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Investigator™ Template Files For GeneMapper® ID User Guide

For analysis of Investigator Human Identification PCR Kits with GeneMapper ID Software

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Introduction

About this user guide

This user guide provides information about the functions and features of Investigator Template Files used in conjunction with GeneMapper ID Software. Please refer to the *GeneMapper ID Software User Guide* for complete information.

This user guide describes the features of the software and associated tools and enables the user to manage and modify files and analyses.

This user guide provides information about GeneMapper ID Software in the following sections:

- Introduction
- Template Files for Investigator Human Identification PCR Kits
- Setup on the Analysis Computer
- Calibration Using Allelic Ladders
- Evaluation of Analysis Data
- Checking Analysis Data
- Print Options and Page Setup
- Troubleshooting Guide

Throughout this document, analysis of PCR products generated using the Investigator ESSplex Kit, ABI PRISM® 3130 Genetic Analyzer, and DNA Size Standard 550 (BTO) are shown as an example. Analyses were performed using GeneMapper ID Software version 3.2.1 and Investigator ESSplex Template Files.

About Investigator Template Files and GeneMapper ID Software

GeneMapper ID Software is a flexible genotyping software package that provides DNA sizing and quality allele calls for all Applied Biosystems® electrophoresis-based genotyping systems. This software specializes in multi-application functionality, including amplified fragment length polymorphism (AFLP®), loss of heterozygosity (LOH), microsatellite, and single-nucleotide polymorphism (SNP) genotyping analysis. GeneMapper ID Software can help users increase data processing efficiency. The software uses process quality values (PQVs) for automated identification that reduces data review time for high-throughput genotyping.

GeneMapper ID Software combines the features of GeneScan® and Genotyper® software. It is specifically designed for human identification applications and enables highly accurate allele calls, including analysis of tri-, tetra-, penta-, and hexa-nucleotide repeat motifs.

Investigator Template Files are software sets for the GeneMapper ID Software in order to simplify data analysis. Investigator Template Files may be used with ABI PRISM® single- and multi-capillary instruments of Applied Biosystems.

GeneMapper ID Software (used in conjunction with Investigator Template Files) assigns the analyzed DNA fragments relative to their length to the allele designation of the short tandem repeat (STR) loci. Optionally, the corresponding fragment length of peaks in base pairs (bp) or peak height in relative fluorescent units (RFU) can be indicated. These data (genotypes) can be tabulated and exported.

Investigator Template Files are available for all Investigator Human Identification PCR Kits for GeneMapper ID Software version 3.1.

Validity for human identification products

Investigator Human Identification PCR Kits require calibration with an allelic ladder. Therefore, the software used must be compatible with human identification (HID) products for forensic applications. Investigator Template Files are valid with the GeneMapper ID Software.

About Investigator IDproof Software and Investigator IDproof Mixture Software

An alternative to the GeneMapper ID Software is Investigator IDproof Software or Investigator IDproof Mixture Software, which do not require the use of template files.

Investigator IDproof Software has been developed specifically for the analysis of kinship case samples, population studies, and chimerism monitoring. It is used to analyze complex studies ranging from raw data analysis to statistical computation.

Investigator IDproof Mixture Software has been developed specifically for the evaluation of mixed samples. It provides the necessary functions to analyze complex DNA mixtures ranging from the analysis of raw data to biostatistical calculations based on the guidelines of the International Society for Forensic Genetics (ISFG) and the German DNA Profiling Group.

Both softwares provide optimized size calling and allele calling algorithms and supports the automated analysis of commercially available and in-house designed multiplex PCR for both autosomal and gonosomal markers. They support the analysis of raw data generated by ABI equipment and software. In addition, they provides extensive quality assurance information, such as artifact identification (e.g. stutter peaks) and contamination inquiries.

For more information about Investigator IDproof Software and Investigator IDproof Mixture Software, visit www.qiagen.com/IDproof or www.qiagen.com/IDproofMixture.

Template Files for Investigator Human Identification PCR Kits

This section is divided into the following subsections:

Size standards

- SST-BTO_60-500bp
- SST-ROX_50-500bp

Panels and BinSets

- Panels files are specific for each kit (e.g., ESSplex_Panels_v1)
- BinSet files are specific for each kit (e.g., ESSplex_Bins_v1)

Analysis methods

- Analysis_HID_310
- Analysis_HID_3130
- Analysis_HID_310_50rfu
- Analysis_HID_3130_50rfu

Plot settings

- Plots_2dyes
- Plots_3dyes
- Plots_4dyes
- PlotsBT5_4dyes
- Plots_5dyes

Table settings

- Table for 2 Alleles
- Table for 10 Alleles

Note: Panels and BinSets are essential for the use of Investigator Human Identification PCR Kits. Use of additional Investigator Template Files is optional.

Size standards

SST-BTO

To check the correct assignment of the labels to the sample, click the color panel of the size standard (orange icon) in the upper toolbar of the GeneMapper ID Software. The orange panels (DNA Size Standard) of all samples are then displayed.

Compare the sample fragments sizes with the sizes of the DNA size standard 550 (BTO), which should be: 60, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 250, 260, 280, 300, 320, 340, 360, 380, 400, 425, 450, 475, 500, 525, and 550 bp.

If sizes differ, further analysis should be performed using GeneMapper ID Software. If necessary, create a new size standard definition within the GeneMapper ID Software, see next section.

Adjustment of the basic template

The basic template, SST-BTO_60-500 bp, defines all fragments from 60 bp to 500 bp (Figure 1). If, for example, only 475 bp are necessary for analysis of a particular test kit, define fragment length only up to 475 bp. The new template may be saved as, (for example) "SST-BTO_60-475bp" and used for further analyses.

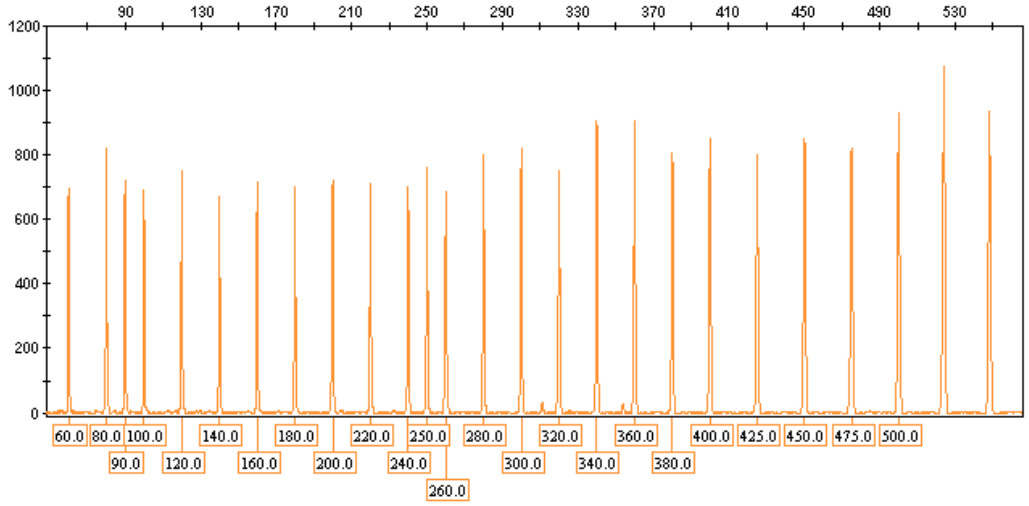


Figure 1. Electropherogram of the DNA size standard 550 (BTO). Fragment lengths are shown in base pairs.

Definition of a new size standard

To define a new size standard, perform the following steps:

1. **Go to "Tools" and choose "GeneMapper Manager".**
2. **Select the Size Standards tab.**
3. **Choose "New" and select "Advanced".**
4. **Assign a name (e.g., SST-BTO_60–500bp) and define the size range on the lines of the analysis range of the corresponding test kit.**
   For further information, see the "*GeneMapper ID Software User Guide*".

SST-ROX

For Investigator Test Kits in 4 Color Assay with the fluorescent labels 6-FAM®, HEX™, NED™, and ROX™ (matrix DS-30), the DNA Size Standard 550 (ROX) in the red panel is necessary. Use DNA Size Standard 550 (ROX) template SST-ROX_50-500bp to define fragment lengths for this test kit.

Panels and BinSets

Panels

The following features of the test kit's markers (short tandem repeat [STR]-loci) are shown in Figure 2:

- Marker name (STR Locus)
- Dye color
- Minimum and maximum size (upper and lower allelic range in bp)
- Control alleles (alleles of the control DNA)
- Marker repeat (units of the repeats in bp)
- Marker-specific stutter Ratio ("Stutter filter", 0.13 corresponds to 13% of the marker's peak height)
- Comments
- Ladder alleles (alleles of the allelic ladder)

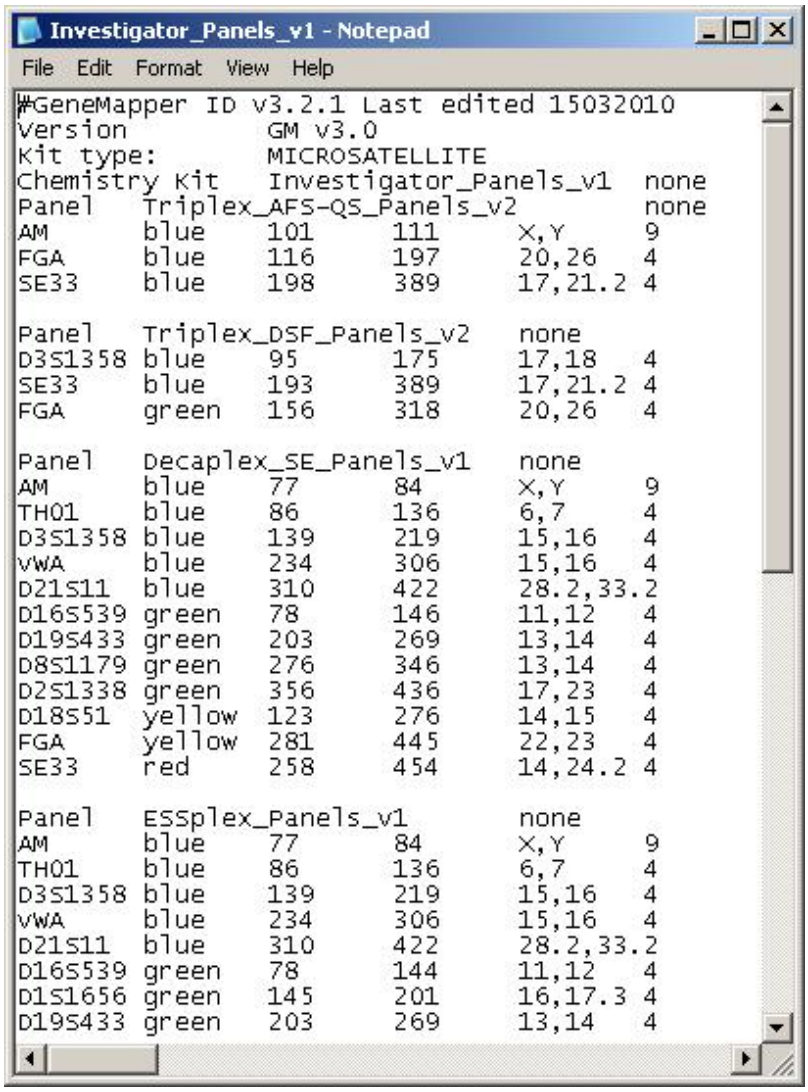


Figure 2. Panel structure.

BinSets

The following features of the allelic designation are shown in Figure 3

- Name (number of the allele)
- Length of fragments (in bp)
- Upper and lower range of tolerance (in bp)

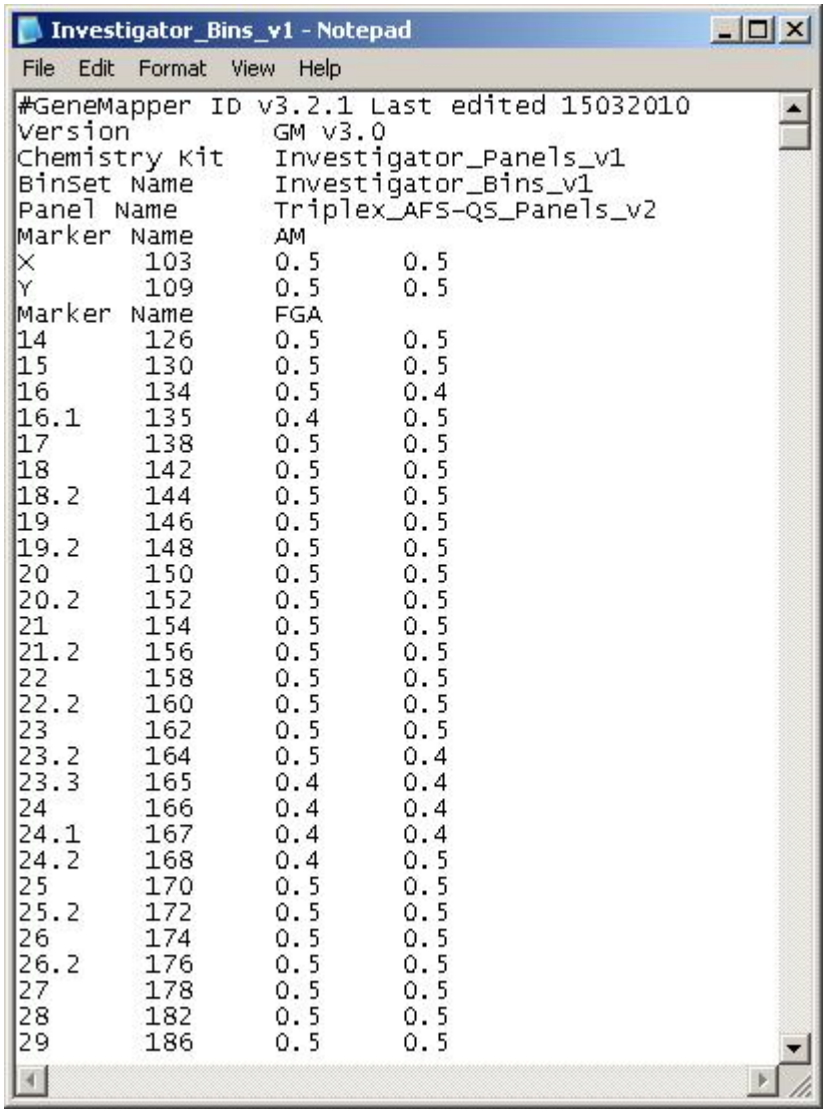


Figure 3. Structure of a BinSet.

Analysis methods

Different analysis methods are available depending on whether an ABI PRISM single-capillary (e.g., the ABI 310) or a multi-capillary (e.g., ABI 3130) instrument is used.

The choice of the analysis method may have a significant impact on the quality of the analysis. Many problems occurring in connection with the import or analysis of raw data can be solved by adjusting the analysis method.

- **Analysis_HID_310**: HID analysis method with 20% cut-off filter for samples from a single source
- **Analysis_HID_310_50rfu**: Sensitive HID analysis method for stains and DNA mixtures
- **Analysis_HID_3130**: HID analysis method with 20% cut-off filter for samples from a single source
- **Analysis_HID_3130_50rfu**: Sensitive HID analysis method for stains and DNA mixtures

Note: This guide focuses on the HID_3130 and HID_3130_50rfu examples.

HID analysis method with 20% cut-off filter for samples from a single source

Analysis_HID_3130

- For analysis of samples from a single source; unsuitable for DNA mixtures
- Label peaks according to filter values used. Peaks will be designated with the appropriate allele
- Filter value pre-selection: Labels lower then 20% of the highest peak height of a marker (STR-locus) will not be displayed (cut-off filter)
- In plot-window (Sample Plot), the labeled color panels are displayed (Figure 4)

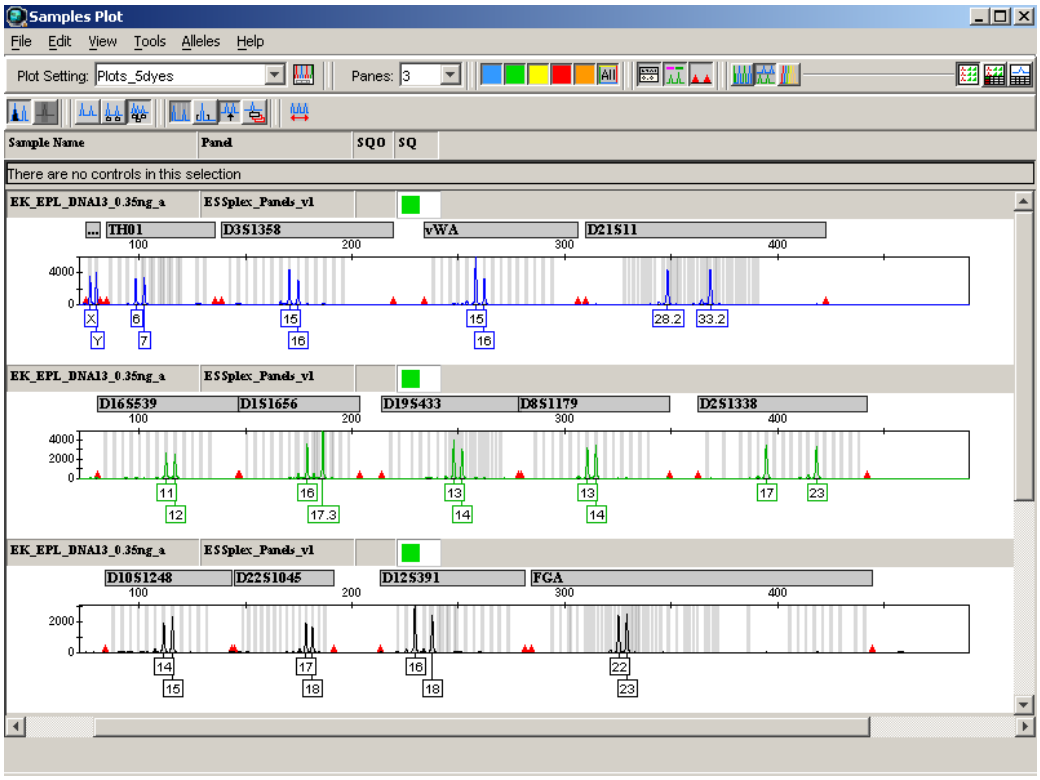


Figure 4. Example sample plot using method HID_3130.

Problems with importing analysis methods

To resolve any problems with import, analysis methods can be created manually in the GeneMapper ID Software by entering the values shown in Figures 5–9.

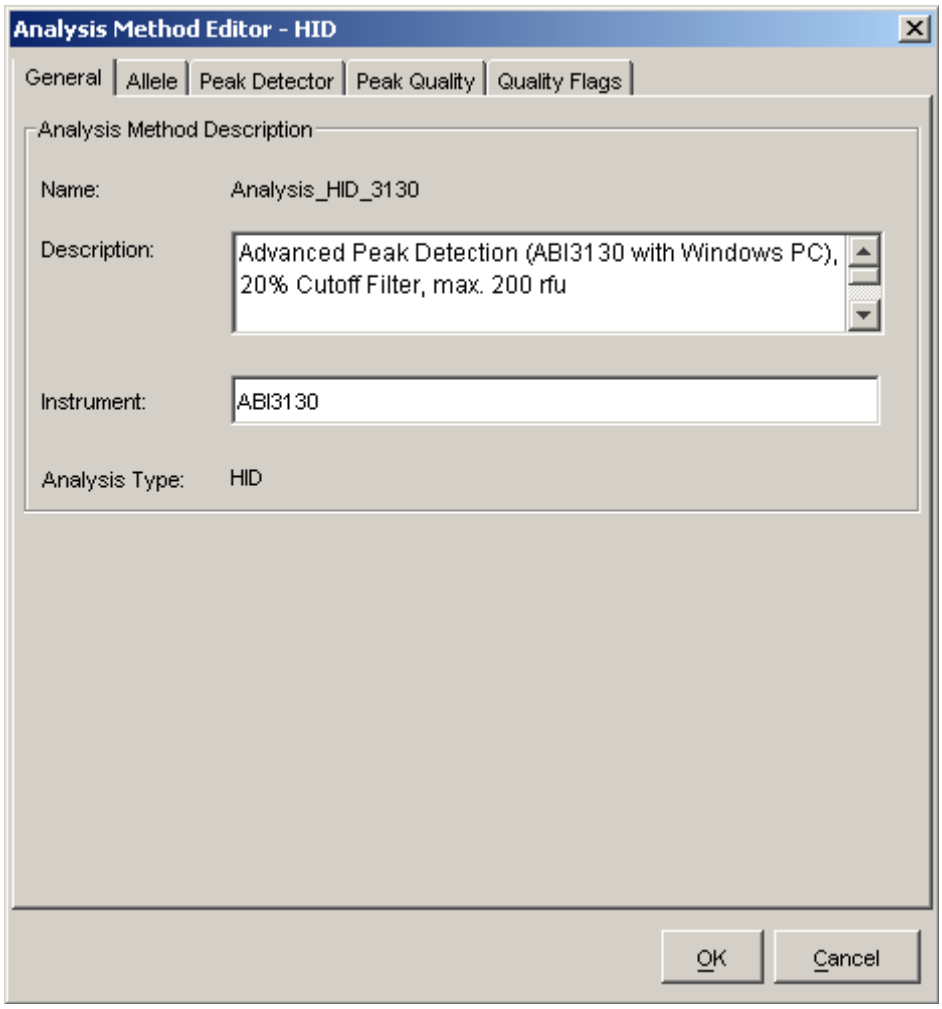


Figure 5. The General tab describes the analysis method.

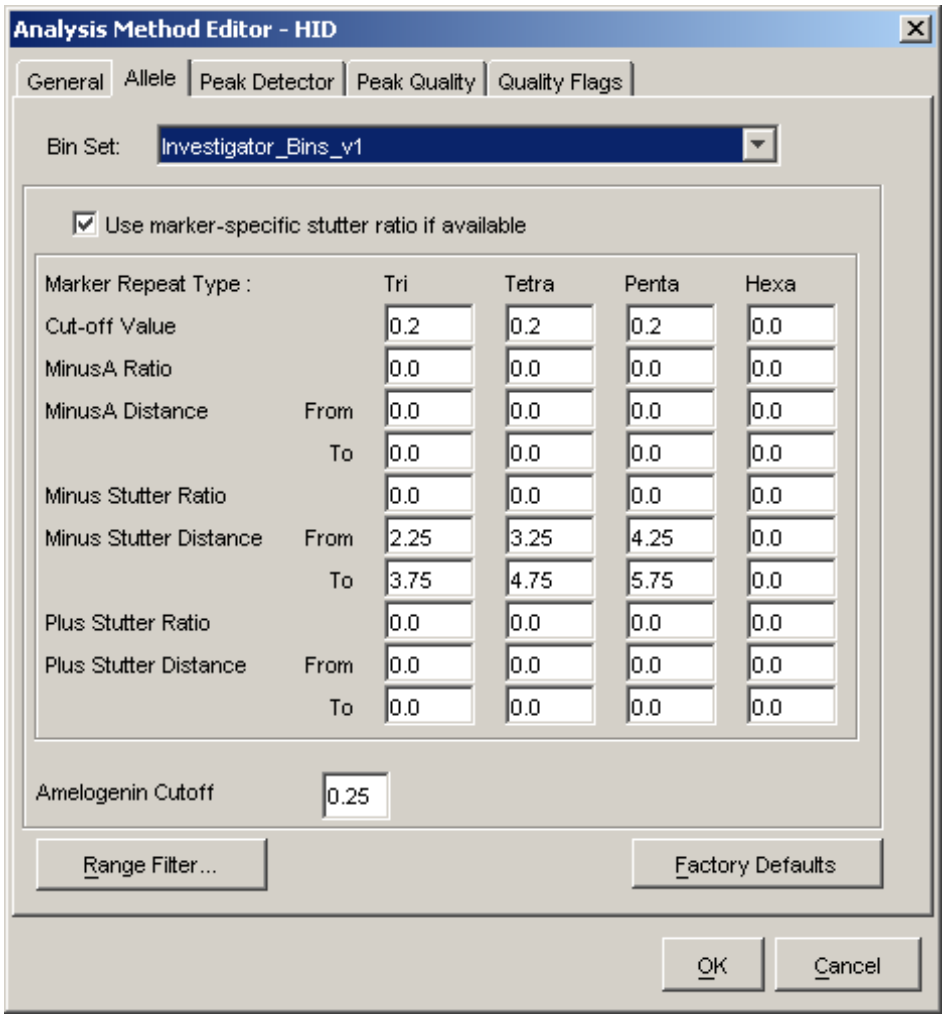


Figure 6. The Allele tab.

Cut-off value

A cut-off value of 0.2 corresponds to a 20% filter compared with the highest peak of a marker. With this setting, all signals with peak heights <20% will not be displayed in plots and tables.

Stutter distance

For peaks falling within stutter positions, as defined by the Stutter Distance settings.

Method limitation

The methods Analysis_HID_310 and Analysis_HID_3130 contain a 20% cut-off filter and should not be used for the analysis of DNA mixtures.

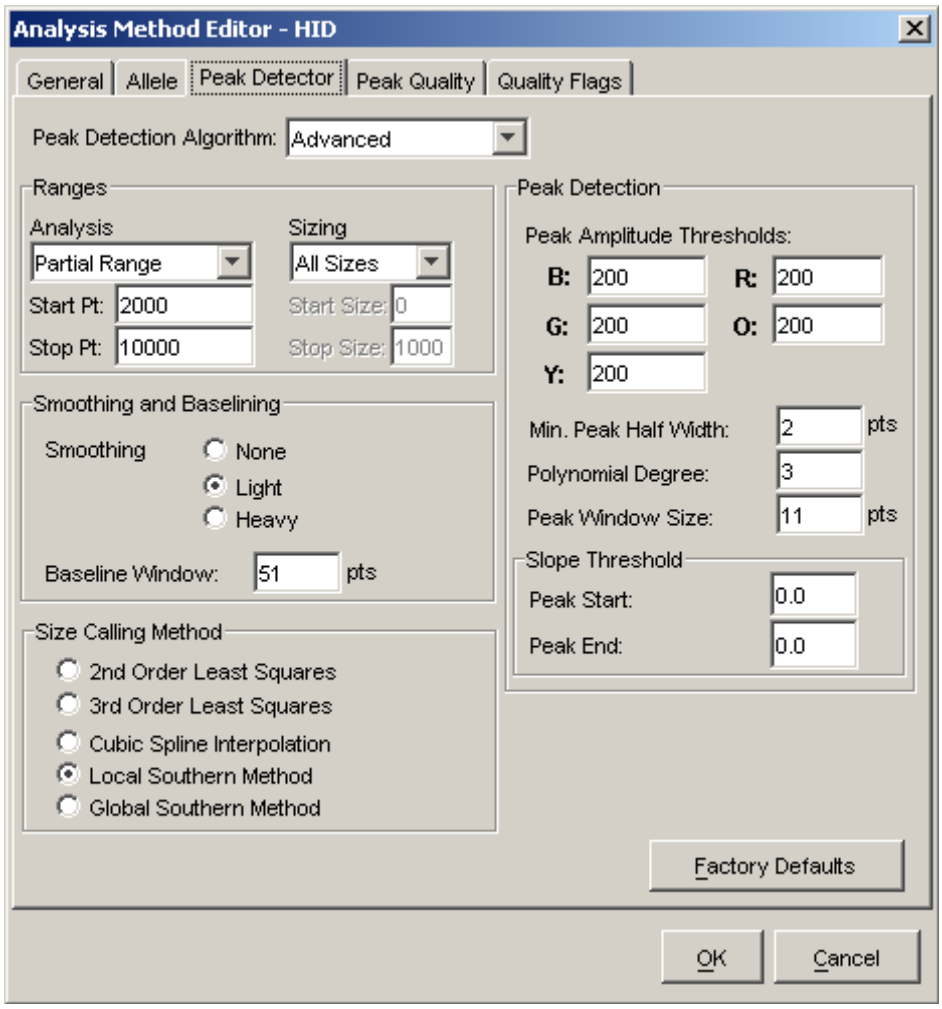


Figure 7. The Peak Detector tab.

For improved peak detection and particularly detection of point alleles (i.e. alleles with at least 1 bp difference to the next integer allele), the value for the Peak Window Size can be minimized to 11 points. Only the setting for Peak Window Size is different to defaults from Applied Biosystems for HID analysis.

Note: New Analysis Methods should be created using advanced settings, such as the Peak Detection Algorithm in the GeneMapper ID Software (see figure above).

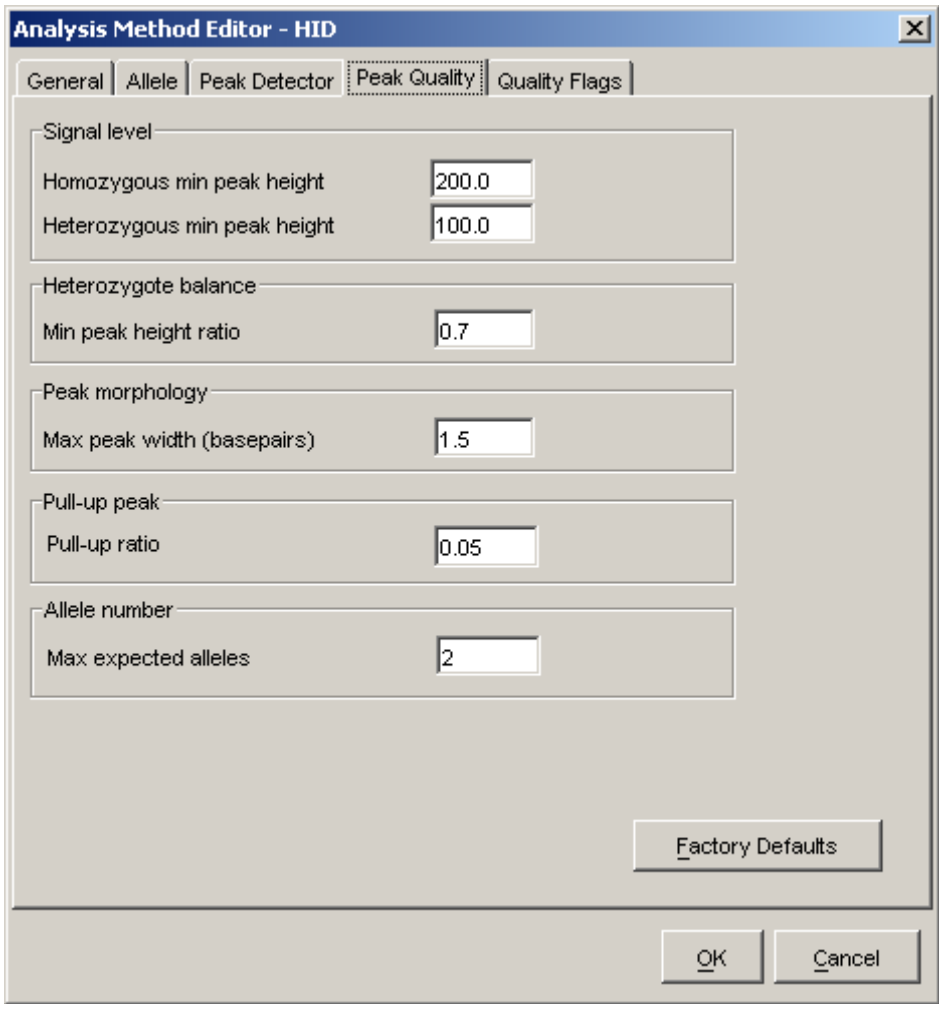


Figure 8. The Peak Quality tab. Peak Quality is used to adjust peak quality parameters.

All settings shown are defaults from Applied Biosystems for HID analysis.

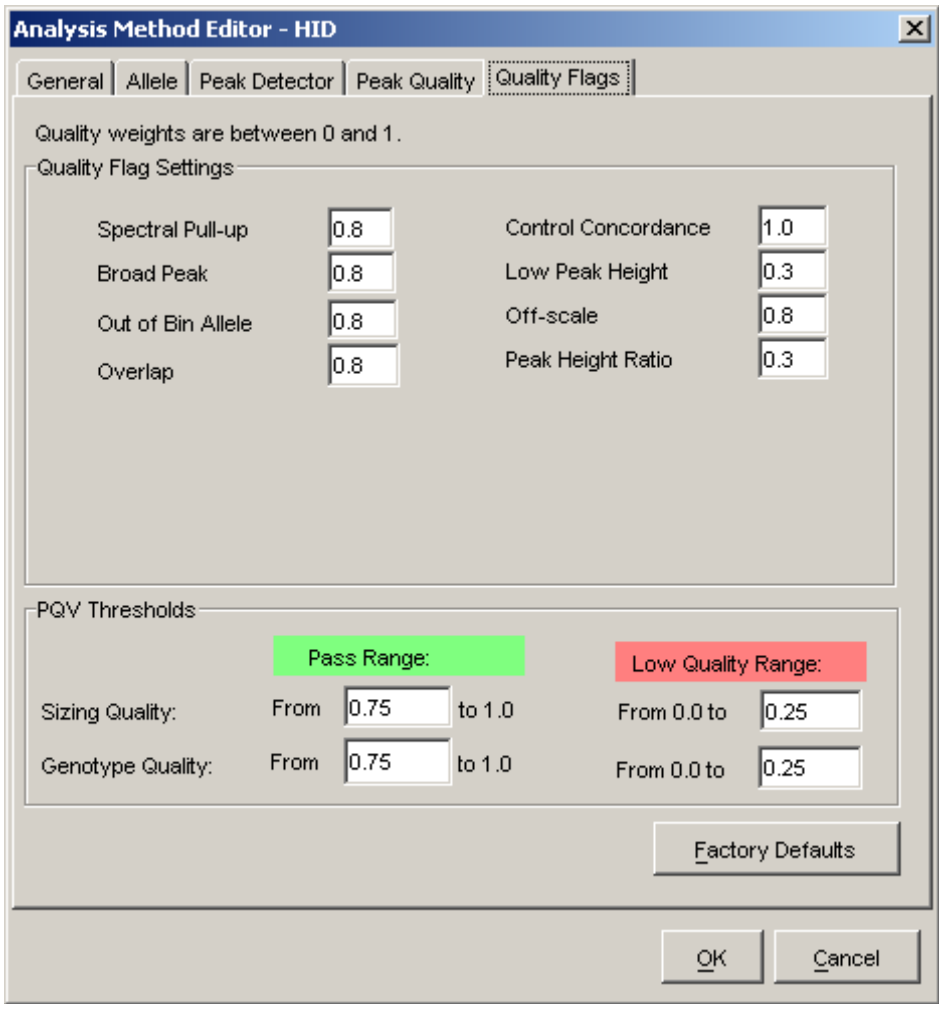


Figure 9. The Quality Flags tab is for weighting of the peak quality parameters.

All settings shown are defaults from Applied Biosystems for HID analysis.

Sensitive HID analysis for stains and DNA mixtures

Analysis_HID_3130_50rfu

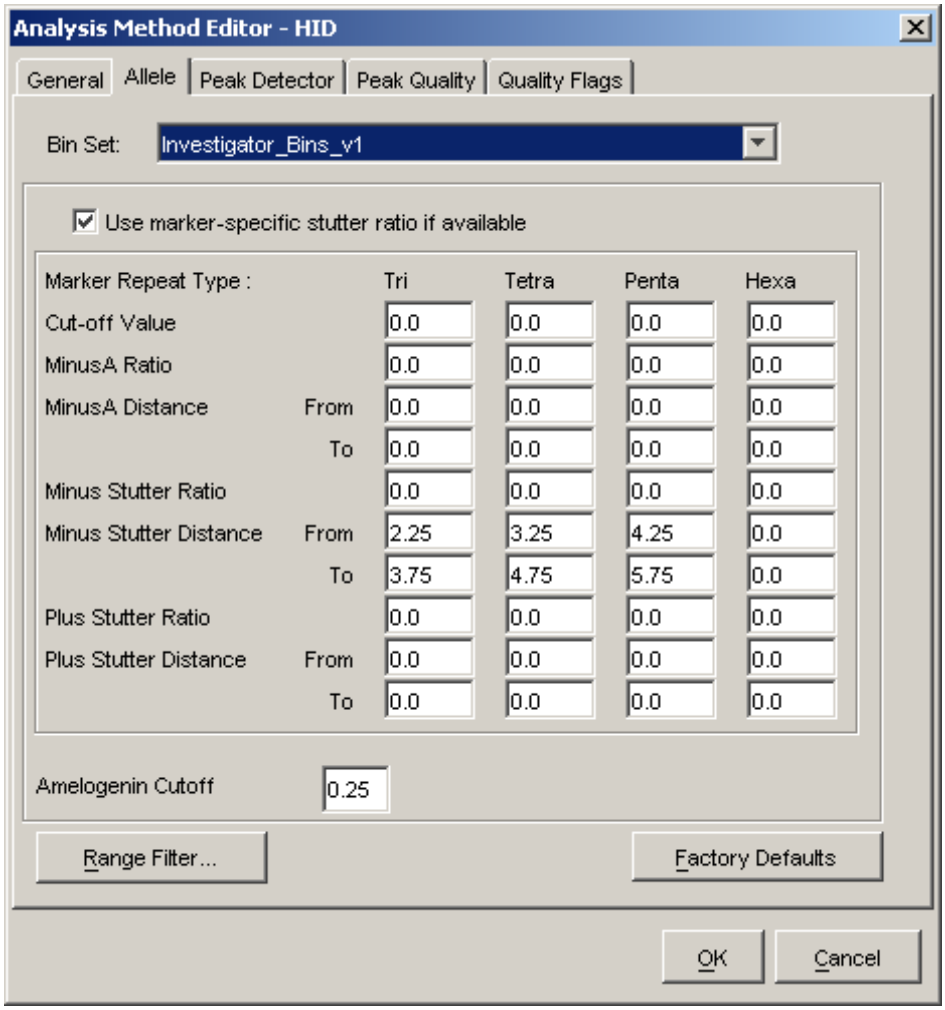


Figure 10. The Allele tab is used to adjust filter values, (preselection: 0% cut-off filter).

Changing the filter value in the Allele tab

To change the lower threshold, e.g., for forensic case work, perform the following steps:

1. **Go to "Tools" and select "GeneMapper Manager".**
2. **Select "Analysis Method" and double-click to open it for editing.**
   "Cut-off Value", "MinusA", "Minus Stutter", and "Plus Stutter" are the filter values that can be amended (Figure 10).
3. **Select the value to change and save changes to the analysis method with a new filename.**

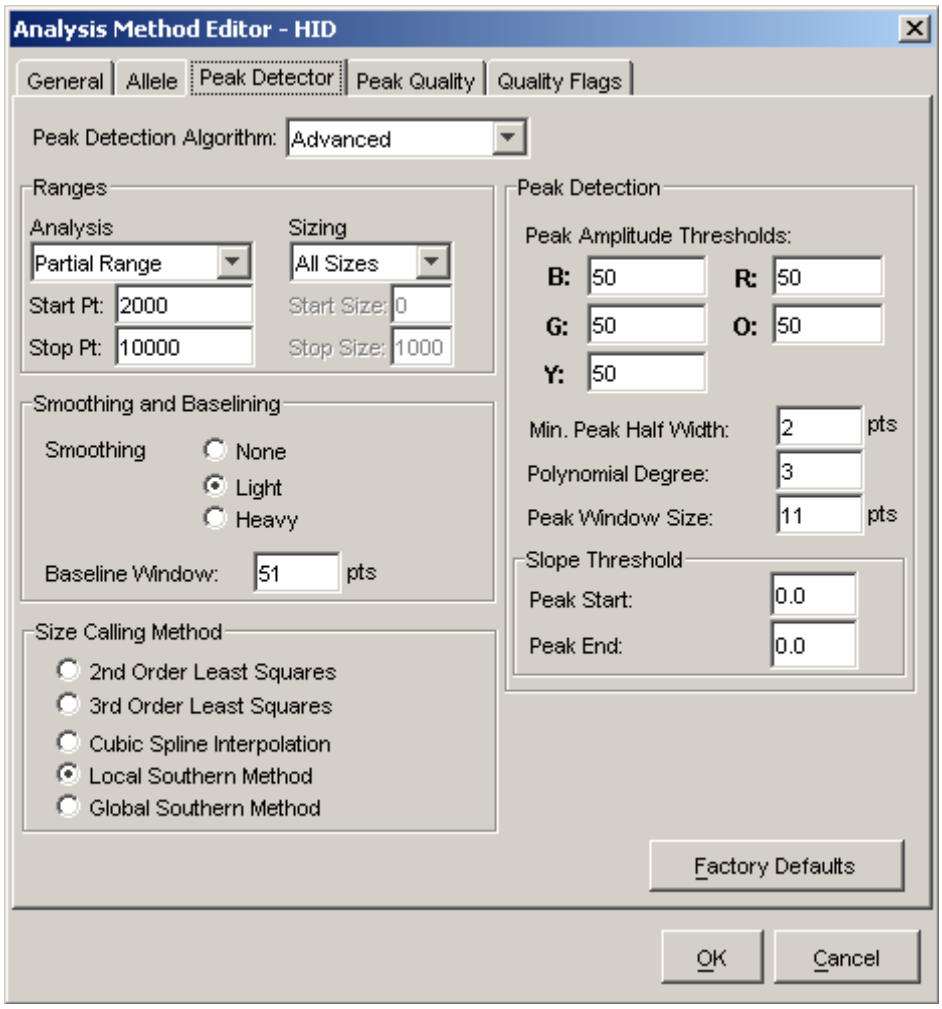


Figure 11. The Peak Detector tab (pre-selection: 50rfu filter).

Changing the filter value in the Peak Detector tab

- Values for peak detection can be changed in the Peak Detector tab.
- Peak Amplitude Threshold describes the minimal peak height which can be detected with the GeneMapper ID Software. Common values are 50–200 relative fluorescent units (RFU) and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times higher then the background noise of the baseline.

Plot Settings

- Plots_2dyes: Display of two-color panels (B, R)
- Plots_3dyes: Display of three-color panels (B, G, R)
- Plots_4dyes: Display of four-color panels (B, G, Y, R)
- PlotsBT5_4dyes: Display of four-color panels (B, G, Y, O)
- Plots_5dyes: Display of five-color panels (B, G, Y, R, O)

Plot settings:

- Are used to compare samples with the appropriate allelic ladder
- Show the peak designation (e. g. allele) with the chosen analysis method
- Enables changes to allele designations
- Allelic ladders and defined control samples are fixed in the upper part of the window. DNA samples are displayed in the lower part
- Zoom the appropriate area in order to simplify allocation (see Figure 17, page 33)
- Initialized without tables

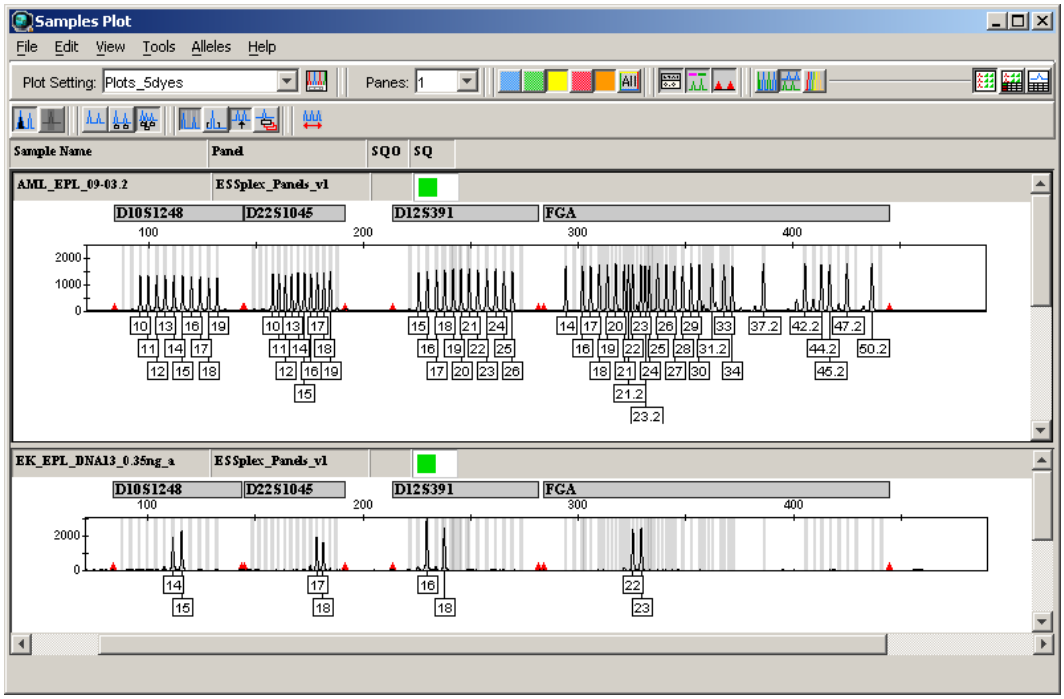
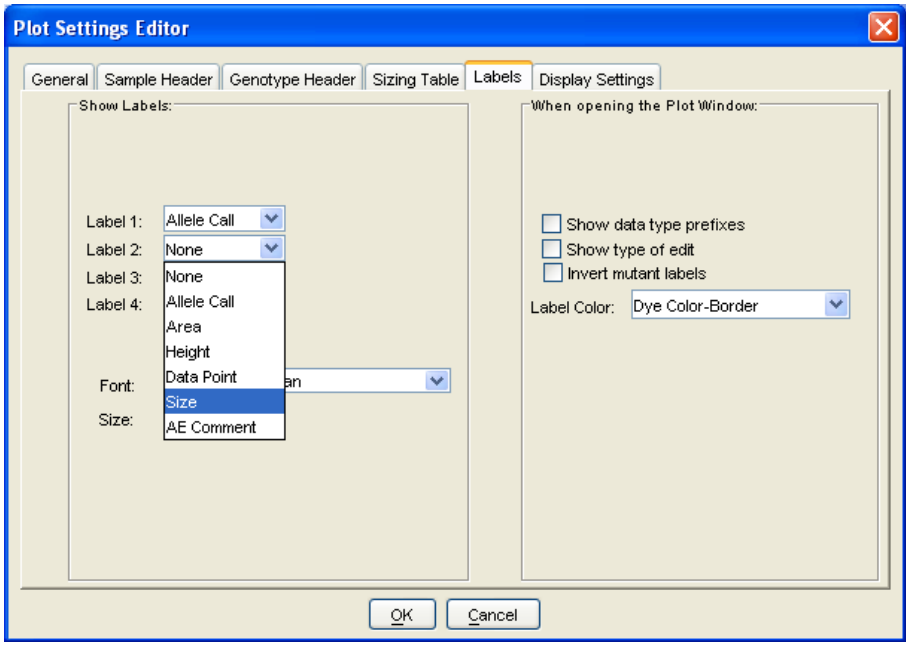


Figure 12. Plot window showing an allelic ladder and DNA sample.

New peak designations (labels)

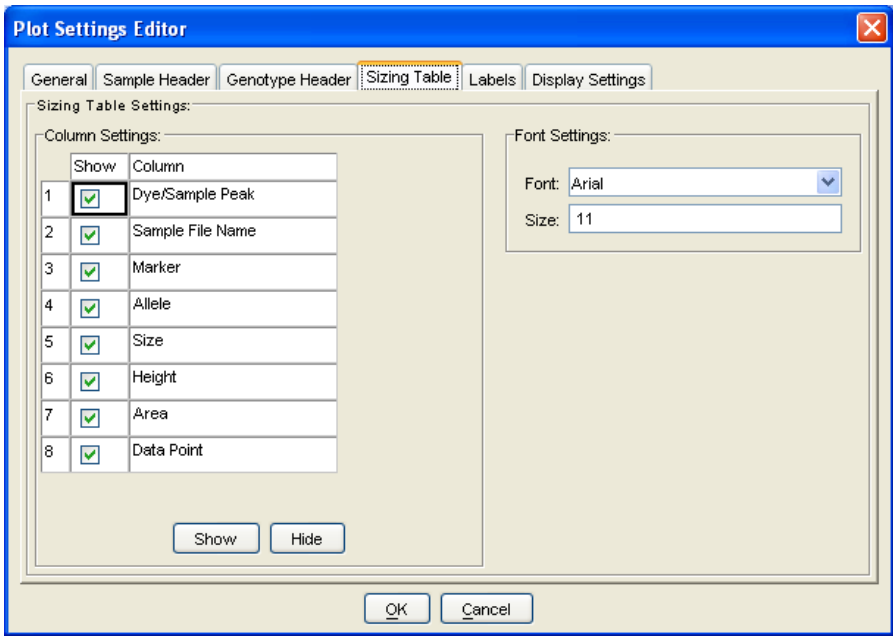
To change peak designation, please perform the following steps:

1. **Go to "Tools" and select "GeneMapper Manager".**
2. **Select "Plot Setting" and double-click to open it for editing.**
   Allele Call, Height, and Size can be edited in the Labels tab (see screenshot).
3. **Select the value to change or add and save the file with a new filename.**
4. **Select the value to change or add and save by clicking "OK".**



The Labels tab is used for setting which labels are displayed for each peak in an electropherogram.

5. **The designation of peaks to be displayed in the table is defined in the Sizing Table tab (see screenshot, next page)**



The Sizing Table tab is used to generate the table display.

Table Settings

- Use Table for 2 Alleles for samples with one DNA
- Use Table for 10 Alleles for samples with DNA mixtures

Table for 2 Alleles

- Are used to analyze samples of one DNA, e.g., for a DNA database
- Display the allele designation of two peaks of each marker
- Generates a table where the analysis of each marker (STR locus) is displayed in one line (e.g., for ESSplex: All markers x 2 alleles) (Figure 13)
- The Genotypes tab displays the following columns: Sample Name, Marker, Allele 1, and Allele 2. In addition, the PQVs shown in Table 2 are displayed.

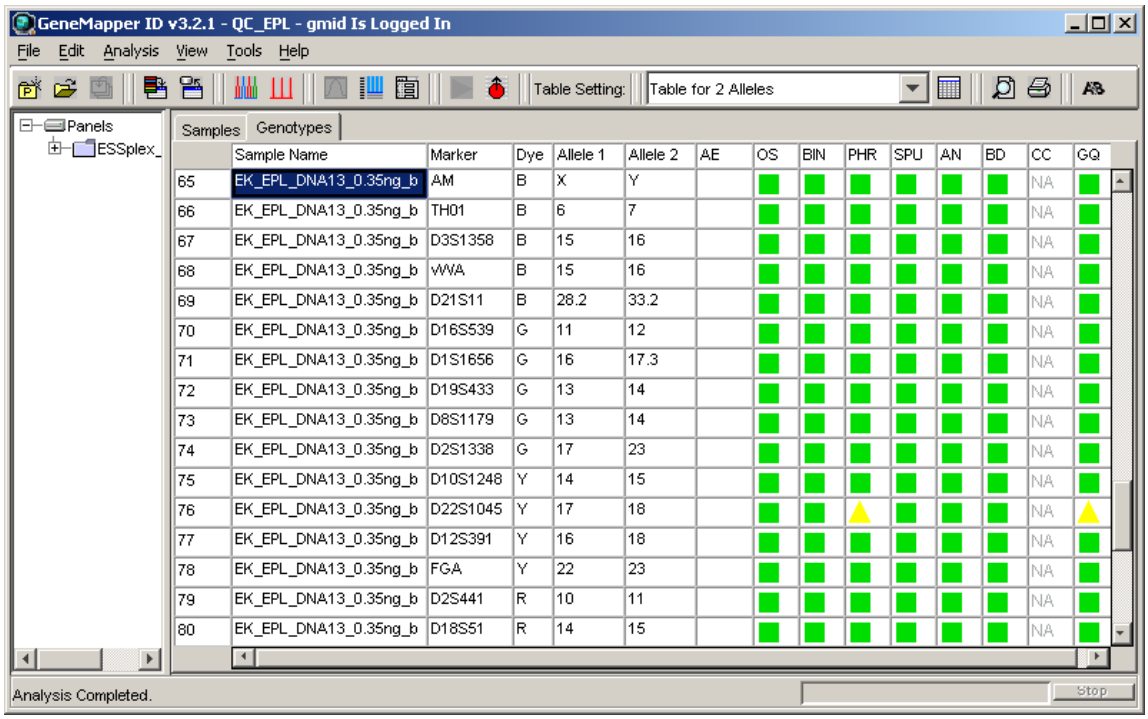


Figure 13. Example output file after Table for 2 Alleles.

- In GeneMapper ID Software, all quality criteria are pre-adjusted by Applied Biosystems for HID products; when a color signal (Check, Low Quality) appears, a PQV was not held.
- Check the reason for the out-of-range range PQV, see Table 2.

Table 1. Peak quality assessment via different signals

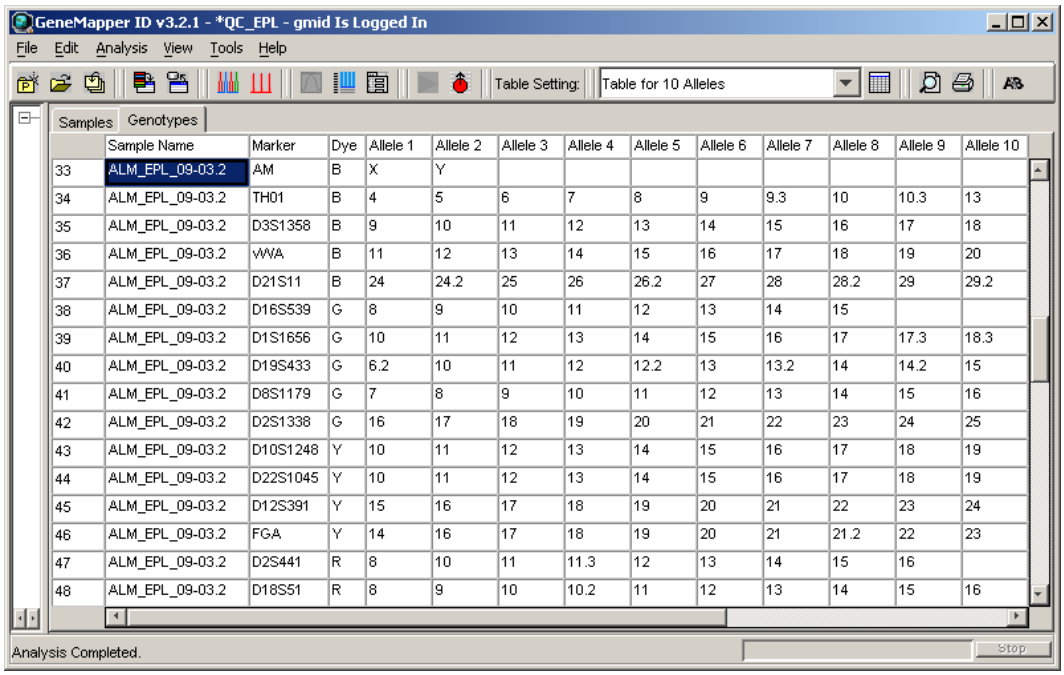
Signal	Table
Pass	Green square icon
Check	Yellow triangle icon
Low quality	Red “stop” icon

Table 2. Process quality values

PQV	Definition
AE	Allele edit
OS	Out of bin allele (BIN), Peak Height Ratio (PHR), indicates if the peak height ratio between the lowest and highest peak is less than as it was defined in the analysis method
SPU	Spectral pull-up: Spectral pull-peaks in another color channel
AN	Allele number
BD	Broad peaks: The allele peak is wider than expected (default 1.5 bp)
CC	Control concordance: Comparison with an internal control, e.g., control DNA
GQ	Genotype quality: Quality of the DNA profile

Table for 10 Alleles

- Are used to analyze stains or sample mixtures
- Display the allele designation for up to 10 peaks per marker
- Generates a table where the analysis of each marker (STR locus) is displayed in one line (e. g. ESSplex: all markers x 10 alleles)
- The Genotypes tab displays the following columns: Sample Name, Marker, and Allele 1 through to Allele 10. In addition, the following PQVs are displayed: AE, OS, BIN, PHR, SPU, BD, CC, and GQ (Figure 14)



	Sample Name	Marker	Dye	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9	Allele 10
33	ALM_EPL_09-03.2	AM	B	X	Y								
34	ALM_EPL_09-03.2	TH01	B	4	5	6	7	8	9	9.3	10	10.3	13
35	ALM_EPL_09-03.2	D3S1358	B	9	10	11	12	13	14	15	16	17	18
36	ALM_EPL_09-03.2	vWA	B	11	12	13	14	15	16	17	18	19	20
37	ALM_EPL_09-03.2	D21S11	B	24	24.2	25	26	26.2	27	28	28.2	29	29.2
38	ALM_EPL_09-03.2	D16S539	G	8	9	10	11	12	13	14	15		
39	ALM_EPL_09-03.2	D1S1656	G	10	11	12	13	14	15	16	17	17.3	18.3
40	ALM_EPL_09-03.2	D19S433	G	6.2	10	11	12	12.2	13	13.2	14	14.2	15
41	ALM_EPL_09-03.2	D8S1179	G	7	8	9	10	11	12	13	14	15	16
42	ALM_EPL_09-03.2	D2S1338	G	16	17	18	19	20	21	22	23	24	25
43	ALM_EPL_09-03.2	D10S1248	Y	10	11	12	13	14	15	16	17	18	19
44	ALM_EPL_09-03.2	D22S1045	Y	10	11	12	13	14	15	16	17	18	19
45	ALM_EPL_09-03.2	D12S391	Y	15	16	17	18	19	20	21	22	23	24
46	ALM_EPL_09-03.2	FGA	Y	14	16	17	18	19	20	21	21.2	22	23
47	ALM_EPL_09-03.2	D2S441	R	8	10	11	11.3	12	13	14	15	16	
48	ALM_EPL_09-03.2	D18S51	R	8	9	10	10.2	11	12	13	14	15	16

Figure 14. Example output file after a Table for 10 Alleles is generated.

Table export

1. **The "Sample Plot" showing the table should be open.**
2. **Go to "File" and choose "Export Table".**
3. **Save the table by adding ".txt" for tab-delimited text or ".csv" for comma-delimited text from the dropdown menu on the "Save" screen.**

   Note: If using DIPSorter Freeware in conjunction with the Investigator DIPplex Kit, it is essential to use the table setting "Table for 20 Alleles" to export table data.

   For general instructions about table export, see the chapter Exporting Table Data from "*GeneMapper ID Software User Guide*".

Setup on the Analysis Computer

This manual provides instructions and guidance on the use of the GeneMapper ID Software in a single-user environment. If using GeneMapper ID Software in a server–client environment, refer to the Applied Biosystems *GeneMapper ID-X Reference Guide* (either v1.1 or v1.2) for detailed set-up information about the creation of users, importing panels, bins, stutter, analysis methods, plot settings, table settings, and size standards.

Investigator Template Files for GeneMapper ID Software are available to download free from the online catalog pages of all Investigator Human Identification PCR Kits at the QIAGEN website (www.qiagen.com/InvestigatorIDKits) or on a CD-ROM, on request.

Before using the GeneMapper ID Software for the first time, Investigator Template Files must be saved to the local analysis computer (Windows® PC), by either downloading the files from the Internet or copying the files from a CD-ROM. Panels and BinSets are imported by the Panel Manager. Other template files, such as Analysis Methods, Table Settings, Plot Settings, etc. can also be imported to the local PC using the GeneMapper ID Manager.

Login

1. **Open the GeneMapper ID Software and log in using the following details.**

   Enter the following user name at first login: gmid

   A prompt appears to enter the password for the gmid user. Since this is the first login, no password is required at the first login. Press the "Return" key to proceed.

2. **The password must be changed at first login. Go to "Tools" and select "Options", followed by "Users", and "New User".**

   Steps 1 and 2 must be repeated in order to register each new user.

   The new login details should be used for every subsequent login.

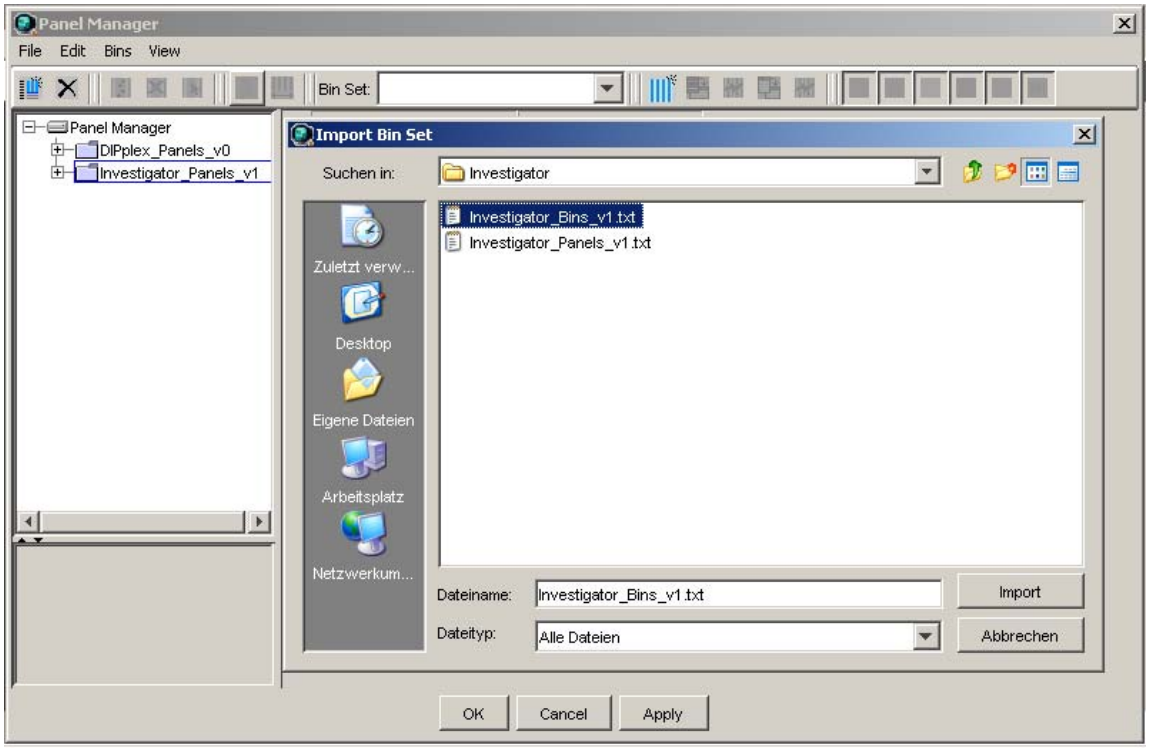
Importing Panels and BinSets

1. **Extract the Investigator files from the archive (downloaded from the QIAGEN web site or CD-ROM) to the desktop or other location. Save these files for future use.**
2. **From the toolbar, select "Tools" and then "Panel Manager".**
3. **Go to "File" and click "Import Panels". Select the folder "Panel Manager" from the left pane, then navigate to the desktop, select Investigator Panels, and click "Import" (see screenshot, next page).**

4. **Go to "File" and click "Import Panels". Select the folder "Panel Manager" from the left pane, then Investigator Panels, and click "Import" (see screenshot, next page).**
5. **In the toolbar, click "File" and then select "Import BinSet". Select the Panel being used from the Panel Manager, highlight the equivalent version of the BinSet file, and import (see screenshot).**

   IMPORTANT: The version number of Panel and BinSet files used must match.
6. **Click "OK".**

   The new Panels and BinSets will be visible in the Panel Manager.



Importing Panels and BinSets.

Importing Analysis Methods, Table Settings, Plot Settings, or Size Standards

1. **Import Analysis Methods, Table Settings, Plot Settings, or Size Standards of the Investigator Human Identification PCR Kits into the GeneMapper ID Software, as detailed in the following steps.**
2. **In the toolbar, select "Tools" and then select "GeneMapper Manager" from the drop-down menu.**
3. **Select the designated tab and click "Import". For each of the following items, navigate to either the Investigator folder created during extraction from the archive or to the CD-ROM containing the Investigator files".**

   Go to "Analysis Method" and click "Import".

   Go to "Table Setting" and click "Import".

Go to “Plot Setting” and click “Import“.

Go to “Size Standard” and click “Import“.

Note: Imported Analysis Methods, Table Settings, Plot Settings, or Size Standards can be redefined and saved by the user.

Note: New Analysis Methods, Table Settings, Plot Settings, or Size Standards can be generated in the GeneMapper Manager.

Calibration Using Allelic Ladders

Analysis with GeneMapper ID Software is performed using related analysis data, i.e. previously analyzed DNA samples, with a known allelic ladder (known as a "Project"). In order to analyze DNA samples using GeneMapper ID Software, calibration with the allelic ladder must first be carried out. The allelic ladder of the Investigator Human Identification PCR Kit should ideally be analyzed before and after the DNA samples under investigation.

For calibration, the measured allele sizes are transferred automatically to the expected sizes within the project. In general, calibration is based upon the most current run of the allelic ladder. If more runs will be used, calibration uses all allelic ladders and the correct assignment of alleles should be verified. If alleles are not assigned correctly, DNA samples should undergo further runs with an appropriate allelic ladder.

Calibration using multi-capillary analyzers

To ensure a reliable allelic assignment on multi-capillary analyzers, a number of allelic ladders should be run on different capillaries.

Room temperature may influence the running performance of PCR products and may result in split peaks — especially at low temperatures — or an altered run velocity of DNA fragments. Ensure that environmental conditions recommended by the instrument manufacturer are maintained at all times.

System parameters

Different analysis instruments, DNA size standards, or polymers may result in different fragment lengths. Thus, DNA samples and allelic ladders from one sample set should be analyzed using the same system parameters.

Evaluation of Analysis Data

1. **Open GeneMapper ID Software and login.**
2. **Go to "Import data".**
3. **Choose "File", followed by "Add Samples to Project".**
4. **Select "Analysis Data Files (.fsa)".**
5. **Select "Add Samples", choose "Add to List", and then click "Add".**
6. **Use the drop-down menu to select "Table Settings".**

   Use "Table for 2 Alleles" for samples containing only one DNA or "Table for 10 Alleles" for mixed DNA samples.
7. **Data Files appear as a new project in the Samples tab.**

   The table sheet displays the following columns: Status, Sample Name, Sample Type, Analysis Method, Panel, Size Standard, Matrix. The PQVs displayed are shown in Table 3.

Table 3. Process quality values (PQV)

PQV	Definition
SQO	Size quality overridden
SFNF	Sample file not found
OS	Off-scales: Signals are outside of the scaling, pull-up peaks in other colors
SQ	Sizing quality: Size calling of the sample
UD1	User-defined comment 1

For more information, see the chapter "Process Quality Values" in the *GeneMapper ID Software User Guide*.

8. **Use the drop-down menu to select the Sample Type, if this was not completed at the time of the CE Run.**

   For example, Sample, Allelic Ladder, Positive Control, Negative Control, etc. (Figure 16).

   Note: Each project must contain at least one allelic ladder.
9. **Use the drop-down menu to select the Analysis Method.**

   For example, Investigator Template: Analysis_HID_3130.

   The analysis method is designed for evaluation of data from Windows PC (Advanced Peak Detection) for ABI PRISM 3130 instruments. There are other methods available for ABI PRISM multi-capillary instruments.

10. **Use the drop-down menu to select the Panel.**
    For example, ESSplex_Panels_v1.
11. **Use the drop-down menu to select the Size Standard.**
    For example, SST-BTO_60-500bp for the DNA Size Standard 550 (BTO).
12. **Use the drop-down menu to select the Table Setting.**
13. **Click the green arrow ▶ icon in order to start the analysis. When prompted, provide a name for the project, and save it. If the analysis was successful, the icon disappears from the "Status" column (Figure 16).**

Important notes

New analysis methods refer to the last BinSets used in the GeneMapper ID Software. For Investigator files, select the required BinSets (e.g., Investigator_Bins_v1) from the Allele tab in the toolbar (Figure 15).

Figure 15. Start analysis.

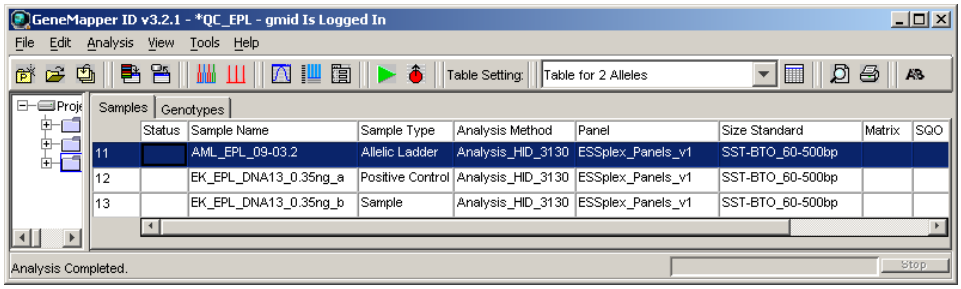


Figure 16. The Allele tab, which is used to adjust the corresponding BinSet.

Use the "Fill Down" function in order to analyze all samples with the same parameters. Select the parameter (e.g., ESSplex_Panels_v1), mark the top of the column using the drop-down menu, and press "Ctrl + D" or click "Edit" and "Fill Down".

Project analysis

1. **To review the analyzed data, click "Edit" followed by "Select All". From the "Analysis" drop-down menu, select "Display Plot".**
2. **Use the drop-down menu to select the Plot Setting.**

   For example, Plot_5dyes (for the blue/green/yellow/red/orange panel).

Scaling of the analysis range

In order to scale up (zoom in) the analysis range of the kit, click the magnifying glass icon above the horizontal scale in front of the first possible allele and drag it behind the last possible allele (Figure 17). To return to basic settings, double-click the scale.

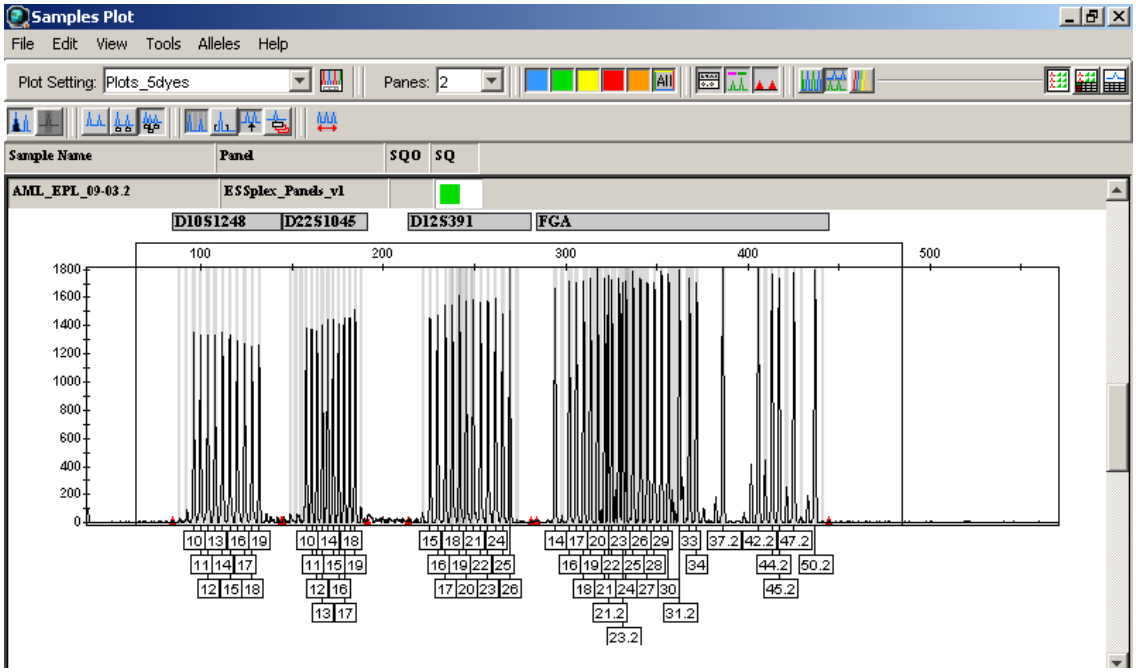


Figure 17. Scaling of the analysis range in Sample Plot.

Checking Analysis Data

The general procedure for analysis is:

- Check size standard
- Check allelic ladder
- Check positive control
- Check negative control
- Review sample data

Checking size calling

The first step in any new project with low Size Quality (PQV=SQ) is to check the size standard for the right fragments (Figure 18), see also chapter “size standards”.

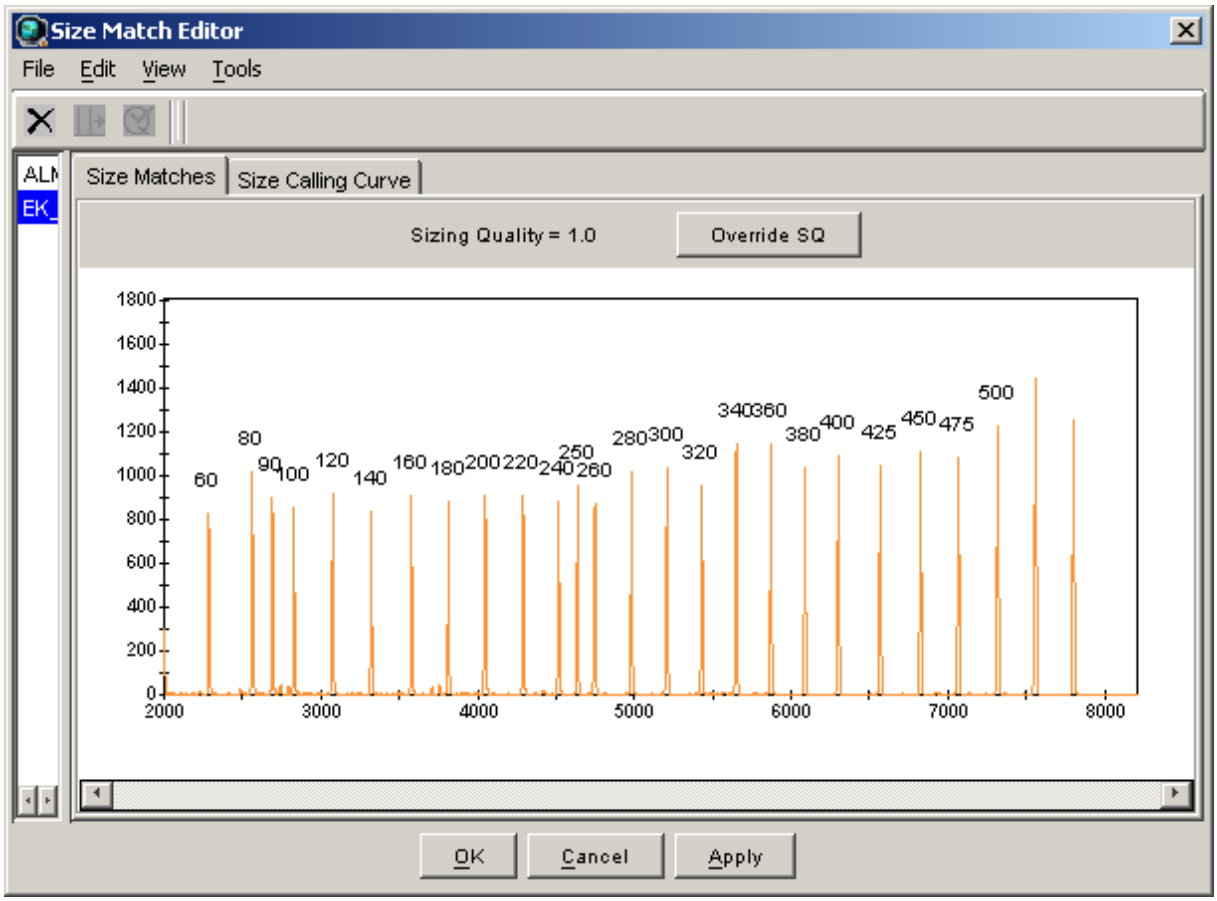


Figure 18. Checking size calling.

Checking allele calling

The second step is to check the allelic ladder for correct allele calling.

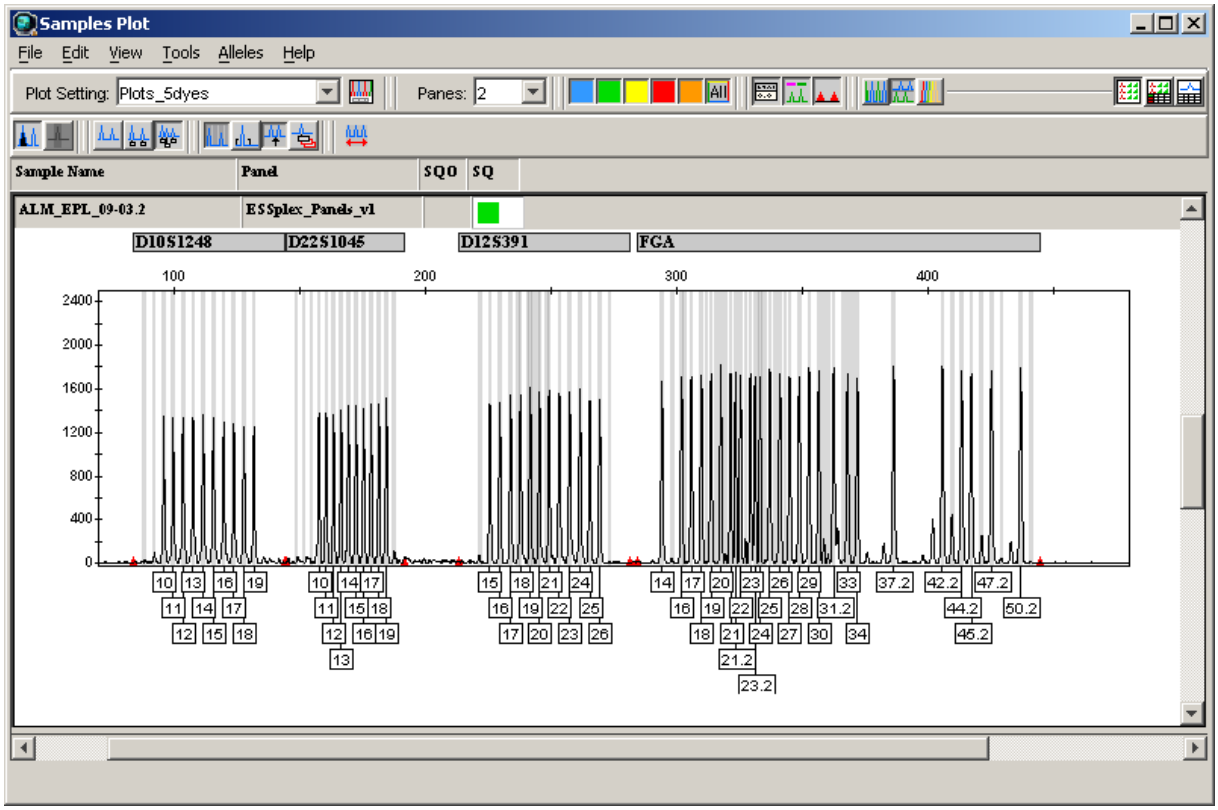


Figure 19. Checking the allelic ladder.

Controls

QIAGEN recommends the following quality value:

- Allele number (AN) for checking the allelic ladder

In order to check allele designations, compare the alleles of the allelic ladder and control DNA of the Investigator Human Identification PCR Kit with data given in the latest version of the kit handbook.

Checking positive control

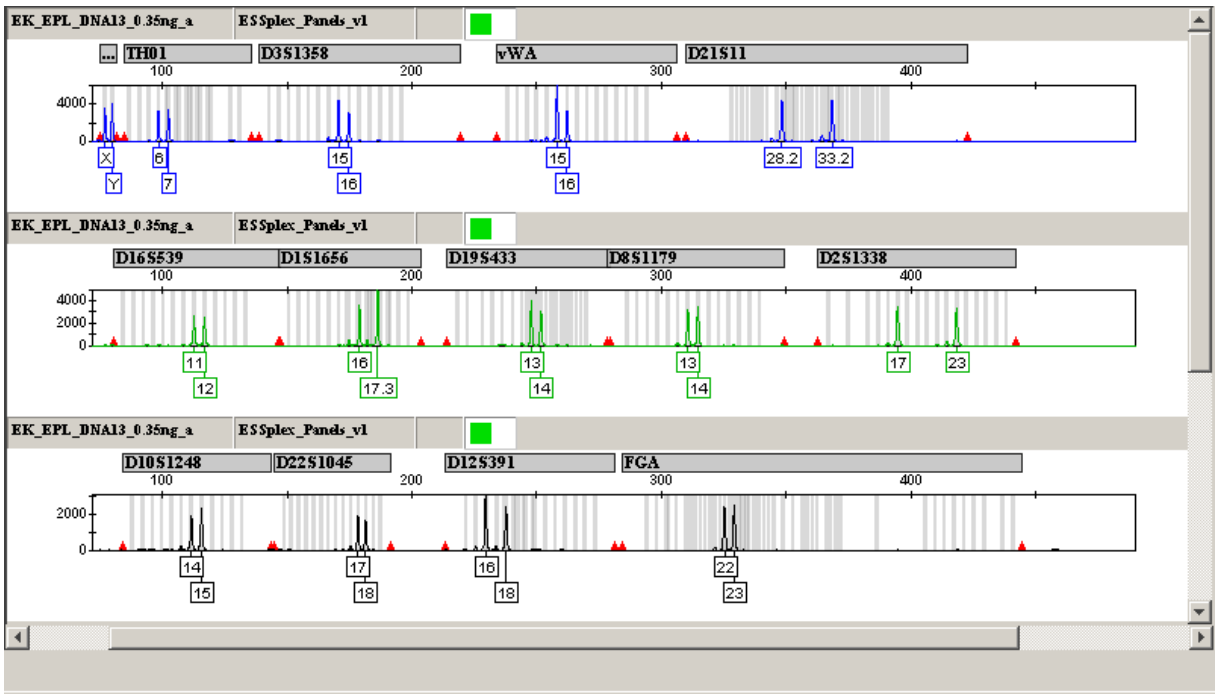


Figure 20. Checking the control DNA.

Controls

QIAGEN recommends the following quality values:

- Control concordance (CC) for the Positive Control
- Negative Control (the negative control contains no DNA and provides information about background signals of the current analysis conditions)

For more information, see the chapter "Process Quality Values" in the "*GeneMapper ID Software User Guide*".

Review sample data

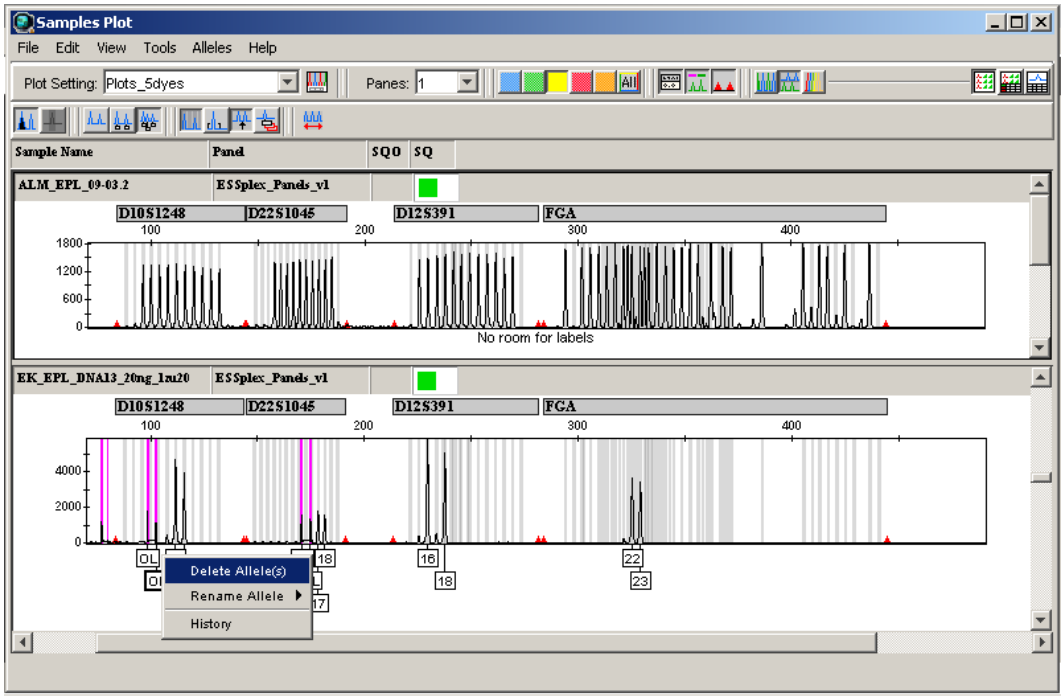


Figure 21. Changing the allele designation.

Off-ladder allele

Peaks labeled with OL (Off-Ladder) could not be assigned to an allele size. These labels must be checked manually and may be deleted or redefined by clicking on them.

Delete allele label

In order to change the allele designation of unrealized peaks, click the OL icon below the peak (the icon turns bold). Open the drop-down menu by right-clicking the OL icon and choose "Delete Allele" (Figure 21). The "Add Allele Comment" window opens so that notes about the changes can be added.

Rename allele label

Note: In the drop-down menu, known alleles of the marker can be chosen from the Bins by clicking "Rename Allele" (Figure 21). A new allele can be defined based on its length by using the "Custom" function found therein.

Print Options and Page Setup

In “Samples Plot”, the following print options can be chosen. Go to “File” and then choose “Page Setup”

- Table to edit (e.g., size or typeface)
- Plot to choose one of four different settings: Honor plots per pane (to print data and/or plots onto one page), small, medium, large (to scale the plot size per page)

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

For general help whilst using the software, go to "Help" and choose "GeneMapper ID 3.2.1 Help" in the GeneMapper ID Software and search by topic.

	Comments and suggestions
Error message during saving	
Country setting	Settings on the computer should be changed to English (US)
Error message during import (Panels and BinSets): "unable to save panel data: java.SQLException: ORA-00001: unique constraint (IFA.CKP_UK) violated."	
a) Path on the computer is incorrect	Delete all Panels in the Panel Manager and then download Panels/BinSets from the web catalog page of the Investigator Human Identification PCR Kit being used.
b) Panels and BinSets are not correctly identified	Compatible versions of Panels and BinSets must be saved within a single folder.
Error message during import (Panels): "Invalid marker repeat value in line # for marker D10S2325. Valid marker repeat values are 2, 3, 4 & 9."	
GeneMapper ID v3.1 does not identify penta repeat motifs (e.g., the marker D10S2325)	Open panel txt.-data in Windows Explorer and change the penta repeat motif of D10S2325 from 5 to 4. Import the new panel data again. Install v3.2 or higher of the GeneMapper ID Software.
Error message during import (Analysis Method, Plot Setting, Table Setting, Size Standard)	
GeneMapper Software is not the same as GeneMapper ID Software	Install GeneMapper ID Software.

	Comments and suggestions
Error message during analysis: "There are samples that do not meet analysis requirements. Please see Error Message in the info view of each sample"	
a) An invalid BinSet is pre-selected in "Analysis Method"	Select the correct BinSet. For Investigator Templates Files (Panels and BinSets) always use the same version (e.g., v3).
b) Data from different DNA analyzers have been stored in one run folder	Always save data from different DNA analyzers in separate run folders.
Injection/file of the allelic ladder is not appropriate	
a) An additional signal can be identified as peak of the allelic ladder because of dysfunctions during the electrophoresis. If peaks of the allelic ladder are miscalled, the ladder can not be used for the analysis	Use a different injection/file of the allelic ladder and check the data of the analyzed sizes from the Size Standard (in bp) of the allelic ladder. Always use the DNA Size Standard 550 for Investigator Human Identification PCR Kits.
b) One peak of the allelic ladder is below the peak detection value (50–200 RFU) of the analysis method used, and thus, is not identified	Repeat electrophoresis using fresh aliquots of ladder and samples. Alternatively, the allelic ladder must be loaded onto the analysis instrument at a higher concentration than samples to be analyzed. The, allelic ladder data can be analyzed with a lower peak detection value in GeneMapper ID Software.
c) One peak of the allelic ladder is not identified because it is outside the expected size range of the software (in bp)	Compare the length of the fragments (in bp) of the first allele in one color of the allelic ladder with the corresponding value in the categories. Then compare it with the other alleles.

	Comments and suggestions
Single marker is not identified	
Various causes	Open the project folder, mark the corresponding sample and check the error message in the Info tab or PQV value.
Many peaks are labeled as off-ladder (OL) alleles in the samples	
a) DNA Size Standard 550 (ROX or BTO) was not defined or identified correctly	Click onto the red "Size Match Editor" icon in the upper toolbar or the GeneMapper ID Software. Check the red or orange fragments of all samples. Always use the DNA Size Standard 550 included in Investigator Human Identification PCR Kits.
b) Signal intensities are too high. If the peak heights of the samples are outside the linear detection range (>4000 RFU/ABI310;>5000 RFU/ABI3130), stutters, split peaks, and artifacts may be increased	Reduce the injection time, reduce the amount of the PCR amplification product for analysis, or reduce the quantity of DNA for PCR.
c) Bubbles in the capillary lead to pull-up peaks in all color panels ("spikes") that result in allele misnomer	Repeat electrophoresis to confirm results.
d) Differences in the run performance among the capillaries of a multi-capillary analyzer may result in allelic assignment shift	For reliable allelic assignment on multi-capillary analyzers, a number of allelic ladders should be run. For further information, see the handbook of the relevant Investigator Human Identification PCR Kit.
Point alleles are not found	

	Comments and suggestions
Point alleles were not separated in the GeneMapper ID Software	Point alleles are i.e. alleles with at least 1 bp difference to the next integer allele. Check the settings of the analysis method. Lower the Peak Window Size value to 11 points. This effect may result from a number of factors, such as poor temperature control within the laboratory, polymer that is past the expiration date, an array which is nearing the expiration date, etc.
Homozygous alleles are not displayed as duplicates in the table	
Preferences of the GeneMapper ID Software	Adjustments can be made. Go to "Tools" and select "Options". Choose the Analysis tab and the setting "Duplicate homozygous alleles", so that a homozygote allele is displayed twice (e.g., 18 would be displayed as 18/18).

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
Investigator Template Files	All template files for Investigator Human Identification PCR Kits for use with GeneMapper ID, GeneMapper ID-X, and Genotyper software, as well as DIPSorter freeware (CD-ROM)	389900
Related products		
Investigator IDplex Kit (100)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	381615
Investigator ESSplex SE Kit (100)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	381525
Investigator Nonaplex ESS Kit (100)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	381315
Investigator Hexaplex ESS Kit (100)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	380615
Investigator HDplex Kit (25)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	381213
Investigator ESSplex Kit (100)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	381515
Investigator Triplex AFS QS Kit (100)*	Primer mix including internal control (QS), reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	380315

* Larger kit size available; please inquire.

Product	Contents	Cat. no.
Investigator Triplex DSF Kit (100)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	380325
Investigator Decaplex SE Kit (100)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	381025
Investigator Argus X-12 Kit (25)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	383213
Investigator Argus Y-12 QS Kit (100)*	Primer mix including internal control (QS), reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	383615
Investigator DIPplex Kit (25)*	Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water	384013
Matrix Standard BT5 single cap. (5 x 25)	Matrix standards 6-FAM, BTG, BTY, BTR, and BTO for single-capillary analyzers	386113
Matrix Standard BT5 multi cap. (25)	Matrix standards 6-FAM, BTG, BTY, BTR, and BTO for multi-capillary analyzers	386123
Matrix Standard BT5 multi cap. (50)	Matrix standards 6-FAM, BTG, BTY, BTR, and BTO for multi-capillary analyzers	386125

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* Larger kit size available; please inquire.

Notes

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Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 021-3865-3865 ■ Fax 021-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

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Italy ■ Orders 02-33430-420 ■ Fax 02-33430-426 ■ Technical 800-787980

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Korea (South) ■ Orders 1544 7145 ■ Fax 1544 7146 ■ Technical 1544 7145

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Singapore ■ Orders 65-67775366 ■ Fax 65-67785177 ■ Technical 65-67775366

Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

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