

April 2010

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# Investigator Template Files for Genotyper<sup>®</sup> User Guide

For analysis of Investigator Human  
Identification PCR Kits with Genotyper Software



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Sample & Assay Technologies

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# Contents

<b>Product Warranty and Satisfaction Guarantee</b>	<b>4</b>
<b>Technical Assistance</b>	<b>4</b>
<b>Introduction</b>	<b>5</b>
About this user guide	5
About Investigator Template Files and Genotyper Software	5
<b>Setup on the Analysis Computer</b>	<b>7</b>
Calibration using multi-capillary analyzers	8
System parameters	8
<b>Evaluation of Analysis Data</b>	<b>9</b>
<b>Macros for Investigator Human Identification PCR Kits</b>	<b>10</b>
Size standards	11
Calibrate ladder	12
Label 20% Filter	13
Label 10% Filter	15
Blue, green, yellow, and red panels	17
Horizontal Table for 2 Alleles	18
Vertical Table for 10 Alleles	19
Calculation of category offsets	20
<b>Adjustment of Categories</b>	<b>20</b>
<b>Troubleshooting Guide</b>	<b>21</b>
<b>References</b>	<b>24</b>
<b>Ordering Information</b>	<b>25</b>

## Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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## Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding Genotyper Software or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at [www.qiagen.com/Support](http://www.qiagen.com/Support) or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://www.qiagen.com)).

# Introduction

## About this user guide

This user guide provides information about the functions and features of Investigator Template Files used in conjunction with Genotyper Software. Please refer to the *Genotyper 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 Genotyper Software in the following sections:

- Introduction
- Setup on the Analysis Computer
- Calibration using Allelic Ladders
- Evaluation of Analysis Data
- Macros for Investigator Human Identification PCR Kits
- Adjustment of Categories
- Troubleshooting Guide

Throughout this document, analysis of PCR products generated using the Investigator ESSplex Kit, ABI PRISM<sup>®</sup> 3130 Genetic Analyzer, and DNA Size Standard 550 (BTO) are shown as an example. Analyses were performed using Genotyper Software version 3.7 for Windows<sup>®</sup> and Investigator ESSplex Template Files.

## About Investigator Template Files and Genotyper Software

Genotyper Software is a flexible genotyping software package that provides DNA sizing and quality allele calls for all Applied Biosystems<sup>®</sup> electrophoresis-based genotyping systems. This software specializes in multi-application functionality, including amplified fragment length polymorphism (AFLP<sup>®</sup>), loss of heterozygosity (LOH), microsatellite, and single-nucleotide polymorphism (SNP) genotyping analysis. Genotyper 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.

GeneScan<sup>®</sup> and Genotyper software together form a software package for HID products. 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 (macros) for the Genotyper Software in order to simplify data analysis. Investigator Template Files may be used with ABI PRISM<sup>®</sup> single- and multi-capillary instruments of Applied Biosystems.

Genotyper 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 Genotyper Software version 3.7 (Windows<sup>®</sup>) and version 2.5 (Macintosh<sup>®</sup>).

**Note:** Both Genotyper and GeneScan Software applications from Applied Biosystems have been discontinued and are unavailable for purchase (as of June 1, 2004). Applied Biosystems support for these products ended June 1, 2009.

### **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 Genotyper Software.

## Setup on the Analysis Computer

Investigator Template Files for Genotyper Software are available to download free from the online catalog pages of all Investigator Human Identification PCR Kits at the QIAGEN Web site ([www.qiagen.com](http://www.qiagen.com)) or on a CD-ROM, on request.

Before using the Genotyper 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. Macintosh users should use only the CD-ROM in order to avoid non-conformities during conversion.

### Using Genotyper Software and Investigator Template Files

- 1. Install Genotyper Software according to the manufacturer's instructions.**
- 2. To use GTA-templates of the Investigator Human Identification PCR Kits with Genotyper Software, an "Investigator folder" should be created on the local hard drive.**
- 3. Choose the current template file of the Investigator Human Identification PCR Kit. Double-click the GTA-template and the Genotyper Software will open automatically.**

## **Calibration Using Allelic Ladders**

Analysis with Genotyper Software is performed using related analysis data, i.e., previously analyzed DNA samples, with a known allelic ladder as a GTA-file. In order to analyze DNA samples using Genotyper Software, calibration with the allelic ladder must first be carried out. The allelic ladder of the Investigator Human Identification PC 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 the template file of the Investigator Human Identification PCR Kit; Genotyper Software will open automatically.
2. Go to "Import data".
3. Select "File", followed by "Import" and then "From GeneScan File".
4. Select "GeneScan Analysis Data Files: Add Sample Files".
5. Select "Add", and then click "Finish".  
The data files will appear at the top of the window.
6. To import, choose the dye colors of the Investigator Human Identification PCR Kit and color for the DNA Size Standard (e.g., B, Y, G, R, O). Go to "Edit", choose "Set Preferences", followed by "Import Colors".
7. To run the macro, highlight or double-click the appropriate macro (found in the bottom left corner of the main window). Click "Run Macro".

**Note:** Before analyzing data, always run the Macro Calibrate Ladder first, see page 12.

The general procedure for analysis is:

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

## Macros for Investigator Human Identification PCR Kits

This section is divided into the following subsections:

- Size standards
- Calibrate ladder
- Label 20% Filter
- Label 10% Filter
- Blue, green, yellow, and red panels
- Horizontal table for 2 alleles
- Vertical table for 10 alleles
- Calculation of category offsets

**Note:** The macro “Calibrate Ladder” is essential for the analysis results from Investigator Human Identification PCR Kits. Use of additional Investigator Template Files is optional.

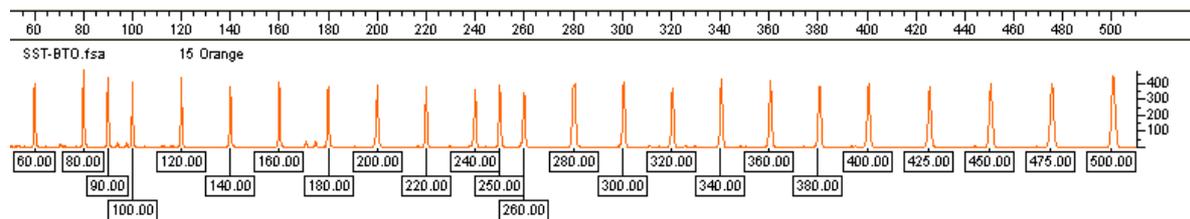
## Size standards

### SST-BTO

To check the correct assignment of the labels to the sample, click the  icon) in the upper left of the main window. The red panels of all samples are then displayed in the plot window.

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 GeneScan Software. If necessary, create a new size standard definition within the GeneScan Software.



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

### SST-ROX

For Investigator Human Identification PCR Kits in 4 Color Assay with the fluorescent labels 6-FAM<sup>®</sup>, HEX, NED, and ROX (matrix DS-30), the DNA Size Standard 550 (ROX) in the red panel ( icon) is necessary.

## Calibrate ladder

- The shortcut key to initiate the macro “Calibrate Ladder” is Ctrl + O
- Use this macro to calibrate the current project (GTA-file) with an allelic ladder
- After calibration, the macro “Label 20% Filter” will run automatically

## Identification of the allelic ladder

All colors of the imported allelic ladder must be defined as “ladder” in the “Dye/Lanes” window. Go to “Views” and select “Show Dye/Lanes Window”. Then, add the term “ladder” to every color in the “Sample Info” text field (Figure 2).

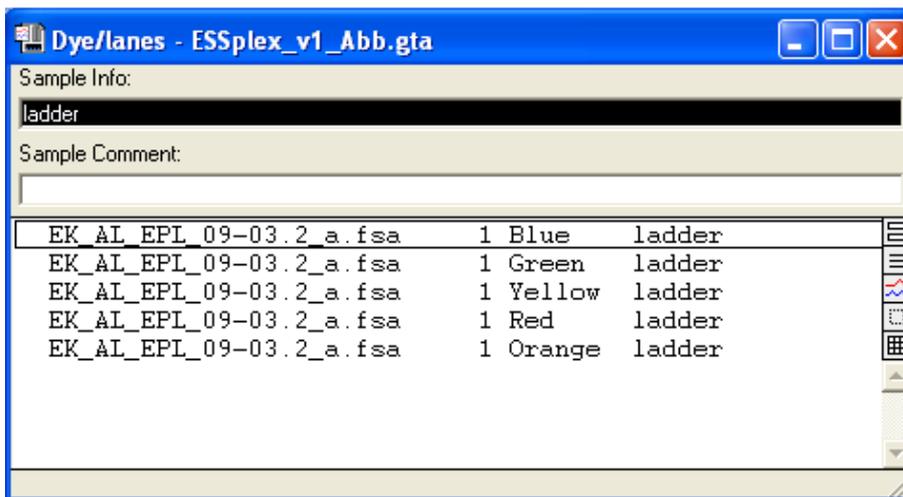


Figure 2. Dye/Lanes window.

**Note:** Peaks in the allelic range that are not identified by the GeneScan software cannot be labeled by the GeneScan Software.

## Controls

In order to check allele designations, compare the alleles of the allelic ladder and control DNA of the test kit with data from the latest version of the Handbook corresponding to the Investigator Human Identification PCR Kit being used.

## Label 20% Filter

- The shortcut keys to initiate the macro “Label 20% Filter” are Ctrl + 1; Ctrl + 2; Ctrl + 3
- Use this macro for analysis of samples containing one DNA; unsuitable for DNA mixtures
- Using the macro “Label “20% Filter”, peaks are indicated with their allele designation (Figure 3)
- Using the macro “Label 20% Filter incl. bp”, peaks are indicated with their allele designation and their size in base pairs
- Using the macro “Label 20% Filter incl. peak height”, peaks are indicated with their allele designation and their peak height in relative fluorescence units (RFU)
- A plot window will open up automatically with the following entries:
  - DNA samples
  - Allelic ladder
  - DNA size standard, if imported

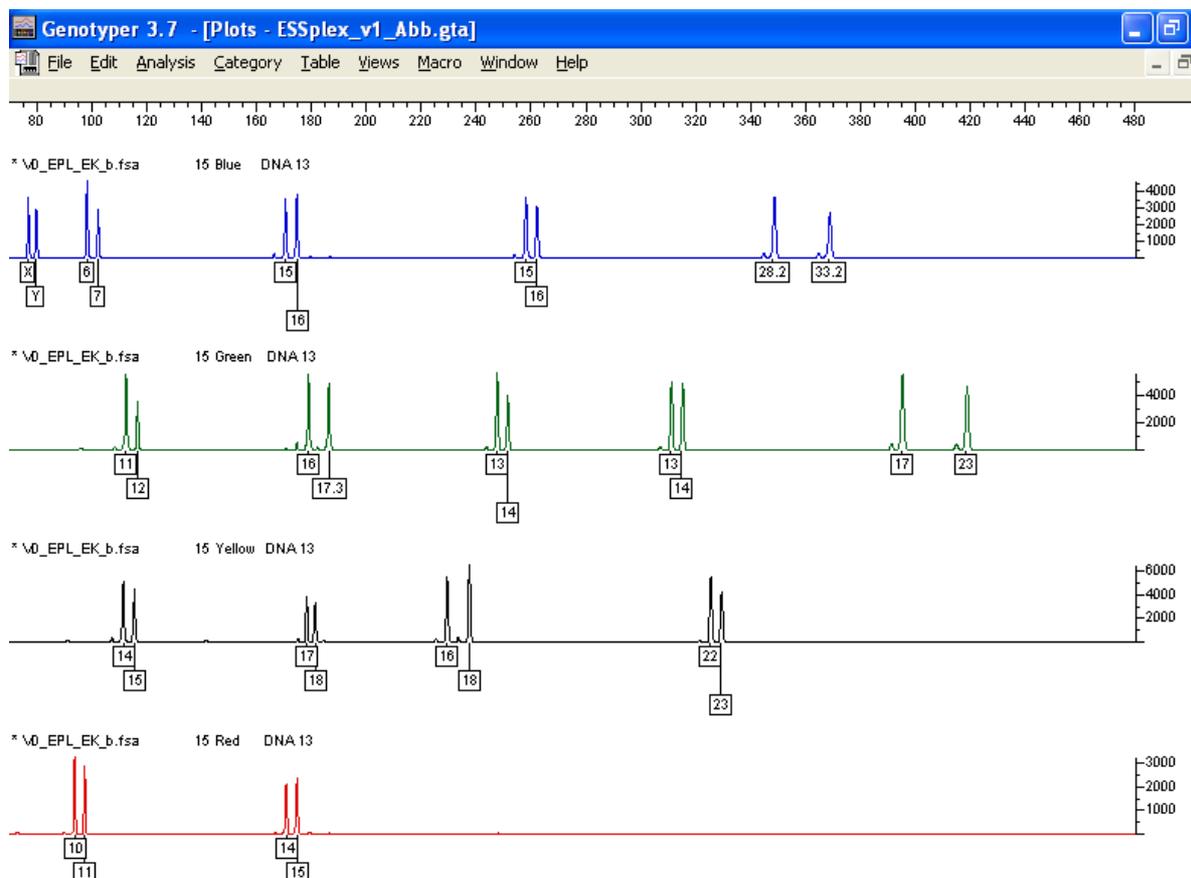


Figure 3. Plot Window after execution of Macro “Label 20% Filter”.

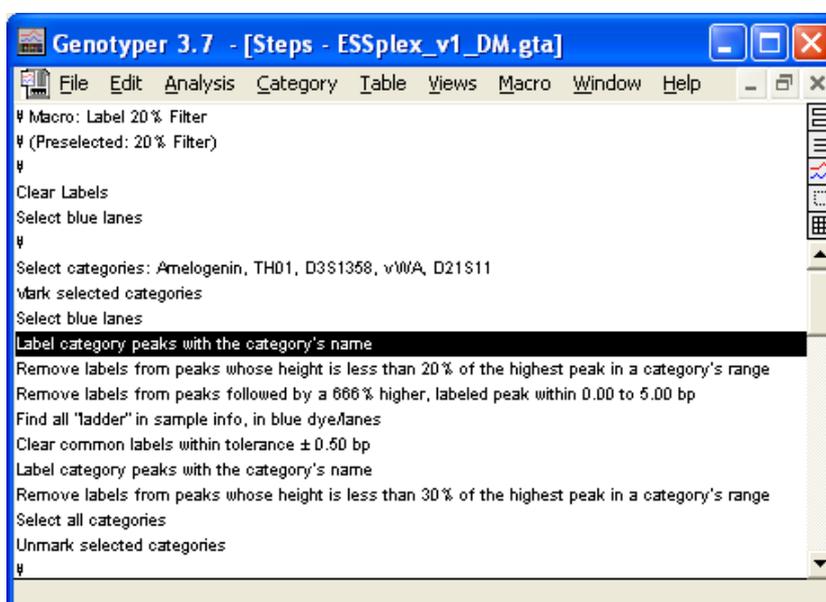
## Labeling of all relevant peaks

- Peak designations below 20% of the height of the highest peak in the category (STR loci) are not shown in the macro "Label 20% Filter". To change the preselected 20% filter of the macro see "Changing Filter Value", page 16.
- Filter options are integrated into the macro in order to avoid stutter peaks and template-independent nucleotide addition (minusA peaks).
- Peaks in the allelic range labeled with "allele?" can not be assigned to an allele size. These labels have to be checked manually and may be deleted by clicking on it (see 'Many peaks of the sample are termed "allele?"' in the troubleshooting guide", page 23). If necessary, scale down the window.

## New peak designations (labels)

Perform the following steps to change peak designation:

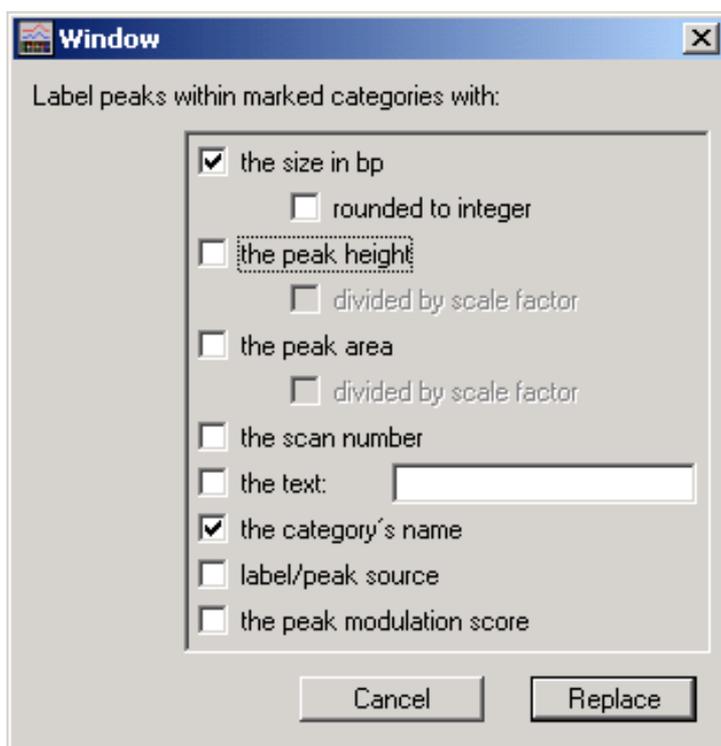
1. **Unlock the Template File by going to "File" and selecting "Unlock".**
2. **Select the macro to be adjusted.**
3. **Go to "View" and select "Show Step Window" to open the step window.**
4. **Select the step to be adjusted by clicking on the appropriate line in the step window (e.g., Label category peaks, see screenshot below).**



"The "Labels" tab is used for designation of the peaks.

5. **Go to "Macro" and select "Edit Step" to edit this step**
6. **Click the desired peak designation (e.g., size in bp, peak height, peak area) on the window that appears and confirm the changes by**

clicking "Replace".



Edit peak designations.

7. Repeat the process with all categories to be adjusted.
8. When all changes are complete, lock the Template File by going to "File" and selecting "Lock".
9. Click "Save As" and save the file with a new filename.

**Note:** The Macro "Label 20% filter" is unsuitable for the analysis of DNA mixtures.

New filter options must be defined by the user. Alternatively, the macro "Label 10% Filter" can be used.

## Label 10% Filter

- The shortcut key to initiate the macro "Label 10% Filter" is Ctrl + 4
- Use this macro for the analysis of stains or samples containing more than one DNA (e.g., for forensic casework)
- Peaks will be labeled with the appropriate allele
- A plot window will open up automatically with the following entries:
  - DNA samples

- Allelic ladder
- DNA size standard, if imported

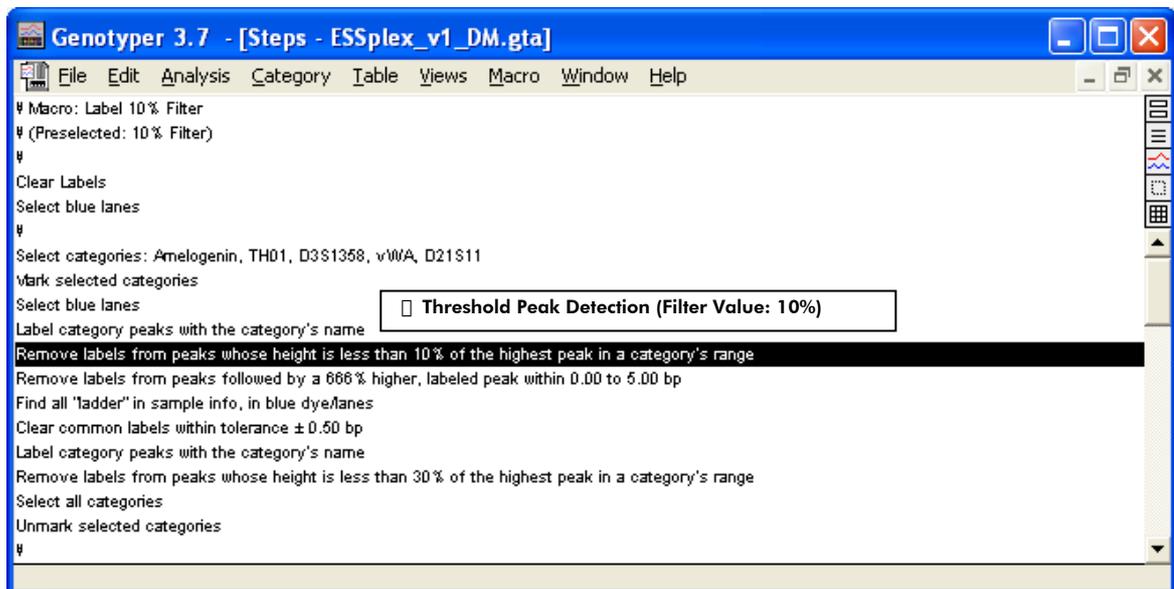
### Basic setting for improved peak detection

- In the macro “Label 10% Filter”, all peak designations higher than 10% of the highest peak height in the category are labeled thereby improving peak detection (preselected: 10% filter).
- Filter options are already integrated into the macro in order to avoid stutter peaks and template-independent nucleotide addition (minusA peaks).

### Changing Filter Value

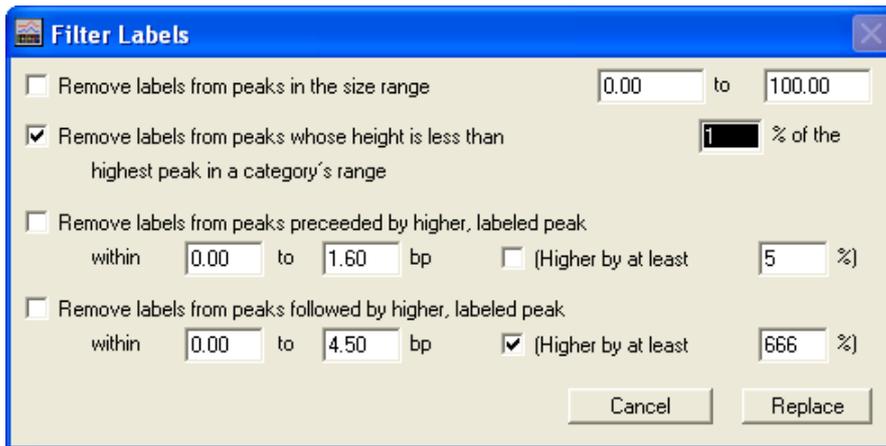
Perform the following steps to change the lower threshold for the peak detection (preselection filter 20%) in the Genotyper macro.

1. Go to “File” and select “Unlock” to unlock the Template File.
2. Select the macro to be adjusted.
3. Go to “Views” and select “Show Step Window”.
4. For improved detection of categories (STR-loci) select the step “Remove Labels...” by clicking on the appropriate line in the step window.



Step Window of Macro “Label 10% Filter”.

5. Go to “Macro” and select “Edit Step”, to edit the step.
6. Go to “Filter Labels” and amend the value for the lower threshold of the filter (see black shaded field in screenshot, next page)



Edit Filter Values.

7. When all changes are complete, lock the Template File by going to "File" and selecting "Lock".
8. Click "Save As" and save the file with a new filename.

## Blue, green, yellow, and red panels

- The shortcut keys required are Ctrl + 5, Ctrl + 6, Ctrl + 7, Ctrl + 8
- Used to compare samples with the appropriate allelic ladder
- Shows the labels generated with the label macro used (see "Label 20% Filter", page 13 and "Label 10% Filter", page 15)
- To simplify allocation, zoom in on the appropriate area: Go to "Views" and click "Zoom", followed by "Zoom to" (e.g., 70–220 bp)

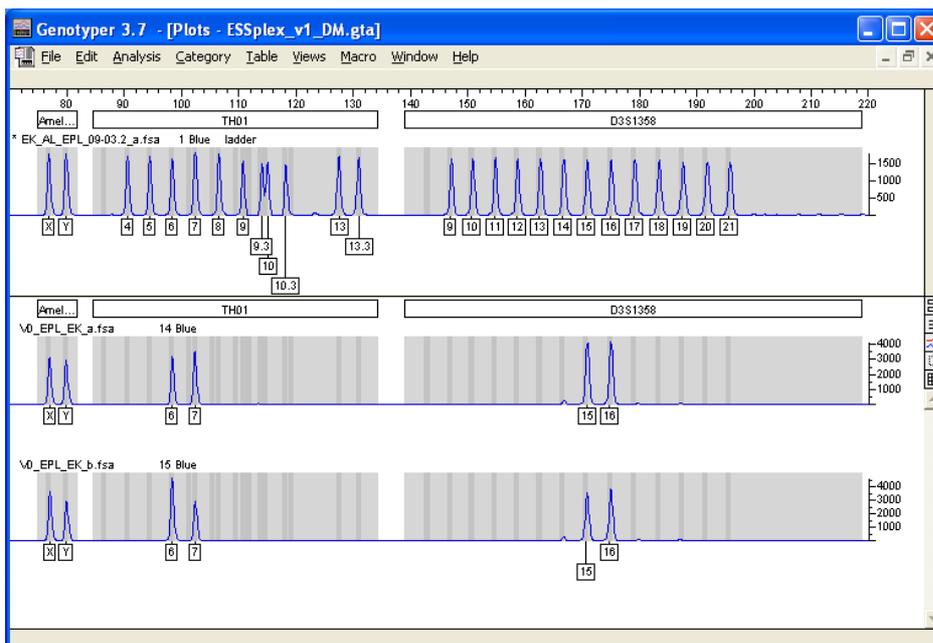


Figure 4. Plot Window after execution of the Macro "Blue Panels" with allelic ladder and DNA samples.

## Show categories

To display all categories (STR loci) in the panel of one color, check the “Category boundaries” check box.

Go to “Views” select “Plot Options”, followed by “Plot Window, Upper Panel” and “Plot Window, Lower Panel”.

In “Plot Window, Upper Panel”, the allelic ladder is fixed at the top of the window. In “Plot Window, Lower Panel”, the DNA samples are shown at the bottom of the window. Genotyper Software is unable to show categories when different panel colors are displayed one below the other.

## Horizontal Table for 2 Alleles

- The shortcut key to initiate the macro “Horizontal table for 2 alleles” is Ctrl + 9
- Use this macro to analyze samples of one DNA, e.g., for a DNA database
- This macro displays the allele designation of two peaks of each marker
- This macro generates a table after the label macro has been performed
- This macro displays the allele designation of up to 2 peaks per category (STR locus) for each sample in one line
- For genetically homozygote peaks, the allele designation will be displayed twice
- In order to discriminate between individual samples, the “Sample Info” field for each sample color must be completed with an appropriate description. Go to “Views” and select “Show Dye/Lanes Window”
- Use “Table” and “Update Table” to copy the current allele designation for peaks to the table. Misnomers resulting from pull-up peaks between different panels can be deleted manually by clicking on the peak.



The screenshot shows a window titled "Genotyper 3.7 - [Table - ESSplex\_v1\_DM.gta]". The window contains a table with the following data:

Sample Info	Amelogenin 1	Amelogenin 2	TH01 1	TH01 2	D3S1358 1	D3S1358 2	vWA 1	vWA 2
ladder	X	Y	4	5	9	10	11	12
	X	Y	6	7	15	16	15	16
DNA 13	X	Y	6	7	15	16	15	16

Figure 5. Output File after execution of the macro “Horizontal Table for 2 Alleles”.

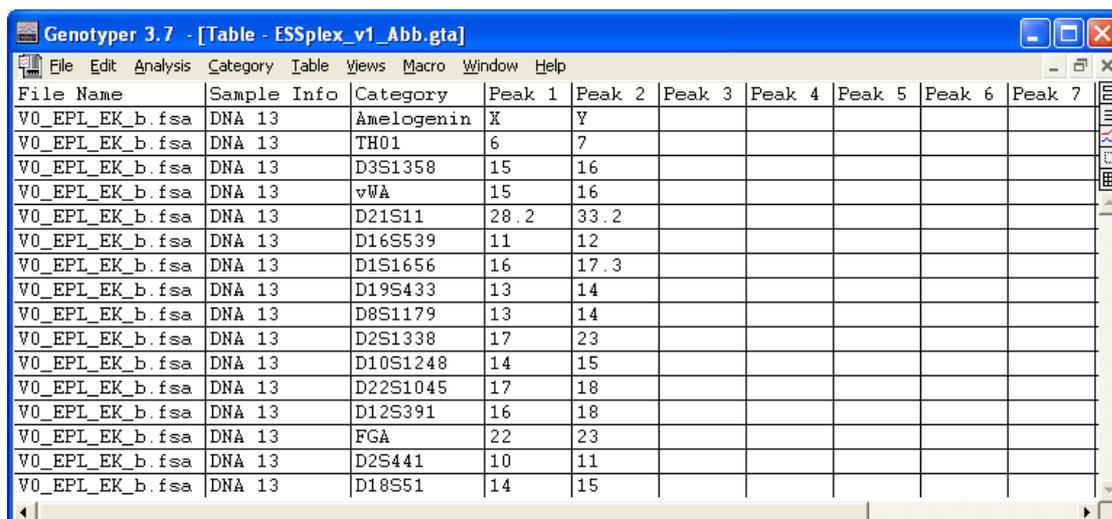
## Table export

Perform the following steps to export the table to Microsoft® Excel®.

1. **Go to “Table” and choose “Export to File”.**  
Save the file with the extension “.xls”.
2. **Open Microsoft Excel software and load the table.**
3. **The conversion procedure opens automatically. Follow the conversion procedure.**

## Vertical Table for 10 Alleles

- Use this macro to analyze stains or mixed samples of different DNAs
- This macro displays the allele designation of up to 10 peaks for each marker
- This macro generates a table after the label macro has been performed
- This macro displays the allele designation of up to 10 alleles per category (STR locus) and generates a vertical table. The categories are all listed in one line (e.g., ESSplex: all categories x 10 alleles)
- In “Sample Info”, the filename of the sample is displayed automatically
- For genetically homozygote peaks, the allele designation will be displayed twice
- Use “Table” and “Update Table” to copy the current allele designation for peaks to the table. Misnomers resulting from pull-up peaks between different panels can be deleted manually by clicking on the peak.



File Name	Sample Info	Category	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
V0_EPL_EK_b.fsa	DNA 13	Amelogenin	X	Y					
V0_EPL_EK_b.fsa	DNA 13	TH01	6	7					
V0_EPL_EK_b.fsa	DNA 13	D3S1358	15	16					
V0_EPL_EK_b.fsa	DNA 13	vWA	15	16					
V0_EPL_EK_b.fsa	DNA 13	D21S11	28.2	33.2					
V0_EPL_EK_b.fsa	DNA 13	D16S539	11	12					
V0_EPL_EK_b.fsa	DNA 13	D1S1656	16	17.3					
V0_EPL_EK_b.fsa	DNA 13	D19S433	13	14					
V0_EPL_EK_b.fsa	DNA 13	D8S1179	13	14					
V0_EPL_EK_b.fsa	DNA 13	D2S1338	17	23					
V0_EPL_EK_b.fsa	DNA 13	D10S1248	14	15					
V0_EPL_EK_b.fsa	DNA 13	D22S1045	17	18					
V0_EPL_EK_b.fsa	DNA 13	D12S391	16	18					
V0_EPL_EK_b.fsa	DNA 13	FGA	22	23					
V0_EPL_EK_b.fsa	DNA 13	D2S441	10	11					
V0_EPL_EK_b.fsa	DNA 13	D18S51	14	15					

Figure 6. Output file after execution of the macro “Vertical Table for 10 Alleles”.

## Calculation of category offsets

- Macros for calibration of individual categories
- This macro must be used with the macro "Calibrate Ladder".

## Adjustment of Categories

Perform the following steps to adjust categories:

1. **Open the Template File corresponding to the Investigator Human Identification PCR Kit being used. Genotyper Software will open automatically.**
2. **Go to "File" and select "Unlock" to unlock the Template File (see lock symbol at the lower left sidebar).**
3. **Go to "Views" and select "Show Categories", followed by "Open categories".**
4. **Go to "Category" and then "Edit Category". Select the allele to be adjusted from the table.**
5. **Change the desired allele features (tolerance range, fragment length).**
6. **Click "Replace" to confirm the changes.**
7. **Got to "File" and click "Save As" to save the file with a new filename.**

**Note:** Additional categories for newly found (intermediate) alleles can be created in the same way. Here, a tolerance range of  $\pm 0.45$  bp should be chosen. However, these added categories are not evaluated by the automatic calibration of the macro "Calibrate Ladder".

QIAGEN assumes no liability for changes made to Investigator Template Files. The actual versions of Investigator Template Files for all test kits are available at the Web catalog page of each Investigator Human Identification PCR Kit on the QIAGEN Web site ([www.qiagen.com](http://www.qiagen.com)).

## 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](http://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](http://www.qiagen.com)).

### Comments and suggestions

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#### **Error message during program start: "Could not start the application because there is not enough memory."**

Both Genotyper and GeneScan Software applications from Applied Biosystems have been discontinued and are unavailable for purchase (as of June 1, 2004).

Applied Biosystems support for these products ended June 1, 2009.

Use an appropriate Windows-based computer.

A computer with less than 1 GB memory may not work with Genotyper software.

#### **The template file cannot be opened**

a) Genotyper Software is not the most recent version (Macintosh version 2.5; Windows version 3.7)

Upgrade the Genotyper Software.

The old Macintosh Genotyper version 2.1 can be updated via a free download, available from Applied Biosystems.

b) Genotyper Software was not installed on the local computer

Install Genotyper Software on the local Computer.

c) Template Files or the CD-ROM were damaged

Download the files again from [www.qiagen.com](http://www.qiagen.com) or reorder the CD-ROM.

## Comments and suggestions

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### **Error message during analysis: "Could not complete your request because the labeled peak could not be found."**

- |   |  |
|---|--|
| a) An additional signal can be identified as a peak of the allelic ladder because of malfunction during the electrophoresis. If peaks of the allelic ladder are miscalled, the ladder can not be used for the analysis. | Use a different injection of the allelic ladder and check the data of the analyzed sizes from the Size Standard (in bp) of the allelic ladder.<br><br>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 as a peak   | The allelic ladder must be loaded onto the analysis instrument at a higher concentration than samples to be analyzed.<br><br>Alternatively, allelic ladder data can be analyzed with a lower peak detection value in Genotyper or GeneScan Software. |
| d) 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.   |
| e) Deviant fragment lengths due to unsuitable size standard   | Always use the DNA Size Standard 550 for Investigator Human Identification PCR Kits.   |

### **Error message during analysis "Could not complete your request because no dye/lanes are selected."**

- |   |  |
|---|--|
| a) The allelic ladder file was not loaded     | Import the file with the allelic ladder.   |
| b) The allelic ladder file was not identified | For every color of the allelic ladder, the term "ladder" must be listed. Go to "Views" and select "Show Dye/Lanes Window". Add "ladder" in the text field "Sample Info". |

## Comments and suggestions

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- c) Not all five dye colors (blue, green, yellow, red, orange) have been imported      Go to "Edit" and choose "Select preferences". Choose all dye colors and import them again.

### Many peaks of the sample are termed "allele?"

- a) Bubbles in the capillary lead to pull-up peaks in all color panels ("spikes") that result in allele misnomer      Repeat electrophoresis to confirm results.
- 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), the frequency of stutters, split peaks, and artifacts may increase      Reduce the injection time by up to 1 s, reduce the amount of the PCR amplification product for analysis, or reduce the quantity of DNA for PCR.
- c) The DNA size standard 550 (BTO or ROX) was not defined or was identified incorrectly      Click on icon  for BTO or  for ROX panels in the left upper corner of the Genotyper Software and display the orange or red fragments of all samples in the Plot Window.
- d) Data from different analyzers (e.g., ABI 310 and ABI 3130) have been saved within one GTA-file      Data from different analyzers must always be saved in different GTA-files.

### Point alleles are not found

Point alleles were not separated in the GeneScan analysis

Point alleles are 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. Then, analyze the DNA samples again using the new parameters.

## References

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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

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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
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