USER GUIDE

applied biosystems by life technologies™

AuthentiFiler™ PCR Amplification Kit

for use with:
50 reaction kit (Cat. no. 4479566)

Publication Number 4479553
Revision C

For Cell Line Authentication use excluding, Forensic, Paternity, Diagnostic, and Therapeutic applications.

life technologies™

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Contents

About This Guide

IMPORTANT! Before using this product, read and understand the information the "Safety" appendix in this document.

Revision history

Revision	Date	Description
A	November 2012	New document
B	February 2013	• Remove 3100/3100 Avant instrument information • Add information on: – Optimum PCR cycle and peak height guidance – Validate DNA extraction and quantification methods are validated for use with PCR amplification
C	March 2013	Perform PCR: Add statement to perform internal validation studies to optimize the DNA template input quantity for STR amplification to obtain optimal peak height.

Purpose

The *AuthentiFiler™ PCR Amplification Kit User Guide* provides information about the Applied Biosystems® instruments, chemistries, and software associated with the AuthentiFiler™ PCR Amplification Kit.

1 Overview

Overview

Purpose

The AuthentiFiler™ PCR Amplification Kit is a short tandem repeat (STR) multiplex PCR assay that amplifies 9 unique STR loci (8 loci comprise tetranucleotide repeat units and one locus trinucleotide) and the Amelogenin gender-determining marker in a single PCR amplification. The kit uses more loci concentrated in the shorter fragments (or smaller amplicons) region of the profile to improve performance on degraded samples, and an improved process for synthesis and purification of the amplification primers to deliver a much cleaner electrophoretic background.

Note: The quality of sample, the DNA extraction method used, and the quality of DNA extract may affect the data quality of profiles.

The AuthentiFiler™ Kit is intended for use in human cell identification in various research based applications requiring cell discrimination and identity confirmation, such as human cell line authentication testing and induced pluripotent stem cell (iPSC) genetic confirmation testing.

Following extraction of gDNA and amplification of STR loci, the amplified PCR fragments are run along with allelic ladder size standards to generate genotypes for each amplified loci. The combination of genotypes creates a unique and highly discriminatory pattern that can differentiate the cell with a probability of identity of approximately 7.75×10^{-12}.

Product description

The AuthentiFiler™ PCR Amplification Kit contains all reagents necessary (PCR Master Mix, PCR Oligo Mix) for the amplification of target loci from 2 ng of human gDNA. In addition, the kit contains control gDNA of known genotype in the form of a positive control and an allelic ladder, which is run alongside unknown fragments to enable genotype determination on the Capillary Electrophoresis instrument using GeneMapper® Software.

The reagents are designed for use with the following Life Technologies instruments:

- Applied Biosystems® 3130/3130*xl* Genetic Analyzer
- Applied Biosystems® 3500/3500xL Genetic Analyzer
- GeneAmp® PCR System 9700 with the Silver 96-Well Block
- GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block
- Veriti® 96-Well Thermal Cycler

About the primers

The AuthentiFiler™ Kit uses the same primer sequences as the AmpFℓSTR® NGM SElect™ Kit for all loci except D6S1043 which uses the same primer sequences as AmpFℓSTR® Sinofiler™ Kit and D2S1338 which uses the same primer sequences as AmpFℓSTR® MiniFiler™ Kit, and benefits from the same primer synthesis and purification improvements. These improvements enhance the assay signal-to-noise ratio and simplify the interpretation of results.

Loci amplified by the kit

The following table shows the loci amplified, their chromosomal locations, and the corresponding fluorescent marker dyes. The AuthentiFiler™ Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder and the genotype of the AuthentiFiler™ DNA Control 007 are also listed in the table.

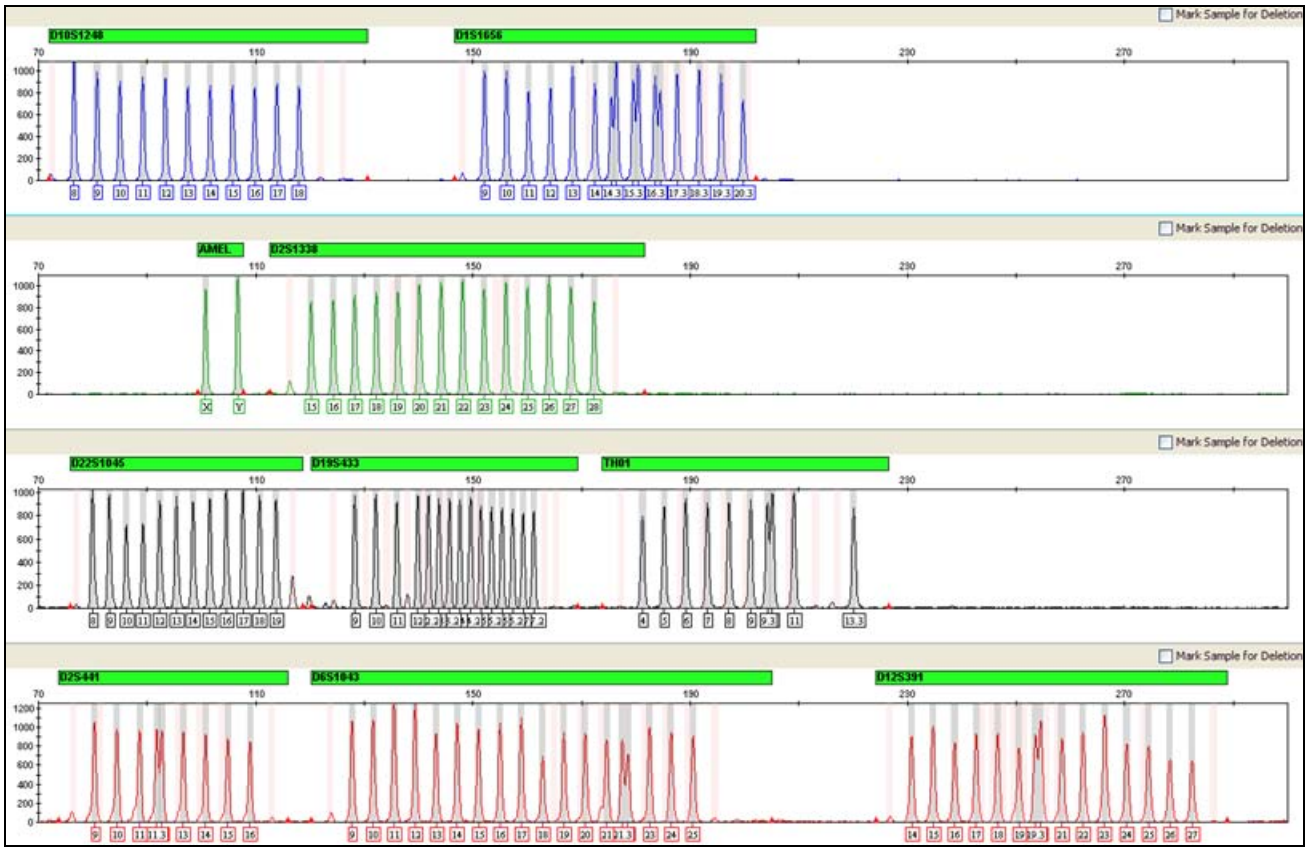
Table 1 AuthentiFiler™ PCR Amplification Kit loci and alleles

Locus designation	Chromosome location	Category; Repeat motif	Alleles included in AuthentiFiler™ Allelic Ladder	Dye label	DNA Control 007
D10S1248	10q26.3	Simple; GGAA	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	6-FAM™	12, 15
D1S1656	1q42.2	Compound; TAGA	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3	6-FAM™	13, 16
Amelogenin	X: p22.1-22.3 Y: p11.2		X, Y	VIC®	X, Y
D2S1338	2q35	Compound; TGCC/TTCC	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28	VIC®	20, 23
D22S1045	22q12.3	Simple; ATT	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	NED™	11, 16
D19S433	19q12	Compound; AAGG/TAGG	9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	NED™	14, 15
TH01	11p15.5	Simple; TCAT	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3	NED™	7, 9.3
D2S441	2p14	Compound; TCTA/TCAA	9, 10, 11, 11.3, 12, 13, 14, 15, 16	PET®	14, 15
D6S1043	6q16.1	Compound; AGAT/AGAC	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 21.3, 22, 23, 24, 25	PET®	12, 14
D12S391	12p13.2	Compound; AGAT/AGAC	14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27	PET®	18, 19

Allelic ladder profile

Figure 1 shows the allelic ladder for the AuthentiFiler™ Kit. See "Allelic ladder requirements" on page 23 for information on ensuring accurate genotyping.

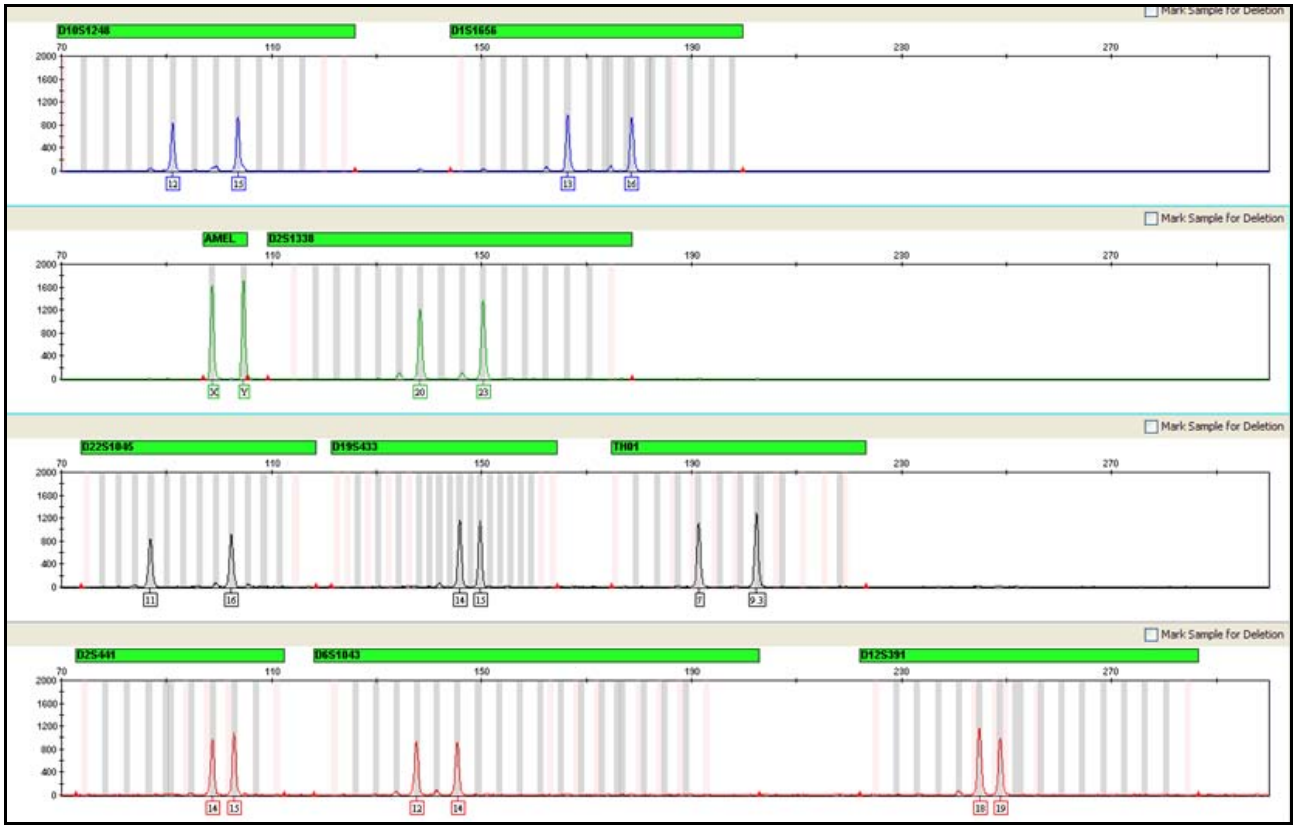
Figure 1 GeneMapper® *ID-X* Software plot of the AuthentiFiler™ Allelic Ladder



AuthentiFiler™ DNA Control 007 profile

Figure 2 shows amplification of DNA Control 007 using the AuthentiFiler™ Kit.

Figure 2 2 ng of AuthentiFiler™ DNA Control 007 amplified at 27 PCR cycles with the AuthentiFiler™ Kit and analyzed on the Applied Biosystems® 3130*xl* Genetic Analyzer



Workflow overview

Perform PCR

Extract DNA

Quantify DNA

Prepare reactions

AuthentiFiler™ PCR Amplification Kit

Perform PCR

GeneAmp® PCR System 9700 Cycler

Veriti® 96-Well Thermal Cycler

Perform electro-phoresis

3130/3130*xl*
Genetic Analyzer

3500/3500xL
Genetic Analyzer

Analyze data

GeneMapper® *ID-X*, GeneMapper® *ID* Software, or GeneMapper® v4.1 Software (and higher)

Instrument and software overview

This section provides information about the Data Collection Software versions required to run the AuthentiFiler™ Kit on specific instruments.

Data Collection and GeneMapper® *ID*, GeneMapper® *ID-X*, or GeneMapper® Software

The Data Collection Software provides instructions to firmware running on the instrument and displays instrument status and raw data in real time. As the instrument measures sample fluorescence with its detection system, the Data Collection Software collects the data and stores it. The Data Collection Software stores information about each sample in a sample file (.fsa files for 3130 instruments and .hid files for 3500 instruments), which is then analyzed by the GeneMapper® *ID*, GeneMapper® *ID-X*, or GeneMapper® Software.

Instrument and software compatibility

Table 2 Software specific to each instrument

Instrument	Operating system	Data Collection Software	Analysis software
3500/3500xL	• Windows® XP • Windows Vista®	3500 Series Data Collection Software v1.0	GeneMapper® *ID-X* Software v1.2 or higher
3130/3130*xl*†	Windows® XP	3.0	• GeneMapper® *ID* Software v3.2.1 *or* • GeneMapper® *ID-X* Software v1.0.1 or higher *or* • GeneMapper® Software v4.1 or higher

† We conducted validation studies for the AuthentiFiler™ Kit using this configuration.

About multicomponent analysis

Our fluorescent multi-color dye technology allows the analysis of multiple loci, including loci that have alleles with overlapping size ranges. Alleles for overlapping loci are distinguished by labeling locus-specific primers with different colored dyes.

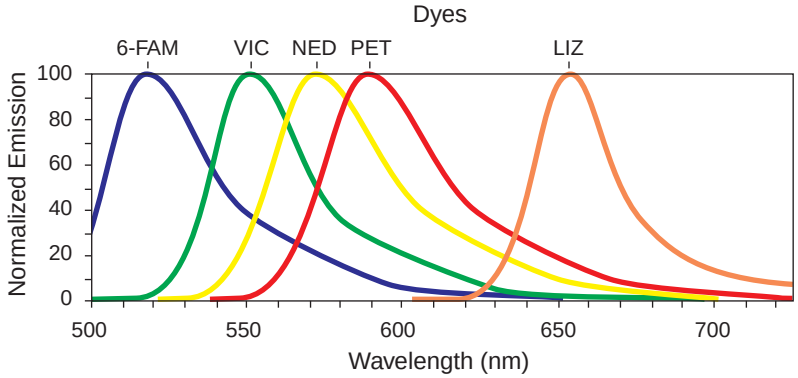
Multicomponent analysis is the process that separates the 5 different fluorescent dye colors into distinct spectral components allowing for fragment size resolution on Applied Biosystems® Genetic Analyzers. The four dyes used in the AuthentiFiler™ PCR Amplification Kit to label samples are 6-FAM™, VIC®, NED™, and PET® dyes. The fifth dye, LIZ®, is used to label the GeneScan™ 500 LIZ® Size Standard or GeneScan™ 600 LIZ® Size Standard v2.0.

How multicomponent analysis works

Each of these fluorescent dyes emits its maximum fluorescence at a different wavelength. During data collection on the Applied Biosystems® instruments, the fluorescence signals are separated by diffraction grating according to their wavelengths and projected onto a charge-coupled device (CCD) camera in a predictably spaced pattern. The 6-FAM™ dye emits at the shortest wavelength and it is displayed as blue, followed by the VIC® dye (green), NED™ dye (yellow), PET® dye (red), and LIZ® dye (orange).

Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes (Figure 3). The goal of multicomponent analysis is to correct for spectral overlap.

Figure 3 Emission spectra of the five dyes used in the AuthentiFiler™ Kit



Materials and equipment

Kit contents and storage

The AuthentiFiler™ Kit contains materials sufficient to perform 50 amplifications (Cat. no. 4479566) at a 25 µL reaction volume.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standards from light when not in use. Keep freeze-thaw cycles to a minimum.

Table 3 Kit contents and storage

Component	Description	50× Volume	Storage
AuthentiFiler™ Primer Set	Contains forward and reverse primers to amplify human DNA targets.	1 tube, 0.25 mL	–15 to –25°C on receipt, 2 to 8 °C after initial use; protect from light
AuthentiFiler™ Allelic Ladder	Contains amplified alleles. See Table 1 on page 10 for a list of alleles included in the allelic ladder.	1 tube, 0.025 mL	
AuthentiFiler™ Master Mix	Contains enzyme, salts, dNTPs, carrier protein, and 0.05% sodium azide.	1 tube, 0.5 mL	–15 to –25°C on receipt, 2 to 8 °C after initial use
AuthentiFiler™ DNA Control 007	Contains 2 ng/µL human male DNA in 0.05% sodium azide and buffer†. See Table 1 on page 10 for profile.	1 tube, 0.025 mL	

† The AuthentiFiler™ DNA Control 007 is included at a concentration appropriate to its intended use as an amplification control (to provide confirmation of the capability of the kit reagents to generate a profile of expected genotype). The AuthentiFiler™ DNA Control 007 is not designed to be used as a DNA quantitation control, and laboratories may expect to see variation from the labelled concentration when quantitating aliquots of the AuthentiFiler™ DNA Control 007.

Standards for samples

For the AuthentiFiler™ Kit, the panel of standards needed for PCR amplification, PCR product sizing, and genotyping are:

- **AuthentiFiler™ DNA Control 007** — A positive control for evaluating the efficiency of the amplification step and STR genotyping using the AuthentiFiler™ Allelic Ladder.
- GeneScan™ 500 LIZ® Size Standard **(GS 500)** or GeneScan™ 600 LIZ® Size Standard v2.0 **(GS 600 v2.0)** — Used for obtaining sizing results. These standards, which have been evaluated as internal size standards, yield precise sizing results for AuthentiFiler™ Kit PCR products. Order the GeneScan™ 500 LIZ® Size Standard (Cat. no. 4322682) or the GeneScan™ 600 LIZ® Size Standard v2.0 (Cat. no. 4408399) separately.
- **AuthentiFiler™ Allelic Ladder** — Allelic ladder developed by Life Technologies for accurate characterization of the alleles amplified by the AuthentiFiler™ Kit. The AuthentiFiler™ Allelic Ladder contains most of the alleles reported for the 9 autosomal loci. See Table 1 on page 10 for a list of the alleles included in the AuthentiFiler™ Allelic Ladder.

2 PCR Amplification

Required user-supplied materials and reagents

In addition to the AuthentiFiler™ Kit reagents, the use of low-TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) is recommended. You can prepare the buffer as described in the procedure below or order it from Teknova (Cat. no. T0223).

To prepare low-TE buffer:

1. Mix together:
   - 10 mL of 1 M Tris-HCl, pH 8.0
   - 0.2 mL of 0.5 M EDTA, pH 8.0
   - 990 mL glass-distilled or deionized water

   Note: Adjust the volumes based on your specific needs.
2. Aliquot and autoclave the solutions.
3. Store at room temperature for appropriate period.

DNA extraction

Note: The quality of sample, the DNA extraction method used, and the quality of DNA extract may affect the data quality of profiles.

Extract DNA using a method you have validated in your laboratory.

IMPORTANT! Validate DNA extraction methods for use with PCR amplification before preparing samples for use with the AuthentiFiler™ Kit.

DNA quantification

Extract DNA using a method you have validated in your laboratory.

IMPORTANT! Validate DNA quantification methods for use with PCR amplification before preparing samples for use with the AuthentiFiler™ Kit.

Importance of DNA quantification

Quantifying the amount of extracted gDNA in a sample before amplification allows you to determine whether or not sufficient DNA is present to permit amplification and to calculate the optimum amount of DNA to add to the reaction. The optimum amount of DNA for the AuthentiFiler™ Kit is 2.0 ng in a maximum input volume of 10 µL amplified for 27 cycles.

Perform internal validation studies to optimize the DNA template input quantity for STR amplification to obtain optimal peak height.

If too much DNA is added to the PCR reaction, then the increased amount of PCR product that is generated can result in:

- Fluorescence intensity that exceeds the linear dynamic range for detection by the instrument ("off-scale" data). Off-scale data are problematic because:
  - Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause the corresponding stutter peak to appear higher in relative intensity, thus increasing the calculated percent stutter.
  - Multicomponent analysis of off-scale data is not accurate, and it results in poor spectral separation ("pull-up").
- Incomplete A-nucleotide addition.

When the total number of allele copies added to the PCR is extremely low, allelic dropout can occur, resulting in a partial profile.

Our studies indicate the optimum PCR cycle number should generate profiles with the following heterozygote peak heights, with no instances of allelic dropout and minimal occurrence of off-scale allele peaks:

Instrument	Heterozygous peak height
3130 Series	1500–3000 RFU
3500 Series	3000–6000 RFU

Methods of quantifying DNA

We recommend using a human-specific quantification method such as found in Quantifiler® Kits for better correlation between the quantity of template DNA used for amplification and the electrophoresis results (rfu of allele peaks). We provide several kits for quantifying DNA in samples. See the references cited in the following table for details about these kits.

Additional DNA quantification methods can be used, for example, spectrophotometer/ NanoDrop™ 1000 Spectrophotometer, etc.

Product	Description	References
Quantifiler® Human DNA Quantification Kit (Cat. no.4343895)	**Properties:** The Quantifiler® Human Kit is highly specific for human DNA and detects total human DNA. The kits detects amplifiable DNA. **How it works:** The Quantifiler® DNA Quantification Kit consists of target-specific and internal control 5' nuclease assays. The Quantifiler® Human Kit consists of a human DNA target-specific assay that contains two locus-specific PCR primers and one TaqMan® MGB probe labeled with 6-FAM™ dye for detecting the amplified sequence. The kit contains a separate internal PCR control (IPC) assay that consists of an IPC template DNA (a synthetic sequence not found in nature), two primers for amplifying the IPC template DNA, and one TaqMan® MGB probe labeled with VIC® dye for detecting the amplified IPC DNA.	*Quantifiler® Human DNA Quantification Kits User's Manual* (Pub. no. 4344790)
Quantifiler® Duo DNA Quantification Kit (Cat. no. 4387746)	**Properties:** The Quantifiler® Duo Kit is highly specific for human DNA and combines the detection of both total human and human male DNA in one PCR reaction.The kit detects amplifiable DNA. **How it works:** The Quantifiler® Duo DNA Quantification Kit consists of target-specific and internal control 5' nuclease assays. The Quantifiler® Duo Kit combines two human-specific assays in one PCR reaction (for total human DNA and human male DNA). The two human DNA specific assays each consist of two PCR primers and a TaqMan® probe. The TaqMan® probes for the human DNA and human male DNA assays are labeled with VIC® and 6-FAM™ dyes, respectively. In addition, the kit contains an internal PCR control (IPC) assay similar in principle to that used in the other Quantifiler® Kits, but labeled with NED™ dye.	*Quantifiler® Duo DNA Quantification Kit User's Manual* (Pub. no. 4391294)

Prepare the amplification kit reactions

1. Calculate the volume of each component needed to prepare the reactions, using the table below.

DNA sample	Volume per reaction
AuthentiFiler™ Master Mix	10.0 µL
AuthentiFiler™ Primer Set	5.0 µL

Note: Include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

2. Prepare reagents. Thaw the AuthentiFiler™ Master Mix and the AuthentiFiler™ Primer Set, then vortex the tubes for 3 seconds and centrifuge them briefly before opening.

IMPORTANT! Thawing is required only during first use of the kit. After first use, reagents are stored at 2–8°C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

3. Pipet the required volumes of components into an appropriately sized polypropylene tube.
4. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
5. Dispense 15 µL of reaction mix into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate or each MicroAmp® tube.
6. Prepare the DNA samples:

DNA sample	To prepare...
Negative control	Add 10 µL of low-TE buffer (10mM Tris, 0.1mM EDTA, pH 8.0).
Test sample	Dilute a portion of the test DNA sample with low-TE buffer so that 2.0 ng of total DNA is in a final volume of 10 µL. Add 10 µL of the diluted sample to the reaction mix.
Positive control	Add 1 µL of AuthentiFiler™ DNA Control 007 (2.0 ng/µL) to provide 2.0 ng of total DNA in the positive control reaction. Add 9 µL of low-TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) to get a final volume of 10 µL.

The final reaction volume (sample or control plus reaction mix) should be 25 µL.

7. Seal the MicroAmp® Optical 96-Well Reaction Plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film, or cap the tubes.
8. Centrifuge the tubes or plate at 3000 rpm for about 20 seconds in a tabletop centrifuge (with plate holders if using 96-well plates) to remove bubbles.

9. Amplify the samples in a GeneAmp® PCR System 9700 with the Silver 96-well block, or a GeneAmp® PCR System 9700 with the Gold-plated Silver 96-well block, or a Veriti® 96-well Thermal Cycler.

IMPORTANT! The AuthentiFiler™ Kit is not validated for use with the GeneAmp® PCR System 9700 with the Aluminium 96-well block. Use of this thermal cycling platform may adversely affect the performance of the AuthentiFiler™ Kit.

Perform PCR

1. Program the thermal cycling conditions.
   - When using the GeneAmp® PCR System 9700 with either 96-well silver or gold-plated silver block, select the **9600 Emulation Mode**.
   - When using the Veriti® 96-Well Thermal Cycler, refer to the following document for instructions on how to configure the Veriti instrument to run in the 9600 Emulation Mode: *User Bulletin: Veriti® 96-Well Thermal Cycler AmpFℓSTR® Kit Validation* (Pub. no. 4440754).

Initial incubation step	Cycle (27 cycles)		Final extension	Final hold
	Denature	Anneal and Extension		
HOLD	CYCLE		HOLD	HOLD
95°C 11 min	94°C 20 sec	59°C 3 min	60°C 10 min	4°C ∞

IMPORTANT! The optimum conditions for the AuthentiFiler™ Kit are 27 cycles of amplification with a 2 ng input DNA concentration. Perform internal validation studies to evaluate kit performance at each cycle number intended for operational use.

Perform internal validation studies to optimize the DNA template input quantity for STR amplification to obtain optimal peak height.

Note: If using genomic DNA isolated from FFPE tissues, use 2 ng DNA input and above mentioned PCR cycling conditions with 29 cycles.

2. Load the plate or tubes into the thermal cycler and close the heated cover.

IMPORTANT! If using the 9700 thermal cycler with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, be sure to place a MicroAmp® Compression Pad (Cat. no. 4312639) on top of the plate to prevent evaporation during thermal cycling.

3. Start the run.

4. On completion of the run, store the amplified DNA and protect from light.

If you are storing the amplified DNA...	Then place at...
< 1 week	2 to 8°C
> 1 week	–15 to –20°C

Note: The signal strength of the VIC channel artifact increases with storage of the amplification plate at 4°C, most commonly when plates are left at 4°C for a few days. We recommend storing amplification products at –20°C.

IMPORTANT! Store the amplified products so that they are protected from light.

3 Electrophoresis

Allelic ladder requirements

To accurately genotype samples, you must run an allelic ladder sample along with the unknown samples.

Instrument	Number of allelic ladders to run	One injection equals	Number of samples per allelic ladder(s)
3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3130*xl*	1 per injection	16 samples	15 samples + 1 allelic ladder
3500	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
3500xL	1 per injection	24 samples	23 samples + 1 allelic ladder

IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between both single- and multiple-capillary runs (with larger size variations seen between samples injected in multiple-capillary runs). We recommend the above frequency of allelic ladder injections, which should account for normal variation in run speed. However, during internal validation studies, verify the required allelic ladder injection frequency to ensure accurate genotyping of all samples in your laboratory environment.

It is critical to genotype using an allelic ladder run under the same conditions as the samples, because size values obtained for the same sample can differ between instrument platforms because of different polymer matrices and electrophoretic conditions.

Section 3.1 3130/3130*xl* instruments

Set up the 3130/3130*xl* instruments for electrophoresis

Reagents and parts

Appendix B, "Ordering Information" on page 87 lists the required materials not supplied with the AuthentiFiler™ Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use. Keep freeze-thaw cycles to a minimum.

Electrophoresis software setup and reference documents

The following table lists data collection software and the run modules that can be used to analyze AuthentiFiler™ Kit PCR products. For details on the procedures, refer to the documents listed in the table.

Genetic Analyzer	Data Collection Software	Operating System	Run modules and conditions	References
Applied Biosystems® 3130/3130*xl*	3.0†	Windows® XP	• HIDFragmentAnalysis36_POP4_1 Injection conditions: – 3130 = 3 kV/5 sec – 3130*xl* = 3 kV/10 sec • Dye Set G5	Applied Biosystems® *3130/3130xl Genetic Analyzers Using Data Collection Software v3.0, Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin* (Pub. no. 4363787)

† We conducted validation studies for the AuthentiFiler™ Kit using this configuration.

Prepare samples for electrophoresis on the 3130/3130*xl* instruments

Prepare the samples for electrophoresis on the 3130/3130*xl* immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and size standard needed to prepare the samples:

Reagent	Volume per reaction
GS 500 LIZ® Size Standard *or* GS 600 LIZ® Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	9.5 µL

Note: Include additional samples in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Pipet the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each well of a MicroAmp® Optical 96-Well Reaction Plate, add:
   - 10 µL of the formamide:size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   Note: For blank wells, add 11 µL of Hi-Di™ Formamide.
5. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
6. Heat the reaction plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Prepare the plate assembly on the autosampler.
9. Start the electrophoresis run.

Note: Expected heterozygote peak heights are 1500–3000 RFU.

Section 3.2 3500/3500xL instruments

Set up the 3500/3500xL instrument for electrophoresis

Reagents and parts

Appendix B, "Ordering Information" on page 87 lists the required materials not supplied with the AuthentiFiler™ Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use. Keep freeze-thaw cycles to a minimum.

Electrophoresis software setup and reference documents

The following table lists Data Collection Software and the run modules that can be used to analyze AuthentiFiler™ Kit PCR products. For details on the procedures, refer to the documents listed in the table.

Genetic Analyzer	Data Collection Software	Operating System	Run modules and conditions	References
Applied Biosystems® 3500	3500 Data Collection Software v1.0	Windows® XP *or* Windows Vista ®	• HID36_POP4 Injection conditions: 1.2kV/15 sec • Dye Set G5	*Applied Biosystems® 3500/ 3500xL Genetic Analyzer User Guide* (Pub. no. 4401661) *3500 and 3500xL Genetic Analyzers Quick Reference Card* (Pub. no. 4401662)
Applied Biosystems® 3500xL			• HID36_POP4 Injection conditions: 1.2kV/24 sec • Dye Set G5	

Prepare samples for electrophoresis on the 3500/3500xL instrument

Prepare the samples for capillary electrophoresis on the 3500/3500xL instrument immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and Size Standard needed to prepare the samples, using the table below.

Reagent	Volume per reaction
GeneScan™ 600 LIZ® Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	9.5 µL

Note: Include additional samples in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your results and experiments.

2. Pipet the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each well of a MicroAmp® Optical 96-Well Reaction Plate, or each MicroAmp® optical strip tube, add:
   a. 10 µL of the formamide: size standard mixture
   b. 1 µL of PCR product or Allelic Ladder

   Note: For blank wells, add 11 µL of Hi-Di™ Formamide.
5. Seal the reaction plate or strip tubes with the appropriate septa, then centrifuge to ensure that the contents of each well are collected at the bottom.
6. Heat the reaction plate or strip tubes in a thermal cycler for 3 minutes at 95°C.
7. Immediately put the plate or strip tubes on ice for 3 minutes.
8. Prepare the plate assembly, then put it onto the autosampler.
9. Ensure that a plate record is completed and link the plate record to the plate.
10. Start the electrophoresis run.

Note: Expected heterozygote peak heights are 3000–6000 RFU.

4 Data Analysis

Section 4.1 GeneMapper® *ID* Software

Overview of GeneMapper® *ID* Software

GeneMapper® *ID* Software is an automated genotyping software for forensic casework, databasing, and paternity data analysis.

After electrophoresis, the Data Collection Software stores information for each sample in an .fsa file. Using GeneMapper® *ID* Software v3.2.1, you can then analyze and interpret the data from the .fsa files.

Instruments

See "Instrument and software overview" on page 14 for a list of compatible instruments.

Before you start

When using GeneMapper® *ID* Software v3.2.1 to perform human identification (HID) analysis with STR kits, be aware that:

- HID analysis requires at least one allelic ladder sample per run folder. Perform the appropriate internal validation studies if you want to use multiple ladder samples in an analysis.

  For multiple ladder samples, the GeneMapper® *ID* Software calculates allelic bin offsets by using an average of all ladders that use the same panel within a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run.

  When the software imports multiple run folders into a project, only the ladder(s) within their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples must be labeled as "Allelic Ladder" in the Sample Type column in a project. Failure to apply this setting for ladder samples results in failed analysis.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples, to ensure proper allele calling.
- Alleles that are not in the allelic ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ±0.5-nt bin window of any known allelic ladder allele or virtual bin.

  Note: If a sample allele peak is called as an off-ladder allele, verify the sample result according to your laboratory's protocol.

Set up GeneMapper® *ID* Software for data analysis

File names

The file names shown in this section may differ from the file names you see when you download or import files. If you need help determining the correct files to use, contact your local Life Technologies Human Identification representative, or go to **www.lifetechnologies.com**.

Overview

To analyze sample (.fsa) files using GeneMapper® *ID* Software v3.2.1 for the first time:

1. Import panels and bins into the Panel Manager, as explained in "Import panels and bins" on page 31.
2. Create an analysis method, as explained in "Create an analysis method" on page 34.
3. Create a size standard, as explained in "Create a size standard" on page 38.
4. Define custom views of analysis tables.

   Refer to the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Pub. no. 4335523) for more information.
5. Define custom views of plots.

   Refer to the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Pub. no. 4335523) for more information.

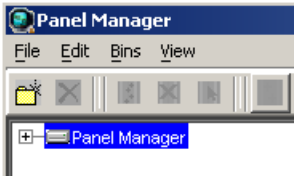
Import panels and bins

To import the AuthentiFiler™ Kit panel and bin set from **www.lifetechnologies.com** into the GeneMapper® *ID* Software v3.2.1 database:

1. Download and open the file containing panels and bins:
   a. From the Support menu of **www.lifetechnologies.com**, select **Support ▸ Software Downloads, Patches & Updates ▸ GeneMapper® *ID* Software v 3.2 ▸ Updates & Patches**, and download the file **AuthentiFiler Analysis Files GMID**.
   b. Unzip the file.
2. Start the GeneMapper® *ID* Software, then log in with the appropriate user name and password.

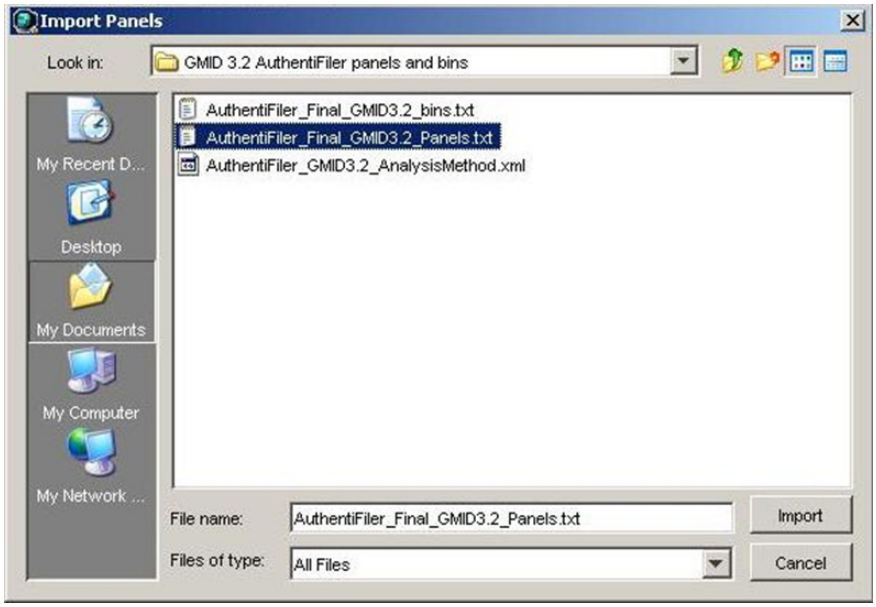
   IMPORTANT! For logon instructions, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (Pub. no. 4338775).

3. Select **Tools ▸ Panel Manager**.
4. Find, then open the folder containing the panels, bins, and marker stutter:
   a. Select **Panel Manager** in the navigation pane.
   b. Select **File ▸ Import Panels** to open the Import Panels dialog box.
   c. Navigate to, then open the **AuthentiFiler Analysis Files GMID** folder that you unzipped in step 1 above.



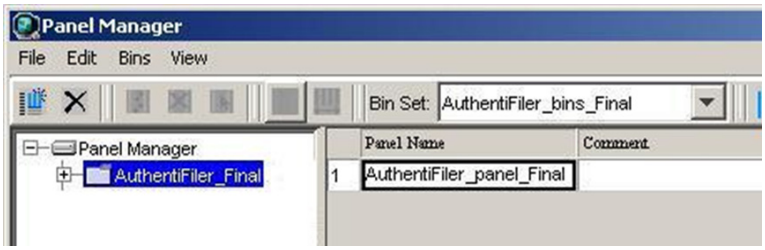
5. Select **AuthentiFiler_Final_GMID3.2_Panels.txt**, then click **Import**.

   Note: Importing this file creates a new folder in the navigation pane of the Panel Manager, AuthentiFiler_Final. This folder contains the panel and associated markers.



6. Import AuthentiFiler_Final_GMID3.2_bins.txt:

a. Select the **AuthentiFiler_Final** folder in the navigation pane.



b. Select **File ▸ Import Bin Set** to open the Import Bin Set dialog box.

c. Navigate to, then open the **AuthentiFiler Analysis Files GMID** folder.

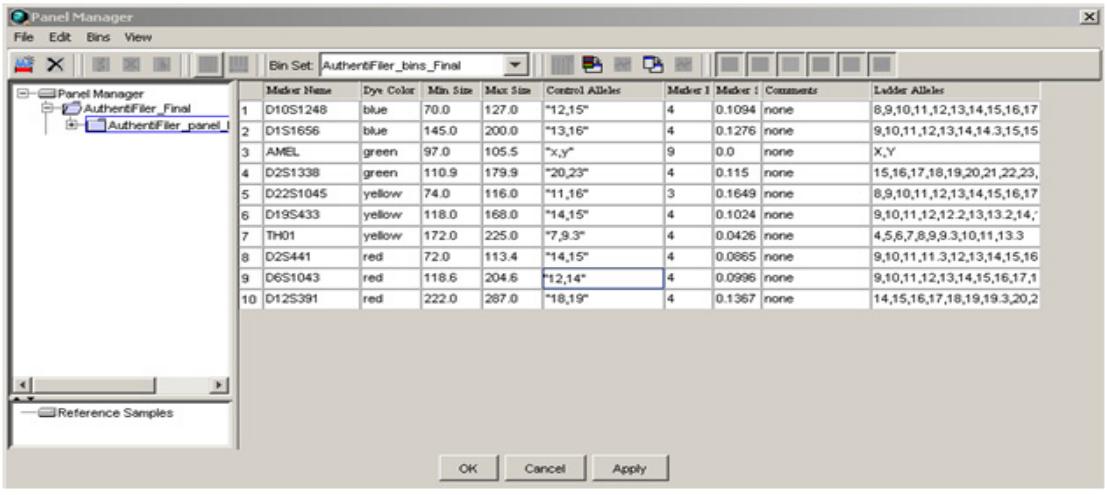
d. Select **AuthentiFiler_Final_GMID3.2_bins.txt**, then click **Import**.

Note: Importing this file associates the bin set with the panels in the AuthentiFiler_Final folder.

7. View the imported panels in the navigation pane:

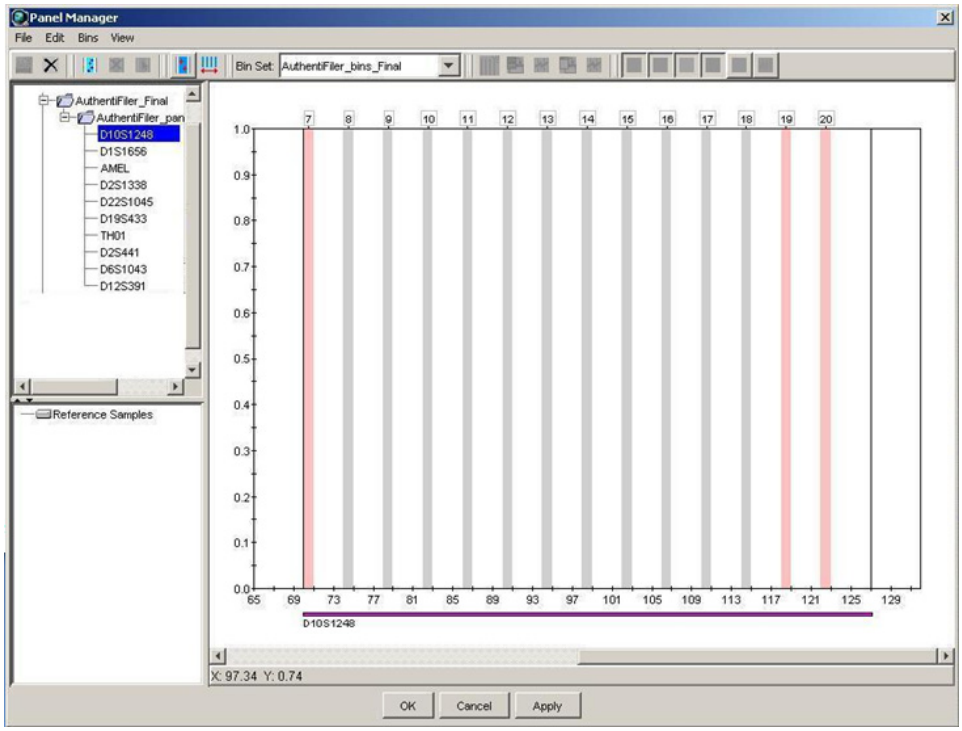
a. Double-click the **AuthentiFiler_Final** folder to view the **AuthentiFiler_panel_Final** folder.

b. Double-click the **AuthentiFiler_panel_Final** folder to display the panel information in the right pane.



	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker I	Marker I	Comments	Ladder Alleles
1	D10S1248	blue	70.0	127.0	"12,15"	4	0.1094	none	8,9,10,11,12,13,14,15,16,17
2	D1S1656	blue	145.0	200.0	"13,16"	4	0.1276	none	9,10,11,12,13,14,14.3,15,15
3	AMEL	green	97.0	105.5	"x,y"	9	0.0	none	X,Y
4	D2S1338	green	110.9	179.9	"20,23"	4	0.115	none	15,16,17,18,19,20,21,22,23,
5	D22S1045	yellow	74.0	116.0	"11,16"	3	0.1649	none	8,9,10,11,12,13,14,15,16,17
6	D19S433	yellow	118.0	168.0	"14,15"	4	0.1024	none	9,10,11,12,12.2,13,13.2,14,
7	TH01	yellow	172.0	225.0	"7,9.3"	4	0.0426	none	4,5,6,7,8,9,9.3,10,11,13.3
8	D2S441	red	72.0	113.4	"14,15"	4	0.0865	none	9,10,11,11.3,12,13,14,15,16
9	D6S1043	red	118.6	204.6	"12,14"	4	0.0996	none	9,10,11,12,13,14,15,16,17,1
10	D12S391	red	222.0	287.0	"18,19"	4	0.1367	none	14,15,16,17,18,19,19.3,20,2

8. Select **D10S1248** to display the Bin view for the marker in the right pane.



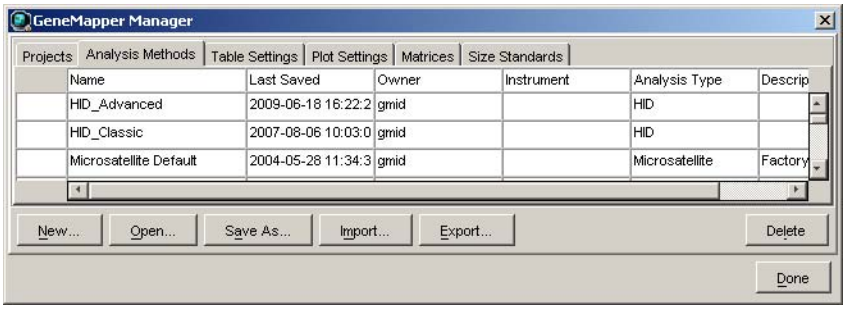
9. Click **Apply**, then **OK** to add the AuthentiFiler™ Kit panel and bin set to the GeneMapper® *ID* Software database.

IMPORTANT! If you close the Panel Manager without clicking OK, the panels and bins are not imported into the GeneMapper® *ID* Software database.

Create an analysis method

Use the following procedure to create an HID analysis method for the AuthentiFiler™ Kit.

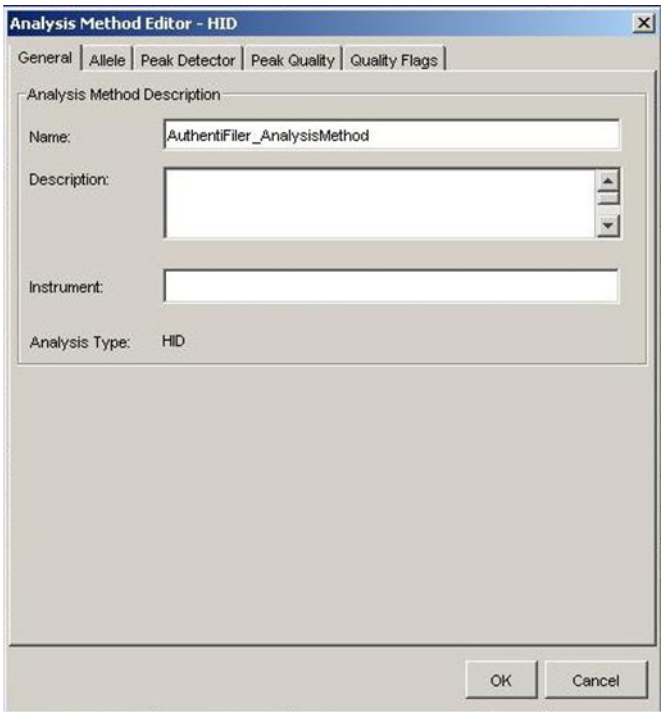
1. Select **Tools ▸ GeneMapper Manager** to open the GeneMapper Manager.



2. Select the **Analysis Methods** tab, then click **New** to open the New Analysis Method dialog box.
3. Select **HID** and click **OK** to open the Analysis Method Editor with the General tab selected.

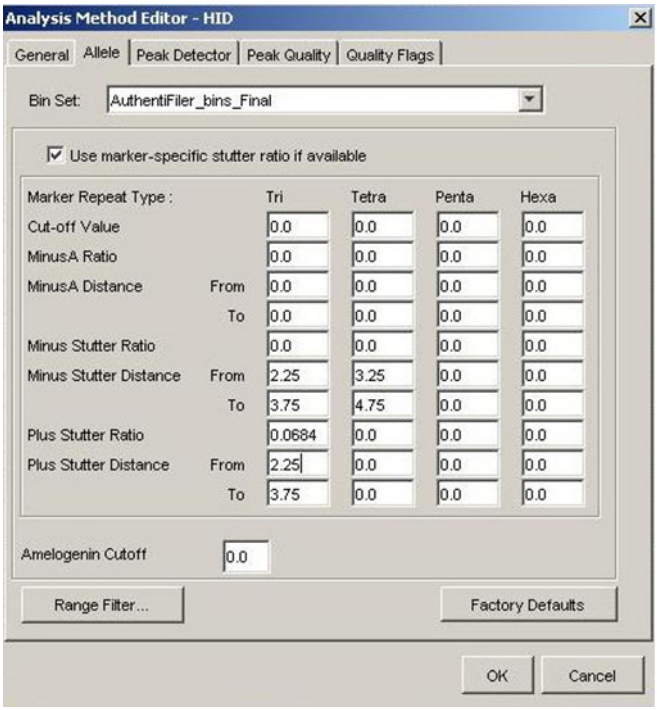
   Enter the settings shown in the figures on the following pages.
4. After you enter settings in all tabs, click **Save**.

General tab settings



In the Name field, either type the name as shown or enter a name of your choosing. The Description and Instrument fields are optional.

Allele tab settings



- In the Bin Set field, select the **AuthentiFiler_bins_Final** bin set imported previously and configure the stutter distance parameters as shown.
- GeneMapper® *ID* Software v3.2.1 allows you to specify four types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- The "Use marker-specific stutter ratio if available" check box is selected by default. Consequently, the software applies the stutter ratio filters supplied in the AuthentiFiler_Final_GMID3.2_Panels.txt file. GeneMapper® *ID* Software v3.2.1 specifies locus-specific filter ratios for minus stutters, but not for –2 nt and plus stutters, in the panel file. However, validation studies with the AuthentiFiler™ Kit show that the trinucleotide repeat D22S1045 locus produces a relatively large amount of plus stutter compared to tetranucleotide repeat loci. The relatively large amount of stutter may cause the stutter peak to be labeled during routine analysis.

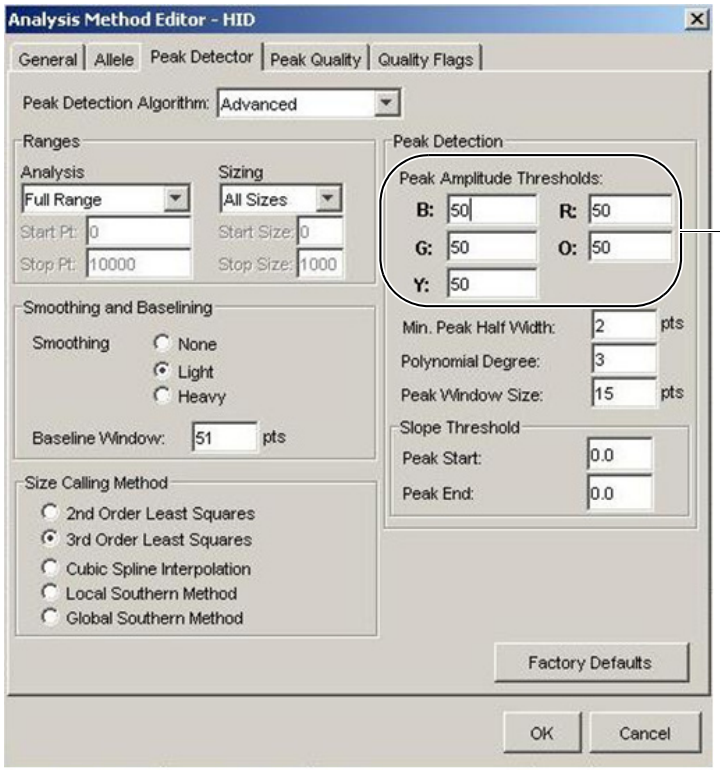
Plus stutters were also observed for the loci D10S1248 and D1S1656. Additionally, and –2 nt stutter was also observed at the D1S1656 locus. The stutter percentages observed during internal validation is reported in the stutter table in Chapter 5. Users are asked to take note of these stutter percentages when analyzing data and follow laboratory guidelines to determine if an allele peak located in a plus stutter position can be discarded as a stutter peak.

- The plus stutter at the D22S1045 locus can be filtered by assigning a global plus stutter filter for trinucleotide repeat loci in the Analysis Parameter file. Because D22S1045 is the only trinucleotide repeat locus in the AuthentiFiler™ Kit, this stutter filter setting is applied only to plus stutter peaks at the D22S1045 locus. The settings shown above resulted in little or no labeling of D22S1045 plus stutter peaks during our validation studies. Perform internal validation studies to determine the settings to use in your laboratory.

  Note: Plus stutter values for tetranucleotide repeats based on stutter percentages for D10S1248 and D1S1656 were not included in the allele tab, as this will interfere with data analysis at other tetranucleotide loci in the multiplex.

  Note: We do not recommend the use of a global filter for analysis.

Peak Detector tab settings



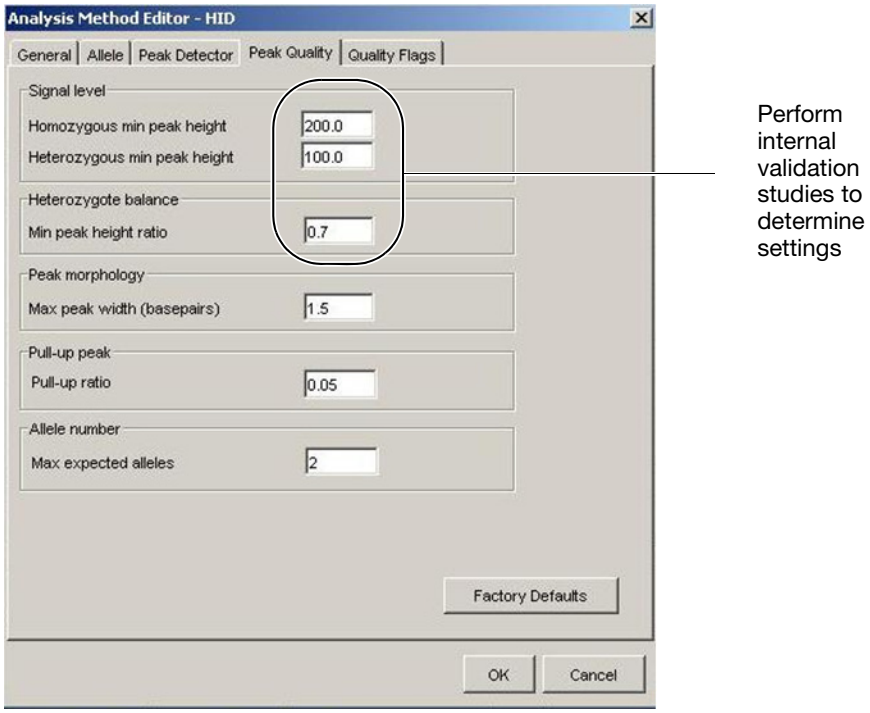
Perform internal validation studies to determine settings

IMPORTANT! Perform the appropriate internal validation studies to determine the peak amplitude thresholds for interpretation of AuthentiFiler™ Kit data.

Fields include:

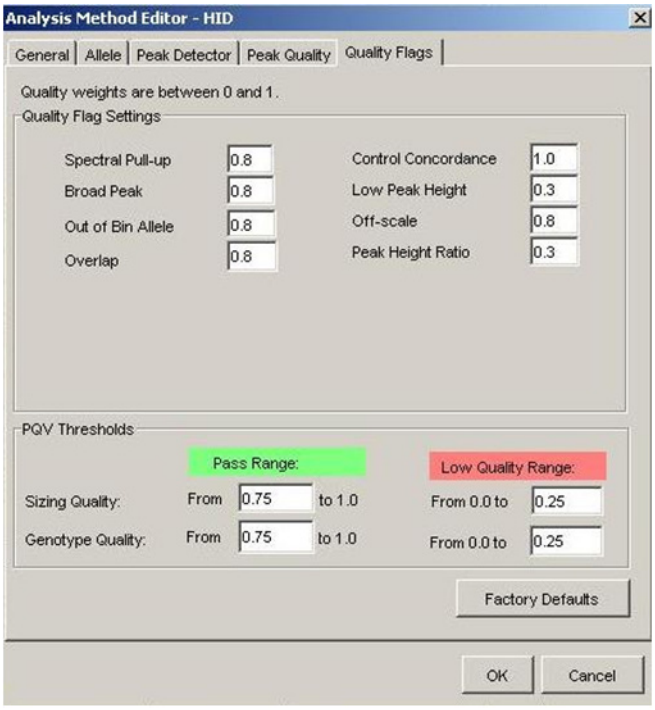
- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper® *ID* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
- **Size calling method** – The AuthentiFiler™ Kit has been validated using the 3rd Order Least Squares sizing method in combination with the GeneScan™ 500 LIZ® Size Standard or the GeneScan™ 600 LIZ® Size Standard v2.0. Select alternative sizing methods only after you perform the appropriate internal validation studies.

Peak Quality tab settings



IMPORTANT! Perform the appropriate internal validation studies to determine the heterozygous and homozygous minimum peak height thresholds and the minimum peak height ratio threshold that allow for reliable interpretation of AuthentiFiler™ Kit data.

Quality Flags tab settings



IMPORTANT! The values shown are the software defaults and are the values we used during developmental validation. Perform the appropriate internal validation studies to determine the appropriate values to use in your laboratory.

Create a size standard

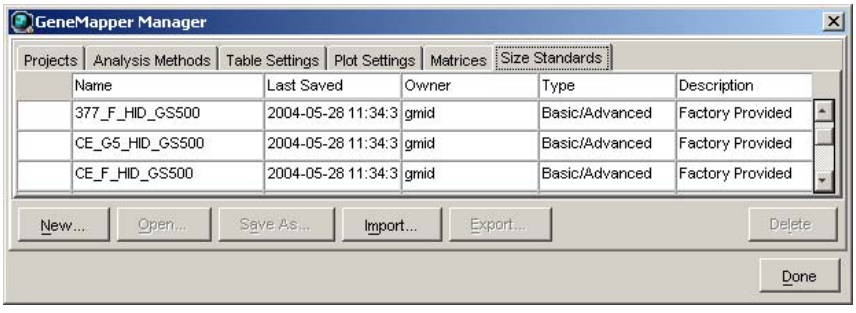
The size standards for the AuthentiFiler™ Kit uses the following size standard peaks in their definitions:

GeneScan™ 500 LIZ® Size Standard	GeneScan™ 600 LIZ® Size Standard v2.0
75, 100, 139, 150, 160, 200, 300, 350, 400, and 450	80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, and 400

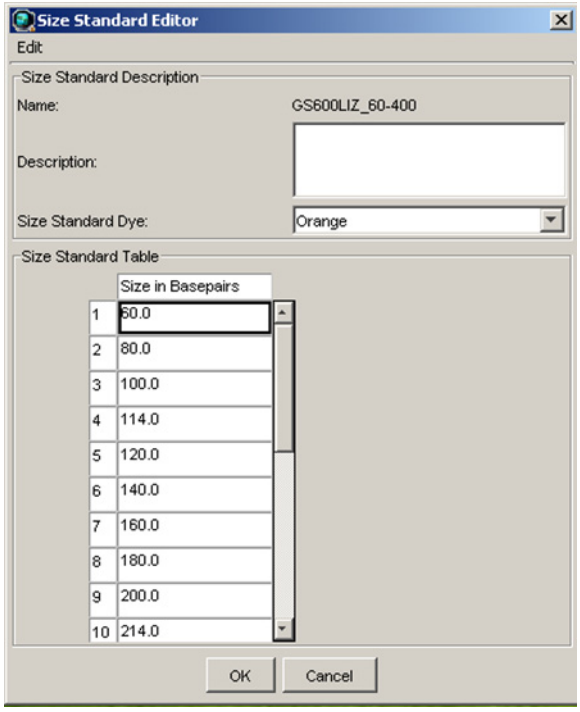
Note: The 250-nt and the 340-nt peak are not included in the GeneScan™ 500 LIZ® Size Standard definition, though present in the reagent formulation. These peaks can be used as an indicator of precision within a run.

Use the following procedure to create the size standard for the AuthentiFiler™ Kit.

1. Select **Tools ▸ GeneMapper Manager** to open the GeneMapper Manager.



2. Select the **Size Standards** tab, then click **New**.
3. Enter a name as shown below or enter a name of your choosing. In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the sizes specified in on page 38. The example below is for the GeneScan™ 600 LIZ® Size Standard v2.0.



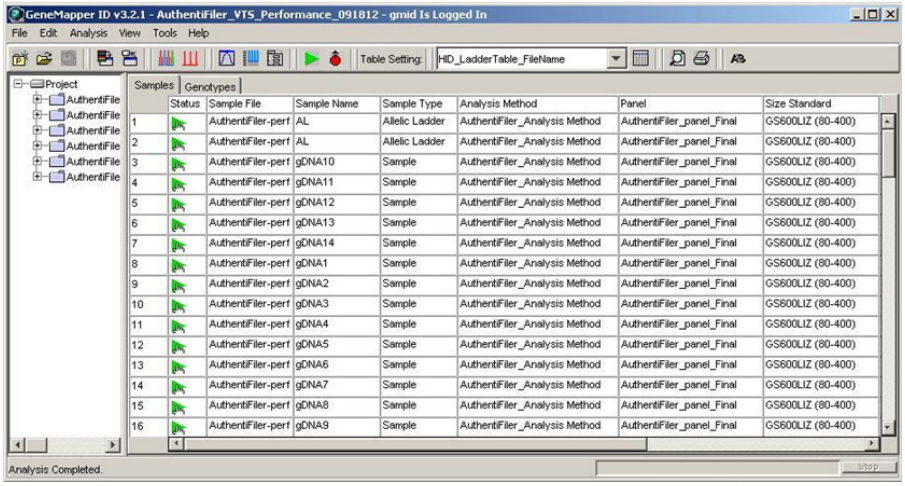
Analyze and edit sample files with GeneMapper® *ID* Software

1. In the Project window, select **File ▸ Add Samples to Project**, then navigate to the disk or directory containing the sample files.
2. Apply analysis settings to the samples in the project. The names of the settings shown are the names suggested in the sections above.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	AuthentiFiler_AnalysisMethod (or the name of the analysis method you created)
Panel	AuthentiFiler_panel_Final
Size Standard	GS600LIZ_60-400 (or the name of the size standard you created)

For more information about how the Size Caller works, refer to the *ABI PRISM® GeneScan® Analysis Software for the Windows NT® Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (Pub. no. 4335617). For additional information about size standards, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (Pub. no. 4338775).

3. Click (Analyze), enter a name for the project (in the Save Project dialog box), then click **OK** to start analysis.
   - The status bar displays the progress of analysis as both:
     - A completion bar extending to the right with the percentage completed indicated
     - With text messages on the left
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
   - The Genotypes tab becomes available after analysis.



Examine and edit a project

You can display electropherogram plots from the Samples and Genotypes tabs of the Project window to examine the data. These procedures start with the Samples tab of the Project window (assuming the analysis is complete).

For more information

For details about GeneMapper® *ID* Software features, allele filters, peak detection algorithms, and project editing, refer to:

- *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Pub. no. 4335523)
- *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (Pub. no. 4338775)
- *Installation Procedures and New Features for GeneMapper® ID Software Version v3.2 User Bulletin* (Pub. no. 4352543)

Section 4.2 GeneMapper® *ID-X* Software

Overview of GeneMapper® *ID-X* Software

GeneMapper® *ID-X* Software is an automated genotyping software for forensic casework, databasing, and paternity data analysis.

After electrophoresis, the Data Collection Software stores information for each sample in an .fsa file. Using GeneMapper® *ID-X* Software v1.0.1 or higher, you can then analyze and interpret the data from the .fsa or .hid files.

Note: The .hid files can only be analyzed using GeneMapper® *ID-X* Software v1.2 or higher.

Instruments

See "Instrument and software overview" on page 14 for a list of compatible instruments.

Before you start

When using GeneMapper® *ID-X* Software v1.0.1 or higher to perform human identification (HID) analysis with STR kits, be aware that:

- HID analysis requires at least one allelic ladder sample per run folder. Perform the appropriate internal validation studies if you want to use multiple ladder samples in an analysis.

  For multiple ladder samples, the GeneMapper® *ID-X* Software calculates allelic bin offsets by using an average of all ladders that use the same panel within a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run.

  When the software imports multiple run folders into a project, only the ladder(s) within their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples must be labeled as "Allelic Ladder" in the Sample Type column in a project. Failure to apply this setting for ladder samples results in failed analysis.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples to ensure proper allele calling.
- Alleles that are not in the allelic ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ±0.5-nt bin window of any known allelic ladder allele or virtual bin.

  Note: If a sample allele peak is called as an off-ladder allele, verify the sample result according to your laboratory protocol.

Set up GeneMapper® *ID-X* Software for data analysis

File names

The file names shown in this section may differ from the file names you see when you download or import files. If you need help determining the correct files to use, contact your local Life Technologies Human Identification representative, or go to **www.lifetechnologies.com**.

Overview

To analyze sample (.fsa) files using GeneMapper® *ID-X* Software v1.0.1 or higher for the first time:

1. Import panels, bins, and marker stutter into the Panel Manager, as explained in "Import panels, bins, and marker stutter" below.
2. Create an analysis method, as explained in "Create an analysis method" on page 47.
3. Create a size standard, as explained in "Create a size standard" on page 52.
4. Define custom views of analysis tables.

   Refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574) for more information.
5. Define custom views of plots.

   Refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574) for more information.

Import panels, bins, and marker stutter

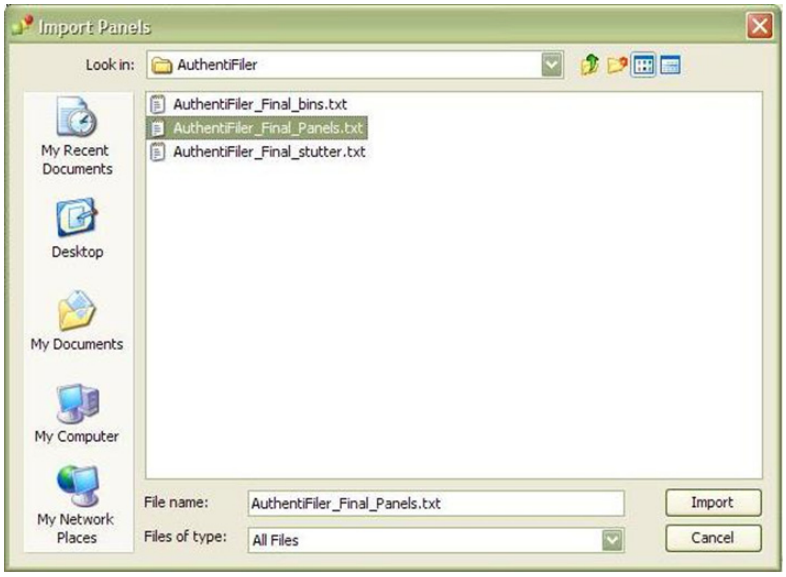
To import the AuthentiFiler™ Kit panels, bin sets, and marker stutter from **www.lifetechnologies.com** into the GeneMapper® *ID-X* Software database:

1. Download and open the file containing panels, bins, and marker stutter:
   a. From the Support menu of **www.lifetechnologies.com**, select **Support ▸ Software Downloads, Patches & Updates ▸ GeneMapper® *ID-X* Software ▸ Updates & Patches**, and download the file **AuthentiFiler Analysis Files GMIDX**.
   b. Unzip the file.
2. Start the GeneMapper® *ID-X* Software, then log in with the appropriate user name and password.

   IMPORTANT! For logon instructions, refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574).
3. Select **Tools ▸ Panel Manager**.
4. Find, then open the folder containing the panels, bins, and marker stutter:
   a. Select **Panel Manager** in the navigation pane.
   b. Select **File ▸ Import Panels** to open the Import Panels dialog box.
   c. Navigate to, then open the **AuthentiFiler Analysis Files GMIDX** folder that you unzipped in step 1 of this section.

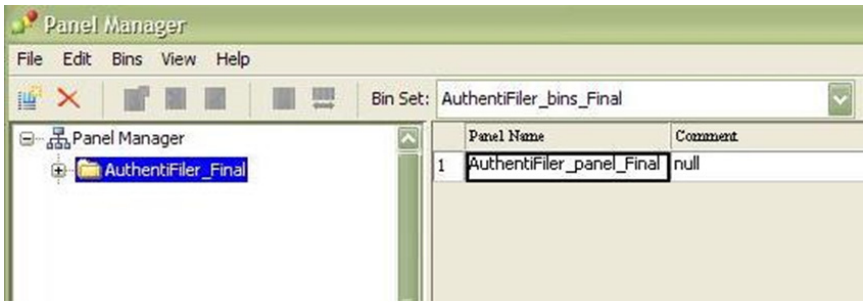
5. Select **AuthentiFiler_Final_Panels.txt**, then click **Import**.

   Note: Importing this file creates a new folder in the navigation pane of the Panel Manager "AuthentiFiler_Final". This folder contains the panel and associated markers.



6. Import AuthentiFiler_Final_bins.txt:

   a. Select the **AuthentiFiler_Final** folder in the navigation pane.

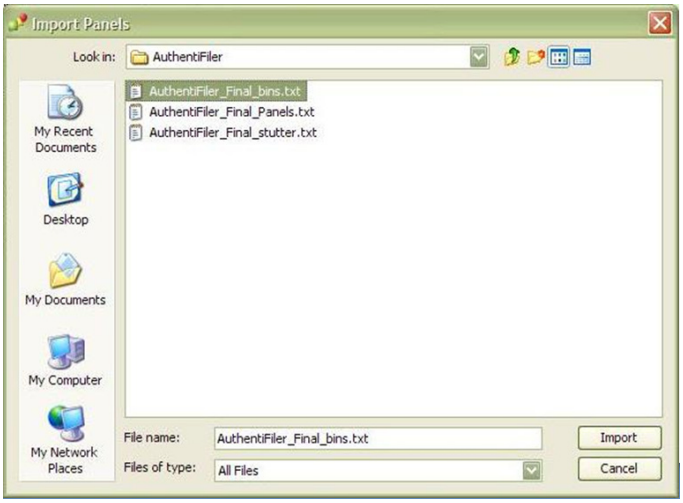


   b. Select **File** ▸ **Import Bin Set** to open the Import Bin Set dialog box.

   c. Navigate to, then open the **AuthentiFiler Analysis Files GMIDX** folder.

d. Select **AuthentiFiler_Final_bins.txt**, then click **Import**.

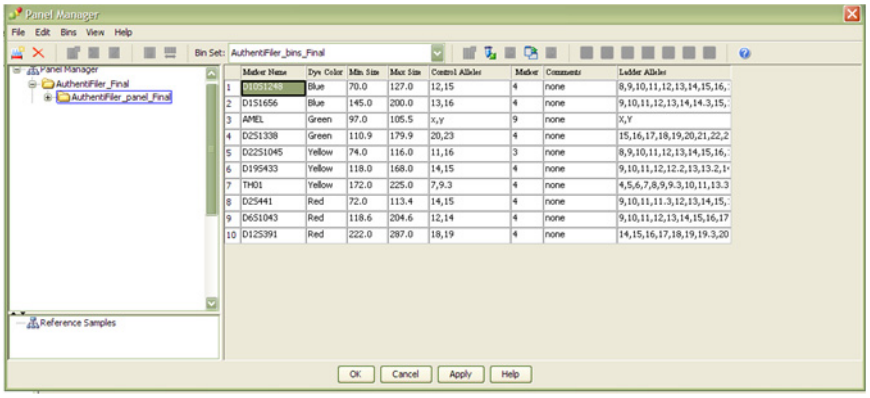
Note: Importing this file associates the bin set with the panels in the AuthentiFiler_Final folder.



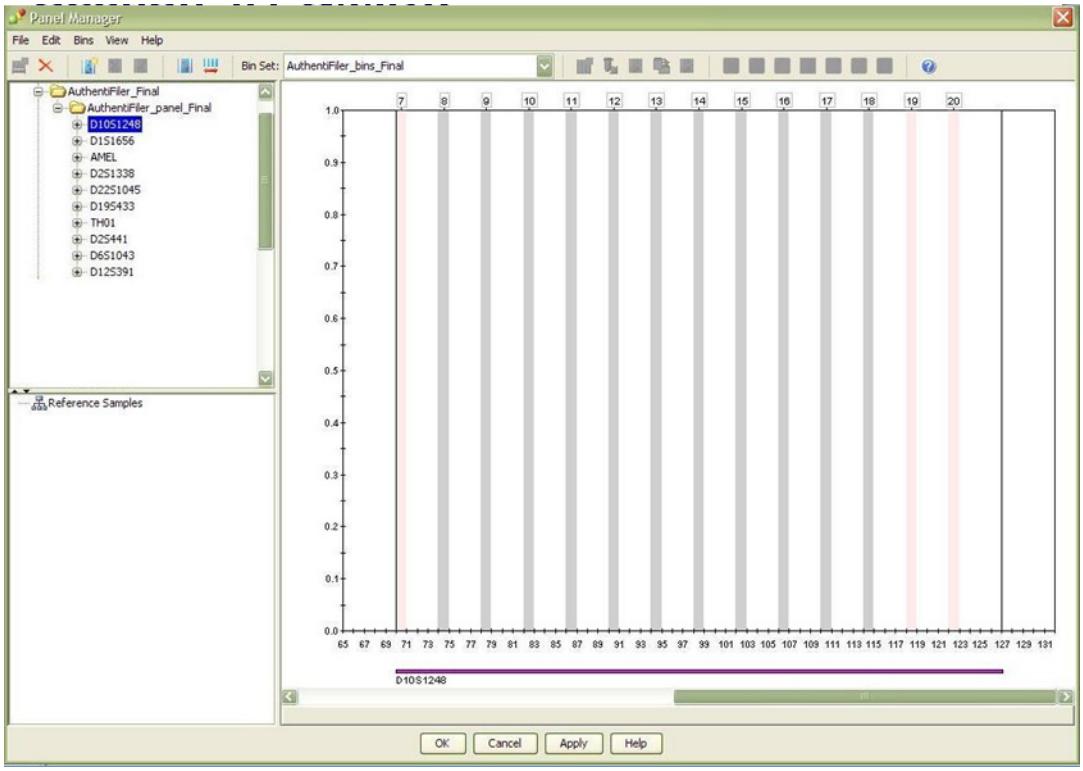
7. View the imported panels in the navigation pane:

a. Double-click the **AuthentiFiler_Final** folder.

b. Double-click the **AuthentiFiler_panel_Final** folder to display the panel information in the right pane and the markers below it.

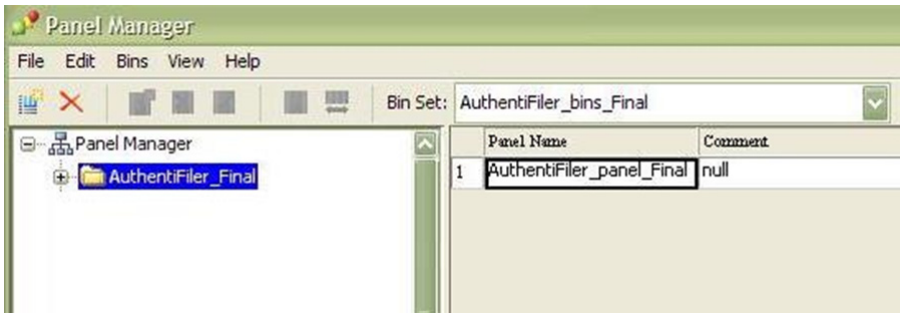


8. Select **D10S1248** to display the Bin view for the marker in the right pane.



9. Import AuthentiFiler_Final_stutter.txt:

   a. Select the **AuthentiFiler_Final** folder in the navigation panel.

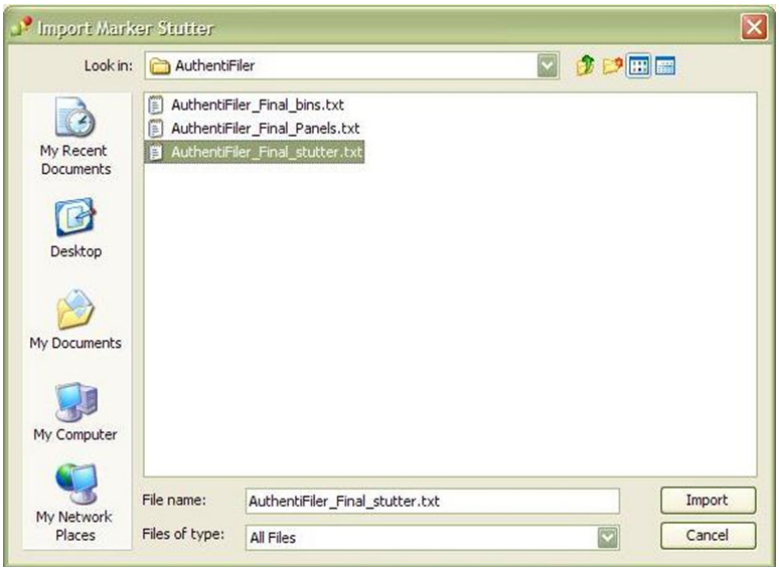


   b. Select File ▸ **Import Marker Stutter** to open the Import Marker Stutter dialog box.

   c. Navigate to, then open the **AuthentiFiler Analysis Files GMIDX** folder.

d. Select **AuthentiFiler_Final_stutter.txt**, then click **Import**.

Note: Importing this file associates the marker stutter ratio with the bin set in the AuthentiFiler_bins_Final folder.



10. View the imported marker stutters in the navigation pane:

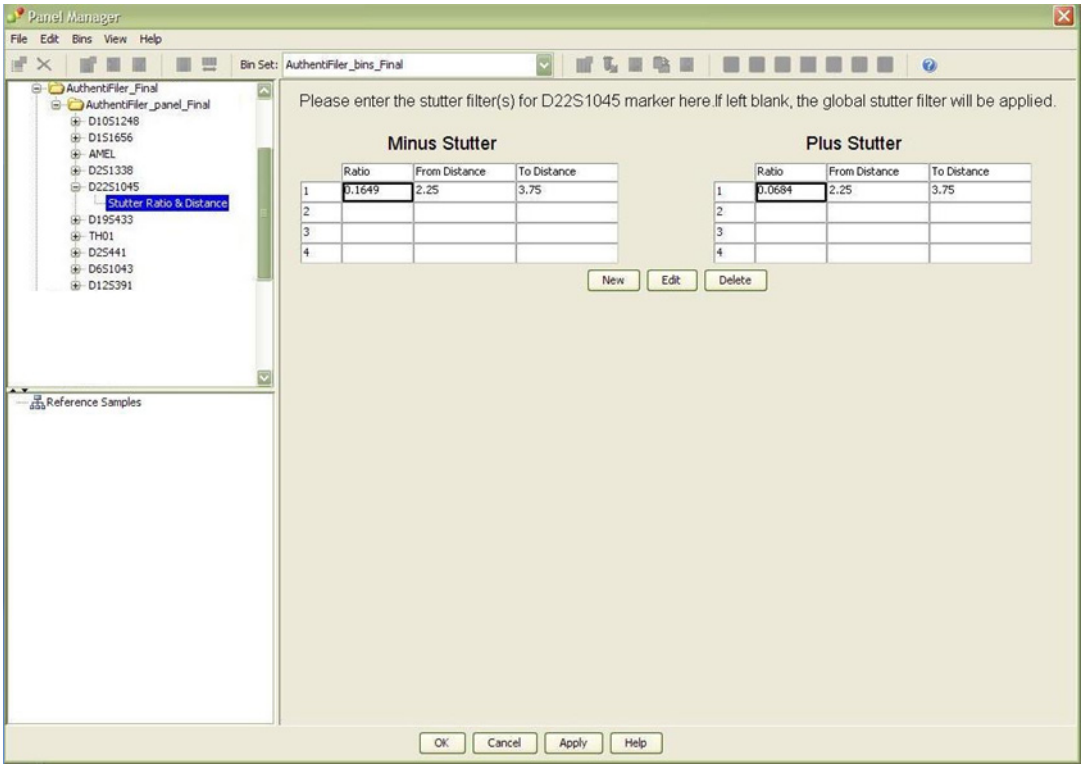
a. Select the **AuthentiFiler_Final** folder to display its list of markers in the right pane.

b. Double-click the **AuthentiFiler_panel_Final** folder to display its list of markers below it.

c. Double-click **D22S1045** to display the Stutter Ratio & Distance view for the marker in the right pane.

Because D22S1045 has a trinucleotide repeat unit, it produces a higher level of plus stutter than tetranucleotide markers, and so requires the use of a plus stutter filter. The settings for the D22S1045 plus stutter filter can be seen in the table in the right pane. In addition to D22S1045, during internal validation, plus stutters were observed for two tetranucleotide markers,

D10S1248 and D1S1656. Plus stutter filters are provided in the stutter file for these markers as well and are displayed in a table in Chapter 5. Other markers may not require a plus stutter filter, in which case the settings for plus stutter are left blank.



11. Click **Apply**, then **OK** to add the AuthentiFiler™ Kit panels, bin sets, and marker stutter to the GeneMapper® *ID-X* Software database.

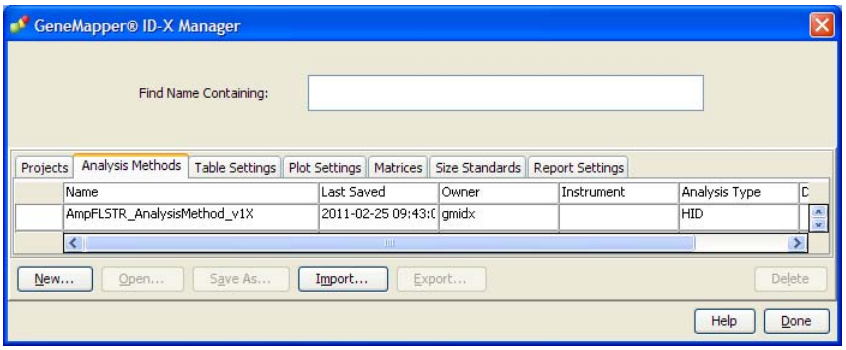
IMPORTANT! If you close the Panel Manager without clicking **Apply**, the panels, bin sets, and marker stutter will not be imported into the GeneMapper® *ID-X* Software database.

Create an analysis method

Use the following procedure to create an analysis method for the AuthentiFiler™ Kit.

IMPORTANT! Analysis methods are version-specific, so you must create an analysis method for each version of the software. For example, an analysis method created for GeneMapper® *ID-X* Software version 1.2 is not compatible with earlier versions of GeneMapper® *ID-X* Software, or with GeneMapper® *ID* Software version 3.2.1.

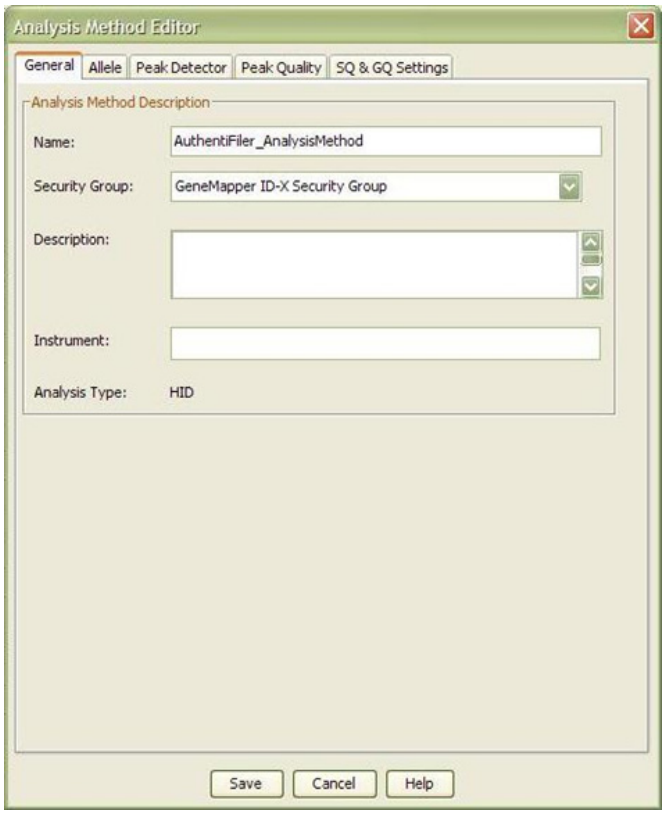
1. Select **Tools ▸ GeneMapper® *ID-X* Manager** to open the GeneMapper® *ID-X* Manager.



2. Select the **Analysis Methods** tab, then click **New** to open the Analysis Method Editor with the **General** tab selected.
3. Enter the settings shown in the figures on the following pages.

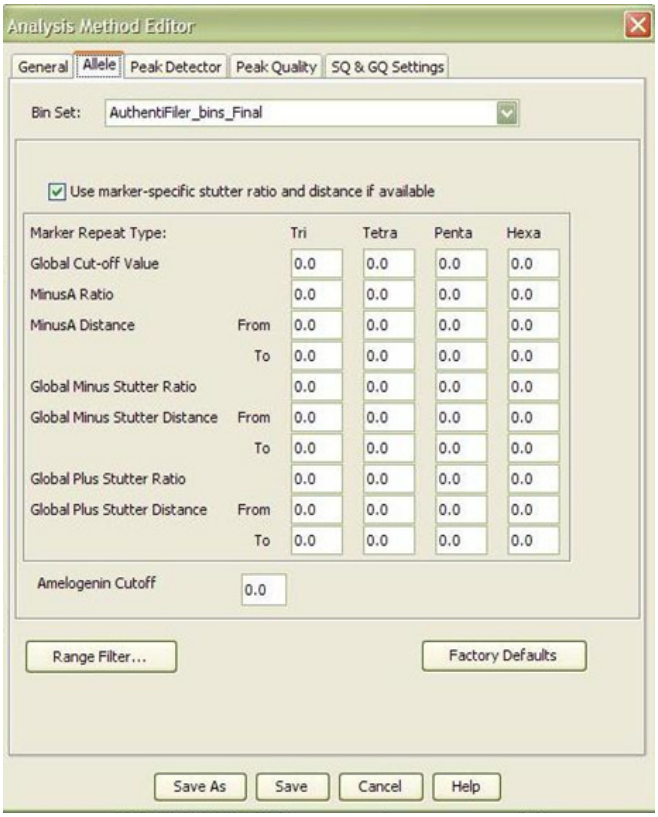
   Note: The Analysis Method Editor closes when you save your settings (see step 4 on page 48). To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.
4. After you enter the settings on all tabs, click **Save**.

General tab settings



Enter a name as shown below or enter a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the drop-down list. The Description and Instrument fields are optional.

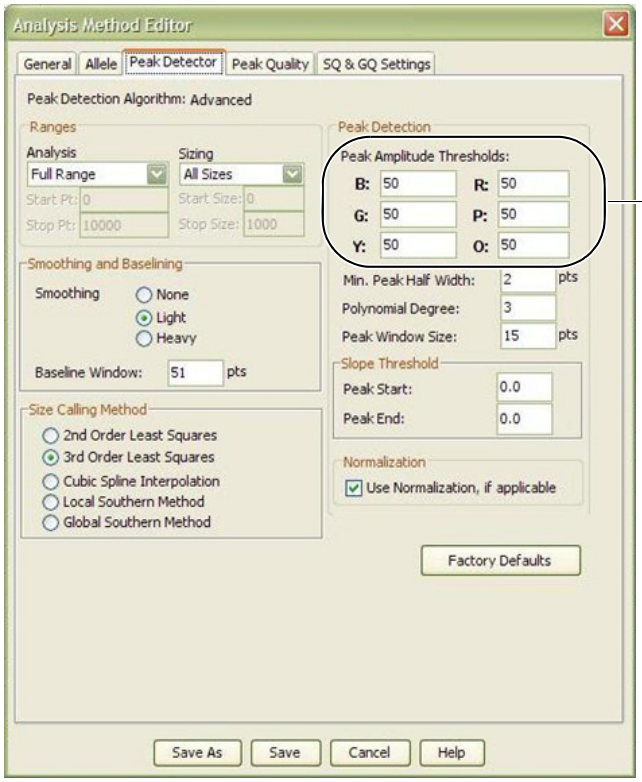
Allele tab settings



- In the Bin Set field, select the **AuthentiFiler_bins_Final** bin set imported previously and configure the stutter distance parameters as shown.
- GeneMapper® *ID-X* Software allows you to specify four types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- The "Use marker-specific stutter ratio and distance if applicable" check box is selected by default. When this box is checked, the software applies the stutter ratio filters in the AuthentiFiler_Final_stutter.txt file.

  Note: We do not recommend the use of global filter for analysis.

Peak Detector tab settings



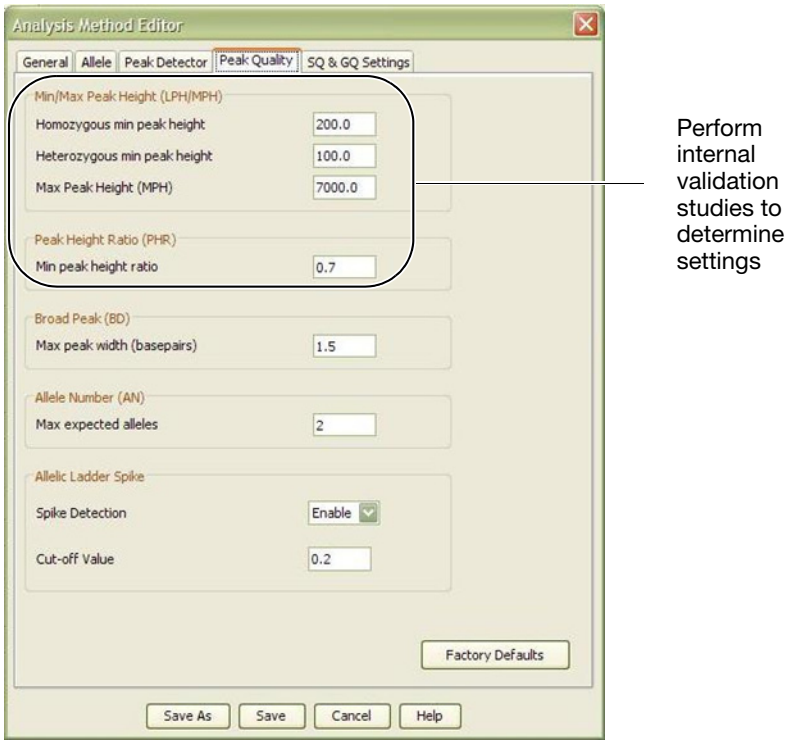
Perform internal validation studies to determine settings

IMPORTANT! Perform the appropriate internal validation studies to determine the appropriate peak amplitude thresholds for interpretation of AuthentiFiler™ Kit data.

Fields include:

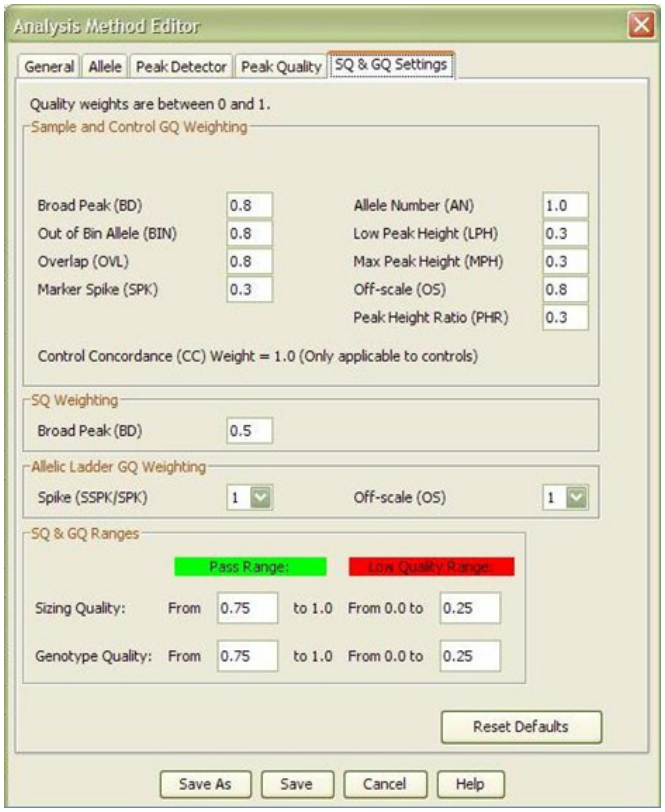
- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper® *ID-X* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
- **Size calling method** – The AuthentiFiler™ Kit has been validated using the 3rd Order Least Squares sizing method with the GeneScan™ 500 LIZ® Size Standard or the GeneScan™ 600 LIZ® Size Standard v2.0. Select alternative sizing methods only after you perform the appropriate internal validation studies.
- **Normalization** (v1.2 or higher) – For use with 3500 data. Perform internal validation studies to determine whether to use the Normalization feature for analysis of AuthentiFiler™ Kit data.

Peak Quality tab settings



IMPORTANT! Perform the appropriate internal validation studies to determine the heterozygous and homozygous minimum peak height thresholds, maximum peak height threshold, and the minimum peak height ratio threshold for interpretation of AuthentiFiler™ Kit data.

SQ & GQ tab settings



IMPORTANT! The values shown are the software defaults and are the values we used during developmental validation. Perform appropriate internal validation studies to determine the appropriate values to use.

Create a size standard

The size standards for the AuthentiFiler™ Kit uses the following size standard peaks in their definitions:

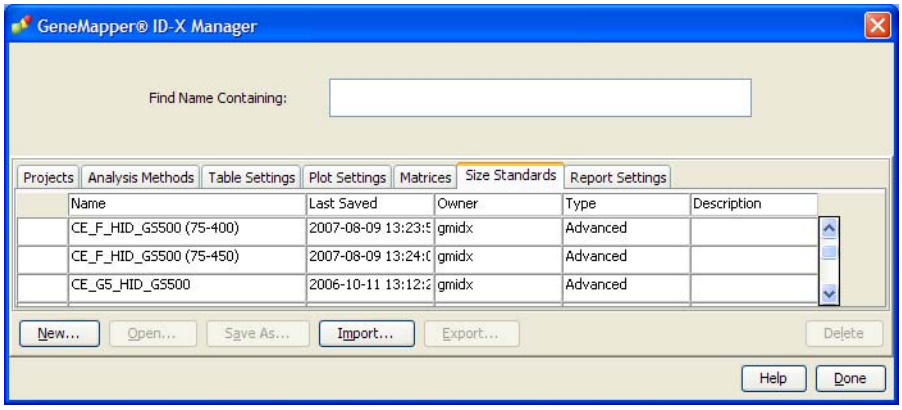
GeneScan™ 500 LIZ® Size Standard	GeneScan™ 600 LIZ® Size Standard v2.0
75, 100, 139, 150, 160, 200, 300, 350, 400, and 450	80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, and 400

Note: The 250-nt and the 340-nt peaks are not included in the GeneScan™ 500 LIZ® Size Standard definition, though present in the reagent formulation. These peaks can be used as an indicator of precision within a run.

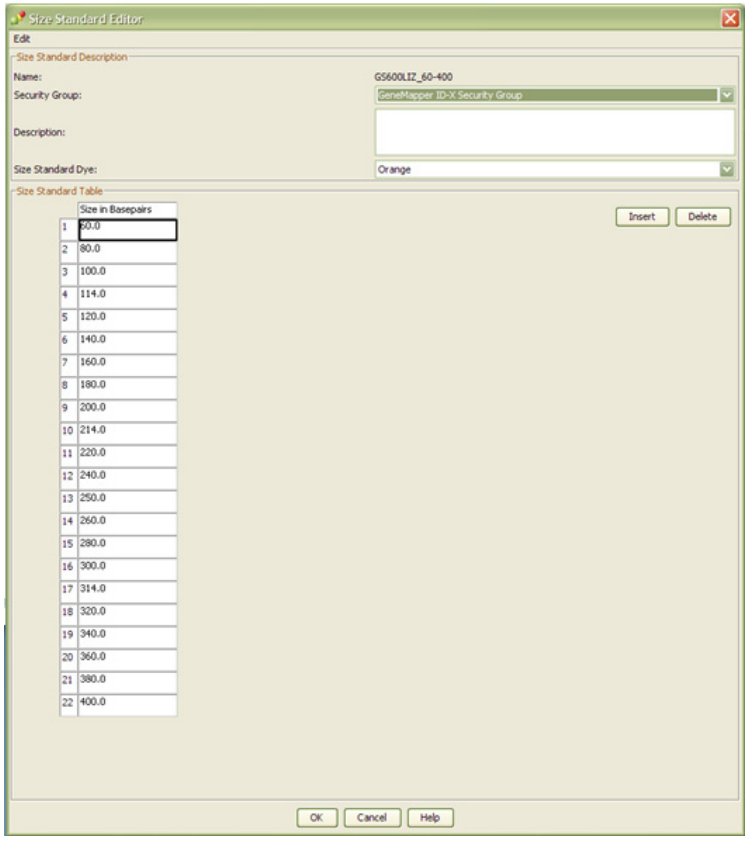
Use the following procedure to create the size standard for the AuthentiFiler™ Kit.

1. Select **Tools ▸ GeneMapper® *ID-X* Manager** to open the GeneMapper® *ID-X* Manager.

2. Select the **Size Standards** tab, then click **New**.



3. Enter a name as shown below or enter a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the drop-down list. In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the sizes specified on page 52. The example below is for the GeneScan™ 600 LIZ® Size Standard v2.0.



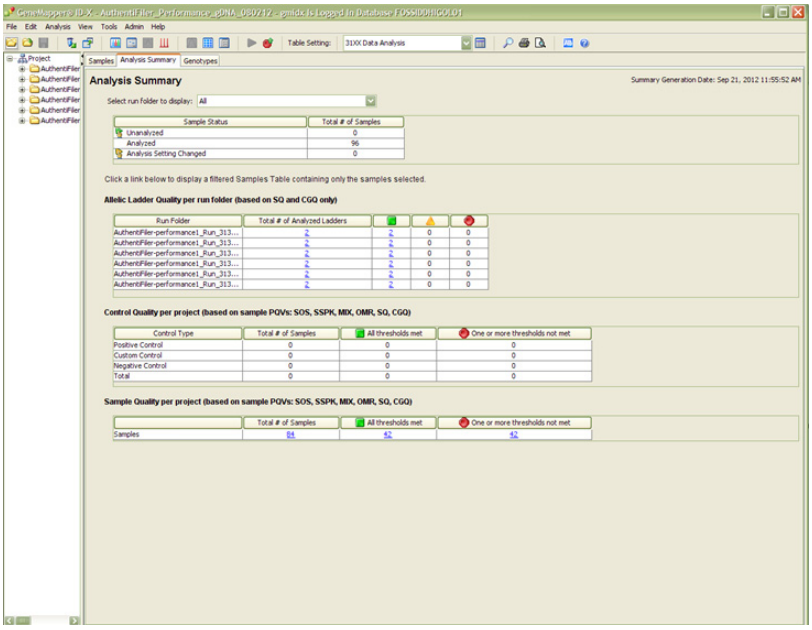
Analyze and edit sample files with GeneMapper® *ID-X* Software

1. In the Project window, select **File ▸ Add Samples to Project**, then navigate to the disk or directory containing the sample files.
2. Apply analysis settings to the samples in the project. The names of the settings shown are the names suggested in the sections above. If you named the settings differently, select the names you specified.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	AuthentiFiler_AnalysisMethod (or the name of the analysis method you created)
Panel	AuthentiFiler_panel_Final
Size Standard	GS600LIZ_60-400 (or the name of the size standard you created)

   For more information about how the Size Caller works, or about size standards, refer to the *GeneMapper® ID-X Software v1.2 Reference Guide* (Pub. no. 4426481).
3. Click ▶ (Analyze), enter a name for the project (in the Save Project dialog box), then click **OK** to start analysis.
   - The status bar displays the progress of analysis as a completion bar extending to the right with the percentage completed indicated.
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
   - The Analysis Summary tab is displayed upon completion of the analysis.

     The figure below shows the analysis summary window after analysis.



Examine and edit a project

You can display electropherogram plots from the Samples and Genotypes tabs of the Project window to examine the data. These procedures start with the Analysis Summary tab of the Project window (assuming the analysis is complete).

For more information

For more information about any of these tasks, refer to:

- For quick set-up instructions, refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574).
- For details about GeneMapper® *ID-X* Software features, allele filters, peak detection algorithms, and project editing, refer to:
  - *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574)
  - *GeneMapper® ID-X Software Version 1.0 Quick Reference Guide* (Pub. no. 4375670)
  - *GeneMapper® ID-X Software Version 1.0 Reference Guide* (Pub. no. 4375671)
  - *GeneMapper® ID-X Software Version 1.1 (Mixture Analysis Tool) Getting Started Guide* (Pub. no. 4396773)
  - *GeneMapper® ID-X Software Version 1.1 (Mixture Analysis Tool) Quick Reference Guide* (Pub. no. 4402094)
  - *GeneMapper® ID-X Software Version 1.2 Quick Reference Guide* (Pub. no. 4426482)
  - *GeneMapper® ID-X Software Version 1.2 Reference Guide* (Pub. no. 4426481)

Section 4.3 GeneMapper® Software

Overview of GeneMapper® Software

GeneMapper® Software is a flexible genotyping software package that provides DNA sizing and quality allele calls for all Applied Biosystems® electrophoresis-based genotyping systems.

After electrophoresis, the Data Collection Software stores information for each sample in an .fsa file. Using GeneMapper® Software v4.1, you can then analyze and interpret the data from the .fsa files.

Instruments

See "Instrument and software overview" on page 14 for a list of compatible instruments.

Before you start

When using GeneMapper® Software v4.1 to perform human identification (HID) analysis with STR kits, be aware that:

- HID analysis requires at least one allelic ladder sample per run folder. Perform the appropriate internal validation studies if you want to use multiple ladder samples in an analysis.

  For multiple ladder samples, the GeneMapper® Software calculates allelic bin offsets by using an average of all ladders that use the same panel within a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run.

  When the software imports multiple run folders into a project, only the ladder(s) within their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples must be labeled as "Allelic Ladder" in the Sample Type column in a project. Failure to apply this setting for ladder samples results in failed analysis.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples to ensure proper allele calling.
- Alleles that are not in the AmpFℓSTR® Allelic Ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ±0.5-nt bin window of any known allelic ladder allele or virtual bin.

  Note: If a sample allele peak is called as an off-ladder allele, verify the sample result according to your laboratory protocol.

Set up GeneMapper® Software for data analysis

File names

The file names shown in this section may differ from the file names you see when you download or import files. If you need help determining the correct files to use, contact your local Life Technologies Human Identification representative, or go to **www.lifetechnologies.com**.

Overview

To analyze sample (.fsa) files using GeneMapper® Software v4.1 for the first time:

1. Import panels and bins into the Panel Manager, as explained in "Import panels and bins" below.
2. Create an analysis method, as explained in "Create an analysis method" on page 61.
3. Create a size standard, as explained in "Create a size standard" on page 66.
4. Define custom views of analysis tables.

   Refer to the *GeneMapper® Software Version 4.1 Getting Started Guide for Microsatellite Analysis* (Pub. no. 4403672) for more information.
5. Define custom views of plots.

   Refer to the *GeneMapper® Software Version 4.1 Getting Started Guide for Microsatellite Analysis* (Pub. no. 4403672) for more information.

Import panels and bins

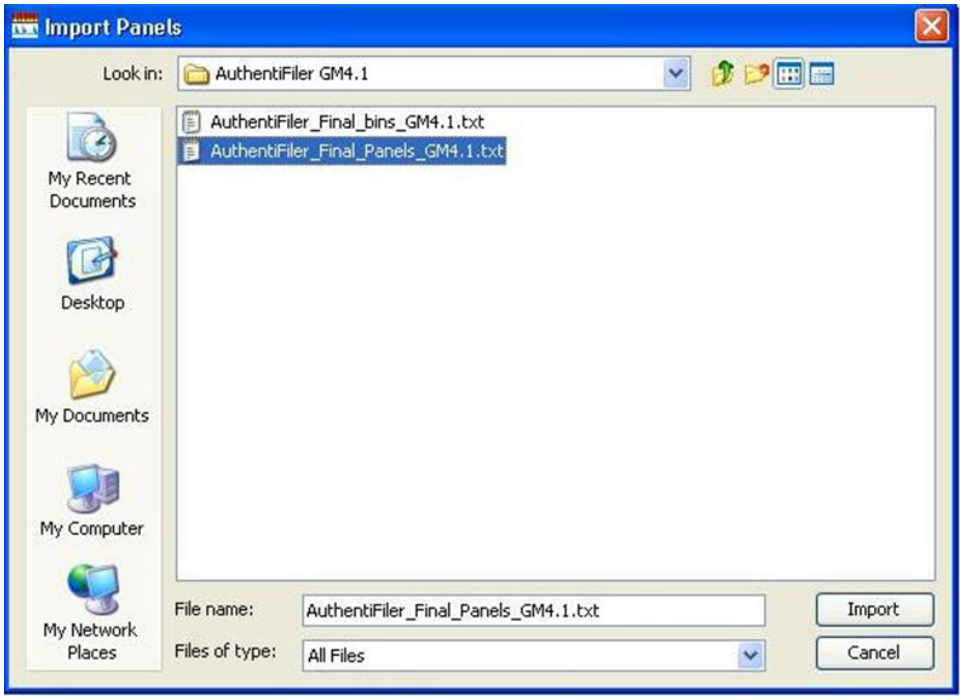
To import the AuthentiFiler™ Kit panel and bin set from **www.lifetechnologies.com** into the GeneMapper® Software v4.1 database:

1. Download and open the file containing panels and bins:
   a. From the Support menu of **www.lifetechnologies.com**, select **Support ▸ Software Downloads, Patches & Updates ▸ GeneMapper® Software v4.1 ▸ Updates & Patches**, and download the file **AuthentiFiler Analysis Files GM4.1**.
   b. Unzip the file.
2. Start the GeneMapper® Software, then log in with the appropriate user name and password.

   IMPORTANT! For logon instructions, refer to the *GeneMapper® Software Version 4.1 Getting Started Guide for Microsatellite Analysis* (Pub. no. 4403672) for more information.
3. Select **Tools ▸ Panel Manager**.
4. Find, then open the folder containing the panels and bins:
   a. Select **Panel Manager** in the navigation pane.
   b. Select **File ▸ Import Panels** to open the Import Panels dialog box.
   c. Navigate to, then open the **AuthentiFiler Analysis Files GM4.1** folder that you unzipped in step 1 above.

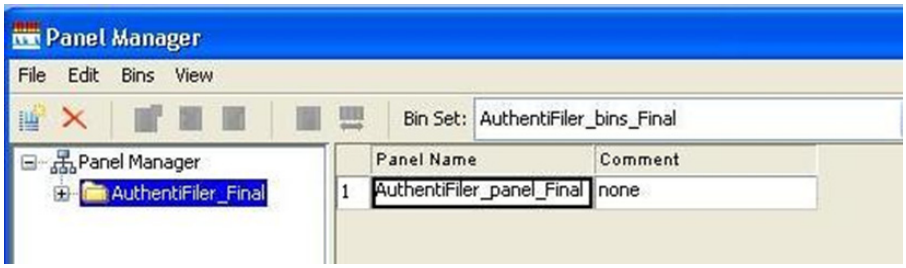
5. Select **AuthentiFiler_Final_Panels_GM4.1.txt**, then click **Import**.

   Note: Importing this file creates a new folder in the navigation pane of the Panel Manager "AuthentiFiler_Final". This folder contains the panel and associated markers.



6. Import **AuthentiFiler_Final_bins_GM4.1.txt**:

   a. Select the **AuthentiFiler_Final** folder in the navigation pane.

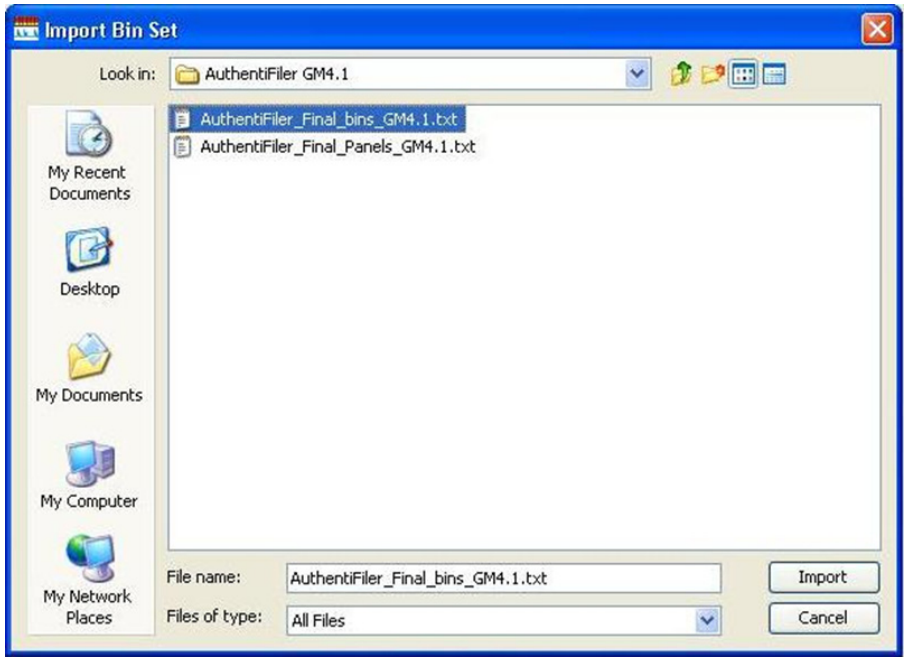


   b. Select **File ▸ Import Bin Set** to open the Import Bin Set dialog box.

   c. Navigate to, then open the **AuthentiFiler Analysis Files GM4.1** folder.

d. Select **AuthentiFiler_Final_bins_GM4.1.txt**, then click **Import**.

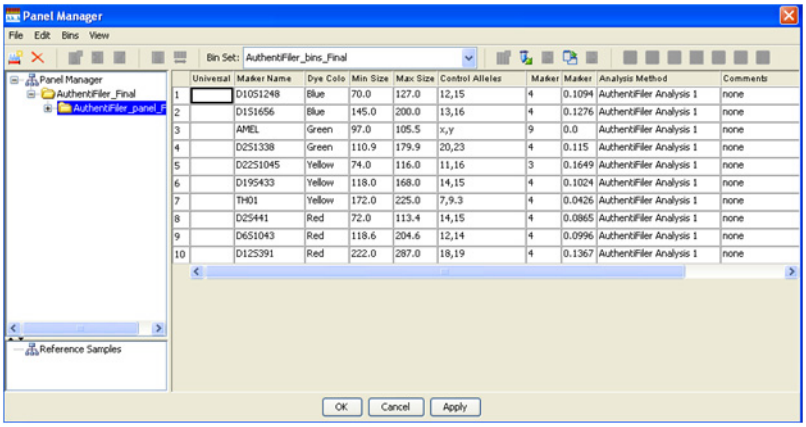
Note: Importing this file associates the bin set with the panels in the AuthentiFiler_Final folder.



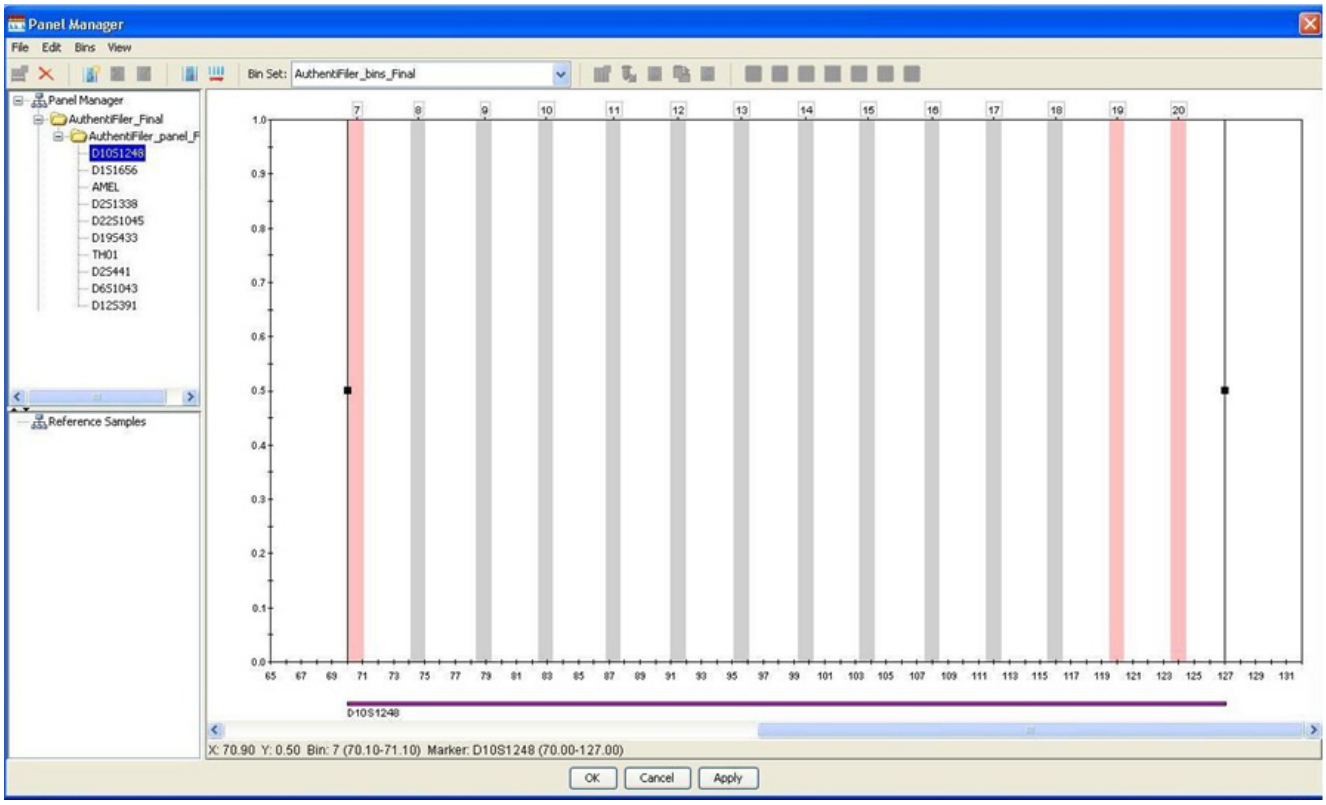
7. View the imported panels in the navigation pane:

a. Double-click the **AuthentiFiler_Final** folder.

b. Double-click the **AuthentiFiler_panel_Final** folder to display the panel information in the right pane.



8. Select **D10S1248** to display the Bin view for the marker in the right pane.



9. Click **Apply**, then **OK** to add the AuthentiFiler™ Kit panel and bin set to the GeneMapper® Software database.

IMPORTANT! If you close the Panel Manager without clicking **Apply**, the panels and bins are not imported into the GeneMapper® Software database.

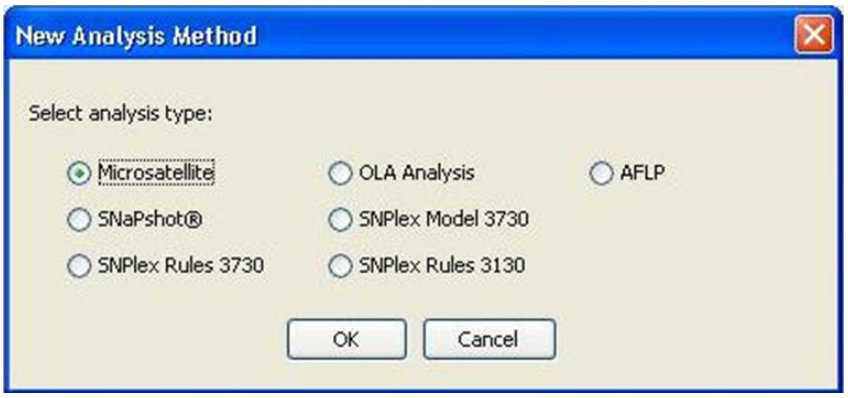
Create an analysis method

Use the following procedure to create an HID analysis method for the AuthentiFiler™ Kit.

IMPORTANT! Analysis methods are version-specific, so you must create an analysis method for each version of the software. For example, an analysis method created for GeneMapper® *ID-X* Software version 1.2 is not compatible with earlier versions of GeneMapper® *ID-X* Software, or with GeneMapper® *ID* Software version 3.2.1.

1. Select **Tools** ▸ **GeneMapper Manager** to open the GeneMapper Manager.
2. Select the **Analysis Methods** tab, then click **New** to open the New Analysis Method dialog box.

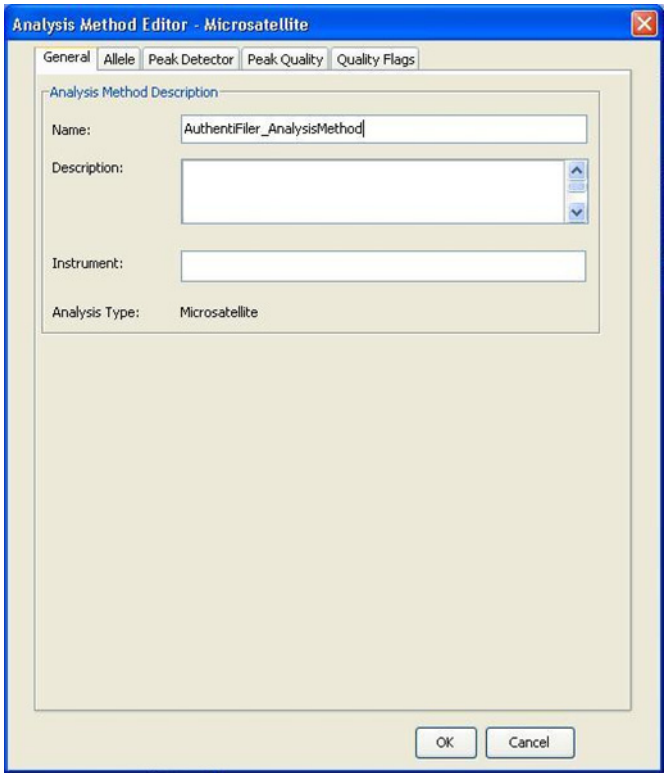
3. Select HID and click OK to open the Analysis Method Editor with the General tab selected.



Enter the settings shown in the figures on the following pages.

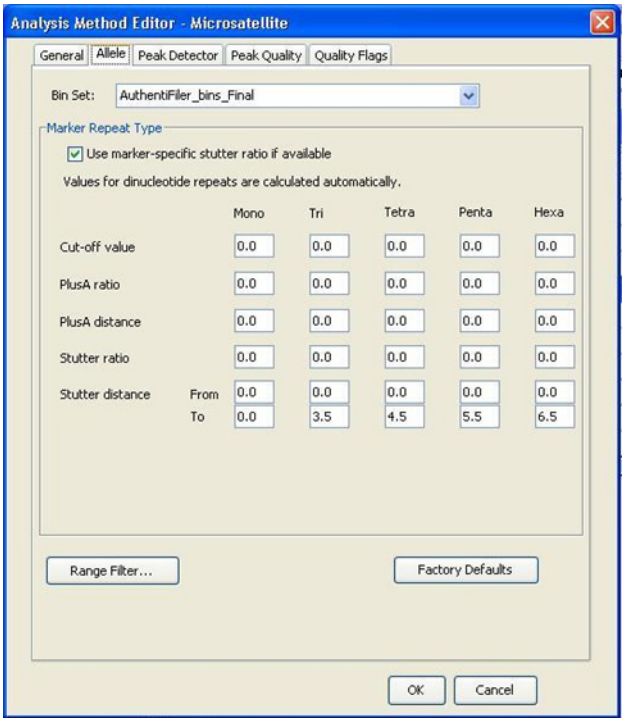
4. After you enter the settings on all tabs, click **Save**.

General tab settings



In the Name field, either type the name as shown or enter a name of your choosing. The Description and Instrument fields are optional.

Allele tab settings



- In the Bin Set field, select the **AuthentiFiler_bins_Final** bin set imported previously and configure the stutter distance parameters as shown.
- GeneMapper® Software v4.1 allows you to specify four types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- The "Use marker-specific stutter ratio and distance if applicable" check box is selected by default. Consequently, the software applies the stutter ratio filters supplied in the AuthentiFiler_Final_Panels_GM4.1.txt file. GeneMapper® Software v4.1 specifies locus-specific filter ratios for minus stutters, but not for plus and –2 nt stutters, in the panel file.

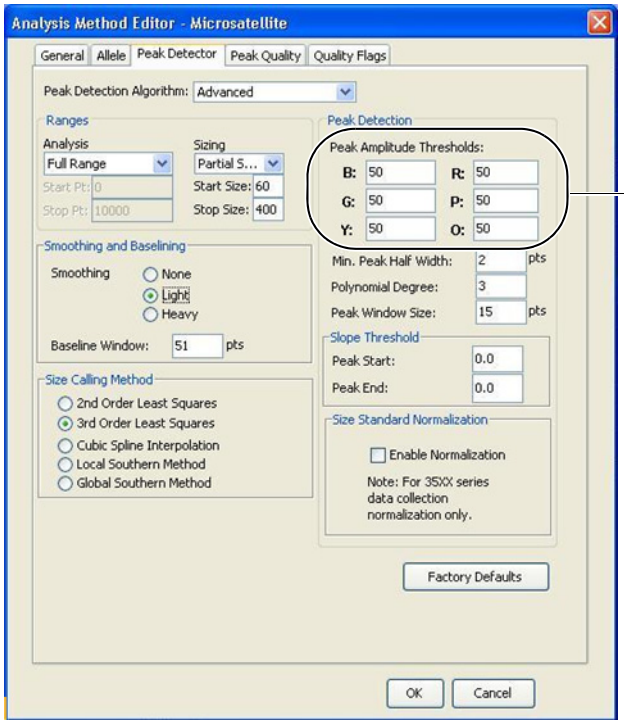
  However, validation studies with the AuthentiFiler™ Kit show that the trinucleotide repeat D22S1045 locus produces a relatively large amount of plus stutter compared to tetranucleotide repeat loci. The relatively large amount of stutter may cause the stutter peak to be labeled during routine analysis. Plus stutters were also observed for the loci D10S1248, and D1S1656. Additionally, and –2 nt stutter was also observed at the D1S1656 locus. The stutter percentages observed during internal validation is reported in the stutter table in Chapter 5.

  Users are asked to take note of these stutter percentages when analyzing data and follow laboratory guidelines to determine if an allele peak located in a plus stutter position can be discarded as a stutter peak.

  Note: Plus stutter and –2 nt stutter (D1S1656 only) values for trinucleotide and tetranucleotide repeats based on stutter percentages for D22S1045, D10S1248, and D1S1656 could not be included in the panel file due to functional limitations in the software.

  Note: We do not recommend the use of global filter for analysis.

Peak Detector tab settings



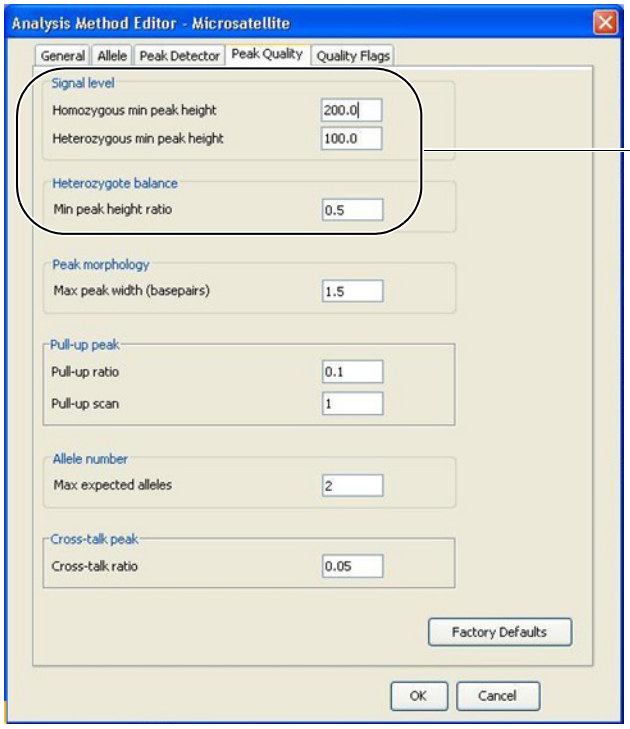
Perform internal validation studies to determine settings

IMPORTANT! Perform the appropriate internal validation studies to determine the appropriate peak amplitude thresholds for interpretation of AuthentiFiler™ Kit data.

Fields include:

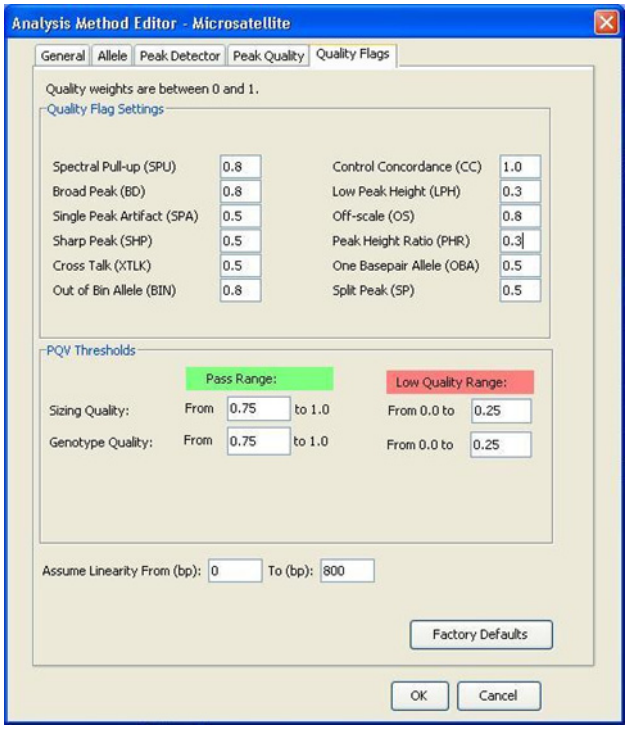
- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper® Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
- **Size calling method** – The AuthentiFiler™ Kit has been validated using the 3rd Order Least Squares sizing method with the GeneScan™ 500 LIZ® Size Standard or the GeneScan™ 600 LIZ® Size Standard v2.0. Select alternative sizing methods only after you perform the appropriate internal validation studies.

Peak Quality tab settings



IMPORTANT! Perform the appropriate internal validation studies to determine the heterozygous and homozygous minimum peak height thresholds, maximum peak height threshold, and the minimum peak height ratio threshold for interpretation of AuthentiFiler™ Kit data.

SQ & GQ tab settings



IMPORTANT! The values shown are the software defaults and are the values we used during developmental validation. Perform appropriate internal validation studies to determine the appropriate values to use.

Create a size standard

The size standards for the AuthentiFiler™ Kit uses the following size standard peaks in their definitions:

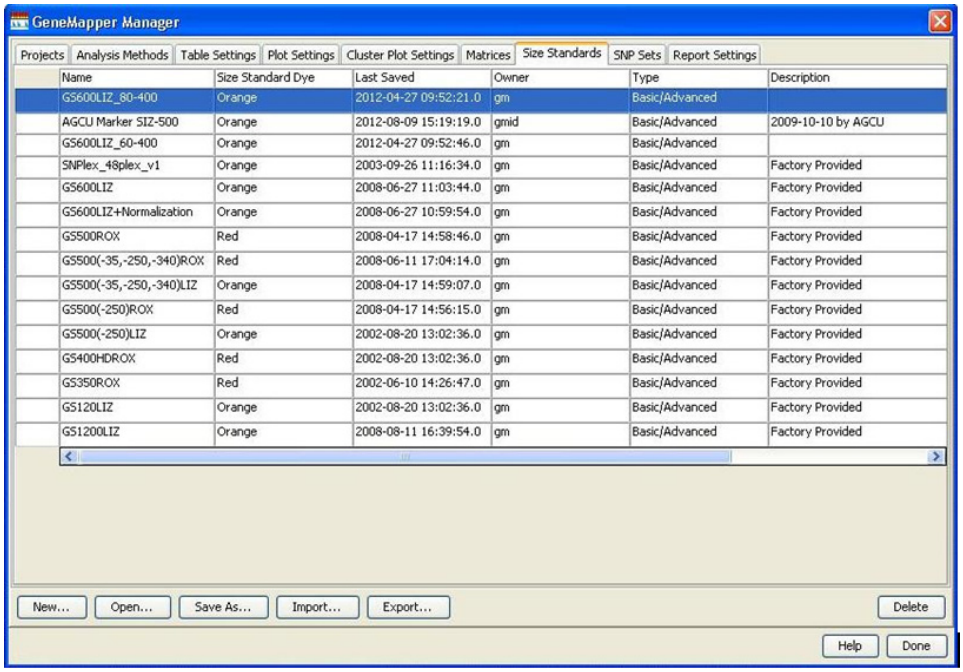
GeneScan™ 500 LIZ® Size Standard	GeneScan™ 600 LIZ® Size Standard v2.0
75, 100, 139, 150, 160, 200, 300, 350, 400, and 450	80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, and 400

Note: The 250-nt and the 340-nt peaks are not included in the GeneScan™ 500 LIZ® Size Standard definition, though present in the reagent formulation. These peaks can be used as an indicator of precision within a run.

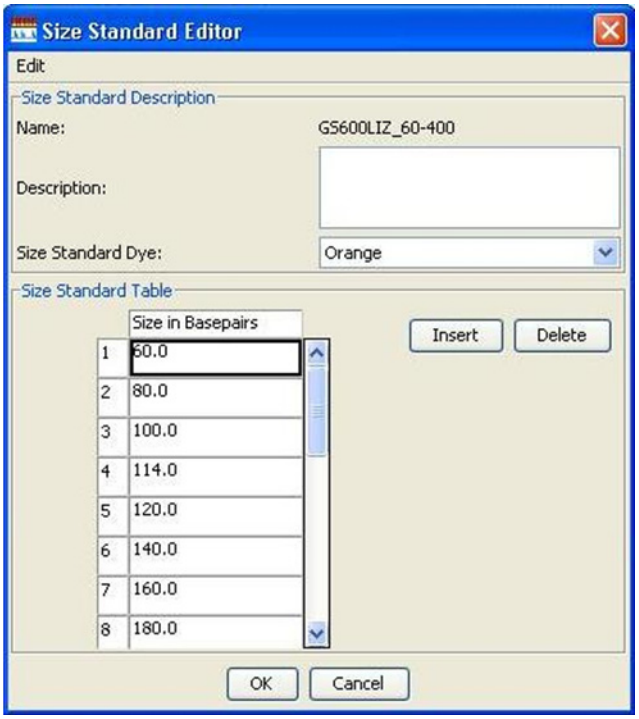
Use the following procedure to create the size standard for the AuthentiFiler™ Kit.

1. Select **Tools ▸ GeneMapper Manager** to open the GeneMapper Manager.

2. Select the **Size Standards** tab, then click **New**.



3. Enter a name as shown below or enter a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the drop-down list. In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the sizes specified on page 66. The example below is for the GeneScan™ 600 LIZ® Size Standard v2.0.



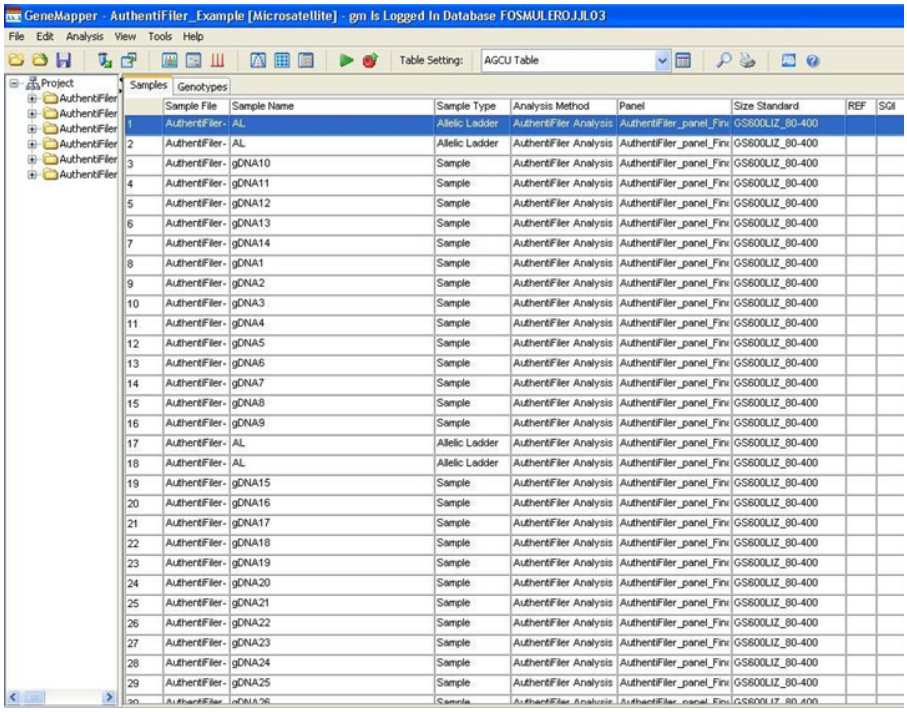
Analyze and edit sample files with GeneMapper® Software

1. In the Project window, select **File ▸ Add Samples to Project**, then navigate to the disk or directory containing the sample files.
2. Apply analysis settings to the samples in the project. The names of the settings shown are the names suggested in the sections above. If you named the settings differently, select the names you specified.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	AuthentiFiler_AnalysisMethod (or the name of the analysis method you created)
Panel	AuthentiFiler_panel_Final
Size Standard	GS600LIZ_60-400 (or the name of the size standard you created)

   For more information about how the Size Caller works, refer to the *ABI PRISM® GeneScan® Analysis Software for the Windows NT® Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (Pub. no. 4335617). For additional information about size standards, refer to the *GeneMapper® Software Version 4.1 Getting Started Guide for Microsatellite Analysis* (Pub. no. 4403672).
3. Click (Analyze), enter a name for the project (in the Save Project dialog box), then click **OK** to start analysis.
   - The status bar displays the progress of analysis as both:
     – A completion bar extending to the right with the percentage completed indicated
     – With text messages on the left

- The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
- The Analysis Summary tab is displayed upon completion of the analysis. The Genotypes tab becomes available after analysis.



Examine and edit a project

You can display electropherogram plots from the Samples and Genotypes tabs of the Project window to examine the data. These procedures start with the Samples tab of the Project window (assuming the analysis is complete).

For more information

For details about GeneMapper® Software features, allele filters, peak detection algorithms, and project editing, refer to:

- ***GeneMapper® Software Version 4.1 Installation and Administration Guide*** (Pub. no. 4403614) — Provides procedures for installing, securing, and maintaining version 4.1 of the GeneMapper® Software.
- ***GeneMapper® Software Version 4.1 Getting Started Guide for Microsatellite Analysis*** (Pub. no. 4403672) — The guide is designed to help you quickly learn to use basic functions of the GeneMapper® Software.
- ***GeneMapper® Software Version 4.1 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® Software window.

- ***GeneMapper® Software Version 4.1 Quick Reference Guide*** (Pub. no. 4403615) — Provides workflows for specific analysis types and lists instruments, software, and analysis applications compatible with the GeneMapper® Software.
- ***GeneMapper® Software Version 4.1 Reference and Troubleshooting Guide*** (Pub. no. 4403673) — Provides reference information such as theory of operation and includes troubleshooting information.

5 Experiments and Results

Overview

Importance of validation

Validation of a DNA typing procedure for human identification applications is an evaluation of the procedure's efficiency, reliability, and performance characteristics. By challenging the procedure with samples commonly encountered in forensic and parentage laboratories, the validation process uncovers attributes and limitations that are critical for sound data interpretation (Sparkes, Kimpton, Watson *et al.*, 1996; Sparkes, Kimpton, Gilbard *et al.*, 1996; Wallin *et al.*, 1998).

Experiment conditions

This chapter discusses many of the experiments performed by us and provides examples of results obtained. We chose conditions that produced optimum PCR product yield and that met reproducible performance standards.

IMPORTANT! Perform internal validation studies before using the AuthentiFiler™ Kit.

Extra peaks in the electropherogram

Causes of extra peaks

Peaks other than the target alleles may be detected on the electropherogram. Causes for the appearance of extra peaks include stutter products, incomplete 3′ A nucleotide addition (at the n-1 position), dye artifacts, and mixed DNA samples (refer to DNA Advisory Board [DAB] Standard 8.1.2.2).

Stutter products

Stutter is a well-characterized PCR artifact that refers to the appearance of a minor peak one repeat unit smaller (or less frequently, one repeat larger) than the target STR allele product (Butler, 2005; Mulero *et al.*, 2006). Sequence analysis of stutter products at tetranucleotide STR loci has revealed that the stutter product is missing a single tetranucleotide core repeat unit relative to the main allele (Walsh *et al.*, 1996).

The proportion of the stutter product relative to the main allele (stutter percent) is measured by dividing the height of the stutter peak by the height of the main allele peak. Peak heights were measured for amplified samples at the loci used in the AuthentiFiler™ Kit:

- 210 genomic DNA samples (2 ng) from four population groups (Caucasian, African-American, Hispanic, and Asian) were amplified using the AuthentiFiler™ Kit and electrophoresed on the Applied Biosystems® 3130*xl* Genetic Analyzer. The data was analyzed for determination of the percentage of the stutter peaks.

All data were generated on the Applied Biosystems® 3130*xl* Genetic Analyzer.

Some conclusions from these measurements and observations are:

- For each AuthentiFiler™ Kit locus, the stutter percentage generally increases with allele length, as shown in Figure 4 to Figure 6 on pages 72 to 73.
- Smaller alleles display a lower level of stutter relative to the longer alleles within each locus.
- Each allele within a locus displays a consistent stutter percentage.
- Stutter filter sets in GeneMapper® *ID*, GeneMapper® *ID-X*, and GeneMapper® software, calculated as the mean stutter for the locus plus three standard deviations, are shown in Table 4 on page 73. Peaks in the stutter position that are above the stutter filter percentage specified in the software are not filtered. Peaks in the stutter position that have not been filtered and remain labeled can be further evaluated.
- The measurement of stutter percentage for allele peaks that are off-scale may be unusually high due to artificial truncation of the main allele peak.

Figure 4 Stutter percentages for D10S1248, D1S1656 and D2S1338 loci. (Blue and green colors indicate loci labeled with 6-FAM™ and VIC® dyes, respectively.)

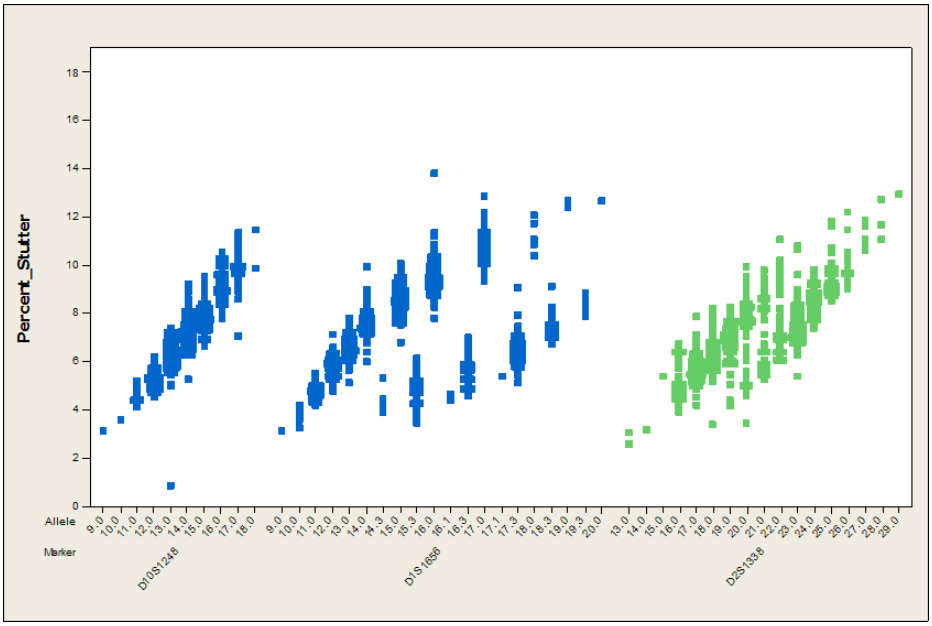


Figure 5 Stutter percentages for D22S1045, D19S433, and TH01 loci. (Black color indicates loci labeled with NED™ dye)

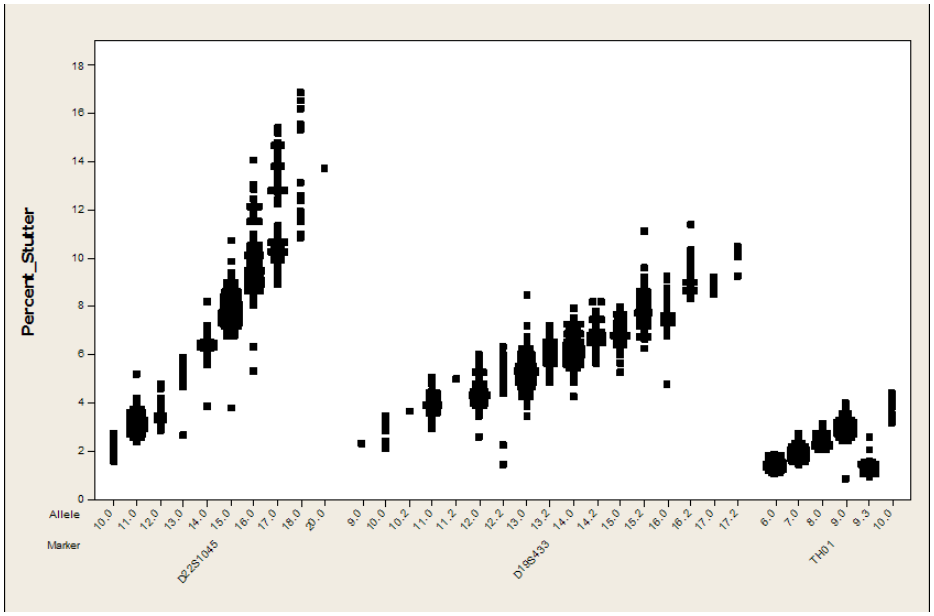


Figure 6 Stutter percentages for D2S441, D6S1043, and D12S391 loci. (Red color indicates loci labeled with PET® dye)

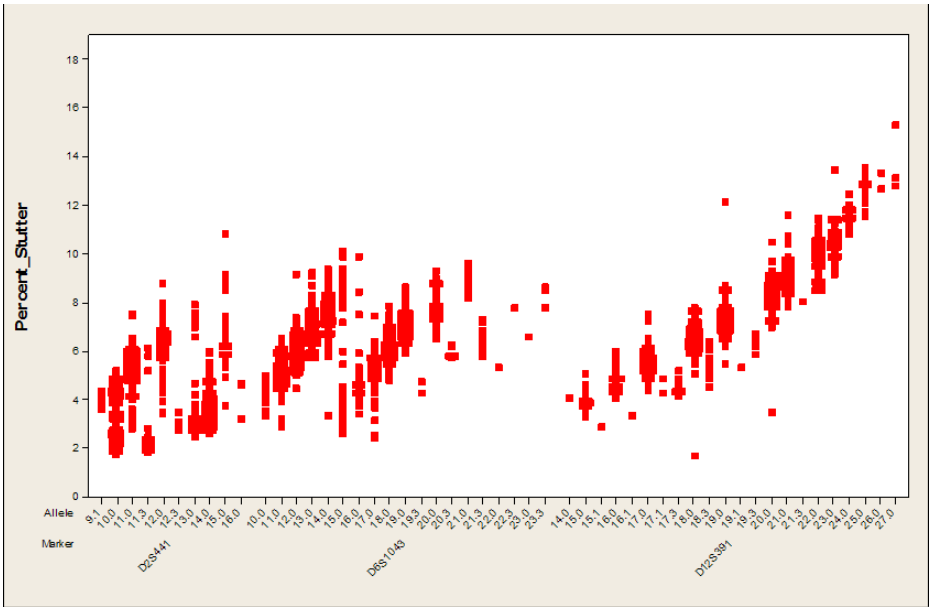


Table 4 Marker-specific stutter filter percentages for AuthentiFiler™ Kit loci

Locus†	% Stutter
D10S1248	10.94
D10S1248 (+4 nt)‡	3.49
D1S1656	12.76
D1S1656 (+4 nt)‡	2.6

Locus†	% Stutter
D1S1656 (–2 nt)‡	3.23
D2S1338	11.50
D22S1045	16.49
D22S1045 (+3 nt)§	6.84
D19S433	10.24
TH01	4.26
D2S441	8.65
D6S1043	9.96
D12S391	13.67

† These percentages are used as stutter filters, in GeneMapper® 4.1 software: AuthentiFiler_Final_Panels_GM4.1.txt; in GeneMapper® *ID* v3.2.1 software: AuthentiFiler_Final_GMID3.2_Panels.txt; and in GeneMapper® *ID-X* software: GeneMapper® *ID-X* AuthentiFiler_Final_stutter.txt

‡ The –2 nt and tetranucleotide plus stutter filters are not included in GeneMapper® *ID* AuthentiFiler_Final_GMID3.2_Panels.txt and GeneMapper® software v4.1 AuthentiFiler_Final_Panels_GM4.1.txt due to functional limitations of the software.

§ The D22S1045 plus stutter can be added in the plus stutter ratio box in the AuthentiFiler_AnalysisMethod, in the "Allele" tab and the plus stutter distances are from 2.25 and 3.75 for the GeneMapper® ID v3.2.1 software. The D22S1046 plus stutter filters cannot be added in GeneMapper® v4.1 Analysis Method due to functional limitations of the software.

IMPORTANT! The values shown are the values we determined during developmental validation studies. We recommend that laboratories perform internal validation studies to determine the appropriate values to use.

Addition of 3′ A nucleotide

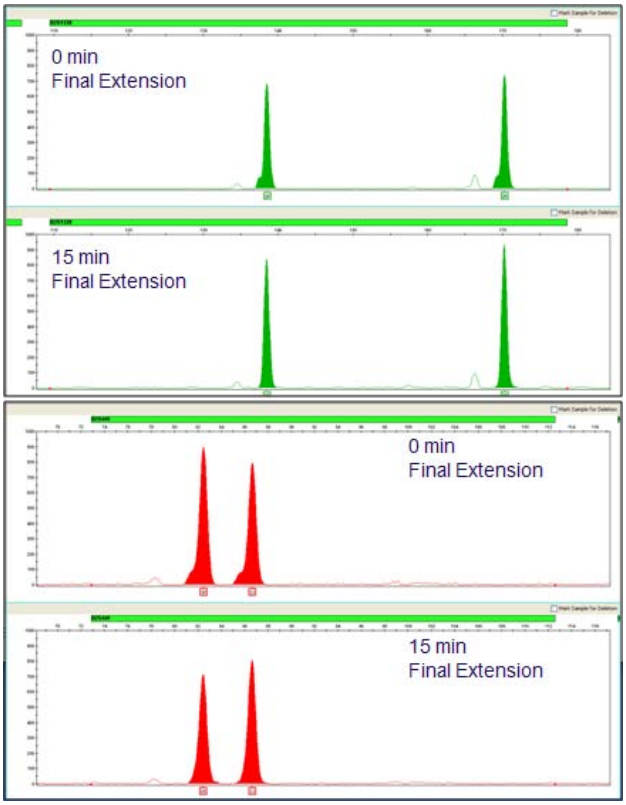
Many DNA polymerases can catalyze the addition of a single nucleotide (predominantly adenosine) to the 3′ ends of double-stranded PCR products (Clark, 1988; Magnuson *et al.*, 1996). This non-template addition results in a PCR product that is one nucleotide longer than the actual target sequence. The PCR product with the extra nucleotide is referred to as the "+A" form.

The efficiency of +A addition is related to the particular sequence of the DNA at the 3′ end of the PCR product. The AuthentiFiler™ Kit includes two main design features that promote maximum +A addition:

- The primer sequences have been optimized to encourage +A addition.
- The new, highly robust PCR chemistry allows complete +A addition with a short final incubation at 60 °C for 10 minutes.

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products. See Figure 7 on page 75 for examples of incomplete and normal +A addition. Final extension incubation for longer than the recommended 10 minutes may result in double +A addition, in which *two* non-template adenosine residues are added to the PCR product. Double +A addition can cause "shoulders" on the right side of main allele peaks, and is therefore to be avoided.

Figure 7 Omitting the final extension step results in shoulders on main allele peaks due to incomplete A nucleotide addition. Examples shown are D2S1338 (green) and D2S441 (red) data from an Applied Biosystems® 3130*xl* Genetic Analyzer using the AuthentiFiler™ Kit.



Due to improved PCR buffer chemistry, the lack of +A addition is generally less an issue with the AuthentiFiler™ Kit than with earlier generation kits. However, "shouldering" of allele peaks may still be observed if the amount of input DNA is greater than that recommended by the AuthentiFiler™ Kit protocol. Amplification of excess input DNA may also result in offscale data.

Artifacts

Artifacts and anomalies are seen in all molecular biological systems. Artifacts are typically reproducible while anomalies are non-reproducible, intermittent occurrences that are not observed consistently in a system (for example, spikes and baseline noise). Due to improvements in PCR primer manufacturing processes, the incidence of artifacts has been greatly reduced in the AuthentiFiler™ Kit. AuthentiFiler™ Kit electropherograms are essentially free of reproducible dye artifacts within the kit's read region of 70–287 nt. Figure 8 on page 76 shows the very low baseline level fluorescence of a typical negative control PCR using the AuthentiFiler™ Kit.

Figure 9 on page 77 shows an example of a non-standard (minus 2-nt) stutter that may be observed in D1S1656 locus that exhibit more complex nucleotide sequences including regions of dinucleotide repeats. In cases where these stutter peaks exceed the peak amplitude threshold (e.g., 50 RFU), they may be detected by analysis software as additional alleles in the profile.

Most STR loci produce minus-stutter peaks as a by-product of PCR amplification. A process of "slippage" has been proposed as a molecular mechanism for stutter, where the Taq DNA polymerase enzyme "slips" on the template DNA during replication and produces a minority PCR product that is shorter than the template strand, usually by

one repeat unit. The stutter process may also occur in the opposite direction to produce amplicon DNA that is usually one repeat unit longer than the template strand, termed plus-stutter. While plus-stutter is normally much less significant than minus-stutter in STR loci with tetranucleotide repeats, the incidence of plus-stutter may be more significant in trinucleotide repeat-containing loci. The D22S1045 locus in the AuthentiFiler™ Kit is a trinucleotide repeat locus, and shows an elevated level of plus-stutter. The D10S1248 and D1S1656 loci in the AuthentiFiler™ Kit are tetranucleotide repeat loci, and show an elevated level of plus-stutter. For example, Figure 10 on page 77 is an electropherogram of the D22S1045 locus showing plus stutter. GeneMapper® *ID-X* analysis files supplied for use with the AuthentiFiler™ Kit contain a plus-stutter filter to prevent these peaks from being called in normal profiles.

It is important to consider possible noise and artifacts when interpreting data from the AuthentiFiler™ Kit on the Applied Biosystems® 3500/3500xL and 3130/3130*xl* Genetic Analyzers. Note that a high degree of magnification is used in the sample electropherograms shown in Figure 8 through Figure 10 on pages 76 to 77.

Using a cycle number that is too high for the DNA input amount may cause artifacts, including:

- Shoulder peaks due to incomplete +A addition.
- Pull-up peaks caused by poor spectral separation when fluorescence signals exceeds the linear dynamic range for detection by the instrument.

Figure 8 Examples of fluorescence background in data with a 31 cycle PCR amplification produced on an Applied Biosystems® 3130*xl* Genetic Analyzer (y-axis 70 RFU).

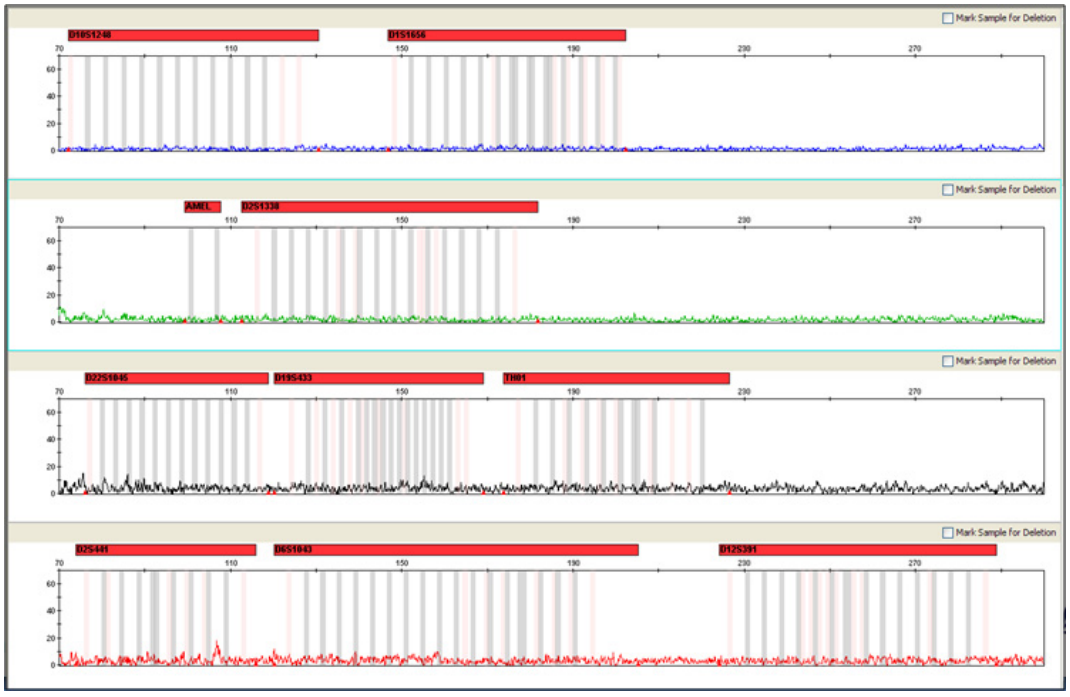


Figure 9 Example of a –2 nt reproducible artifact at the D1S1656 locus. Data produced on an Applied Biosystems® 3130*xl* Genetic Analyzer.

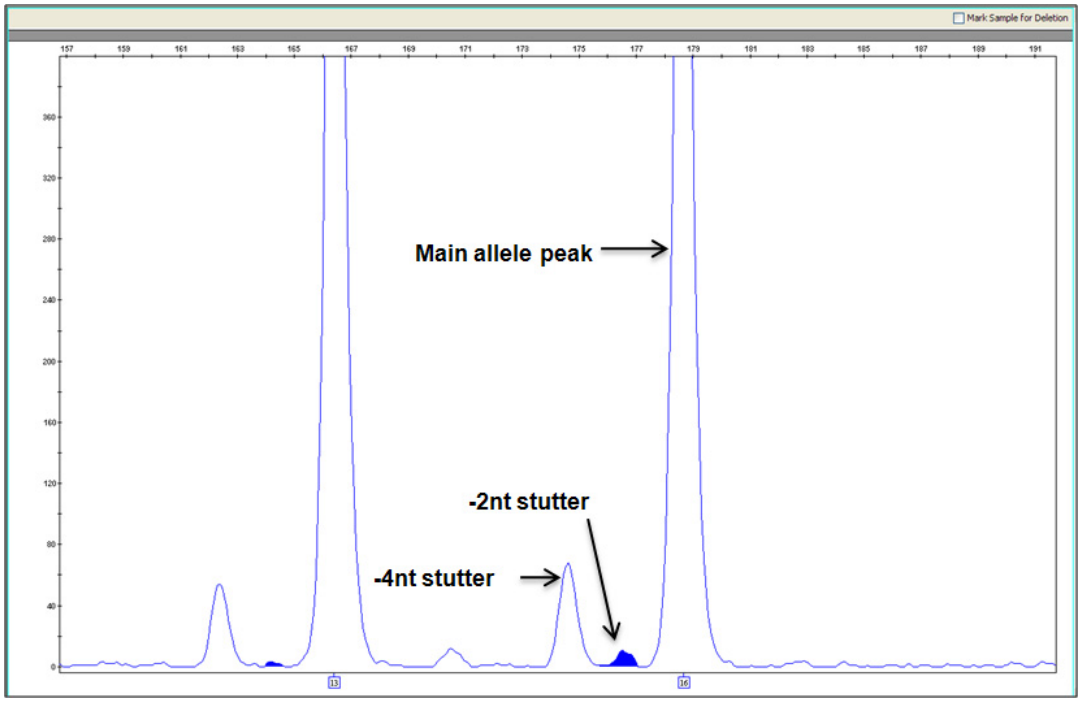
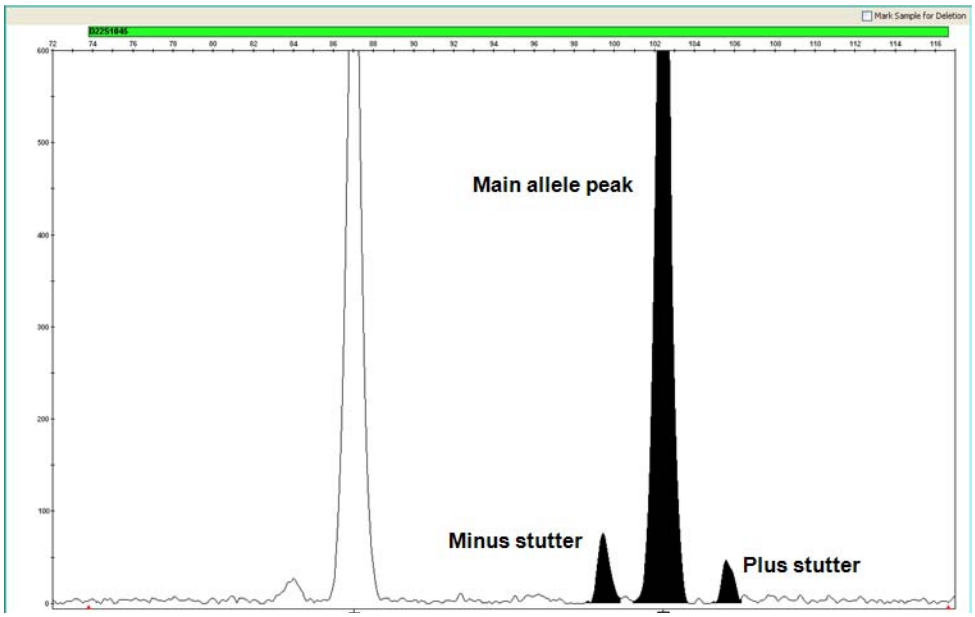


Figure 10 AuthentiFiler™ Kit electropherogram showing plus stutter associated with the D22S1045 STR locus. Data produced on an Applied Biosystems® 3130*xl* Genetic Analyzer.



Sensitivity

Importance of quantification

The recommended amount of input DNA for the AuthentiFiler™ Kit is 2.0 ng based on quantification using either the NanoDrop™ 1000 Spectrophotometer or any other quantification method of choice. Individual laboratories should determine the optimum input DNA amount according to the quantification method in use in the laboratory. For inhibited DNA, it is recommended to dilute the sample and amplify provided that the sample has sufficient quantity of DNA. This is to reduce the concentration of DNA. Adding more quantity of sample increases the quantity of inhibitor as well. In Figure 11 on page 79, the genomic DNA from a Human Fibroblast Cell Line was serially diluted from 4.0 ng to 0.125 ng. Full profiles (32 PCR products) were consistently obtained at 0.125 ng.

Effect of DNA quantity on results

If too much DNA is added to the PCR reaction, the increased amount of PCR product that is generated can result in:

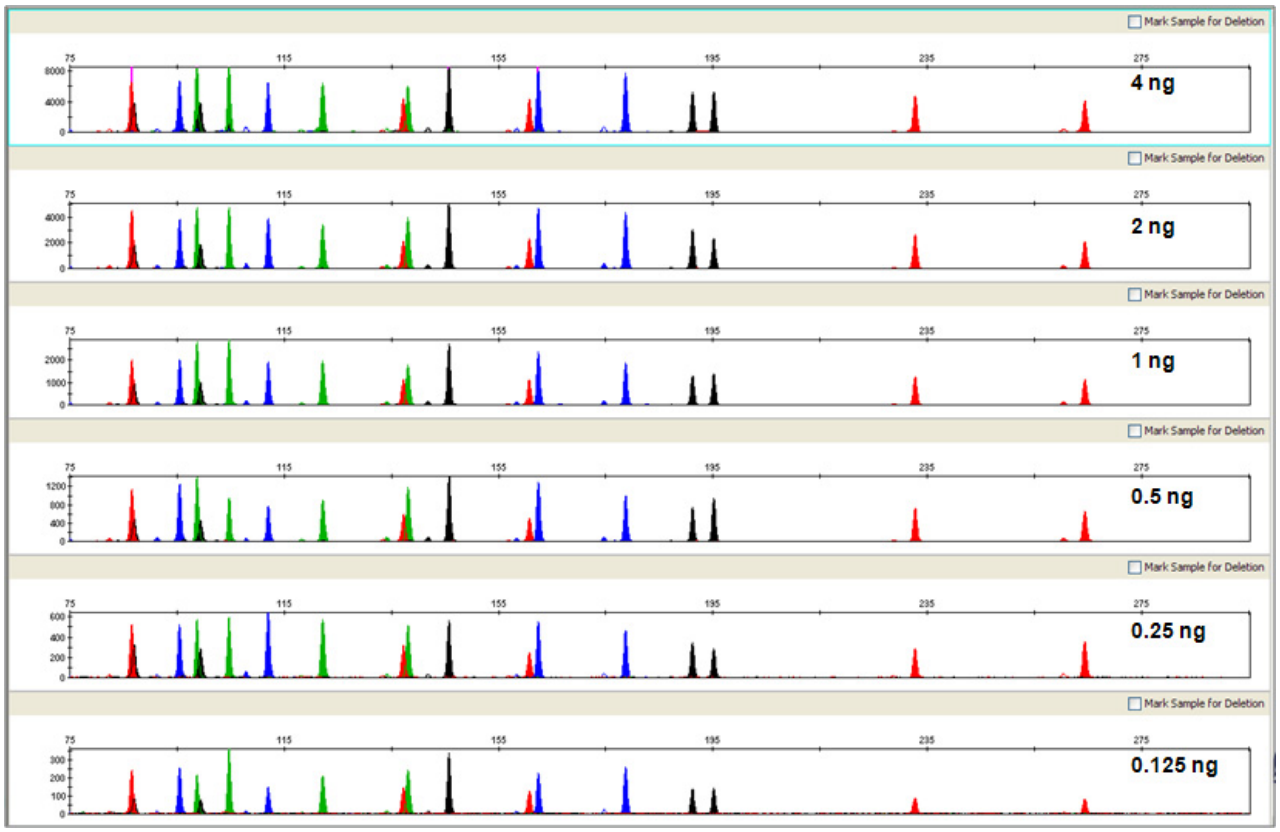
- Fluorescence intensity that exceeds the linear dynamic range for detection by the instrument (“off-scale” data).
- Off-scale data. Off-scale data is a problem because:
  - Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause the corresponding stutter peak to appear higher in relative intensity, thus increasing the calculated percent stutter.
  - Multicomponent analysis of off-scale data is not accurate. This inaccuracy results in poor spectral separation (“pull-up”).
  - Off-scale data can result in “pull-up” peaks in the other colors, which may interfere in the allele call if falls in the fractionation range of a locus.
- Incomplete +A nucleotide addition.

To address these issues, reamplify the sample using less DNA.

When the total number of allele copies added to the PCR is extremely low, unbalanced amplification of the alleles may occur because of stochastic fluctuation.

Individual laboratories may find it useful to determine an appropriate minimum peak height threshold based on their own results and instruments using low amounts of input DNA.

Figure 11 Electropherograms for 27-cycle amplifications using 4 ng, 2 ng, 1 ng, 0.50 ng, 0.25 ng, and 0.125 ng, of genomic DNA from Human Fibroblast Cell Line. Electrophoresis was performed on an Applied Biosystems® 3130*xl* Genetic Analyzer. Note that the y-axis scale is magnified for the smaller input amounts of DNA.



Mixture studies

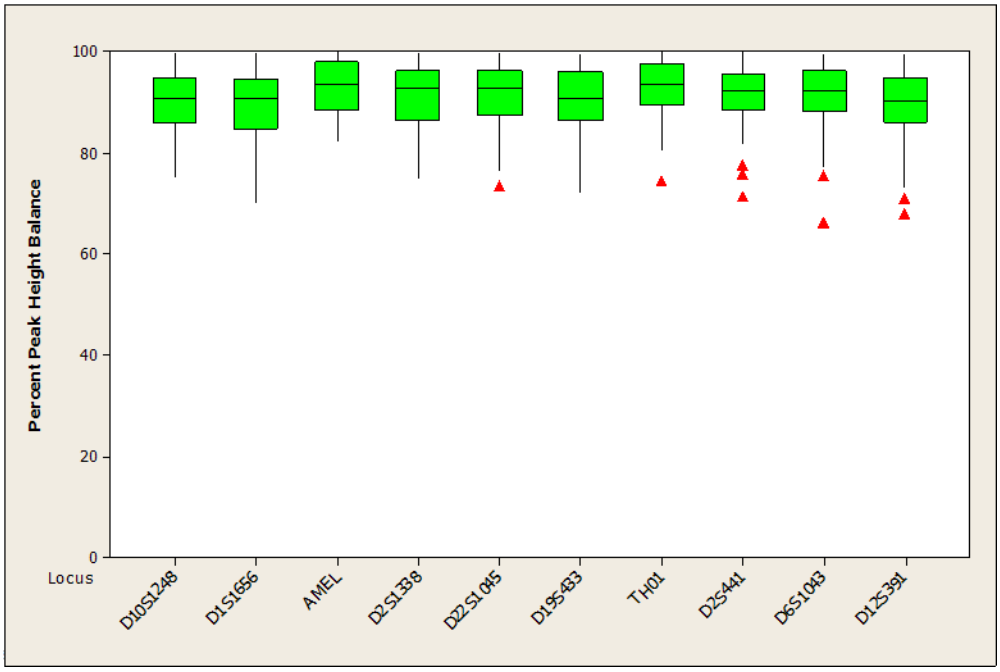
Mixture studies

It is essential to ensure that the DNA typing system is able to detect DNA mixtures. Typically, mixed samples can be distinguished from single-source samples by:

- The presence of more than two alleles at one or more loci
- The presence of a peak at a stutter position that is significantly greater in percentage than typically observed in a single-source sample
- Significantly imbalanced alleles for a heterozygous genotype

The peak height ratio is defined as the height of the lower peak (in RFU) divided by the height of the higher peak (in RFU), expressed as a percentage. Mean, median, minimum, and maximum peak height ratios observed for alleles in the AuthentiFiler™ Kit loci in unmixed human population database samples are shown in Figure 12 below.

Figure 12 Heterozygote ratios for 2 ng of input DNA amplified for 27 cycles with the AuthentiFiler™ Kit. The distribution of intra-locus peak height ratios are expressed as a percent, by locus. Green boxes show the middle 50% or interquartile range (IQR). Box halves below and above median show the second and third quartile, respectively. "Whiskers" indicate 1.5 IQR from the upper and lower margins of the IQR. Red diamonds are outlier data points more than 1.5 IQR from the median.



If an unusually low peak height ratio is observed for one locus, and there are no other indications that the sample is a mixture, the sample may be reamplified and reanalyzed to determine if the imbalance is reproducible. Possible causes of imbalance at a locus are:

- Degraded DNA
- Presence of inhibitors
- Extremely low amounts of input DNA
- A mutation in one of the primer binding sites
- Presence of an allele containing a rare sequence that does not amplify as efficiently as the other allele

Resolution of genotypes in mixed samples

A sample containing DNA from two sources can comprise (at a single locus) any of the seven genotype combinations (see below).

- Heterozygote + heterozygote, no overlapping alleles (four peaks)
- Heterozygote + heterozygote, one overlapping allele (three peaks)
- Heterozygote + heterozygote, two overlapping alleles (two peaks)
- Heterozygote + homozygote, no overlapping alleles (three peaks)
- Heterozygote + homozygote, overlapping allele (two peaks)
- Homozygote + homozygote, no overlapping alleles (two peaks)
- Homozygote + homozygote, overlapping allele (one peak)

Specific genotype combinations and input DNA ratios of the samples contained in a mixture determine whether or not it is possible to resolve the genotypes of the major and minor component(s) at a single locus.

The ability to obtain and compare quantitative values for the different allele peak heights on Applied Biosystems® instruments provides additional valuable data to aid in resolving mixed genotypes.

Ultimately, the likelihood that any sample is a mixture must be determined by the analyst in the context of each particular case, including the information provided from known reference sample(s).

Limit of detection of the minor component

Mixtures of two DNA samples from Human Fibroblast Cell Line were examined at various ratios (1:0, 1:5, 1:10, 1:20). The total amount of genomic input DNA mixed at each ratio was 2.0 ng. The samples were amplified in a GeneAmp® PCR System 9700, then electrophoresed and detected using an Applied Biosystems® 3130*xl* Genetic Analyzer.

The results of the mixed DNA samples are shown in Figure 13 on page 82 where samples A and B were mixed according to the ratios indicated. The minor component allele calls at non-overlapping loci are highlighted. Detection of full profiles for the minor contributor was possible at ratios of 1:5 (0.4:1.6 ng) and 1:10 (0.2:1.8 ng). Generally, 1:20 ratios resulted in partial profiles for the minor component at the tested concentrations. The profiles of these samples are described in Table 5 on page 82.

Figure 13 Amplification of DNA mixtures at various ratios. Panels show electropherograms for (top to bottom): Minor contributor only, 1:5 mixture (minor:major), 1:10 mixture,1:20 mixture, and Major contributor only. The experiment was performed with a 27 cycle amplification.

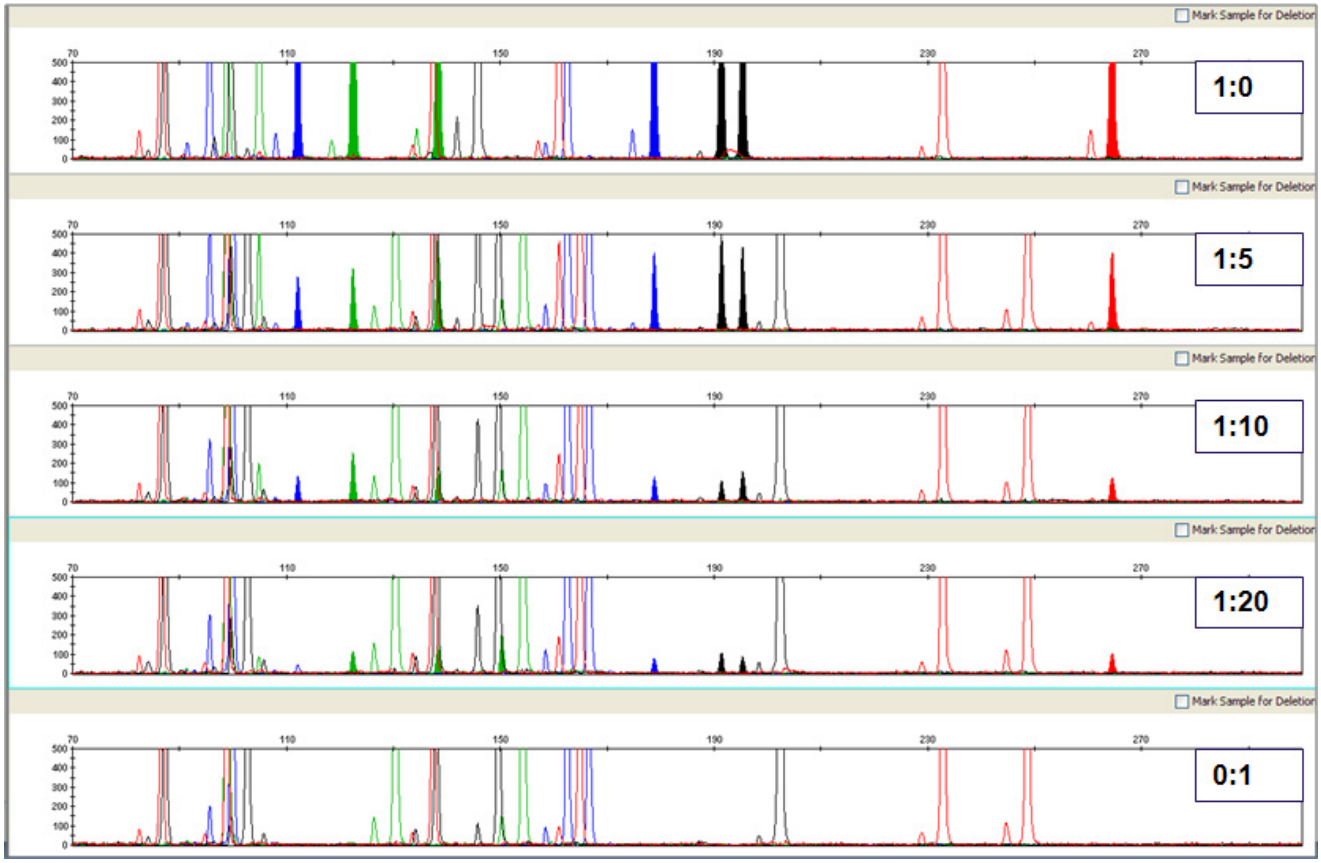


Table 5 Genotypes of mixed DNA samples

Locus	Sample A Genotype	Sample B Genotype
D10S1248	13, 17	14
D1S1656	12, 16	12, 13
Amelogenin	X, Y	X
D2S1338	16, 20	18, 24
D22S1045	11, 15	11, 16
D19S433	14	12, 15
TH01	7, 8	9.3
D2S441	11	11, 14
D6S1043	12, 18	12, 19
D12S391	15, 23	15, 19

A Troubleshooting

Follow the actions recommended in this appendix to troubleshoot problems that occur during analysis.

Observation	Possible causes	Recommended actions
Faint or no signal from both, the AuthentiFiler™ DNA Control 007 and the DNA test samples at all loci	Incorrect volume or absence of either Master Mix or Primer Set	Repeat amplification using correct reagent volumes.
	No activation of enzyme	Repeat amplification, making sure to hold reactions initially at 95°C for 11 min.
	Master Mix not vortexed thoroughly before aliquoting	Vortex Master Mix thoroughly.
	Primer Set exposed to too much light	Store Primer Set protected from light.
	GeneAmp® PCR System malfunction	Refer to the thermal cycler user's manual and check instrument calibration.
	Incorrect thermal cycler parameters	Check the protocol for correct thermal cycler parameters.
	Tubes/plate not seated tightly in the thermal cycler during amplification	Push reaction tubes/plate firmly into contact with block after first cycle. Repeat test.
	Wrong PCR reaction tubes or plate	Use Applied Biosystems® MicroAmp® Reaction Tubes with Caps or the MicroAmp® Optical 96-Well Reaction Plate for the GeneAmp® PCR System 9700 or Veriti® 96-well Thermal Cycler.
	MicroAmp® Base used with tray/ retainer set and tubes in GeneAmp® PCR System 9700	Remove MicroAmp® Base from tray/retainer set and repeat test.
	Insufficient PCR product electrokinetically injected	See Chapter 3, "Electrophoresis" on page 23, for instructions on recommended actions on the 3130/ 3130*xl*, and 3500/ 3500xL.
	Degraded formamide	Check the storage of formamide; do not thaw and refreeze multiple times. Try Hi-Di™ Formamide.

Observation	Possible causes	Recommended actions
Positive signal from AuthentiFiler™ DNA Control 007 but partial or no signal from DNA test samples	Quantity of test DNA sample is below assay sensitivity	Quantify DNA and add 2.0 ng of DNA for a 27 cycle amplification. Repeat test.
	Test sample contains high concentration of PCR inhibitor (for example, heme compounds, certain dyes	Quantify DNA and add minimum necessary volume. Repeat test.
		Wash the sample in a Centricon®-100 centrifugal filter unit. Repeat test.
	Test sample DNA is severely degraded	If possible, evaluate the quality of DNA sample by running an agarose gel. If DNA is degraded, reamplify with an increased amount of DNA.
	Dilution of test sample DNA in water or wrong buffer (for example, TE formula with incorrect EDTA concentration)	Redilute DNA using low-TE Buffer (with 0.1 mM EDTA).
More than two alleles present at a locus	Presence of exogenous DNA	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Amplification of stutter product	Interpret according to laboratory procedures. **Note:** Additional information will be provided on completion of validation.
	Mixed sample	
	Incomplete 3´ A base addition (n-1 nt position)	Addition of excess DNA to the reaction will contribute to the occurrence of incomplete 3' base addition. Quantify DNA and add 1.0 ng of DNA to the reaction. Repeat test. Also be sure to include the final extension step of 60°C for 10 min in the PCR.
	Cell line you are interrogating has genomic instability or genetic duplications	Some cell lines exhibit more than 2 alleles at one or more loci due to genomic instability, which can be a characteristic of the cell line. One can confirm this by comparing the tested sample profile with the reference profile for that cell line. If the profile of the tested sample exhibits an additional allele, the possibility of genetic drift during frequent subculturing should be evaluated.
	Signal exceeds dynamic range of instrument (off-scale data)	Ensure cycle number is optimized according to instructions on page 21. Repeat PCR amplification using fewer PCR cycles or use your laboratory's SOP to analyze off-scale data.
	Poor spectral separation (bad matrix)	Follow the steps for creating a spectral file.
		Confirm that Filter Set G5 modules are installed and used for analysis.
	Too much DNA in reaction	Use recommended amount of template DNA: 2.0 ng at 27 cycles.
	Incomplete denaturation of double stranded DNA	Use the recommended amount of Hi-Di™ Formamide and perform heat denaturation according to instructions in Chapter 3, "Electrophoresis".

Observation	Possible causes	Recommended actions
Poor peak height balance	Incorrect thermal cycler parameters	Check the protocol for correct thermal cycler parameters.
	GeneAmp® PCR System 9700 with Aluminum 96-Well block or third-party thermal cyclers	Use Applied Biosystems® GeneAmp® PCR System 9700 with silver, gold-plated silver blocks or Veriti® 96-well Thermal Cycler only.

B

Ordering Information

Materials and equipment not included

The tables below list optional equipment and materials not supplied with the AuthentiFiler™ Kit. Unless otherwise noted, many of the items are available from major laboratory suppliers (MLS).

Equipment	Cat. number
Applied Biosystems® 3500/3500xL Genetic Analyzer for Human Identification	Contact your local Life Technologies sales representative.
Applied Biosystems® 3130/3130*xl* Genetic Analyzer	
GeneAmp® PCR System 9700 with the Silver 96-Well Block	N8050001
GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block	4314878
Silver 96-Well Sample Block	N8050251
Gold-plated Silver 96-Well Sample Block	4314443
Veriti® 96-well Thermal Cycler	4375786
Tabletop centrifuge with 96-Well Plate Adapters (optional)	MLS

Item	Cat. number
3500/3500xL Analyzer materials	
Anode buffer container (ABC)	4393927
Cathode buffer container (CBC)	4408256
POP-4® polymer (960 samples) for 3500/3500xL Genetic Analyzers	4393710
POP-4® polymer (384 samples) for 3500/3500xL Genetic Analyzers	4393715
Conditioning reagent	4393718
8-Capillary array, 36 cm for 3500 Genetic Analyzers	4404683
24-Capillary array, 36 cm for 3500xL Genetic Analyzers	4404687
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers	4410231
8-Strip Septa for 3500/3500xL Genetic Analyzers	4410701
96-Well Septa for 3500/3500xL Genetic Analyzers	4412614
Septa Cathode Buffer Container, 3500 series	4410715
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Note: For a complete list of parts and accessories for the 3500/3500xL instrument, refer to the *Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide* (Pub. no. 4401661)	

Item	Cat. number
3130/3130*xl* Analyzer materials	
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3130*xl* Genetic Analyzer Capillary Array, 36-cm	4315931
POP-4® Polymer for 3130/3130*xl* Genetic Analyzers	4352755
3130/3130*xl* Genetic Analyzer Autosampler Plate Kit, 96-well	4316471
GeneScan™ 500 LIZ® Size Standard	4322682
Or	*Or*
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Running Buffer, 10X	402824
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp® Optical 96-Well Reaction Plate	N8010560
For a complete list of parts and accessories for the 3130*xl* instrument, refer to Appendix A of the *Applied Biosystems® 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* (Pub. no. 352716).	
PCR Amplification	
MicroAmp® 96-Well Tray	N8010541
MicroAmp® Reaction Tube with Cap, 0.2-mL	N8010540
MicroAmp® 8-Tube Strip, 0.2-mL	N8010580
MicroAmp® 8-Cap Strip	N8010535
MicroAmp® 96-Well Tray/Retainer Set	403081
MicroAmp® 96-Well Base	N8010531
MicroAmp® Clear Adhesive Film	4306311
MicroAmp® Optical Adhesive Film	4311971
MicroAmp® Optical 96-Well Reaction Plate	N8010560
Other user-supplied materials	
Hi-Di™ Formamide, 25-mL	4311320
Aerosol resistant pipette tips	MLS
Microcentrifuge tubes	MLS
Pipettors	MLS
Tape, labeling	MLS
Tube, 50-mL Falcon	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Tris-HCL, pH 8.0	MLS
EDTA, 0.5 M	MLS
Vortex	MLS

C

PCR Work Areas

Work area setup and lab design

The sensitivity of the AuthentiFiler™ Kit (and other PCR-based tests) enables amplification of minute quantities of DNA, necessitating precautions to avoid contamination of samples yet to be amplified (Kwok and Higuchi, 1989).

Also take care while handling and processing samples to prevent contamination by human DNA. Wear gloves at all times and change them frequently. Close sample tubes when not in use. Limit aerosol dispersal by handling sample tubes and reagents carefully.

Note: We do not intend these references for laboratory design to constitute all precautions and care necessary for using PCR technology.

PCR setup work area

IMPORTANT! These items should never leave the PCR Setup Work Area.

- Calculator
- Gloves, disposable
- Marker pen, permanent
- Microcentrifuge
- Microcentrifuge tubes, 1.5-mL, or 2.0-mL, or other appropriate clean tube (for Master Mix preparation)
- Microcentrifuge tube rack
- Pipette tips, sterile, disposable hydrophobic filter-plugged
- Pipettors
- Tube decapper, autoclavable
- Vortex

Amplified DNA work area

IMPORTANT! Place the thermal cyclers in the Amplified DNA Work Area.

You can use the following systems:

- GeneAmp® PCR System 9700 with the Silver 96-Well Block
- GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block

  IMPORTANT! The AuthentiFiler™ Kit is not validated for use with the GeneAmp® PCR System 9700 with the Aluminium 96-Well Block. Use of this thermal cycling platform may adversely affect performance of the AuthentiFiler™ Kit.

- Veriti® 96-well Thermal Cycler

  IMPORTANT! The AuthentiFiler™ Kit is not validated for use with the Veriti® 96-Well Fast Thermal Cycler (Cat. no. 4375305). Use of this thermal cycling platform may adversely affect performance of the AuthentiFiler™ Kit.

D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Specific chemical handling

CAS	Chemical	Phrase
26628-22-8	Sodium Azide	Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Related documentation

The following related documents are shipped with the system:

Document	Pub. number
AuthentiFiler™ PCR Amplification Kit Quick Reference	4479556
Applied Biosystems® 3130/3130xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin	4363787
Applied Biosystems® 3130/3130xl Genetic Analyzers Getting Started Guide	4352715
Applied Biosystems® 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide	4352716
Applied Biosystems® *3130/3130xl Genetic Analyzers Quick Reference Card*	4362825
Applied Biosystems® 3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide	4359472
Applied Biosystems® 3130/3130xl DNA Analyzers User Guide	4331468
Applied Biosystems® 3730/3730xl Genetic Analyzer Getting Started Guide	4359476
Quantifiler® Kits: Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual	4344790
GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide	4338775
GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial	4335523
Installation Procedures and New Features for GeneMapper® ID Software v3.2 User Bulletin	4352543
GeneMapper® ID-X Software Version 1.0 Getting Started Guide	4375574
GeneMapper® ID-X Software Version 1.0 Quick Reference Guide	4375670
GeneMapper® ID-X Software Version 1.0 Reference Guide	4375671
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Getting Started Guide	4396773
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Quick Reference Guide	4402094
GeneMapper® ID-X Software Version 1.2 Quick Reference Guide	4426482
GeneMapper® ID-X Software Version 1.2 Reference Guide	4426481

Note: To open the user documentation, use the Adobe® Reader® software available from **www.adobe.com**

Note: For additional documentation, see "Obtaining support" on page 96.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For HID support:

- **In North America** – Send an email to **HIDTechSupport@lifetech.com**, or call **888-821-4443 option 1**.
- **Outside North America** – Go to **www.lifetechnologies.com/contactus.html** and select the appropriate country from the drop-down menu.

For the latest services and support information for all locations, go to:

www.lifetechnologies.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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