder.
   b. From the list of samples, select a sample that displayed  (Check) or  (Low Quality).
3. Select the **Info**, **Raw Data**, and **EPT Data** tabs as needed to display the sample information for the selected file.
   - **Info tab** – Displays a summary of all information for the associated sample file (see Table 1-2 on page 6).
   - **Raw Data tab** – Displays an electropherogram of spectral data collected during the run of the associated sample. The spectral data is displayed in relative fluorescent units (RFU).
   - **EPT Data tab** – Displays the EPT (electrical, power, and temperature) data for the associated sample throughout the course of the run.

Note: Table 1-2 lists only the information contained in the Info tab that is relevant to troubleshooting. For a complete description of the elements listed in the Info tab, see the *GeneMapper® Software Online Help*.

Table 1-2 Info Tab information relevant to troubleshooting

Group	Description/Information
Sample Information	Describes the source and status of the imported sample data. **Sample Origin Path** – Displays the path to the associated sample file at the time it was imported (provided that the sample was added to the project from a sample file). **Status Message** – Displays information related to any events that occurred when the sample was imported into the project.
Error Message	Displays any errors the software encounters during the analysis of the associated sample. You can use the information in this group to verify the source of several analysis problems.
Current Settings	Describes the analysis settings currently applied to the associated sample. All of the data displayed in this group is useful for troubleshooting problems with the GeneMapper Software. Although, most of the information can be viewed in various parts of the software, the Current Settings group summarizes all the information in a single location for easier access.
Run Information	Provides basic information about the configuration of the compatible Applied Biosystems electrophoresis instrument and the run itself. **Instrument Name** – Displays the name of the instrument used to run the sample. The instrument name is important when diagnosing trends in analysis errors that can be traced back to the instrument used to run the failed sample(s). **Data Collection Ver** – Displays the version number of the Data Collection Software used to run the sample.
Data Collection Settings	Describes the configuration of the Data Collection Software at the time the sample was run. The data displayed in this group can be used to troubleshoot problems caused by modifications to run modules. By comparing the Data Collection Settings information from passing and failing runs, you can identify any changes made to the run module (intentional or unintentional) that may have caused or contributed to the failure. **Note:** The data displayed in the EPT tab provides a log of the actual parameters throughout the run.
Capillary Information	Provides the basic specifications of the capillary array used to run the sample. **Capillary Number** – Displays the number of the capillary used to run the associated sample. Like the instrument name, the capillary number is important when diagnosing trends in analysis errors that can be traced back to the capillary used to run the failed sample(s).

Displaying Numeric PQV Metrics

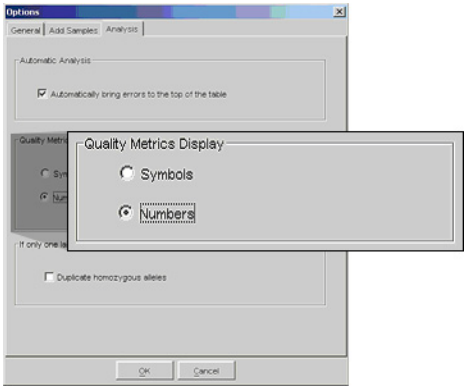
By default, the GeneMapper Software displays (Pass), (Check), or (Low Quality) in some PQV columns to represent the numeric score of the associated quality metric. When troubleshooting quality errors, it is often more useful to configure the software to display numeric representations of the quality values.

For example, the Sizing Quality (SQ) PQV evaluates the similarity between the fragment pattern defined by the size standard definition and the actual size standard peak distribution pattern in the sample data. The Sizing Quality metric yields a value between 1 and 0 that represents a combination of statistical measures for the size calling method used to perform the analysis. Based on the PQV Threshold settings of the Quality Flags tab, the software displays (Pass), (Check), or (Low Quality) to indicate the result of the Sizing Quality calculation.

Sizing Quality Representation	Samples Table Example
Symbols (default)	Run Name | SFNF | SNF | OS | SQ | WELLQ DGB_SNPlex_ | ■ | ■ | ■ | ■ | ■ DGB_SNPlex_ | ■ | ■ | ■ | ■ | ■
Numbers (recommended for troubleshooting)	Run Name | SFNF | SNF | OS | SQ | WELLQ DGB_SNPlex_ | ■ | ■ | ■ | 1.0 | 1.0 DGB_SNPlex_ | ■ | ■ | ■ | 1.0 | 1.0

To display numerical representations of the quality metrics:

1. Select **Tools ▸ Options**, then select the **Analysis** tab.
2. In the Quality Metrics Display settings, select **Numbers**.



3. Click **OK** to apply the settings.

ADO (Allele Display Overflow)

Description/ Function

The ADO PQV indicates that the number of alleles called for the associated sample at the specified marker exceeds the Allele Setting in the Genoptypes tab of the table setting. Because the software is configured to display fewer alleles than are present, the data for the additional allele is hidden from view.

Note: For each allele detected by the software, the Genotypes tab displays six columns: name, size, height, area, mutation, and comments.

Expected Values

✕ – Indicates that the associated sample contains a number of alleles at the specified marker that is greater than the user-defined limit.

Troubleshooting

Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the affected sample for miscalled peaks.

AE (Allele Edit)

Description/ Function

The AE PQV indicates whether or not a user modified the allele call for the associated genotype.

Note: Allele calls can be modified in the Samples Plot, the Genotypes Plot, and the Cluster Plot.

Expected Values

✕ – Indicates that the associated genotype call has been edited.

AN (Allele Number)

Description/ Function

The AN PQV indicates that the associated sample contains either:

- A number of alleles at the specified marker that exceeds the Max Expected Alleles setting (in the Peak Quality tab of the analysis method)

  or

- No alleles are present at the specified marker

Expected Values

(Pass) or (Check)

Troubleshooting

Select the affected genotype(s), click (**Analysis ▸ Display Plots**), then review the sample data at the affected marker for additional peaks or for the absence of peaks.

BD (Broad Peak)

Description/ Function

The BD PQV indicates that the width of the peak for the associated genotype exceeds the Max peak width setting (in the Peak Quality tab of the analysis method).

Note: When the BD PQV is triggered, the software reduces the GQ PQV by 50% because the default multiplier is 0.5.

Expected Values

(Pass) or (Check)

Troubleshooting

Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the associated peak for irregularities.

BIN (Out of Bin Allele)

Description/ Function

The BIN PQV indicates that the apex of the peak for the associated genotype is outside of the boundaries that define the associated bin.

Note: When the BIN PQV is triggered, the software reduces the GQ PQV by 80% because the default multiplier is 0.8.

Note: For human identification (HID) analysis, the BIN PQV is displayed as the OL (Off-Ladder Alleles) PQV.

Expected Values

(Pass) or (Check)

Troubleshooting

Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the allele(s) at the appropriate bin location.

Symptom	Possible Cause	Solution
BIN PQV displays (Check)	After using Auto Bin to generate bins, the software did not create a bin for an allele peak because it considered the peak to be a single peak artifact. The SPA flag was triggered because the software did not detect any stutter peaks to the left of the allele peak; a result of the minimum fragment length for the marker being set too high	Correct the SPA flag by editing the marker minimum fragment length, then reanalyze and perform the Auto Bin again.
	The GeneMapper Software detected an allele peak that did not fit into any of the defined bins because the bins were not calibrated to the allelic ladder; a result of a sample file containing an allelic ladder that is not designated as an allelic ladder in the Samples tab.	In the Samples tab of the GeneMapper window, set the Sample Type of the sample containing the allelic ladder to **Allelic Ladder**.
	You generated bins using the Auto Bin function but the GQ value for a marker was less than the Minimum Quality Value of 0.1 (as set in the Auto Bin dialog box).	View the allele peak(s) for the marker in the Genotypes Plot window. Determine if the allele peaks(s) are valid. If so, manually create bin(s) for the peak(s).

CC (Control Concordance)

Description/ Function

The CC PQV indicates that the associated control sample does not exactly match the defined alleles for the related marker.

IMPORTANT! Applied Biosystems recommends running the control sample at least once for every panel.

Note: The CC PQV serves primarily as an internal control for quality assurance.

Expected Values

(Pass) or (Check)

Troubleshooting

Select the affected control sample in the Samples tab of the GeneMapper window, click (**Analysis ▸ Display Plots**), then review the positions of the peaks relative to the bins.

Symptom	Possible Cause	Solution
CC PQV displays (Check)	The allele calls of the sample defined as the Positive Control in the Samples tab do not match the Positive Control allele calls in the marker definition because the well contains the incorrect positive control sample.	Run the correct positive control and add the sample file to the project, then define the sample as the Positive Control in the Samples tab of the GeneMapper window.
	The allele calls of the sample defined as the Positive Control in the Samples tab do not match the positive control allele calls in the marker definition because the alleles were defined incorrectly.	Edit the Positive Control allele calls in the marker definition in the Panel Manager.
	The sample defined as the Negative Control contains an “allele peak” due to the presence of a spike caused by dust or a gas bubble.	Rerun the negative control and add the sample file to the project, then define the sample as the Negative Control in the Samples tab of the GeneMapper window.

DP (Double Peak)

Description/ Function

The DP PQV indicates that the peak for the associated genotype:

- Resides in a bin with another peak of the same dye color

  and

- The ratio of the peak height (minor/major peak height) is greater than the Double Peak setting in the Peak Quality tab of the Analysis Method

Expected Values

(Pass) or (Check)

Troubleshooting

Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the sample data at the appropriate bin for additional peaks.

Symptom	Possible Cause	Solution
DP PQV displays (Check)	A problem with the chemistry is causing peaks from two different markers not to resolve, possibly because either of the primers are too similar in length or the mobilities of the two primer fragments are similar.	Check primer lengths and electrophoresis conditions and adjust as necessary.

GQ (Genotype Quality)

Description/ Function

The GQ PQV provides a summary of the quality metrics for each genotype. The GQ value is a calculated combination of the relevant, weighted PQVs and the Marker Quality value for the genotype.

Calculation of the Genotype Quality (GQ) Metric

The formulas used by the GeneMapper Software to calculate the GQ value are analysis-specific, and differ largely based on the PQVs supported by each application. The following general formula describes the genotype quality calculation:

$$GQ = MQ \times ((1 - BD) \times (1 - OS) \times \ldots \times (1 - SPU))$$

where the Marker Quality (MQ) value is modified by the user-defined PQVs to generate the final GQ value, and the PQVs are weighted from 0 to 1. The actual value of each PQV in the equation is 1 minus the weight assigned in the Quality Flags tab of the analysis method used to analyze the data.

PQV Weight	Net Effect on GQ Calculation
0	No effect on the GQ calculation The initial value of 1 minus the weight of 0 yields a PQV of 1. When used in the GQ calculation, the PQV has no effect since 1 × MQ = MQ.
1	Reduces the GQ value to 0 The initial value of 1 minus the weight of 1 yields a PQV of 0. When used in the GQ calculation, the PQV automatically causes the GQ to fail since 0 × MQ = 0.
$0 < x < 1$	Reduces the GQ value to the fraction specified by the weight. The higher the value, the greater the effect on GQ.

IMPORTANT! The filtering of individual PQVs is controlled by the threshold settings in the Peak Quality tab of the analysis method. Also, the PQVs remain fully functional regardless of the weights used.

Calculation of the Marker Quality Metric

Figure 1-1 shows how the GeneMapper Software generates a Marker Quality (MQ) value from sample peak data with assigned Allele Quality (AQ) values. AQ values are a function of quality value assignments for: sizing quality, allele calling quality, bin assignment quality, and bin quality.

Note: When analyzing SNPlex™ System sample data, the GeneMapper Software calculates GQ values depending on the method (Model or Rules) selected to perform allele calling. The following figure illustrates the derivation of GQ values using the Rules method.

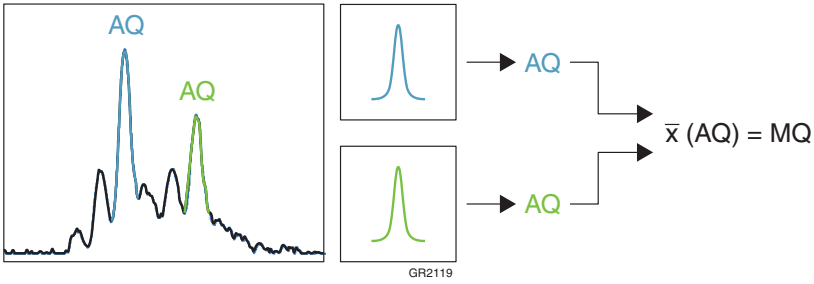


Figure 1-1 Calculation of the Marker Quality metric

Expected Values

(Pass), (Check), or (Fail)

Note: The software assigns the GQ PQV flags based on the PQV threshold settings in the Quality Flags tab of the analysis method.

Troubleshooting

Review the PQV for the affected genotype to determine the metric that is causing the GQ PQV to fail.

Note: To better determine how individual PQV contribute to the GQ PQV, configure the software to display the PQV numerically, as explained in "Displaying Numeric PQV Metrics" on page 7.

LPH (Low Peak Height)

Description/ Function

The LPH PQV indicates that the height of the peak for the associated genotype is lower than the associated heterozygous or homozygous height limit that is specified in the analysis method. You can set homozygous value (default is 200) and heterozygous value (default is 100) in the Peak Quality tab of the analysis method.

Note: When the LPH PQV is triggered, the software reduces the GQ PQV by 50% (the default multiplier is "0.5").

Expected Values

(Pass) or (Check)

Troubleshooting

Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the associated peak for irregularities.

MNF (Matrix Not Found)

Description/ Function

The MNF PQV indicates whether or not the GeneMapper Software can access the matrix specified in the Matrix column of the Samples tab for the associated sample.

IMPORTANT! Because recent models of Applied Biosystems instruments save matrix data to the sample files they create, the MNF flag is applicable only to sample files created by the ABI PRISM® 310 and 377 instruments.

Expected Values

(Pass) or (Check)

Troubleshooting

Determine the name of the missing matrix file(s) by reviewing the sample information for the affected samples, as explained in "Displaying Sample Information/Raw and EPT Data" on page 5.

Symptom	Possible Cause	Solution
MNF PQV displays (Check)	The software could not access the matrix file specified in the Matrix column for the associated sample file.	Locate the missing matrix file, then import it as explained in "Importing a Matrix File (Windows® Only)" on page 16
		If the sample files for the matrix standards used to create the missing matrix are available, re-create the matrix as explained in "Generating a Matrix" on page 17.

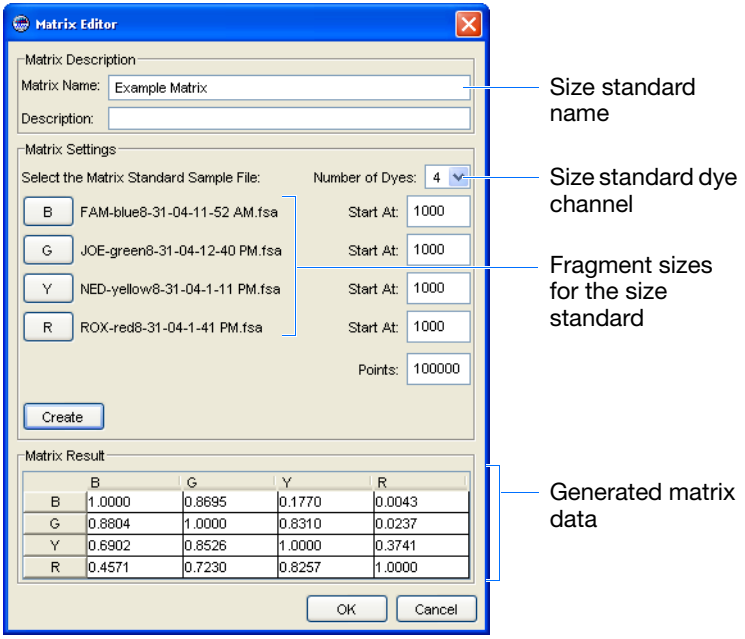
Importing a Matrix File (Windows® Only)

IMPORTANT! You must convert matrix files created by Macintosh® computers before importing them. The conversion utility is free and available from the software support section of the Applied Biosystems website (**www.appliedbiosystems.com/support/software/**).

1. Click (**Tools ▸ GeneMapper Manager**).
2. In the GeneMapper Manager, select the **Matrices** tab, then click **Import**.
3. In the Importing Matrix dialog box, navigate to and select the matrix file, then click **Import**.
4. Click **Done** to close the GeneMapper Manager, then analyze the project.

Generating a Matrix

1. Click (**Tools ▸ GeneMapper Manager**).
2. In the GeneMapper Manager, select the **Matrices** tab, then click **New**.
3. In the Matrix Editor dialog box:
   a. Type a name and description for the matrix.
   b. In the Number of Dyes drop-down list, select the number of dyes present in the matrix (**4** or **5**).
   c. Click **B**, navigate to and select the sample file for the blue matrix standard, then click **Open**.
   d. Repeat step 3c for the remaining dyes in the matrix (**G**reen, **Y**ellow, **R**ed, and **O**range if applicable).
   e. Click **Create** to create the matrix.
   f. Click **OK**.



4. Click **Done** to close the GeneMapper Manager.
5. In the Matrix column of the Samples tab, select the new matrix, then analyze the project.

NB (Narrow Bin)

Description/ Function The NB PQV indicates that the apex of the associated peak for the associated genotype is present within 0.5 base pairs of a bin that does not contain a peak. This PQV is designed to capture peaks that are outside of bin boundaries because of incorrect bin definitions.

Expected Values (Pass) or (Check)

Troubleshooting Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the peak at the appropriate bin location.

Symptom	Possible Cause	Solution
NB PQV displays (Check)	You created a bin that is too narrow to contain its associated allele peak.	In the Panel Manager, edit the bin width and/or location so that it contains the allele peak.

OBA (One Basepair Allele)

Description/ Function The OBA PQV indicates, for the associated genotype, that the apex of the associated peak is present at a position within 1 base pair of another peak.

Expected Values (Pass) or (Check)

Troubleshooting Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the allele at the appropriate bin location for a microvariant peak or an invalid allele call.

OS (Offscale)

Description/ Function

The OS PQV is displayed in both the Samples and Genotypes tabs of the GeneMapper window, but the function of the OS PQV in each tab differs in the following way:

- **OS PQV for the Samples tab** – The signal associated with the size standard of the specified sample contains one or more peaks that exceed the maximum detectable range.
- **OS PQV for the Genotypes Tab** – The signals associated with the given sample contain one or more peaks that exceed the maximum detectable range.

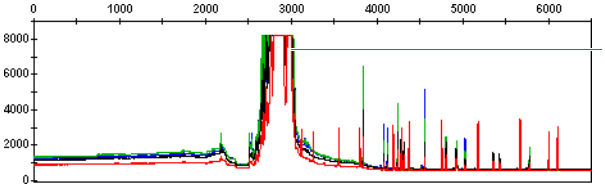
Note: When the OS PQV of the Genotypes tab is triggered, the software reduces the GQ PQV by 50% (the default multiplier is "0.5").

Expected Values

(Pass) or (Check)

Troubleshooting

1. In the GeneMapper window, select the **Samples** tab.
2. In the Navigation Pane, click ⊞ to expand the project folder, then select the sample that displays (Check) in the OS column.
3. Select the **Raw Data** tab to display the electropherogram of normalized spectral data collected during the associated sample run. The spectral data is displayed in Relative Fluorescent Units (RFU).
4. Review the data for offscale peaks.



5. Use Table 1-3 on page 20 to determine an appropriate corrective action.

Table 1-3 OS PQV Troubleshooting

Symptom	Possible Cause	Solution
• MNF PQV in the Samples tab displays ⚠ (Check) • Raw data contains multiple off-scale size standard peaks	Too much size standard injected into the capillary	No action necessary. The data cannot be manipulated to remove the oversized peaks. Decrease the quantity of size standard used in subsequent runs. Also, make sure to use Hi-Di Formamide as the loading reagent. **IMPORTANT!** Water loading can produce artificially high signal and is not recommended.
• MNF PQV in the Genotypes tab displays ⚠ (Check) • Raw data contains multiple off-scale peaks in the signal(s) associated with the sample fragments	Too much sample injected into the capillary	No action necessary. The data cannot be manipulated to remove the oversized peaks. Decrease the quantity of sample used in subsequent runs.

OVL (Overlap)

Description/ Function

The OVL PQV indicates that the peak for the associated genotype has been called twice by the GeneMapper Software. If the ranges of two bins overlap, a peak can reside in both bins and, therefore, be called twice, once for each allele.

Expected Values

■ (Pass) or ⚠ (Check)

Troubleshooting

Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the peak and associated bins at the appropriate location.

PHR (Peak Height Ratio)

Description/ Function

The PHR PQV indicates that the apex of the peak for the associated genotype is:

- Present at a position within 1 base pair of another peak

  and
- The ratio of the height of the lower peak to that of the higher peak is less than the Minimum Peak Height Ratio setting in the Peak Quality tab of the analysis method.

Note: For LMS markers, the ratio is calculated based on the peak heights of the called allele peaks.

Note: For SNaPshot® kit analysis, the ratios are calculated as they are for microsatellite markers except that they span two different colors, and only two peaks are used in the calculation.

Expected Values

(Pass) or (Check)

Troubleshooting

Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the peaks at the appropriate location.

Symptom	Possible Cause	Solution
PHR PQV displays (Check)	The sample has undergone Loss of Heterozygosity (LOH). A difference in peak heights between alleles is expected.	Normal occurrence. No action necessary. Further evaluate the sample for LOH using the Report Settings Editor and Report Manager. For more information, see the *GeneMapper® Software Version 4.0 LOH Analysis Getting Started Guide* (PN 4363081).

SFNF (Sample File Not Found)

Description/ Function

The SFNF PQV indicates whether or not the software can access the sample file (*.fsa) shown in the Sample File column of the associated sample. When the software adds a sample to a project from a sample file, it retains a link to the original file. The software displays ⚠ (Check) in the SFNF column if the sample file is deleted, renamed, or moved.

Expected Values

■ (Pass) or ⚠ (Check)

Troubleshooting

Determine the name and location of the missing sample file:

1. In the GeneMapper window, select the **Samples** tab.
2. In the Navigation Pane of the GeneMapper window, click ⊞ to expand the contents of the project folder, then select the sample that display ⚠ (Check) in the SFNF column.
3. Select the **Info** tab, then note the name (Sample File) and location (Sample Origin Path) of the sample.

Symptom	Possible Cause	Solution
SFNF PQV displays ⚠ (Check)	Sample file has been renamed, moved, or deleted.	Search the local drives of the computer for the sample file, then do one of the following: • If you cannot find the file, no further action can be taken to resolve the PQV flag. • If you find the file, use the Associate Sample feature to direct the software to the new location as follows: a. In the Samples tab of the GeneMapper window, select the affected samples. b. Select **File ▸ Associate Samples**. c. In the Select Folder dialog box, select the folder containing the missing files, then click **Select**.

SHP (Sharp Peak)

Description/ Function

The SHP PQV indicates that the peak for the associated genotype is part of a cluster of peaks with a large, narrow peak in the middle whose width is 50% less than either of the neighboring peaks.

Expected Values

(Pass) or (Check)

Troubleshooting

Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the peak at the appropriate location.

SNF (Size Standard Not Found)

Description/ Function

The SNF PQV indicates whether or not the GeneMapper Software can access the size standard definition specified in the Size Standard column for the associated sample.

Expected Values

(Pass) or (Check)

Troubleshooting

Verify that the software does not contain the desired size standard:

1. In the Samples tab of the GeneMapper window, note the name of the size standard assigned to the affected sample.
2. Click (**Tools ▸ GeneMapper Manager**).
3. In the GeneMapper Manager, select the **Size Standards** tab.
4. Verify that the Size Standard tab does not list the missing size standard, or that it has not been renamed.

Symptom	Possible Cause	Solution
SNF PQV displays (Check)	(Autoanalysis only) The size standard may have been set incorrectly in the plate record of the Data Collection Software.	Do one of the following: • If using an Applied Biosystems size standard, click **Import** to import the definition from the default Panels folder. • Click **New** to create a custom size standard of the same name.
	The size standard definition has been renamed, deleted, or does not exist.	

SP (Split Peak)

Description/ Function The SP PQV indicates that the peak for the associated genotype is part of a pair of overlapping peaks that are less than 0.25 base pairs apart (the horizontal distance between two peak apexes).

Expected Values (Pass) or (Check)

Troubleshooting Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the peak at the appropriate location.

SPA (Single Peak Artifact)

Description/ Function The SPA PQV indicates that no peaks are present within a two-base-pair range before the peak for the associated genotype. This PQV is designed to detect the absence of stutter peaks that accompany microsatellite peaks.

Expected Values (Pass) or (Check)

Troubleshooting Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the peak at the appropriate location.

Symptom	Possible Cause	Solution
SPA PQV displays (Check)	The GeneMapper Software did not detect any stutter peaks to the left of the allele peak because the minimum fragment length for the marker was set too high.	In the Panel Manager, edit the marker minimum fragment length, then reanalyze.

SPU (Spectral Pull-Up)

Description/ Function

The SPU PQV indicates that the apex of the peak for the associated genotype is at a position where the marker signal contains pull-up peaks (also called bleed-through peaks). Pull-up peaks occur when the peak height of the called allele is less than X% of the larger peak that is within ±1 data point.

Expected Values

(Pass) or (Check)

Troubleshooting

Select the affected genotype, click (**Analysis ▸ Display Plots**), then review the peak at the appropriate location.

SQ (Sizing Quality)

Description/ Function

The SQ PQV reports the result of the Sizing Quality test, which gauges the similarity between the fragment pattern defined by the size standard definition, and the actual distribution of size standard peaks in the sample data. The metric of the Sizing Quality test is a combination of several values which measure the success of the algorithms that:

- Identify and eliminate primer peaks based on peak shape
- Perform size matching (ratio matching)
- Make a size-calling curve using the chosen sizing method

The Sizing Quality metric yields a value between 0 and 1. Based on the PQV Threshold settings in the analysis method used to analyze the data, the software translates the metric into the (Pass), (Check), or (Low Quality) flags to indicate the result of the test.

Note: The GeneMapper Software does not complete the analysis of samples that fail the Sizing Quality test (samples that display).

Expected Values

(Pass), (Check), or (Low Quality)

Note: When performing size calling using the Classic sizing method, the software cannot determine Sizing Quality and, therefore, SQ is always (Check).

Troubleshooting

Review the data of the size standards that failed the SQ PQV:

1. In the Samples tab of the GeneMapper window, click (**Analysis ▸ Low Quality to Top**) to sort the data so that the samples that produced errors appear at the top of the table.
2. In the Samples tab, select the rows for the sample(s) that display (Check) or (Fail) in the SQ column.
3. Click (**Analysis ▸ Size Match Editor**) to view the sizing information for the selected sample(s).
4. In the Navigation Pane of the Size Match Editor, select a sample file to display the sizing data for the associated sample.
5. Review the data for the following qualities:
   - **Signal Strength** – The signal strength (peak height) of all peaks must exceed the Peak Detection Threshold defined in the analysis method used to analyze the data.
   - **Correct Size Calls/Labels** – All peaks must be correctly identified by the software. The labels above the peaks must be in sequential order from left to right, least to greatest.
   - **Evenness of Signal Strength** – All peaks should have relatively uniform signal strengths.
   - **Sizing Quality** – The sizing quality of each sample should be within the passing range for your chemistry application.

   Note: To magnify the plot of the Size Matches tab, drag the mouse cursor () across a region of the x- or y-axis.
6. Use Table 1-4 on page 27 to determine an appropriate corrective action.
7. Repeat steps 4 through 6 for each sample file.

Table 1-4 SQ PQV Troubleshooting

Symptom	Possible Cause	Solution
• SQ PQV displays ⚠ or 🛑 • Size Match Editor does not display peak data	The Size Standard Dye setting for the size standard definition is not set to the correct dye.	1. Verify that the correct size standard definition is in use. 2. Open the size standard definition and verify that the: – Dye setting is set to the correct dye – Fragment sizes are correct 3. Modify the size standard definition as necessary.
• SQ PQV displays ⚠ or 🛑 • Peaks do not contain size labels	The fragment sizes of the size standard definition do not match the positions of the detected peaks.	
• SQ PQV displays ⚠ or 🛑 • One or more miscalled peaks	Peak detection threshold associated with the size standard is set too high or low.	Adjust the analysis method so that the peak detection threshold associated with the size standard is greater than the height of the miscalled peak. See "Adjusting Peak Detection Thresholds" on page 29 for more information.
• SQ PQV displays ⚠ or 🛑 • Peaks are clear and distinguishable, but have low signal strength	Peak detection threshold associated with the size standard is set too high or low.	
	Electrophoresis or pipetting error	
• SQ PQV displays ⚠ or 🛑 • Size standard peaks occur within a primer peak	Insufficient cleanup step	Create and analyze the data using a custom size standard that does not include the undetectable peak. See "Correcting Miscalled Peaks" on page 28 for more information.
• SQ PQV displays ⚠ or 🛑 • Size standard peaks are clear and distinguishable, but consistently have low signal strength	Incorrect concentration of size standard in sample loading reagent	Increase the concentration of size standard added to subsequent runs.
	Incorrect injection settings	Review the injection settings of the run module for errors.
• SQ PQV displays ⚠ or 🛑 • Peaks are clear and distinguishable, but have low signal strength • Sizing failures occur in a regular pattern (the same wells fail repeatedly)	• Electrophoresis or pipetting error • Defective capillaries/arrays	See the user manual for your Applied Biosystems electrophoresis instrument for information on troubleshooting defective capillaries/arrays.
• SQ PQV displays ⚠ or 🛑 • Size calling errors occur for different samples on the same capillary over multiple runs	Defective capillary	

Correcting Miscalled Peaks

You can use the Size Match Editor to correct peaks that are miscalled by the GeneMapper Software.

To correct a miscalled size standard:

1. In the Navigation Pane of the Size Match Editor, select the sample file containing the miscalled peak.
2. Remove the label from the miscalled peak:
   a. Select the peak with the label by clicking inside the body of the peak.
   b. Select **Edit ▸ Delete Size Label** (or right-click the peak, then select **Delete**).
3. Apply the label to the correct peak:
   a. Select the correct peak.
   b. Select **Edit ▸ Add Size Label** (or right-click the peak, then select **Add**).
   c. In the Select Size dialog box, double-click the label to apply to the selected peak.
4. Click (**Tools ▸ Check Sizing Quality**) to verify that the sample sizes correctly.
5. Click **Apply** to save the changes, then click **OK**.

IMPORTANT! You must click Apply to reanalyze the software.

Note: Observe that the cell in the Status column for the sample now displays (Analysis Required).

6. Reanalyze the sample using the new setting to verify that the problem is resolved.

Adjusting Peak Detection Thresholds

You can resolve a significant number of sizing failures by adjusting the peak detection thresholds of the analysis method for a project. The software identifies peaks that exceed the threshold for each associated dye channel, but it cannot identify peaks that fall below it. Samples that exhibit low signal intensity (low peak heights) can occasionally fail sizing because one or more peaks fall below the threshold defined in the analysis method. By lowering the threshold of the appropriate dye channel, the software can call the peak(s) correctly.

In contrast, when the signals of the size standard peaks are very high, the software may misidentify a shoulder preceding a peak as the main peak (see Figure 1-2). Because the shoulder peak does not occur at the correct position relative to the other peaks, sizing fails. By adjusting the analysis method so that the threshold value is greater than the height of the shoulder, you can achieve good sizing.

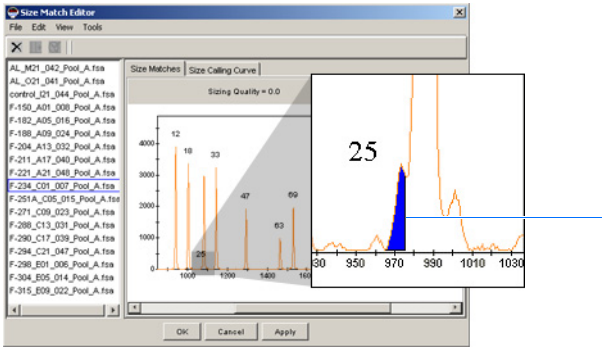


Figure 1-2 Size standard with shoulder incorrectly labeled as a peak

To lower the peak detection thresholds of an analysis method:

1. In the GeneMapper window, click (**Tools ▸ GeneMapper Manager**).
2. In the GeneMapper Manager, select the **Analysis Methods** tab.

3. Do one of the following, to:

   Modify the current analysis method, go to step 4.

   Create a copy of the current analysis method:

   a. Select the current analysis method.

   b. Click **Save As**.

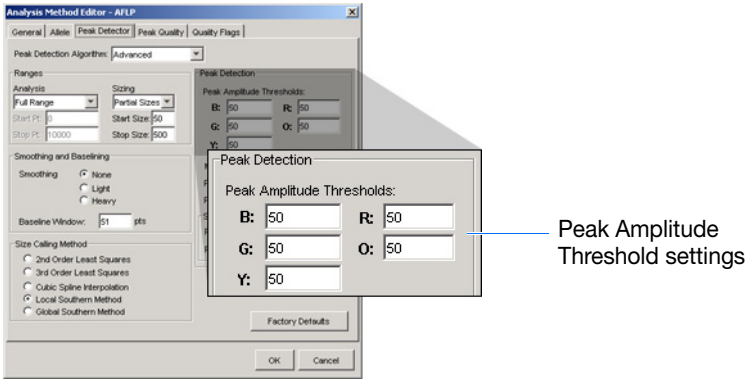
   c. In the Save As dialog box, type a name for the new method, then click **OK**.

4. Select the analysis method you want to modify, then click **Open**.

5. In the Analysis Method Editor, select the **Peak Detector** tab.

6. Modify the appropriate Peak Amplitude Threshold settings as needed. Ideally, you should set the threshold of the appropriate dye channel to a value less than the signal intensity of the shortest size standard peak.

   Note: Applied Biosystems recommends using Peak Amplitude Threshold settings of no less than 50 RFU.



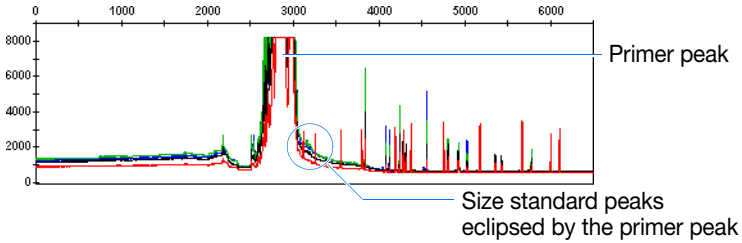
7. Click **OK** to save the analysis method.

8. Click **Done** to close the GeneMapper Manager, then reanalyze the samples using the new analysis method.

Customizing a Size Standard Definition

You can create a custom size standard definition to correct some problems that consistently cause samples to fail sizing. Examples of problems that you can resolve using custom size standards include a series of samples that fail sizing because:

- The primer peak prevents the software from detecting and sizing the peaks of the smaller size standard fragments



- A fragment of a custom size standard does not migrate as expected during electrophoresis

About GeneMapper Software Size Standards

Before the GeneMapper Software can size fragment analysis data, it must contain information about the size standard that was run with the samples. The size standard definition supplies the software with two pieces of information: the color of the dye associated with the size standard, and the sizes (in bp) of the fragments that comprise the size standard. Although the software provides definitions for all Applied Biosystems size standards, you may need to create your own definition if you choose to use a third-party standard, or experience difficulty analyzing your data.

To create a custom size standard definition:

1. In the GeneMapper window, click (**Tools ▸ GeneMapper Manager**).
2. In the GeneMapper Manager, select the **Size Standards** tab.
3. Click **New**.
4. In the Select Dye and Analysis Method dialog box, select **Basic** or **Advanced**, then click **OK**.

5. In the Size Standard Editor:
   a. In the Name field, type a name for the custom standard.
   b. Select **Size Standard Dye** ▸ *<appropriate dye>*.
   c. In the Size Standard table, type the size values for the size standard (press **Enter** after typing each value).

IMPORTANT! After typing the last value, you must press **Enter** to ensure the final value is included in the definition.

IMPORTANT! The values for the Analysis Range and Sizing Range defined in the Allele and Peak Detector tabs of the analysis method must match the peak range defined by the associated size standard.

6. Click **OK** to save the size standard.
7. Click **Done** to close the GeneMapper Manager.
8. In the Samples tab of the GeneMapper window, apply the new size standard to the samples of the project.
9. Reanalyze the sample using the new setting to verify that the problem has been resolved.

XTLK (Cross Talk)

Description/ Function

The XTLK PQV indicates that, at the peak position of the associated genotype, the ratio of the signals collected from the neighboring capillaries exceed the Cross-talk ratio setting in the Peak Quality tab of the analysis method.

Note: When the XTLK PQV is triggered, the software reduces the GQ PQV by 50% (the default multiplier is “0.5”).

Expected Values

(Pass) or (Check)

2 SNPlex™ System Troubleshooting

Chapter 1
Process Quality Values and Basic Troubleshooting

Chapter 2
SNPlex™ System Troubleshooting

Chapter 3
Algorithms

In this chapter:

Overview

Identifying Potential Problems

You can use tools in the GeneMapper® Software to identify potential problems at both the project and study levels of the SNPlex™ System analysis.

Identifying Potential Problems in SNPlex System Studies

Version 4.0 of the GeneMapper Software emphasizes the use of studies for analyzing data generated using the SNPlex™ Genotyping System Chemistries. You can use the tools of the Study Manager to maintain system-wide quality control and visualize potential problems in SNPlex System data. After you identify a potential problem, the software allows you to review the applicable run and resolve the issues that can be corrected.

Note: See the *GeneMapper® Software Version 4.0 SNPlex™ System Analysis Getting Started Guide* (PN 4363077) for a detailed explanation of the study management system.

Identifying Potential Problems in SNPlex Projects

SNPlex projects, like the other analyses supported by the GeneMapper Software, contain a variety of Process Quality Values (PQVs), which can aid you in identifying potential problems. Chapter 1 explains the functions of all PQVs relevant to the analysis of SNPlex System data.

Resolving Problems and Errors

The *SNPlex™ Genotyping System 48-Plex User Guide* (PN 4360856) describes how to resolve common chemistry- and software-related problems. The user guide addresses all aspects of the SNPlex System, not just those issues that are exclusive to the GeneMapper Software analysis.

3 Algorithms

Chapter 1
Process Quality Values and Basic Troubleshooting

Chapter 2
SNPlex™ System Troubleshooting

Chapter 3
Algorithms

In this chapter:

Genotyping Algorithms

Overview

This chapter discusses the following algorithms:

- **Peak Detection** – Uses the Basic, Advanced, or Classic mode algorithms to detect peaks and process data
- **Size-Matching/Calling** – Matches detected peaks to size standards
- **Binning** – Determines bin centers for genotyping
- **Allele-Calling** – Produces a consensus call based on several allele-calling algorithms

Data Flow

Figure 3-3 and Figure 3-4 on page 37 show the data flow in GeneMapper® Software. Standard signal processing is applied to the data before the data are delivered to the GeneMapper Software algorithms.

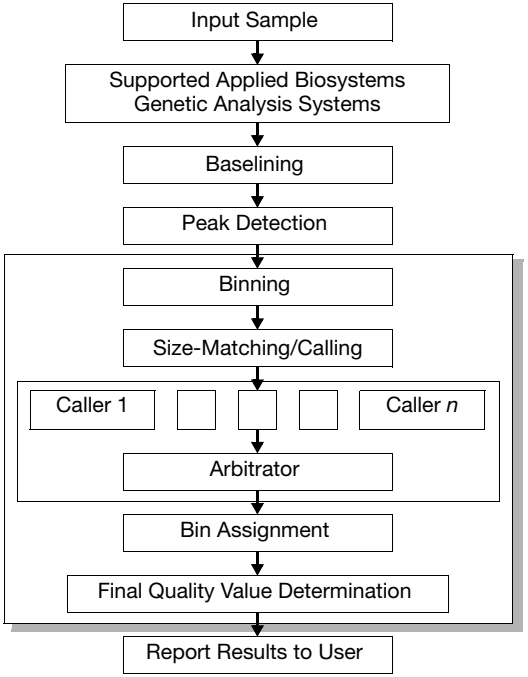


Figure 3-3 Microsatellite analysis data flow

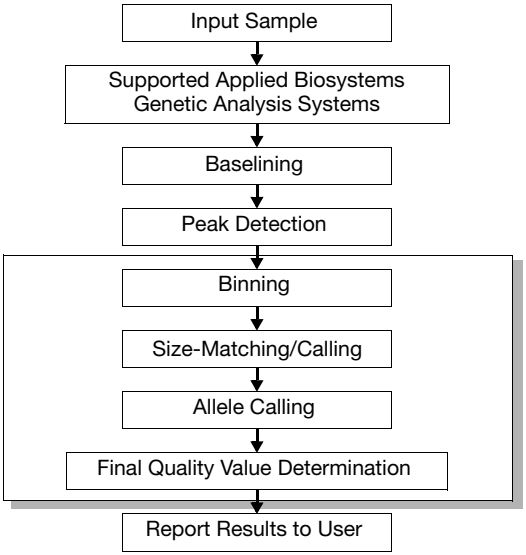


Figure 3-4 SNPlex™ System analysis data flow

Peak Detection

Polynomial Degree and Peak Window Size Parameters

Two peak-detection parameters are used in the polynomial detection algorithm: Polynomial Degree, and Peak Window Size.

Use the Polynomial Degree and the Peak Window Size settings to adjust the sensitivity of the peak detection. You can adjust these parameters to detect a single base pair difference while minimizing the detection of shoulder effects or noise.

Sensitivity increases with larger polynomial degree values and smaller window size values. Conversely, sensitivity decreases with smaller polynomial degree values and larger window size values.

How They Work

The peak window size functions with the polynomial degree to set the sensitivity of peak detection. The peak detector calculates the first derivative of a polynomial curve fitted to the data within a window that is centered on each data point in the analysis range.

Using curves with larger polynomial degree values allows the curve to more closely approximate the signal and, therefore, the peak detector captures more peak structure in the electropherogram.

The peak window size sets the width (in data points) of the window to which the polynomial curve is fitted to data. Higher peak window size values smooth out the polynomial curve, which limits the structure being detected. Smaller window size values allow a curve to better fit the underlying data.

How to Use the Peak Detection Parameters

Use the table below to adjust the sensitivity of detection.

Function	Polynomial Degree Value	Window Size Value
Increase sensitivity	Higher	Lower
Decrease sensitivity	Lower	Higher

Guidelines for Use

To detect well-isolated, baseline-resolved peaks, use polynomial degree values of 2 or 3. For finer control, use a degree value of 4 or greater.

As a guideline, set the peak window size (in data points) to be about 1 to 2 times the full width at half maximum height of the peaks that you want to detect.

Examining Peak Definitions

To examine how GeneMapper Software has defined a peak, select **View ▸ Show Peak Positions**. The peak positions, including the beginning, apex, and end of each peak, are tick-marked in the electropherogram.

Effects of Varying the Polynomial Degree

Figure 3-5 shows peaks detected with a window size of 15 data points and a polynomial curve of degree 2 (green), 3 (red), and 4 (black). The diamonds represent a detected peak using the respective polynomial curves.

Note that the smaller trailing peak is not detected using a degree of 2 (green). As the peak detection window is applied to each data point across the displayed region, a polynomial curve of degree 2 could not be fitted to the underlying data to detect its structure.

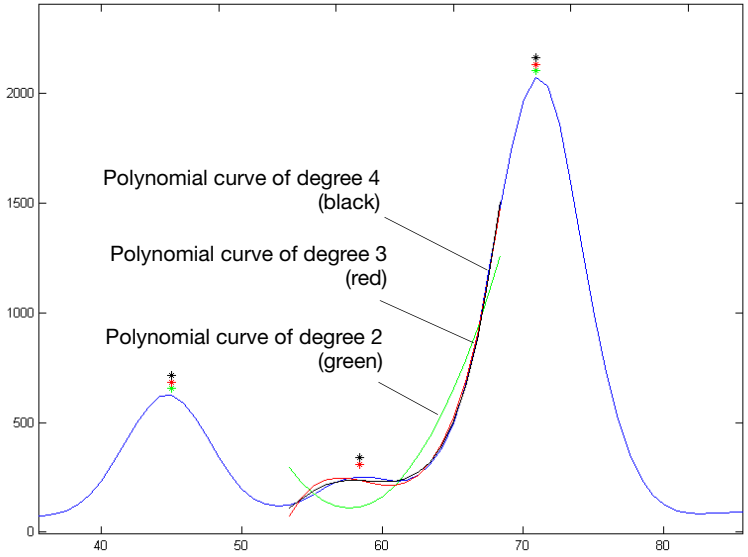


Figure 3-5 Electropherogram showing peaks detected with three different polynomial degrees

Effects of Increasing the Window Size Value

In Figure 3-6 both polynomial curves have a degree of 3 and the window size value was increased from 15 (red) to 31(black) data points.

As the cubic polynomial is stretched to fit the data in the larger window size, the polynomial curve becomes smoother. Note that the structure of the smaller trailing peak is no longer detected as a distinct peak from the adjacent larger peak to the right.

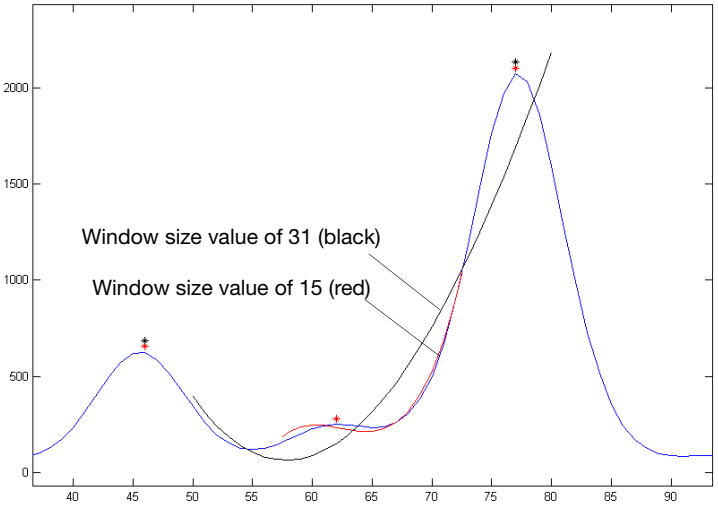


Figure 3-6 Electropherogram showing the same peaks as in the Figure 3-5 after increasing the window size value but keeping the polynomial degree the same

Optimizing Peak Detection Sensitivity

Examples

Example 1: Reducing Window Size

Initial Electropherogram

Figure 3-7 shows two resolved alleles of known fragment lengths (that differ by one nucleotide) detected as a single peak. The analysis was performed using a polynomial degree of 3 and a peak window size of 19 data points.

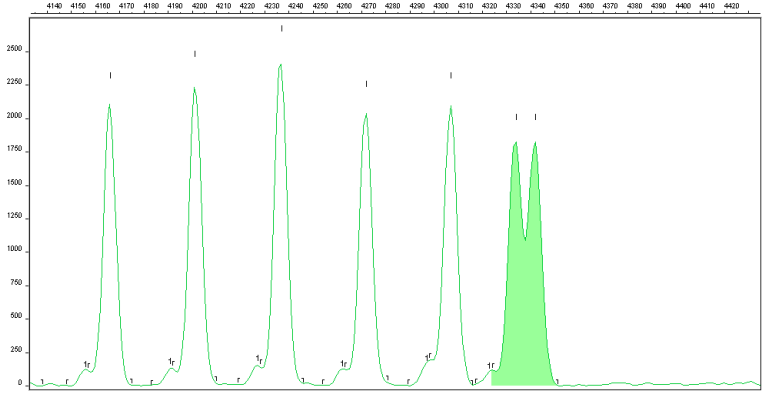


Figure 3-7 Electropherogram showing two resolved alleles detected as a single peak

Note: For information on the tick marks displayed in the electropherogram, see "Examining Peak Definitions" on page 39.

Effects of Reducing

Figure 3-8 shows that both alleles are detected after reanalyzing with the polynomial degree set to 3 and the window size value decreased to 15 (from 19) data points.

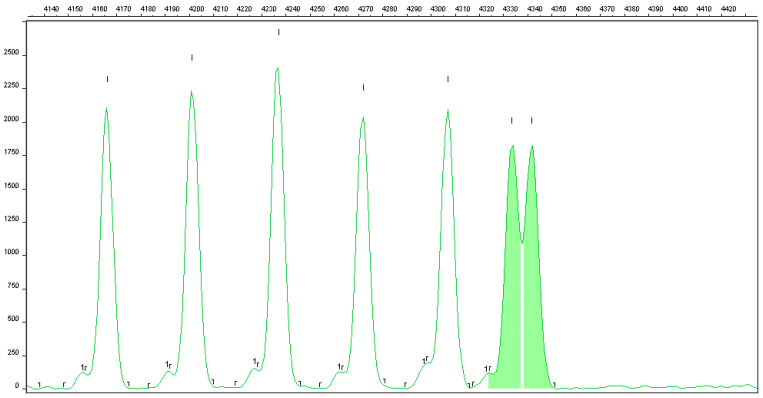


Figure 3-8 Electropherogram showing the alleles detected as two peaks after decreasing the window size value

Example 2: Reducing Window Size/Increasing Polynomial Degree

Initial Electropherogram

Figure 3-9 shows an analysis performed using a polynomial degree of 3 and a peak window size of 19 data points.

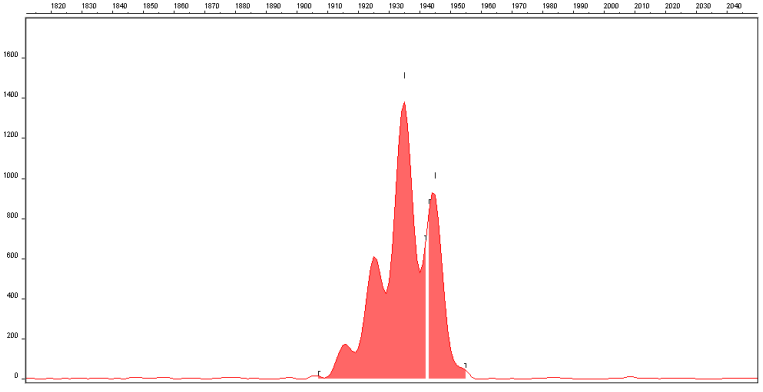


Figure 3-9 Electropherogram showing four resolved peaks detected as two peaks

Effects of Reducing the Window Size Value While Increasing the Polynomial Degree Value

Figure 3-10 shows the data presented in the figure above re-analyzed with a window size value of 10 and polynomial degree value of 5.

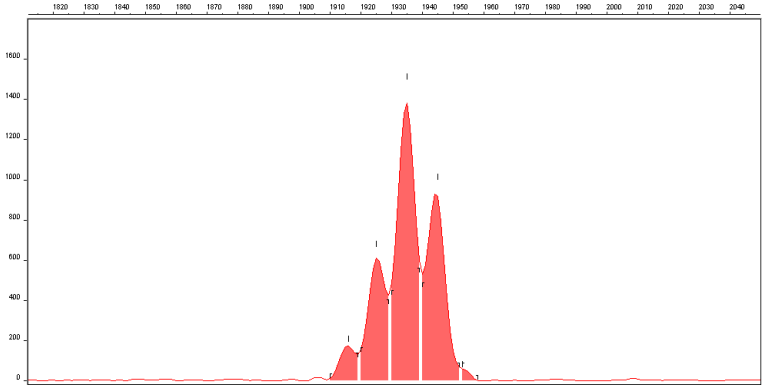


Figure 3-10 Electropherogram showing all four peaks detected after reducing the window size value and increasing the polynomial degree value

Example 3: Extreme Settings

Effects of Extreme Settings

Figure 3-11 shows the result of an analysis using a peak window size value set to 10 and a polynomial degree set to 9. These extreme settings for peak detection caused several peaks to be split and detected as two separate peaks.

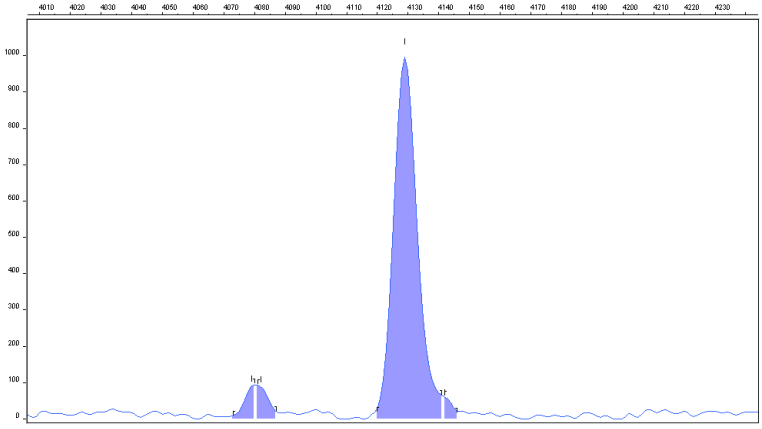


Figure 3-11 Electropherogram showing the result of an analysis using extreme setting for peak detection

Slope Thresholds for Peak Start/End Parameters

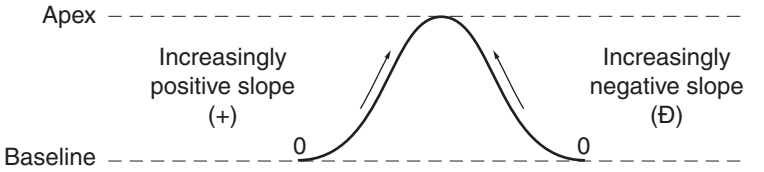
About These Parameters

Use the Slope Threshold for Peak Start and Slope Threshold for Peak End parameters to adjust the start and end points of a peak.

The values assigned to these parameters can be used to better position the start and end points of an asymmetrical peak, or a poorly resolved shouldering peak to more accurately reflect the peak position and area.

How These Parameters Work

In general, from left to right, the slope of a peak increases from the baseline up to the apex. From the apex down to the baseline, the slope becomes decreasingly negative until it returns to zero at the baseline.



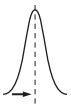
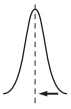
If either of the slope values you enter exceeds the slope of the peak being detected, the software overrides your value and reverts to zero.

Guidelines for Using These Parameters

- For typical or symmetrical peaks, use a value of zero.
- For asymmetrical peaks, select values other than zero to better reflect the beginning and end points.
- A value of zero does not affect the sizing accuracy or precision of an asymmetrical peak.

Using These Parameters

Note: The size of a detected peak is the calculated apex between the start and end points of a peak and does not change based on your settings.

To move the…	Then…	Example
Start point of a peak closer to its apex	Change the Slope Threshold for Peak Start value from zero to a positive number.	
End point of a peak closer to its apex	Change the Slope Threshold for Peak End value to a more negative number.	

Slope Threshold Example

Asymmetrical Peak

Initial Electropherogram

The initial analysis using a value of 0 for both the Slope Threshold for Peak Start and the Slope Threshold for Peak End produces an asymmetrical peak with a noticeable tail on the right side.

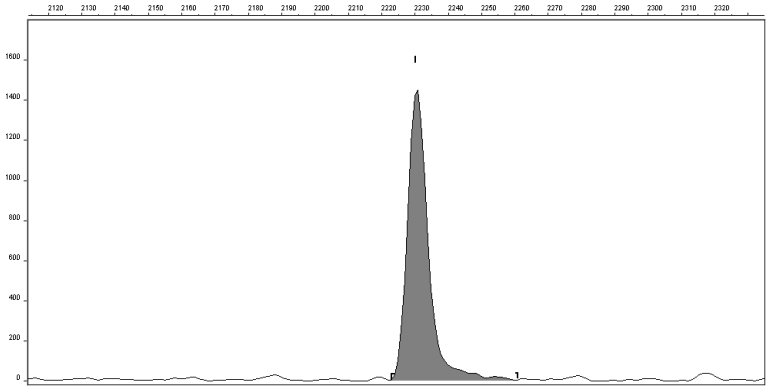


Figure 3-12 Electropherogram showing an asymmetrical peak

Adjusting Slope Threshold for Peak End

After reanalyzing with a value of –35.0 for the Slope Threshold for Peak End, the end point that defines the peak moves closer to its apex, thereby removing the tailing feature. Note that the only change to tabular data is the area (peak size and height are unchanged).

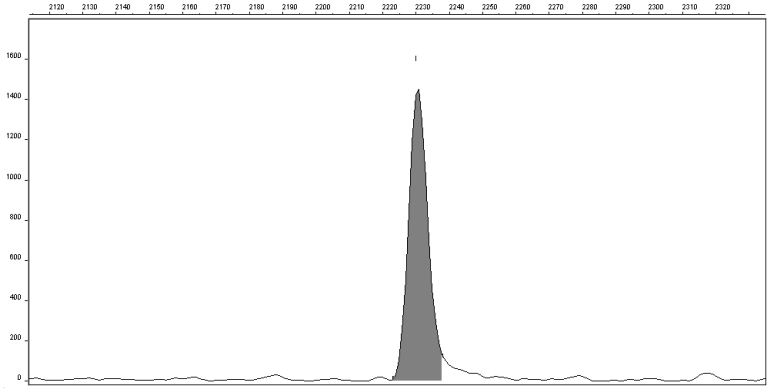


Figure 3-13 Electropherogram showing the effect of changing the slope threshold for peak end

Size-Matching/Size-Calling Algorithm

Size-Matching Size-Calling Algorithm

This algorithm uses a dynamic programming approach that is efficient (runs in low polynomial time and space) and guarantees an optimal solution. It first matches a list of peaks from the electropherogram to a list of fragment sizes from the size standard. It then derives quality values statistically by examining the similarity between the theoretical and actual distance between the fragments.

Size-Matching Algorithm Example

Figure 3-14 shows an example of how the size-matching/calling algorithm works using contaminated GeneScan™ 120 size standard data.

Detected peaks (standard and contamination) are indicated by blue lower bars along the x-axis. The size standard fragments as determined by the algorithm (and their corresponding lengths in base pairs) are designated by the upper green bars. Note that there are more peaks than size standard locations because the standard was purposely contaminated to test the algorithm. The algorithm correctly identifies all the size standard peaks and removes the contamination peaks (indicated by the black triangles) from consideration. The large peak is excluded from the candidate list by a filter that identifies the peak as being atypical with respect to the other peaks.

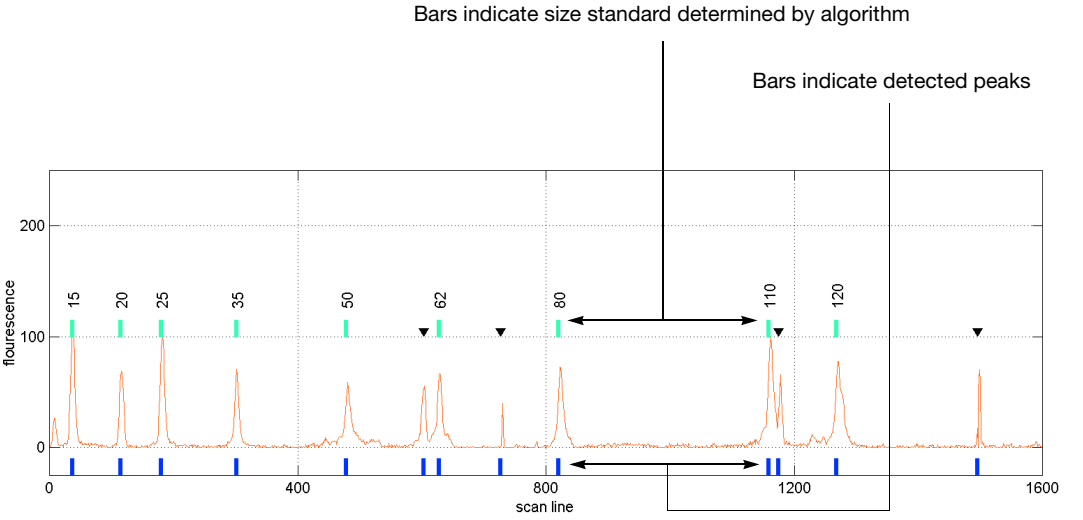


Figure 3-14 Size-matching example

Size-Calling Methods (Classic and Advanced Modes)

Types of Size Calling Methods

The GeneMapper Software provides the following size calling methods:

Least Squares Method

Overview

Both Least Squares methods (2nd-Order and 3rd-Order) use regression analysis to build a best-fit size calling curve. This curve compensates for any fragments that may run anomalously. Consequently, this method typically results in the least amount of deviation 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 or a cubic function. The software uses the known standard fragments and the associated data points to produce a sizing curve based on Multiple Linear Regression.

Advantages

Figures 3-15 and 3-16 on page 48 show that in nearly all instances 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.

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

Note: The graphs in this section were generated using Version 3.5.1 of the GeneScan® Software. The results are similar to those obtained when you use the GeneMapper Software.

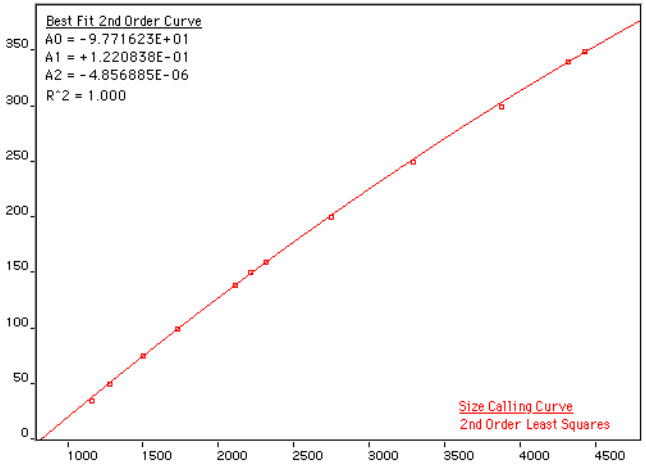


Figure 3-15 2nd-Order Least Squares size calling curve

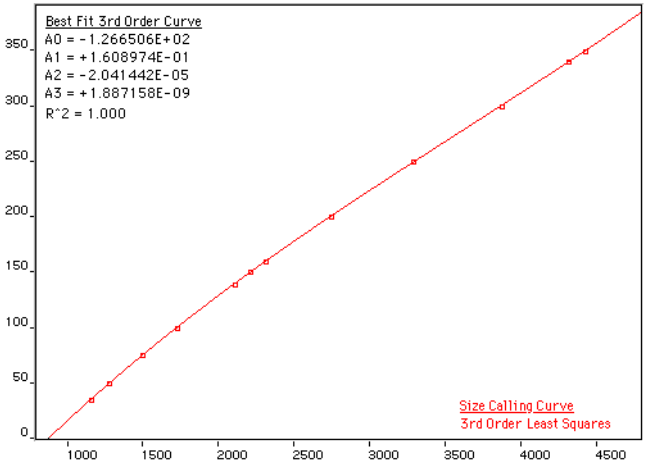


Figure 3-16 3rd-Order Least Squares size calling curve

Cubic Spline Interpolation Method

Overview

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

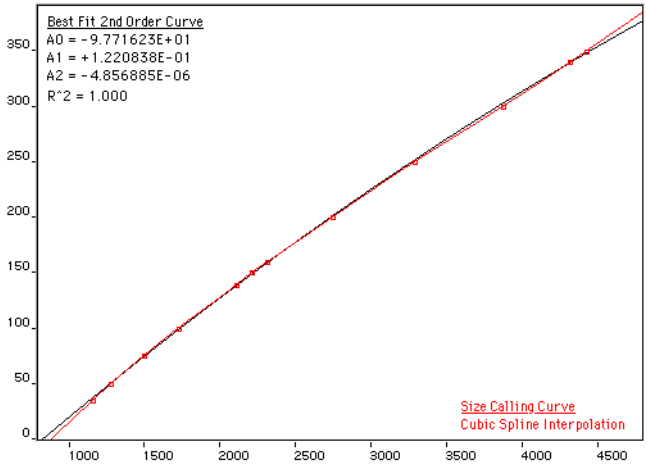


Figure 3-17 Cubic Spline Interpolation Method

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

Overview

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).

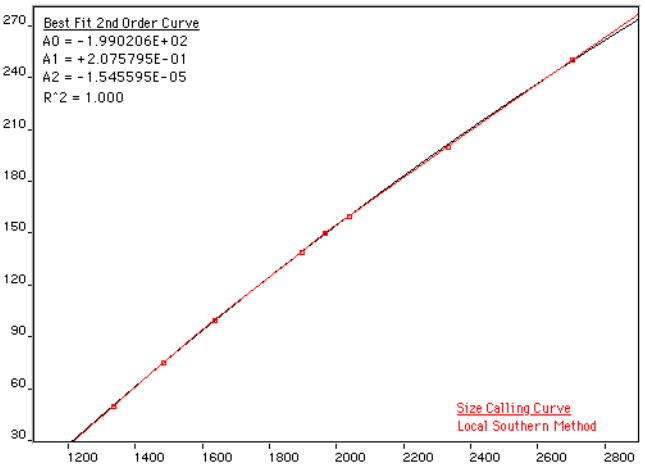


Figure 3-18 Local Southern Method

Local Southern Method Equation

The 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 The Local Southern 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. Only the region of the size ladder near the fragment of unknown length is analyzed.

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

In the Local Southern method:

- 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.
- A curve is then created by using three standard points (two points below and one point above the fragment), then a fragment size is determined.
- Another curve is created by looking at an additional set of three points (one point below and two points above the fragment), then another value is assigned.
- The two size values are averaged to determine the unknown fragment length.

Global Southern Method

Overview

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 on that line to calculate the fragment values.

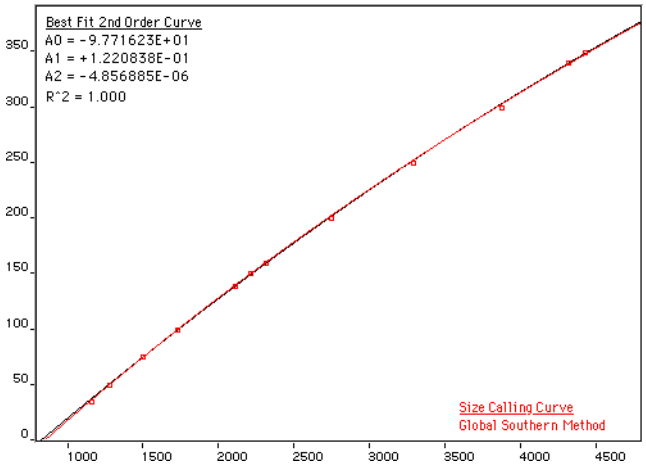


Figure 3-19 Global Southern Method

Global Southern Method Equations

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 left side quantity.

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

Allele-Calling Algorithms

Overview

Final allele calls are based on a consensus between a variety of different allele-calling algorithms. Each caller has a different design philosophy such that it excels in a particular data regime but not in others. Allele-calling algorithms involve envelope detection, optimization of parametric models, and rule-based systems.

Types of Allele-Calling Methods

The GeneMapper Software provides following allele-calling methods:

Microsatellite Analysis Methods

Example Output of Different Allele-Calling Algorithms

Figure 3-20 on page 54 shows an example of three different allele-calling algorithms for 16 samples. User annotations are indicated by the (red) circles, and allele caller outputs are indicated by the (green, black, and blue) asterisks. Note that consensus between multiple callers virtually ensures that the calls are correct. In samples (i) and (p), the algorithms have not made a call because they determined that the data are too complex to act on. Here the blue asterisks show the calls transmitted to the user. Low-quality values are reported because in both cases the first algorithm did not call, and in (i), the black caller does not agree with the blue. Despite these conditions, however, the calls are correct. The low-quality values alert the user to potential problems such as the spurious peak in (i) and the high background in (p).

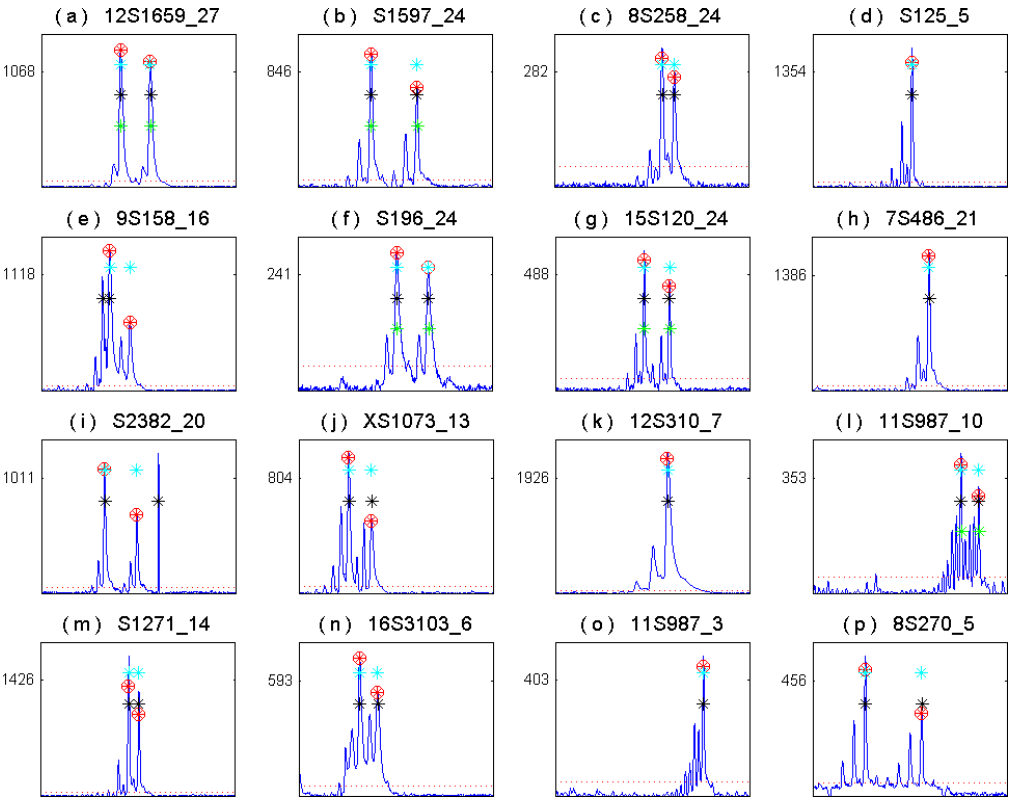


Figure 3-20 The effect of three different allele-calling algorithms on 16 different samples

SNPlex™ System Analysis Methods

Overview

The GeneMapper Software provides the following allele-calling methods for SNPlex System Analysis:

Rules Genotyping Algorithm

The Rules (Maximum-Likelihood or ML) SNP genotyping algorithm consists of several processes: genotype cluster identification, sample cluster classification, and confidence value assignment.

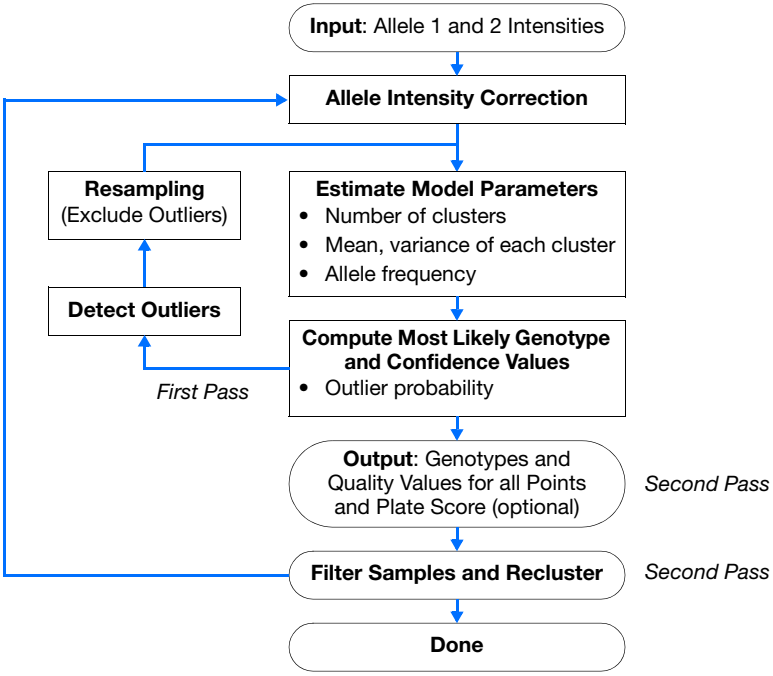


Figure 3-21 Rules algorithm block diagram

Because of small systematic differences in assay performance between the alleles in a SNP, fixed classification boundaries between the clusters do not give accurate results for most, if not all, current SNP detection platforms. Clustering, like all calibration methods, can correct for systematic errors, but not random errors. Random errors are estimated and assigned appropriate p-values.

Genotype Cluster Identification

The cluster identification stage uses a priori knowledge of the intensity relationship and frequency distribution of SNP alleles to construct a most likely model of the SNP assays generating in a given data set. Systematic parameters such as allele assay strength and other chemistry factors are estimated. If the most likely model fit is too poor, the entire data set is rejected.

Sample Cluster Classification and Confidence Value Assignment

The best fit model is used to classify each point into its most likely class, or genotype. The likelihood of class membership (genotype) can be derived in the same step.

Post Processing, Filtering, and Reiterations

Genotype and sample filters, bootstrap resampling, and several reiterations are used to ensure the accuracy of the model fit and classifications.

Model Genotyping Algorithm

The Model genotyping algorithm consists of several processes: normalization, allele calling, and confidence value generation.

Normalization

Normalization is a run-based algorithm that removes systematic variation of peak height by allele and sample. This process is accomplished by comparing the data to a model in which the sum of allele peaks for any given sample is a constant. Over many repeated measurements of a given sample or allele, systematic variations from this a priori model can be quantified and later removed. In the process of normalization, an overall scale factor can be selected, which can be set to one. This utility is demonstrated in Figure 3-22 on page 57 where the output from 20 runs can be overlaid to understand the random, as opposed to systematic, behavior of signals. For each run $2 \times 48 \times 96 = 9216$ measurements were normalized with 193 parameters, or approximately one parameter for each 48 measurements.

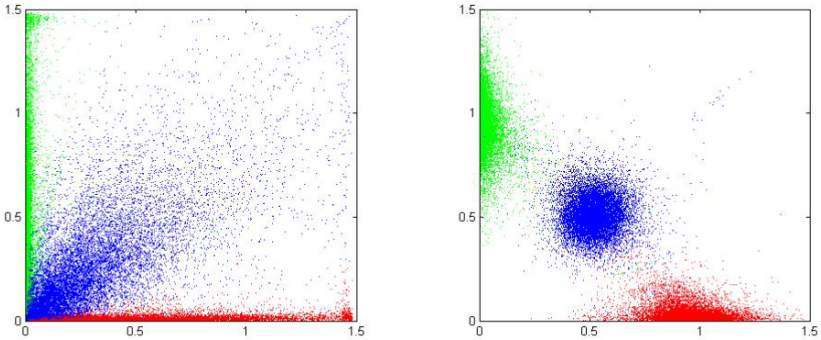


Figure 3-22 Pre- and post- normalization data. Shown are 80,000 measurements of SNPlex System data overlaid in normalized allele coordinates. In the left plot, data are normalized only by run. In the right, the data are fully normalized by sample and allele.

Genotyping

The random error remaining in the data after normalization is relatively small when compared with the separation of the genotype coordinates, as seen in Figure 3-23. Each measurement is assigned the genotype for which it has the highest probability.

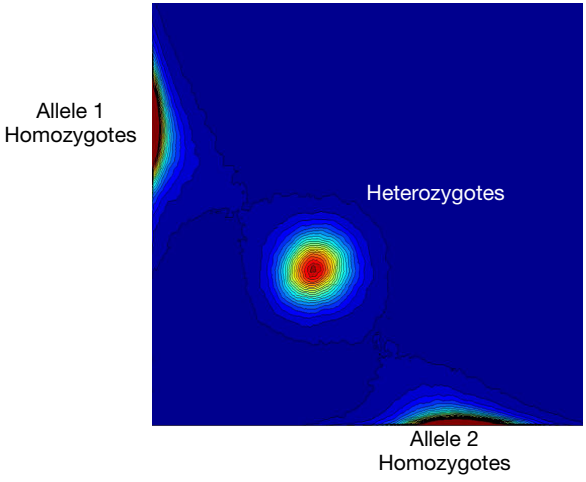


Figure 3-23 Training data distribution

Final Genotyping and Confidence Value Assignment

The confidence value is a quantitative estimate of the probability of a correct call (that is one minus the estimated error rate). This single parameter can be used to optimize the trade-off between throughput and accuracy for a particular data set, as shown in Figure 3-24.

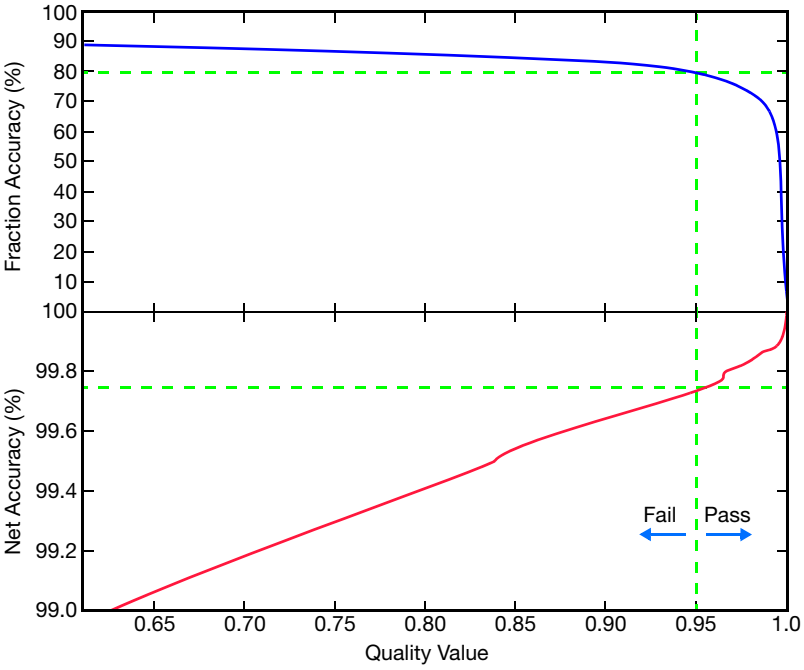


Figure 3-24 Accuracy-throughput trade off

Glossary

ABB (Automatic Bin Builder)	The first step in accurate allele assignment. After sample files are collected, bins are created by the ABB based on the chosen panel information and successive allele calls from sample file collection. As each sample file in the collection is processed, the bin definitions are refined to reflect the actual data.
access control list	(*See* Security Group)
Admin profile	A pre-configured profile that cannot be removed and that has execute access to all functions. Initially associated with the "admin" user. (A user must always have an assigned profile.)
Admin security group	A pre-configured security group that cannot be removed. This security group has been granted all rights to all data, to provide a way for at least one user to have "admin" access to all data.
Admin User Group	A pre-configured user group that cannot be removed and that is always associated with the Admin security group.
Administrator User	A pre-configured user that cannot be removed and that is always associated with the Admin user group.
AFLP	Amplified Fragment Length Polymorphism. A DNA fingerprinting technique that allows the comparison of the DNA from different organisms. DNA fragments of varying lengths are created by cleaving an organism's DNA with restriction enzymes; a specific subset of these fragments are amplified and analyzed for comparison purposes.

Algorithm A set of ordered steps for solving a problem, such as a mathematical formula or the instructions in a program. The terms algorithm and logic are synonymous, where both refer to a sequence of steps to solve a problem. However, an algorithm is an expression that solves a complex problem rather than the overall input-process-output logic of typical business programs.

All User group A user group that contains all users. A user cannot be disassociated from the user group.

allele One of two or more alternate forms of a marker or gene.

Reference alleles are all alleles or bins created in a bin set in the GeneMapper Software. They are denoted by a red cross hatch + in the Panel Manager.

Project alleles are all alleles detected in sample data in a project in the GeneMapper Software. They are denoted by a blue asterisk in the Panel Manager.

allele call Identification of the specific allelic form of a marker.

allele-calling Identification of alleles based on bin definitions; genotyping; GeneMapper® Software analysis

allelic ladder A sample of DNA containing most possible alleles for a specific marker or set of markers. Used to create a sample file that the GeneMapper Software can use to genotype or make allele calls on sample data.

Within the GeneMapper Software, you select a Sample Type of Allelic Ladder for the sample file generated using an allelic ladder.

analysis method A collection of user-defined parameters that determine the bin set and analysis algorithms.

analysis parameters A collection of user-defined settings (including analysis method, size standard, and panel) that determine the sizing and genotyping algorithms used by the GeneMapper Software to analyze all sample files in a project. Also called project settings.

association	Two identifiers combined are said to be associated. A user can be associated with a user group. A user group associated with a security group yields a set of data rights.
audit event	A single permanent change to one or more attributes of an object. Includes creating a new instance of an object or deleting an exiting one.
audit map	An object associated with an object type; used to tell the audit component how to audit an object type.
audit object	A collection of data defined by an application. Also referred to as an object.
audit record	The description of a single audit event.
autopanelizer	A feature that uses reference data generated by the Primer Focus kit to quickly define new SNP markers and bin sets.
bin	Within the GeneMapper Software, a fragment size (bp) or basepair range and dye color that define an allele within a marker. You create a bin for each possible allele associated with a marker.
bin set	Within the GeneMapper Software, a collection of bins (allele definitions), typically specific to a set of experimental conditions, usually an instrument. Bin sets are available inside a kit.
cache	An “in memory” representation of the access control data. The Admin Tool modifies the data in the cache. When the Admin Tool or Admin API issues the “save” command, the data in the cache are written to the data store.
challenge	A term from user authentication indicating that the user is asked to provide identification (typically by entering a password).
chromosome	A long stretch of coiled strands of DNA and proteins containing many genes. Human DNA is contained within 23 pairs of chromosomes.

control — See "positive control" on page 64.

See "negative control" on page 64.

Control Security Group — The security group assigned to an Access Control administrative identifier (user, user group, security group, profile). This security group is used to determine access by a user to the administrative data in the Administrative GUI and API.

data access control — The part of access control that administers access to user data.

Data group — (*See* Security Group)

data rights — Properties that define the type of access a user has to a piece of data.

database — One form of offline storage.

diploid — Having two sets of chromosomes and, therefore, having two alleles per marker or gene. Human cells (other than egg and sperm cells) are diploid.

dye set — A set of four to five different colored dyes. A specific dye set is used to label DNA fragments or markers in matrix standards, installation standards, and chemistry kits.

electropherogram — A graphical representation of the intensity (y-axis) of bands produced in a single gel lane or capillary as a function of time (x-axis).

electrophoresis — A technique used to separate molecules by using an electric field to pass those molecules through a porous matrix.

gene — The basic unit of heredity that carries the genetic information for a given RNA molecule or protein.

genome — All the DNA contained in an organism or cell, including both the chromosomes in the nucleus and the DNA in the mitochondria.

genotype — The set of allele calls for specific markers or genes within an organism. (noun)

To determine the allele calls for specific markers or genes within an organism. (verb)

haploid	Having one set of chromosomes and, therefore, having one allele per marker or gene. Human egg and sperm cells are haploid.
heterozygous	Having two different alleles for a specific marker or gene.
homozygous	Having two identical alleles for a specific marker or gene.
installation standard	A collection of known genetic markers, labeled with dyes from a specific dye set, used to test the function of a genetic analyzer.
kit	Within the GeneMapper Software, a group of panels.
LMS	Linkage Mapping Set; Applied Biosystems chemistry using dinucleotide repeat microsatellite markers
locus	The chromosomal location of a genetic marker or gene.
marker	A known segment of DNA that has two or more allelic forms. A marker exists at a known chromosomal loci and can be a gene or a non-gene. See also microsatellite and SNP. Within the GeneMapper Software: A microsatellite marker is defined by a name, fragment size range (bp), dye color, and repeat length. A SNaPshot kit analysis marker is defined by a name and fragment size range (bp).
matrix standard	A collection of known DNA fragments, labeled with four to five different colored dyes from a specific dye set. The matrix standard is run on a genetic analyzer and used for spectral calibration of sample fragments that are run on the same instrument and labeled with the same dye set.
microsatellite	Microsatellite markers, also known as short tandem repeats (STRs), are polymorphic DNA loci consisting of a repeated nucleotide sequence. The repeat sequence can be from 2 to 7 base pairs long. The number of repeat units varies within a population, thereby creating multiple alleles for a microsatellite locus.

negative control A blank sample that contains no DNA, but all other reagents used in the experiment. It can indicate if any contamination came from sample preparation.

In the Sample tab of GeneMapper Software, you select a Sample Type of Negative Control for the sample file generated from the negative control. Additionally, in the Panel Manager you define the negative control when creating markers.

panel A group of markers. Within the GeneMapper Software, you associate a panel with a bin set to provide bin definitions for the markers.

phenotype The physical manifestation of a genotype.

polymorphism Differences between organisms' or individuals' DNA. Variations of allele calls.

positive control A sample that contains DNA with known alleles for specific markers. Its purpose is to verify that the PCR amplification, electrophoresis, and GeneMapper Software analysis worked correctly.

In the Sample tab of GeneMapper Software, you select a Sample Type of Positive Control for the sample file generated from the positive control. Additionally, in the Panel Manager you define the positive control when creating markers.

primer A single-stranded piece of DNA or RNA that anneals to a complementary section of DNA and serves as a starting point for chain extension by DNA polymerase.

primer focus Within the GeneMapper Software, you select a Sample Type of Primer Focus for the sample file generated using the Primer Focus kit. The GeneMapper Software uses this file to automatically create bins using the Auto Panel feature.

Primer Focus® kit An Applied Biosystems kit containing reagents used to create and amplify all four possible alleles of any SNP marker. The kit allows you to take advantage of the Auto Panel feature in the GeneMapper Software to automatically create bins for each allele in a SNaPshot kit analysis.

probe	A DNA or RNA fragment that has been labeled in some way (for example, fluorescent or radioactive), then used in a molecular hybridization assay to identify DNA or RNA sequences that are the same or closely related to it in sequence.
profile	An identifier that gives an administrator the ability to grant or revoke access to functions.
project	Within the GeneMapper Software, a collection of sample files and the analysis parameters for genotyping them.
project settings	See "analysis parameters" on page 60.
project settings	Parameters set by the user to prepare a project for analysis.
reference samples	All or a subset of the actual samples. The reference samples typically contain all of the alleles present in the sample set and are used to create a bin for each allele within the GeneMapper Software.
rights	Properties that define whether a user has access to data or a function.
Security group	An identifier that can be associated with a user group to confer a set of data rights.
Security ID	The universal identifier of the security group and the preferred name of the column in an application table that holds the security group ID.
SFNF	Sample File Not Found
silent auditing	Automatic audit record creation (without prompting of the user).
size match editor	A window in GeneMapper® Software that allows users to examine size-standard electropherograms, edit the identification of size-standard peaks, and view the size-calling curve.
size standard	A collection of DNA fragments of known lengths within a range (for example, 50 to 400 bp) all tagged with the same dye. The size standard is co-injected into the genetic analyzer capillary with the sample, then used to size the sample data. All Applied Biosystems size standards are labeled with a red or orange dye.

SNaPshot® kit	An Applied Biosystems kit containing reagents used to PCR amplify any SNP markers, using single-base-extension technology. Sample files can then be sized and genotyped by using a SNaPshot analysis in the GeneMapper Software.
SNaPshot® System Multiplex Analysis	Primer extension-based chemistry for SNP validation
SNP	Single Nucleotide Polymorphism. A marker consisting of a single base pair that varies, thereby creating up to four alleles of the marker. In this document, SNP refers to SNaPshot® system markers and SNPlex™ systems
SNP	Single-Nucleotide Polymorphism (used)
SNPlex™ System	High-throughput assay for genotyping.

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Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com**.

Applera is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

06/2005

Applied Biosystems

Part Number 4366831 Rev. A