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AmpFℓSTR™ MiniFiler™ PCR Amplification Kit

USER GUIDE

for use with:
100 reaction kit

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For Forensic or Paternity Use Only.

ThermoFisher SCIENTIFIC

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F	August 2012	• Add 3500 and 3500xL Genetic Analyzer information. Add GeneMapper® ID-X Software information. • Add validation experiments and results for buffer and enzyme kit component changes.
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B	March 2007	Add Experiments and Results chapter.
A	October 2006	New document.

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About This Guide

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

Purpose

The *AmpFℓSTR™ MiniFiler™ PCR Amplification Kit User Guide* provides information about the Life Technologies instruments, chemistries, and software associated with the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit.

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Product overview

Purpose

The AmpFℓSTR™ MiniFiler™ PCR Amplification Kit is an assay optimized for genotyping degraded and/or inhibited DNA samples. It is a short tandem repeat (STR) multiplex assay optimized to allow direct amplification of single-source samples:

The MiniFiler™ Kit amplifies eight autosomal STR loci (D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO and FGA) and the sex-determining marker, Amelogenin, in a single PCR reaction. The loci span a range between 70 to 283 nucleotides with the aid of non-nucleotide linkers to achieve appropriate spacing between loci.

Product description

The MiniFiler™ Kit contains all the necessary reagents for the amplification of human genomic DNA.

The reagents are designed for use with the following instruments:

- Applied Biosystems™ 3100/3100-*Avant* Genetic Analyzer
- Applied Biosystems™ 3130/3130*xl* Genetic Analyzer
- Applied Biosystems™ 3500/3500xL Genetic Analyzer
- Veriti™ 96-Well Thermal Cycler (Part no. 4375786)
- GeneAmp™ PCR System 9700 with the Silver 96-Well Block
- GeneAmp™ PCR System 9700 with the Gold-plated Silver 96-Well Block

About the primers

The MiniFiler™ Kit uses primers closely flanking the STR repetitive regions (miniSTRs) of the DNA. This amplification results in amplicons that are significantly shorter in length than those produced in the AmpFℓSTR™ Identifiler™ and SGM Plus™ PCR Amplification Kits. The comparison is shown in Table 1 on page 12. Several laboratories confirm that MiniSTRs have a higher success rate for DNA analysis of degraded DNA samples (Butler *et al.*, 2003; Chung *et al.*, 2004; Coble and Butler, 2005; Drabek *et al.*, 2004; Grubwieser *et al.*, 2006; Wiegand *et al.*, 2001).

To prevent overlap of the miniSTR amplicons in the multiplex, non-nucleotide linkers are used in primer synthesis for the following loci: CSF1PO, FGA, D16S539, D18S51, Amelogenin, D2S1338, D21S11, and D7S820. For these primers, non-nucleotide linkers are placed between the primers and the fluorescent dye during oligonucleotide synthesis (Butler 2005, Grossman *et al.*, 1994, and Baron *et al.*, 1996). Non-nucleotide

linkers enable reproducible positioning of the alleles to facilitate inter-locus spacing. The combination of a five-dye fluorescent system and the inclusion of non-nucleotide linkers allows for simultaneous amplification and efficient separation of the eight STR loci and Amelogenin during automated DNA fragment analysis.

Locus	MiniFiler Kit Amplicon Length Reduction (nt)
D7S820	–129
D13S317	–99
D21S11	–33
D2S1338	–183
Amelogenin	0
D18S51	–168
D16S539	–157
FGA	–87
CSF1PO	–201

Loci amplified by the kit

Table 1 shows the loci amplified, their chromosomal locations, and the corresponding fluorescent marker dyes. The AmpFℓSTR™ MiniFiler™ Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder, and the genotype of the AmpFℓSTR™ Control DNA 007, are also listed in the table.

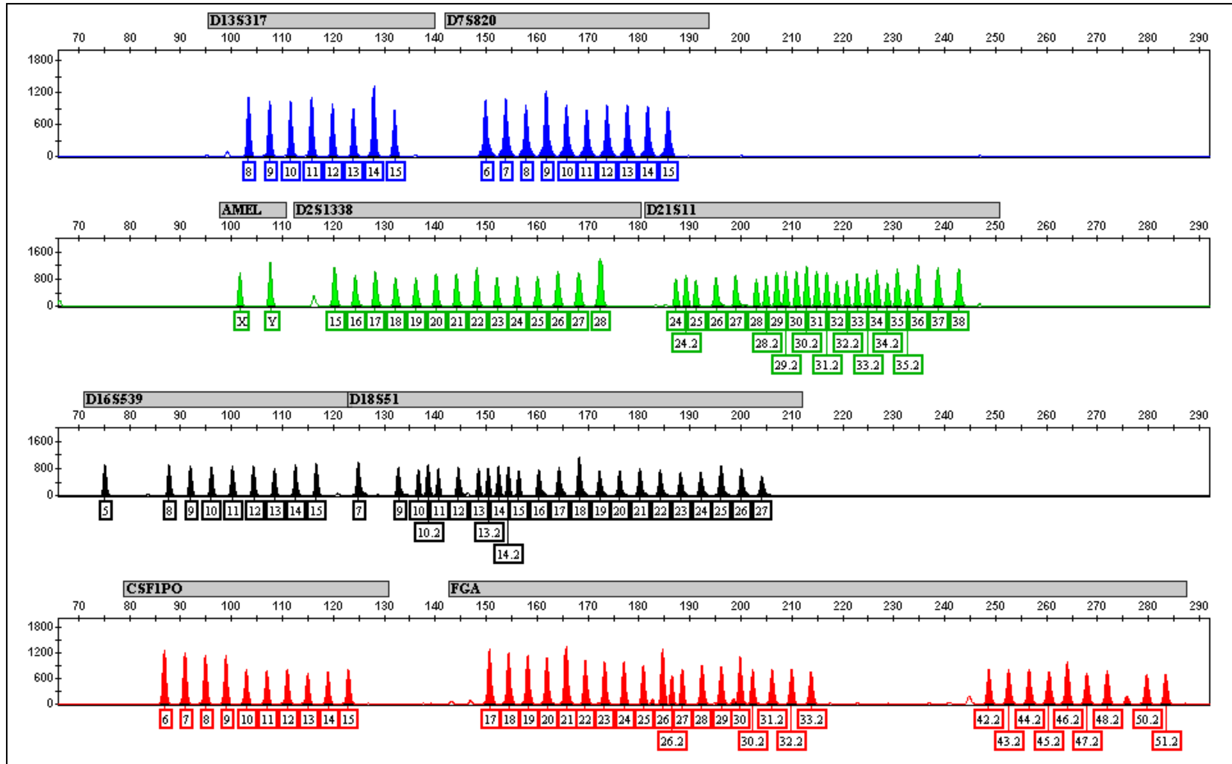
Table 1 AmpFℓSTR™ MiniFiler™ PCR Amplification Kit loci and alleles

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	Control DNA 007
D13S317	13q22-31	8, 9, 10, 11, 12, 13, 14, 15	6-FAM™	11
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		7, 12
Amelogenin	X:p22.1-22.3 Y:p11.2	X, Y	VIC™	X, Y
D2S1338	2q35-37.1	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		20, 23
D21S11	21q11.2-q21	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38		28, 31
D16S539	16q24-qter	5, 8, 9, 10, 11, 12, 13, 14, 15	NED™	9, 10
D18S51	18q21.3	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27		12, 15
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	PET™	11, 12
FGA	4q28	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2		24, 26

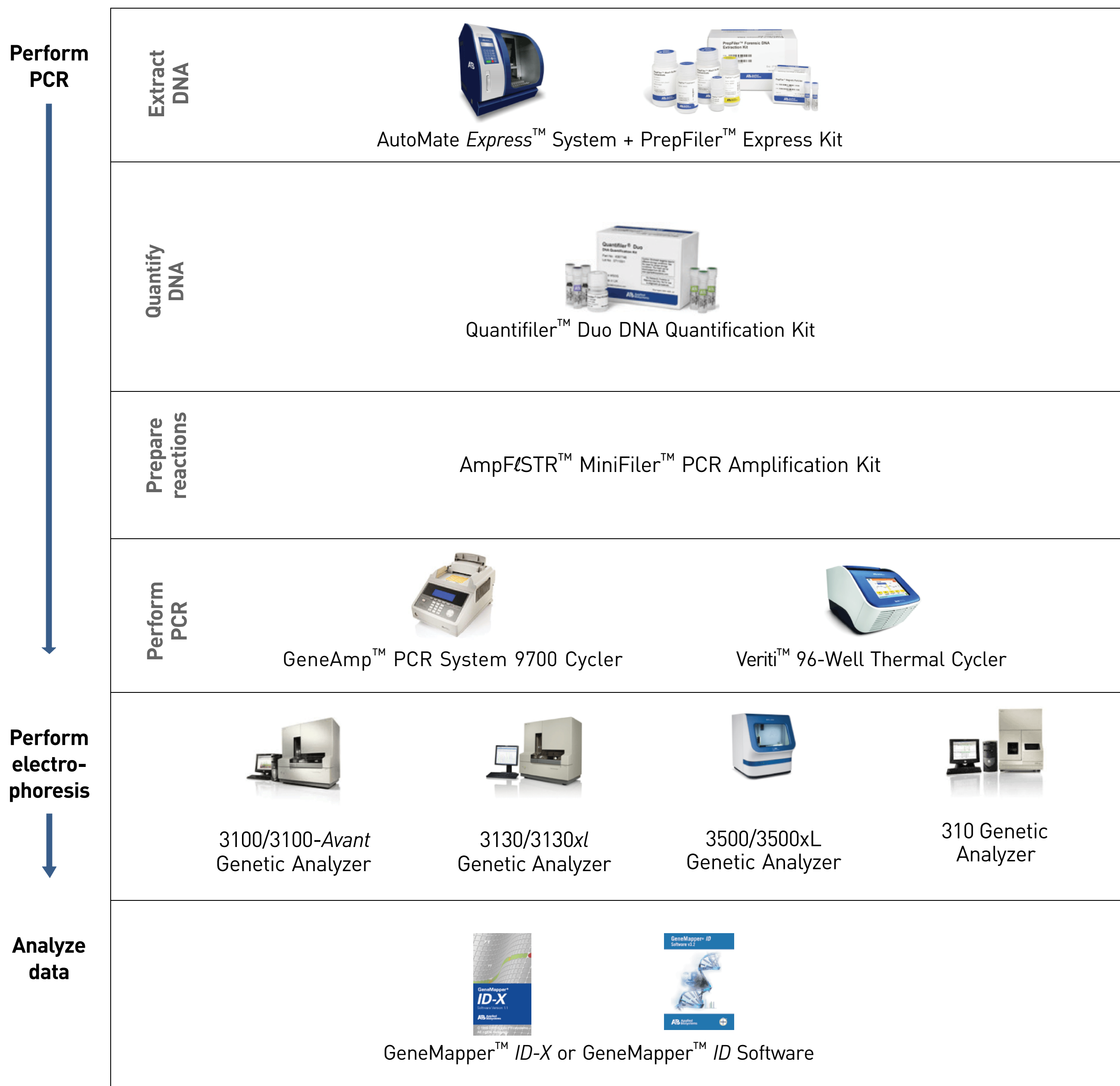
Allelic ladder

Figure 1 shows the allelic ladder for the MiniFiler™ Kit. See "Allelic ladder requirements" on page 26 for information on ensuring accurate genotyping.

Figure 1 GeneMapper™ *ID-X* Software plot of the AmpFℓSTR™ MiniFiler™ Allelic Ladder



Workflow overview



Instrument and software overview

This section provides information about the data collection and analysis software versions required to run the this kit on specific instruments.

Data collection and analysis 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 31xx instruments and .hid files for 3500 instruments), which is then analyzed by the analysis software.

Instrument and software compatibility

Instrument	Operating system	Data collection software	Analysis software
3100/3100-*Avant*	Windows™ NT	1.1 (3100) 1.0 (3100-*Avant*)	• GeneMapper™ *ID* Software v3.2.1 • GeneMapper™ *ID-X* Software v1.0.1 or higher
	Windows™ 2000	2.0	
3130/3130*xl*†	Windows XP	3.0	
3500/3500xL	• Windows™ XP *or* • Windows Vista™	3500 Series Data Collection Software v1.0	GeneMapper™ *ID-X* Software v1.2 or higher
310	Windows XP	3.1	GeneMapper *ID* 3.2
	Windows NT and Windows 2000	3.0	GeneMapper *ID* 3.2

† We conducted validation studies for the MiniFiler™ Kit using these configurations.

About multicomponent analysis

Life Technologies 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.

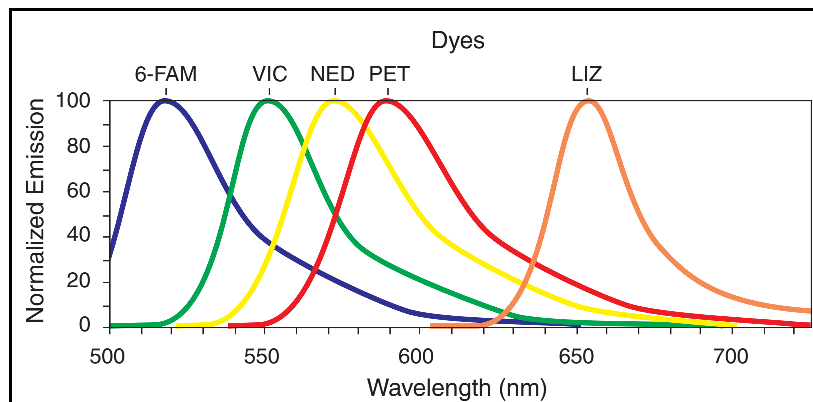
Multicomponent analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. The four dyes used in the MiniFiler™ Kit to label samples are 6-FAM™, VIC , NED™, and PET™ dyes. The fifth dye, LIZ™, is used to label the GeneScan™ 500 LIZ™ Size Standard or the 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 Life Technologies instruments, the fluorescence signals are separated by a 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 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 2). The goal of multicomponent analysis is to correct for spectral overlap.

Figure 2 Emission spectra of the five dyes used in the MiniFiler™ Kit



Materials and equipment

Kit contents and storage

The MiniFiler™ Kit (Part no. 4373872) contains sufficient quantities of the following reagents to perform 100 reactions at 25 µL/reaction:

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.

Table 2 Kit contents and storage

Component	Description	100 reaction	Storage
AmpFℓSTR™ MiniFiler™ Master Mix	Contains enzyme, salts, dNTPs, carrier protein, and 0.05% sodium azide	2 tubes, 0.5 mL/tube	−15 to −25°C upon receipt, 2 to 8°C after initial use
AmpFℓSTR™ Control DNA 007	Contains 0.10 ng/µL human male genomic DNA in 0.05% sodium azide and buffer.† See Table 1 on page 12 for profile.	1 tube, 0.3 mL	
AmpFℓSTR™ MiniFiler™ Primer Set	Contains forward and reverse primers to amplify human DNA targets.	1 tube, 0.5 mL	−15 to −25°C upon receipt. 2 to 8°C after initial use. Store protected from light.
AmpFℓSTR™ MiniFiler™ Allelic Ladder	Contains amplified alleles. See Table 1 on page 12 for a list of alleles included in the allelic ladder.	1 tube, 0.05 mL	

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

Standards for samples

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

- **AmpFℓSTR™ Control DNA 007 –** A positive control for evaluating the efficiency of the amplification step and STR genotyping using the AmpFℓSTR™ MiniFiler™ Allelic Ladder.
- **GeneScan™ 500 LIZ™ Size Standard or GeneScan™ 600 LIZ™ Size Standard v2.0 –** Used for obtaining sizing results. These standards, which have been evaluated as internal size standards, yield precise sizing results for MiniFiler™ Kit PCR products. Order the GeneScan™ 500 LIZ™ Size Standard (Part no. 4322682) or the GeneScan™ 600 LIZ™ Size Standard v2.0 (Part no. 4408399) separately.
- **AmpFℓSTR™ MiniFiler™ Allelic Ladder –** Developed for accurate characterization of the alleles amplified by the MiniFiler™ Kit. The Allelic Ladder contains most of the alleles reported for the 16 autosomal loci. See page 12 for a list of the alleles included in the Allelic Ladder.

2 Perform PCR

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Required user-supplied reagents

In addition to the Identifiler™ 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 # 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 accordingly for specific needs.
2. Aliquot and autoclave the solutions.
3. Store at room temperature.

DNA quantification

Importance of quantification

Quantifying the amount of DNA 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 Identifiler™ Kit is 1.0 ng in a maximum input volume of 10 µL for 28 PCR cycles.

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:
  - Quantitation (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.

Methods of quantifying DNA

Life Technologies provides several kits for quantifying DNA in samples. See the references cited in the following table for details about these kits.

Product	Description
Quantifiler™ Human DNA Quantification Kit (Part no. 4343895) *and* Quantifiler™ Y Human Male DNA Quantification Kit (Part no. 4343906) For more information, see *Quantifiler™ Human DNA Quantification Kits User's Manual* (Pub. no. 4344790)	**Properties:** The Quantifiler™ Human and Quantifiler™ Y Human Male Kits are highly specific for human DNA, and they individually detect total human or male DNA, respectively. The kits detect single-stranded and degraded DNA. **How they work:** The Quantifiler™ DNA Quantification Kits consist of target-specific and internal control 5' nuclease assays. The Quantifiler™ Human and Quantifiler™ Y Human Male Kits contain different target-specific assays (human DNA or human male DNA, respectively) that each consist of two locus-specific PCR primers and one TaqMan™ MGB probe labeled with FAM™ dye for detecting the amplified sequence. The kits each contain a separate internal PCR control (IPC) assay, which consists of an IPC template DNA (a synthetic sequence not found in nature), two primers for amplifying the IPC template, and one TaqMan™ MGB probe labeled with VIC™ dye for detecting the amplified IPC.
Quantifiler™ Duo DNA Quantification Kit (Part no. 4387746) For more information, see *Quantifiler™ Duo DNA Quantification Kit User's Manual* (Pub. no.4391294)	**Properties:** The Quantifiler™ Duo Kit is highly specific for human DNA. This kit combines the detection of both total human and male DNA in one PCR reaction.The kit detects single-stranded and degraded 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 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.

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
AmpFℓSTR™ MiniFiler™ Master Mix	10.0 µL
AmpFℓSTR™ MiniFiler™ Primer Set	5.0 µL

   Note: The volumes indicated above include a slight overfill to account for the loss that occurs during reagent transfers.

2. Prepare reagents. Thaw the PCR Reaction Mix and the Identifiler™ Primer Set, then vortex all reagent tubes, including the enzyme, for 3 seconds and centrifuge briefly before opening the tubes.

   IMPORTANT! Thawing is required only during first use of the Primer Set and PCR Reaction Mix. After first use, these reagents are stored at 2 to 8 °C and do not require subsequent thawing. Do not refreeze these reagents.

3. Prepare the reaction mix: Pipette 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 the 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 0.5 to 0.75 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	Combine 5 µL of control DNA (0.1 ng/µL) with 5 µL of low-TE buffer for a total volume of 10 µL. (The final sample concentration is 0.05 ng/µL.) Add to the reaction mix.

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

7. Seal the plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film, or cap the tubes.
8. Centrifuge the tubes at 3000 rpm for about 20 seconds in a tabletop centrifuge (with plate holders if using 96-well plates).
9. Amplify the samples in a GeneAmp™ PCR System 9700 with the silver or gold-plated silver 96-well blocks or a Veriti™ 96-Well Thermal Cycler.

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	Denature	Anneal	Extend	Final extension	Final hold
HOLD	CYCLE (30)			HOLD	HOLD
95°C 11 min	94°C 20 sec	59°C 2 min	72°C 1 min	60°C 45 min	4°C ∞

2. Load the plate 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 (Part no. 4312639) on top of the plate to prevent evaporation during thermal cycling. The Veriti™ Thermal Cycler does not require a compression pad.

3. Start the run.

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

If you are storing the DNA...	Then place at...
< 2 weeks	2 to 8°C
> 2 weeks	–15 to –25 °C

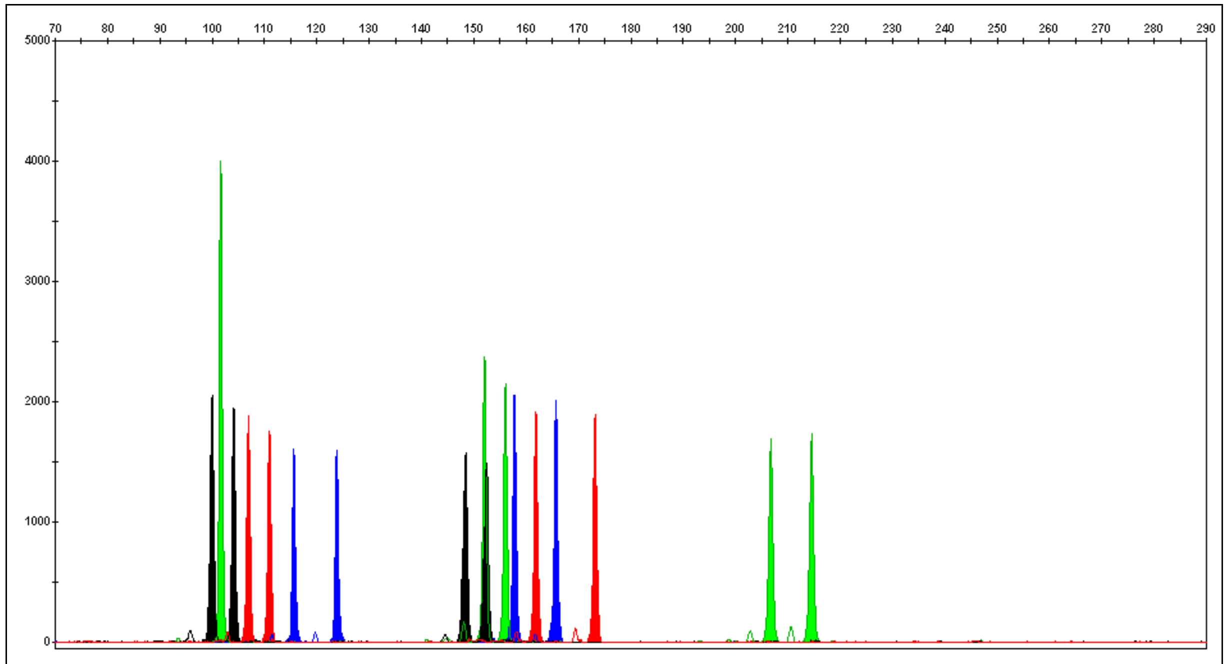
   IMPORTANT! Store the amplified products so that they are protected from light.

Amplification using bloodstained FTA™ cards

FTA™ cards can be useful for collecting, storing, and processing biological samples. A small punch disc of the card containing the sample can be placed directly into an amplification tube, purified, and amplified, without transferring the disc. Our studies indicate that a 1.2-mm bloodstained disc contains approximately 5 to 20 ng DNA. An appropriate cycle number for this high quantity of DNA is 24 cycles as determined by our validation studies. However, it is recommended that each laboratory determine the optimum cycle number based on internal validation studies.

In the example shown in Figure 3, a 1.2-mm disc of a bloodstained FTA™ card was purified using three washes with FTA™ Purification Reagent and two washes with 1X low-TE buffer. The purified punch disc was then amplified in the MicroAmp™ tube for 24 cycles.

Figure 3 MiniFiler™ Kit results from a 1.2-mm FTA™ bloodstain disc (24-cycle amplification), analyzed on the Applied Biosystems 3130*xl* Genetic Analyzer



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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)
3100-*Avant* or 3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3100 or 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 3100/3100-*Avant* and 3130/3130*xl* instruments

Set up the 3100/3100-*Avant* and 3130/3130*xl* instruments for electrophoresis

Reagents and parts

Appendix C, "Ordering Information" on page 117 lists the required materials not supplied with this 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 PCR products generated by this kit. 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™ 3100-*Avant*	1.0	Windows™ NT	• GeneScan36Avb_DyeSetG5Module Injection condition: 3 kV/5sec • GS600v2.0Analysis.gsp	*3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR™ PCR Amplification Kit PCR Products User Bulletin* (Part no. 4332345)
Applied Biosystems™ 3100	2.0	Windows™ 2000	• HIDFragmentAnalysis36_POP4_1 Injection condition: 3kV/10 sec • Dye Set G5	*3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0, Protocols for Processing AmpFℓSTR™ PCR Amplification Kit PCR Products User Bulletin* (Part no. 4350218)
	1.1	Windows™ NT	• GeneScan36vb_DyeSetG5Module Injection condition: 3kV/10 sec • GS600v2.0Analysis.gsp	*3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR™ PCR Amplification Kit PCR Products User Bulletin* (Part no. 4332345)
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* (Part no. 4363787)

† We conducted validation studies for the MiniFiler™ Kit using the 3130*xl* configuration.

Prepare samples for electrophoresis on the 3100/3100-*Avant* or 3130/3130*xl* instruments

Prepare the samples for electrophoresis immediately before loading.

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

Reagent	Volume per reaction
GeneScan™ 500 LIZ™ Size Standard	0.3 µL
Hi-Di™ Formamide	8.7 µL

OR

Reagent	Volume per reaction
GeneScan™ 600 LIZ™ Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	8.5 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:
   - 9 µL of the formamide:size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   Note: For blank wells, add 10 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.

Section 3.2 3500/3500xL instruments

Set up the 3500/3500xL instruments for electrophoresis

Reagents and parts

Appendix C, "Ordering Information" on page 117 lists the required materials not supplied with this 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 you can use to analyze PCR products generated by this kit. 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	1.0	Windows™ XP *or* Windows Vista ™	• HID36_POP4 Injection conditions: 1.2kV/15 sec • Dye Set G5	Applied Biosystems™ *3500/ 3500xL Genetic Analyzer User Guide* (Part no. 4401661) *3500 and 3500xL Genetic Analyzers Quick Reference Card* (Part no. 4401662)
Applied Biosystems™ 3500xL			• HID36_POP4 Injection conditions: 1.2kV/24 sec • Dye Set G5	

Prepare samples for electrophoresis on the 3500/3500xL instruments

Prepare the samples for electrophoresis immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and GeneScan™ 600 LIZ™ Size Standard v2.0 needed to prepare the samples:

Reagent	Volume per reaction
GeneScan™ 600 LIZ™ Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	8.5 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:
   - 9 µL of the formamide:size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   Note: For blank wells, add 10 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 plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Place the sample tray on the autosampler.
9. Start the electrophoresis run.

Section 3.3 310 Instrument

Set up the 310 instrument for electrophoresis

Reagents and parts

"Ordering Information" on page 117 lists the required materials not supplied with the AmpFℓSTR™ Identifiler™ PCR Amplification 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 Identifiler™ Kit PCR products. For details on the procedures, refer to the documents listed in the table.

Data Collection Software	Operating System	Run modules and conditions	References
3.1† *or* 3.0	Windows XP *or* Windows™ NT and Windows 2000	• GS STR POP4 (1mL) G5 v2.md5 Injection condition: 15 kV/5 sec	*310 Genetic Analyzer User's Manual (Windows)* (Pub. no. 4317588) *310 Protocols for Processing* AmpFℓSTR™ *PCR Amplification Kit Products with Microsoft Windows NT Operating System: User Bulletin* (Pub. no. 4341742)

† We conducted concordance studies for the Identifiler™ Kit using this configuration.

Prepare samples for electrophoresis on the 310 instrument

Prepare the samples for electrophoresis immediately before loading.

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

Reagent	Volume per reaction
GeneScan™ 500 LIZ™ Size Standard or GeneScan™ 600 LIZ™ Size Standard	0.5 µL
Hi-Di™ Formamide	24.5 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. Pipette the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each 0.2 mL sample tube, add:
   - 25 µL of the formamide:size standard mixture
   - 1.5 µL of PCR product or allelic ladder

   Note: For blank wells, add 25 µL of Hi-Di™ Formamide.
5. Seal the tubes with the appropriate septa, then briefly centrifuge to ensure that the contents of each tube are mixed and collected at the bottom.
6. Heat the tubes in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the tubes on ice for 3 minutes.
8. Place the sample tray on the autosampler.
9. Ensure that an injection list is prepared.
10. Start the electrophoresis run.

4 Analyze Data

- Section 4.1 GeneMapper™ ID Software 33
  - Overview of GeneMapper™ ID Software 33
  - Set up GeneMapper™ ID Software for data analysis 34
  - Analyze and edit sample files with GeneMapper™ ID Software 44
  - Examine and edit a project 45
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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 software, you can then analyze and interpret the data from the .fsa files.

Instruments

Refer to "Instrument and software overview" on page 16 for a list of compatible instruments.

Before you start

When using GeneMapper™ *ID* Software v3.2.1 to perform human identification (HID) analysis with AmpFℓSTR™ 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 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'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/support** ▸ **Software, Patches & Updates** ▸ GeneMapper™ *ID* Software.

Before using the software for the first time

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

- Import panels and bins into the Panel Manager, as explained in "Import panels and bins" on page 34.
- Create an analysis method, as explained in "Create an analysis method" on page 38.
- Create a size standard, as explained in "Create a size standard" on page 43.
- Define custom views of analysis tables.
  Refer to the *GeneMapper™ ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Part no. 4335523) for more information.
- Define custom views of plots.
  Refer to the *GeneMapper™ ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Part no. 4335523) for more information.

Import panels and bins

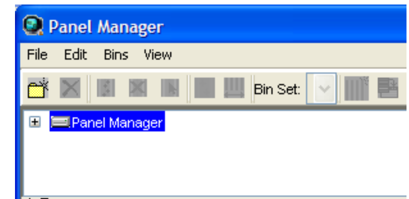
To import the latest panel and bin set from the our web site into the GeneMapper™ *ID* Software v3.2.1 database:

1. Download and open the file containing panels and bins:
   a. Go to **www.lifetechnologies.com/support** ▸ **Software, Patches & Updates** ▸ **GeneMapper™ *ID* Software**. Download the file **GMID_MiniFiler_files**.
   b. Unzip the file.

2. Start the GeneMapper™ *ID* Software, then log in with the appropriate user name and password.

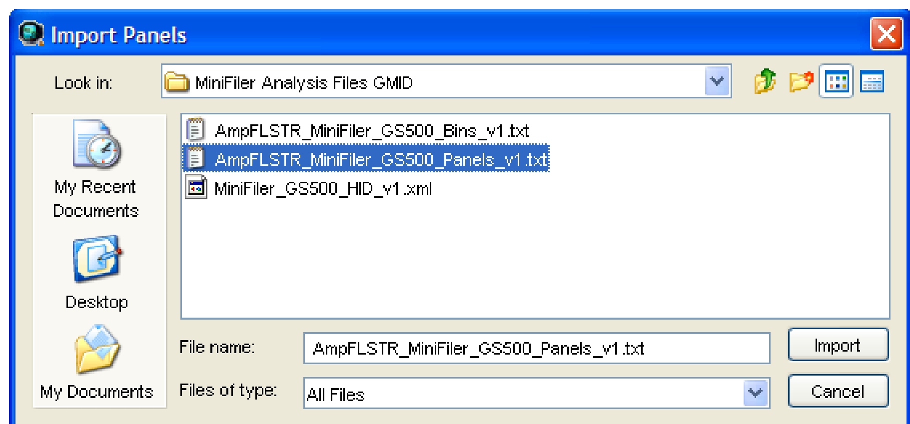
IMPORTANT! For logon instructions, refer to the *GeneMapper™ ID Software Version 3.1 Human Identification Analysis User Guide* (Part no. 4338775).

3. Select **Tools ▸ Panel Manager**.
4. Find, then open the folder containing the panels and bins:
   a. Select **Panel Manager** in the navigation pane.

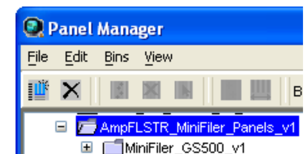


   b. Select **File ▸ Import Panels** to open the Import Panels dialog box.
   c. Navigate to, then open the **MiniFiler Analysis Files GMID** folder that you unzipped in step 1 on page 34.
5. Select **AmpFLSTR_MiniFiler_GS500_Panels_v1.txt**, then click **Import**.

Note: Importing this file creates a new folder in the navigation pane of the Panel Manager, AmpFlSTR_MiniFiler_GS500_Panels_v1. This folder contains the panel and associated markers.



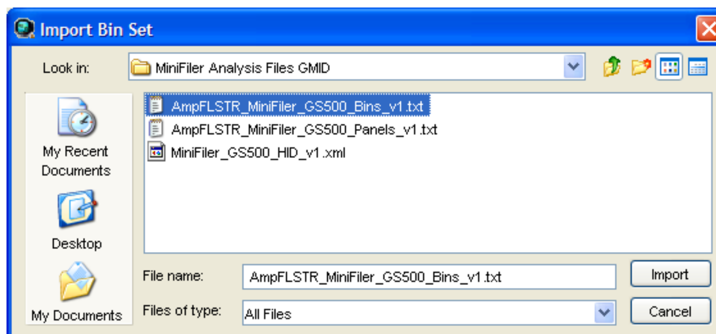
6. Import the bins file:



   a. Select the **AmpFlSTR_MiniFiler_GS500_Panels_v1** 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 **MiniFiler Analysis Files GMID** folder.

d. Select **AmpFLSTR_MiniFiler_GS500_Bins_v1.txt**, then click **Import**.

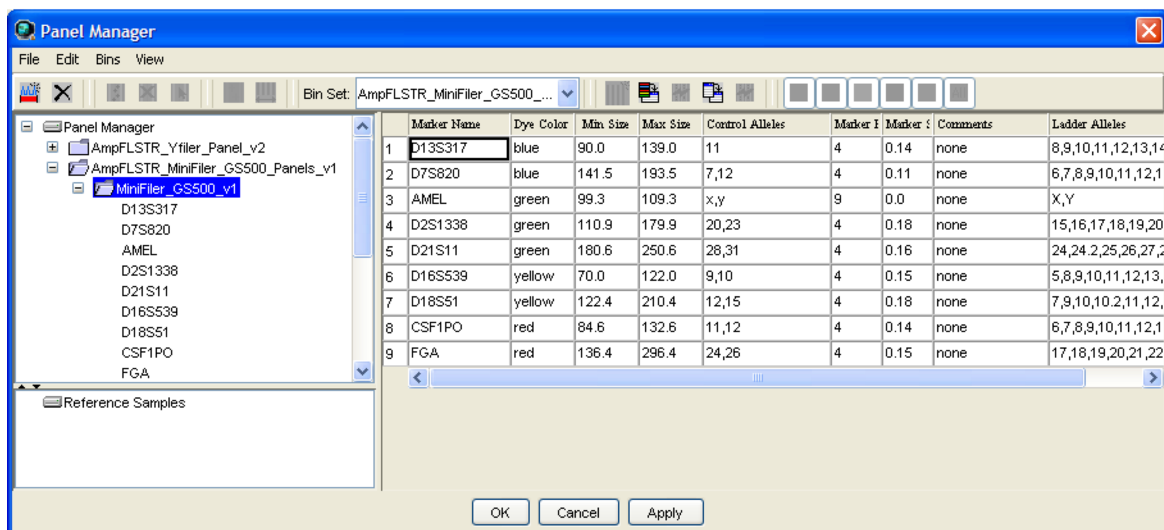
Note: Importing this file associates the bin set with the panels in the AmpFlSTR_MiniFiler_GS500_Panels_v1 folder.



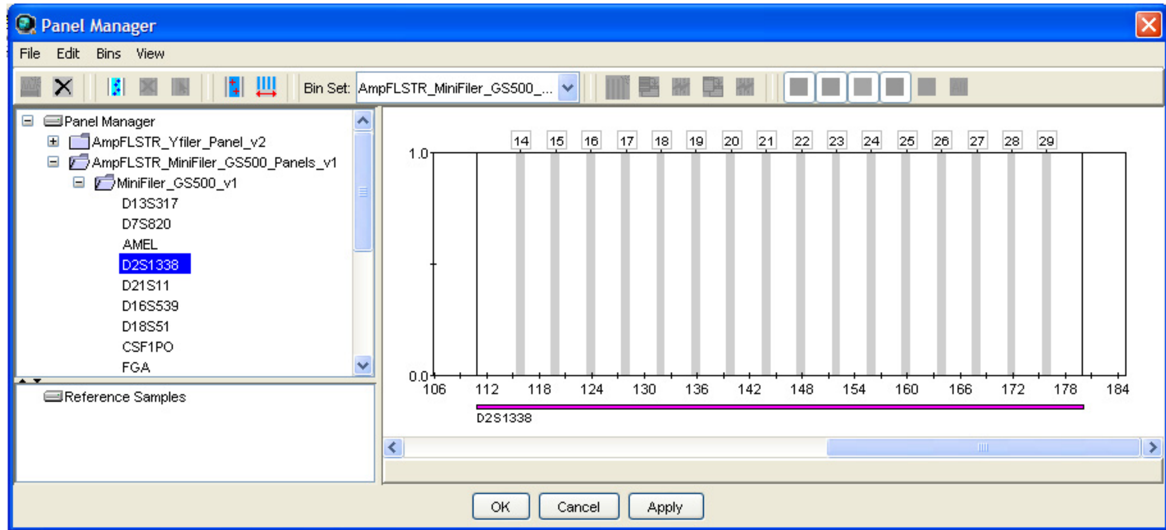
7. View the imported panels in the navigation pane:

   a. Double-click the **AmpFlSTR_MiniFiler_GS500_Panels_v1** folder to view the MiniFiler_GS500_v1 folder.

   b. Double-click the **MiniFiler_GS500_v1** folder to display the panel information in the right pane.



8. Select **D2S1338** to display the Bin view for the marker in the right pane.



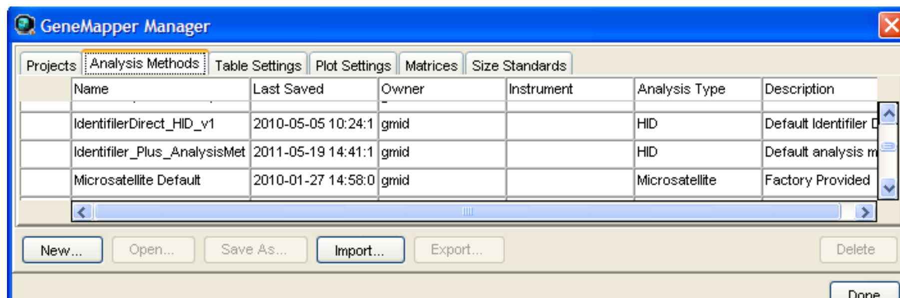
9. Click **Apply**, then **OK** to add the panel and bin set to the GeneMapper™ *ID* Software database.

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 analysis method.

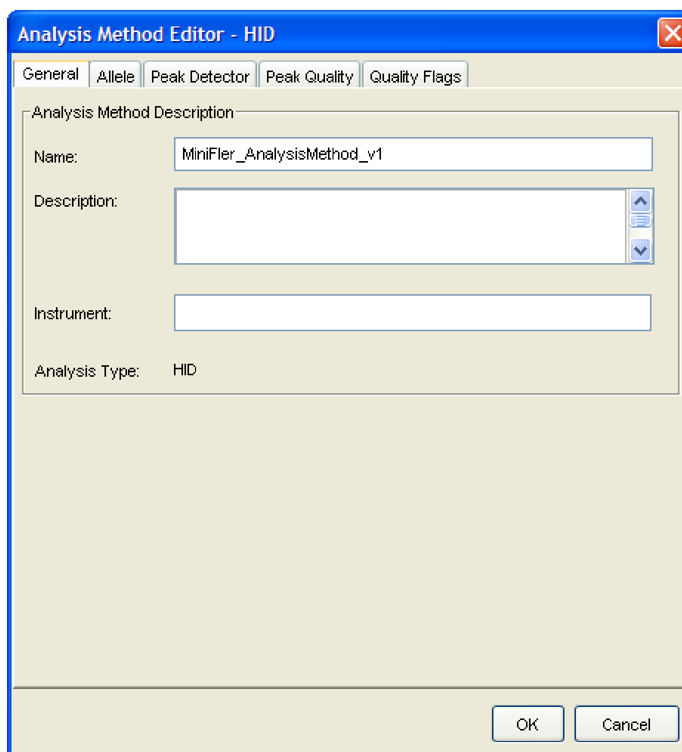
1. Select **Tools ▸ GeneMapper Manager** to open the GeneMapper Manager.



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

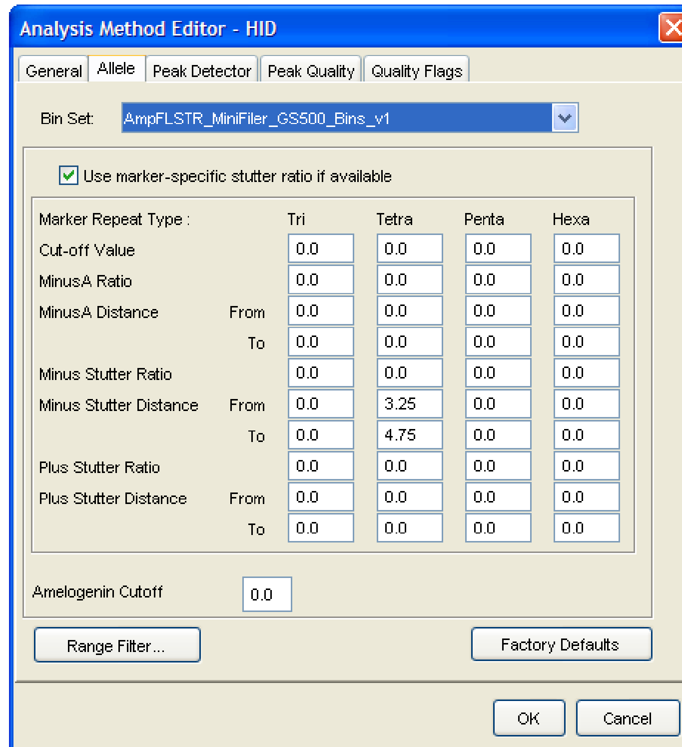
   Note: The Analysis Method Editor closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.
5. After you enter settings in all tabs, click **Save**.

General tab settings



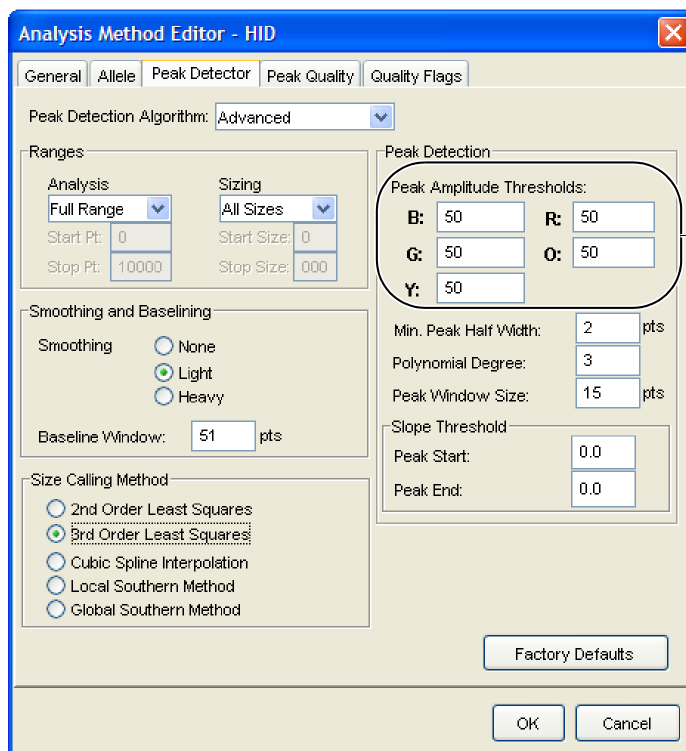
In the Name field, either type the name as shown for consistency with files supplied with other AmpFℓSTR™ kits, or enter a name of your choosing. The Description and Instrument fields are optional.

Allele tab settings



- In the Bin Set field, select the **AmpFLSTR_MiniFiler_Bins_v1** 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.
- Specify the peak filter ratios:
  - To apply the stutter ratios listed in the Allele tab for single-source data, deselect the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.
    Note: Applying global stutter ratios may reduce the editing required for single-source sample data.
  - To apply the stutter ratios contained in the MiniFiler_GS500_v1 file, select the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.
- To specify an Amelogenin Cutoff Ratio, enter the appropriate ratio into the Amelogenin Cutoff field.
  Note: Do not use an Amelogenin cutoff for data that may contain mixtures of male and female DNA

Peak Detector tab settings



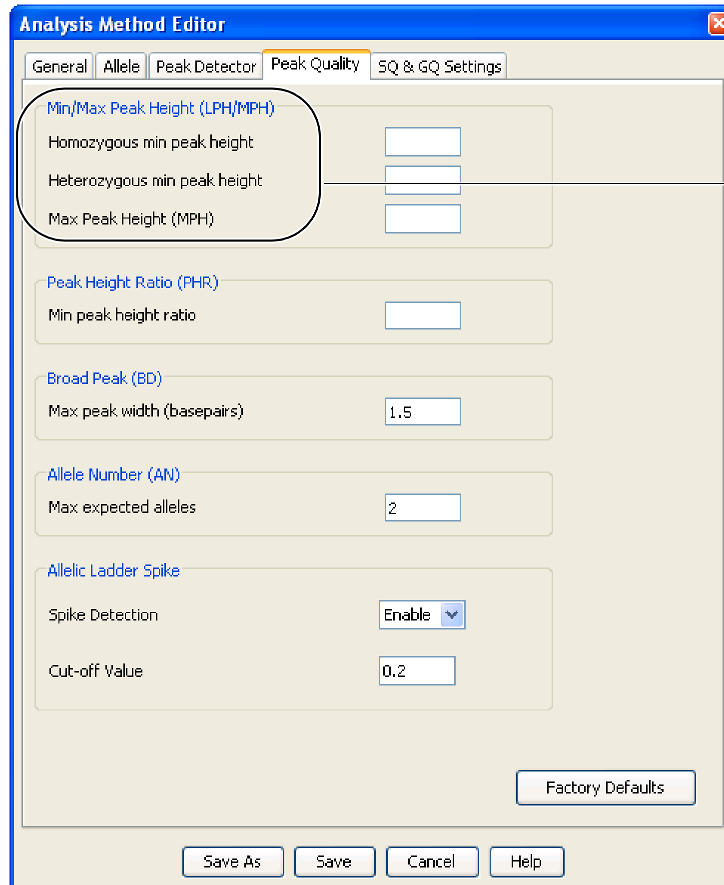
Perform internal validation studies to determine settings

IMPORTANT! Perform the appropriate internal validation studies to determine the peak amplitude thresholds for interpretation of 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** – This kit has been validated using the Local Southern sizing method. Before using alternative sizing methods, perform the appropriate internal validation studies.

Peak Quality tab settings



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

Quality Flags tab settings

Analysis Method Editor - HID

General | Allele | Peak Detector | Peak Quality | Quality Flags

Quality weights are between 0 and 1.

Quality Flag Settings

Spectral Pull-up	0.8	Control Concordance	1.0
Broad Peak	0.8	Low Peak Height	0.3
Out of Bin Allele	0.8	Off-scale	0.8
Overlap	0.8	Peak Height Ratio	0.3

PQV Thresholds

	Pass Range:	Low Quality Range:
Sizing Quality:	From 0.75 to 1.0	From 0.0 to 0.25
Genotype Quality:	From 0.75 to 1.0	From 0.0 to 0.25

Factory Defaults

OK Cancel

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.

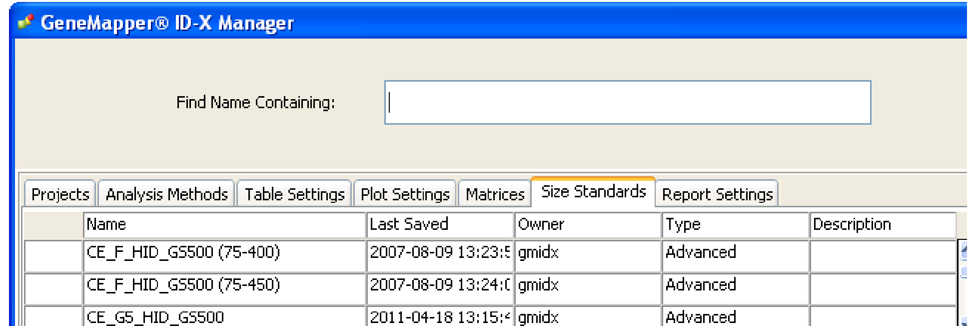
Create a size standard

The GeneScan™ 600 LIZ™ Size Standard v2.0 contains the following size standard peaks:

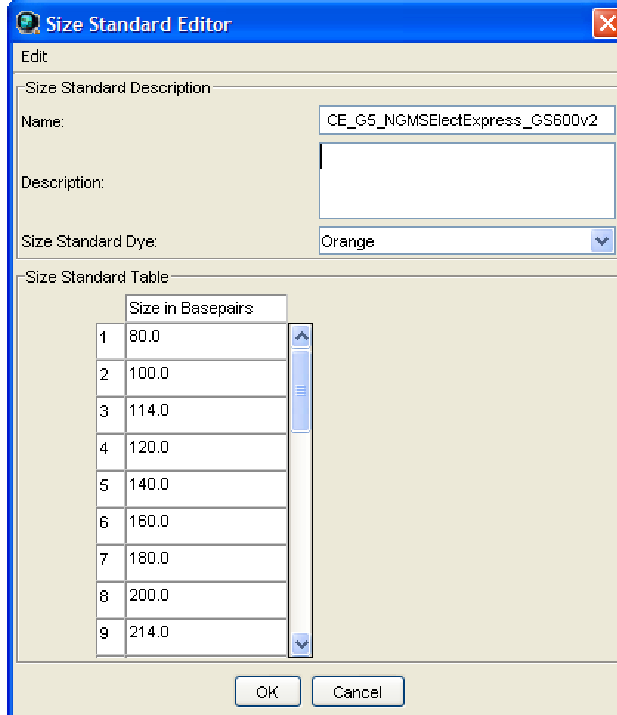
GeneScan™ 600 LIZ™ Size Standard v2.0 peak sizes
60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440 and 460

To create the size standard definition file:

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 above.

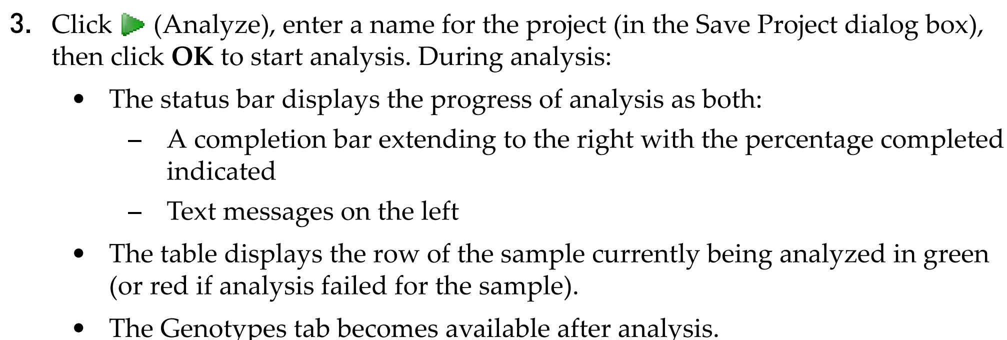


Analyze and edit sample files with GeneMapper™ *ID* Software

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. If you named the settings differently, select the names you specified.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	MiniFiler_AnalysisMethod_v1 (or the name of the analysis method you created)
Panel	MiniFiler_GS500_v1
Size Standard	CE_G5_HID_GS500 (or the name of the size standard you created)

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



3. Click ▶ (Analyze), enter a name for the project (in the Save Project dialog box), then click **OK** to start analysis. During analysis:
   - The status bar displays the progress of analysis as both:
     - A completion bar extending to the right with the percentage completed indicated
     - 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.

The following figure shows the analysis summary window after analysis.

GeneMapper ID v3.2.1 - *Untitled - gmid Is Logged In

File Edit Analysis View Tools Help

Table Setting: AmpFLSTR Table

Project
MiniFiler Example

Samples Genotypes

	Status	Sample File	Sample Type	Analysis Method	Panel	Size Standard	Instrument
1		Allelic Ladder.fsa	Allelic Ladder	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
2		Sample 1.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
3		Sample 10.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
4		Sample 11.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
5		Sample 12.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
6		Sample 13.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
7		Sample 14.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
8		Sample 15.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
9		Sample 16.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
10		Sample 17.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
11		Sample 18.fsa	Positive Control	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
12		Sample 19.fsa	Negative Contrc	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
13		Sample 2.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
14		Sample 3.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
15		Sample 4.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
16		Sample 5.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
17		Sample 6.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
18		Sample 7.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
19		Sample 8.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130
20		Sample 9.fsa	Sample	MiniFiler_AnalysisMetho	MiniFiler_GS500_v1	CE_G5_HID_GS500 (75-450)	ABI3130

Progress Status ... 0% Stop

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 (Part no. 4335523)*
- *GeneMapper™ ID Software Version 3.1 Human Identification Analysis User Guide (Part no. 4338775)*
- *Installation Procedures and New Features for GeneMapper™ ID Software Software Version v3.2 User Bulletin* (Part 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 a .fsa or .hid 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.

Instruments

Refer to "Instrument and software overview" on page 16 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 AmpFℓSTR™ kits, be aware that:

- HID analysis requires at least one allelic ladder sample per run folder. Your laboratory can use multiple ladder samples in an analysis, provided individual laboratories conduct the appropriate validation studies.
  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 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, the sample result needs to be verified according to the laboratory's protocol.

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

Panel, bin, and stutter file version

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/support** ▸ **Software, Patches & Updates** ▸ GeneMapper™ *ID-X* Software.

The instructions and examples in this section refer to the latest version of panel, bin, and stutter file available at the time of publication.

Before using the software for the first time

Before you use GeneMapper™ *ID-X* Software (v1.0.1 or higher for .fsa files, v1.2 or higher for .hid files) to analyze data for the first time, you must do the following:

1. Check the version of panel, bin, and stutter files installed with the GeneMapper™ *ID-X* Software as explained in "Check panel, bin, and stutter file version" below.
2. Check **www.lifetechnologies.com/support** ▸ **Software, Patches & Updates** ▸ GeneMapper™ *ID-X* Software to determine if newer files are available.
3. If updated files are available, download and import the files into the GeneMapper™ *ID-X* Software, as explained in "Import panels, bins, and marker stutter" on page 48.
   Note: When downloading new versions of analysis files, refer to the associated Read Me file for details of changes between software file versions. If you have validated previous file versions for data analysis, conduct the appropriate internal verification studies before using new file versions for operational analysis.
4. Create an analysis method, as explained in "Create an analysis method" on page 53.
5. Define custom views of analysis tables.
   Refer to Chapter 1 of the *GeneMapper™ ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574) for more information.
6. Define custom views of plots.
   Refer to Chapter 1 of the *GeneMapper™ ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574) for more information.

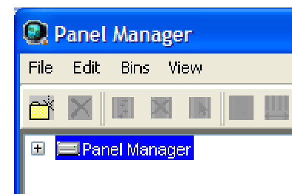
Check panel, bin, and stutter file version

1. 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).

2. Select **Tools** ▸ **Panel Manager**.

3. Check the version of files imported into the Panel Manager:



   a. Select **Panel Manager** in the navigation pane.
   b. Expand the Panel Manager folder and any sub-folders to identify the analysis file version already installed for your kit choice.
4. Check the version of files available for import into the Panel Manager:
   a. Select **Panel Manager**, then select **File ▸ Import Panels** to open the Import Panels dialog box.
   b. Navigate to, then open the Panels folder and check the version of panel, bin, and stutter files installed.
5. If newer versions are available on the website, download and import as described below.

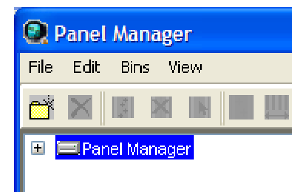
Import panels, bins, and marker stutter

To import the MiniFiler™ Kit panel, bin set, and marker stutter from our web site into the GeneMapper™ *ID-X* Software database:

1. Download and open the file containing panels, bins, and marker stutter:
   a. Go to **www.lifetechnologies.com/support ▸ Software, Patches & Updates ▸ GeneMapper™ *ID-X* Software**. Download the file AmpFLSTR 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).

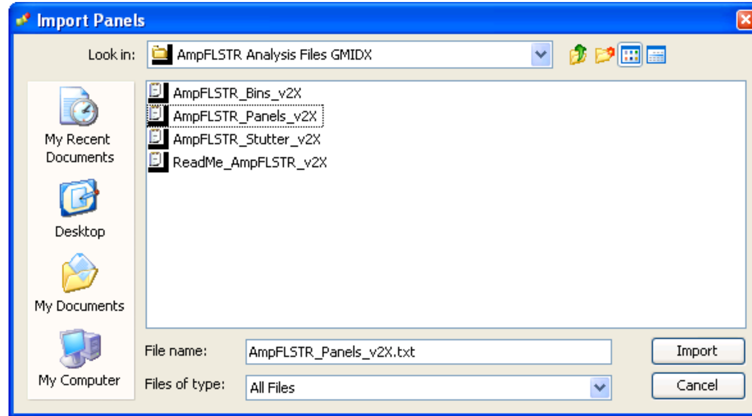
3. Select **Tools ▸ Panel Manager**.
4. Find, then open the folder containing the panels, bins, and marker stutter:
   a. Select **Panel Manager** in the navigation pane.



   b. Select **File ▸ Import Panels** to open the Import Panels dialog box.
   c. Navigate to, then open the **AmpFLSTR Analysis Files GMIDX** folder that you unzipped in step 1 on page 48.

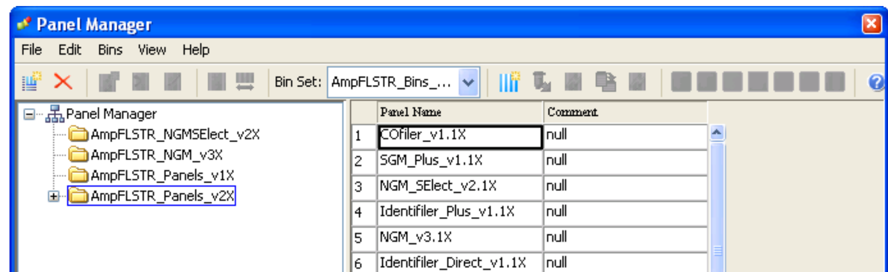
5. Select **AmpFLSTR_Panels_v2X** (or the version you installed), then click **Import**.

   Note: Importing this file creates a new folder in the navigation pane of the Panel Manager "AmpFLSTR_Panels_v2X". This folder contains panels for multiple AmpFℓSTR™ kits and associated markers.



6. Import AmpFLSTR_Bins_v2X.txt:

   a. Select the **AmpFLSTR_Panels_v2X** folder in the navigation pane.

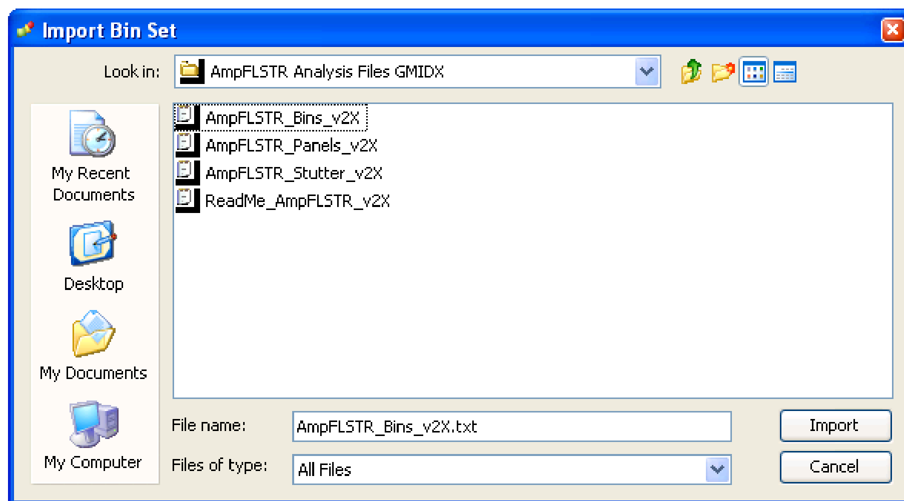


   b. Select **File** ▸ **Import Bin Set** to open the Import Bin Set dialog box.

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

d. Select **AmpFLSTR_Bins_v2X.txt**, then click **Import**.

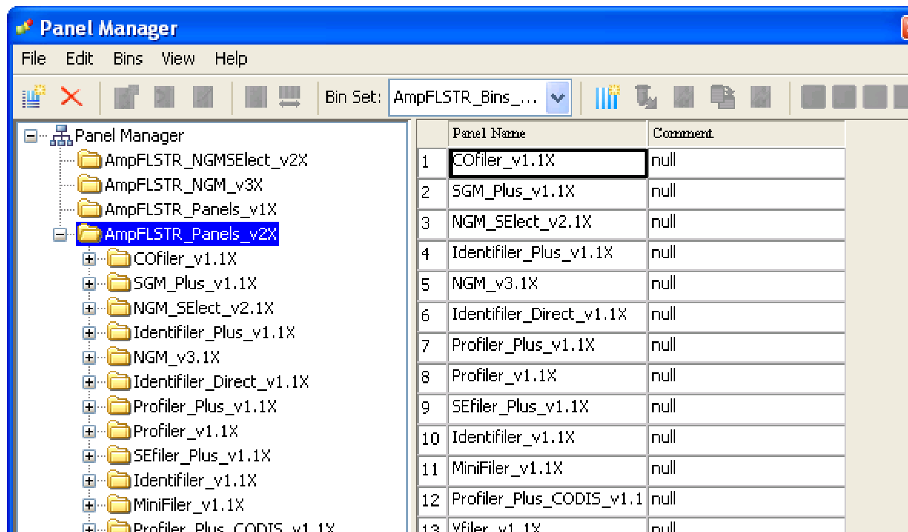
Note: Importing this file associates the bin set with the panels in the AmpFLSTR_Panels_v2X folder.



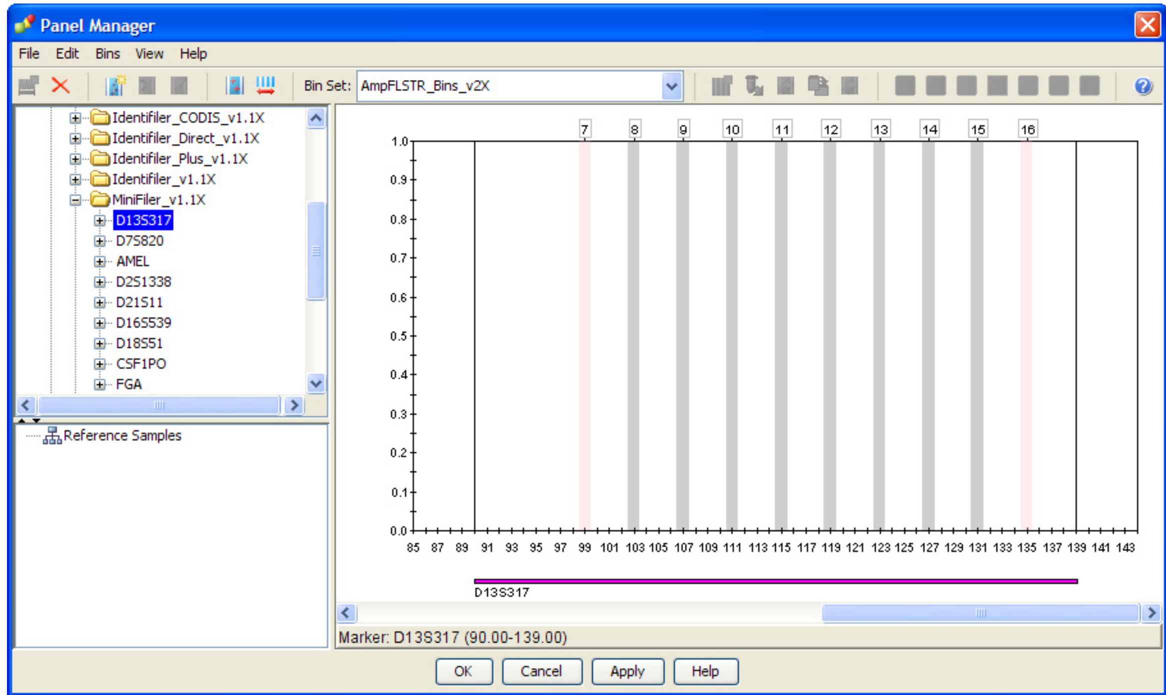
7. View the imported panels in the navigation pane:

a. Double-click the **AmpFLSTR_Panels_v2X** folder.

b. Double-click the **MiniFiler_v1.1X** folder to display the panel information in the right pane.

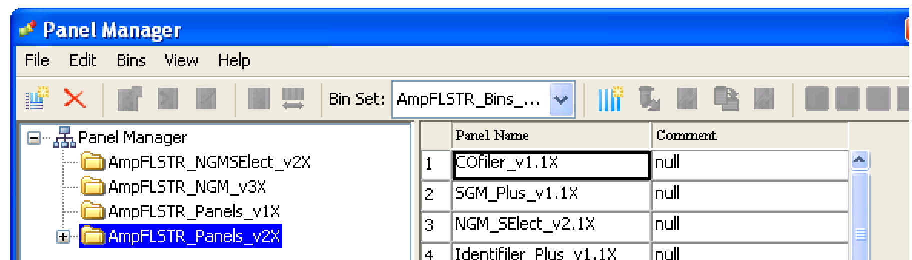


8. Select and expand **MiniFiler_v1.1X** in the navigation pane, then select **D13S317** to display the Bin view for the marker in the right pane.



9. Import AmpFLSTR_Stutter_v2X:

   a. Select the **AmpFLSTR_Panels_v2X** folder in the navigation panel.

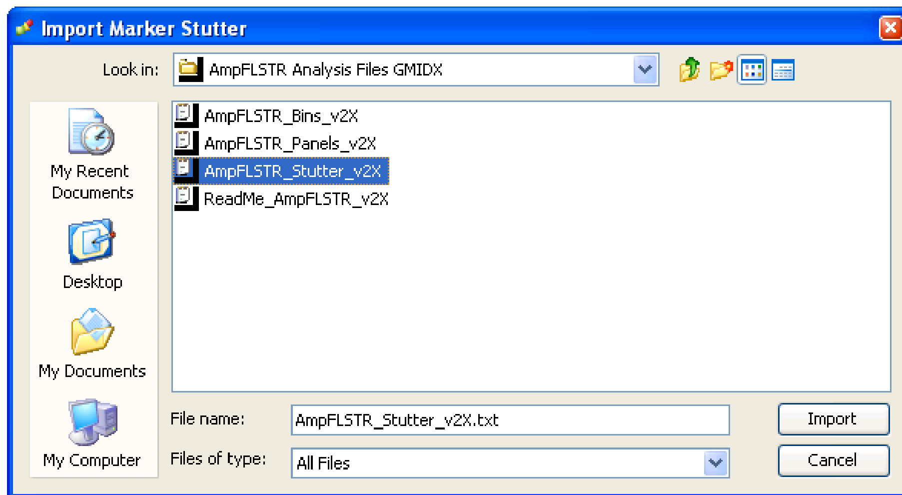


   b. Select File ▸ **Import Marker Stutter** to open the Import Marker Stutter dialog box.

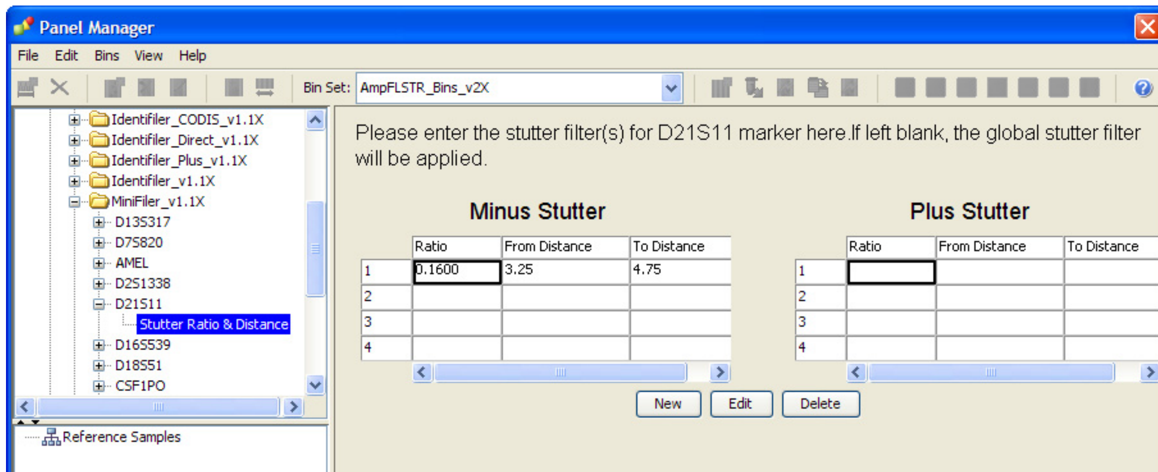
   c. Navigate to, then open the **AmpFLSTR Analysis Files GMIDX** folder.

d. Select **AmpFLSTR_Stutter_v2X**, then click **Import**.

Note: Importing this file associates the marker stutter ratio with the bin set in the AmpFLSTR_Panels_v2X folder.



10. View the imported marker stutters in the navigation pane:
    a. Double-click the **AmpFLSTR_Panels_v2X** folder to display its list of kits in the right pane.
    b. Double-click the **MiniFiler_v1.1X** folder to display its list of markers below it.
    c. Double-click **D21S11** to display the Stutter Ratio & Distance view for the marker in the right pane.



11. Click **Apply**, then **OK** to add the MiniFiler™ Kit panel, bin set, 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.

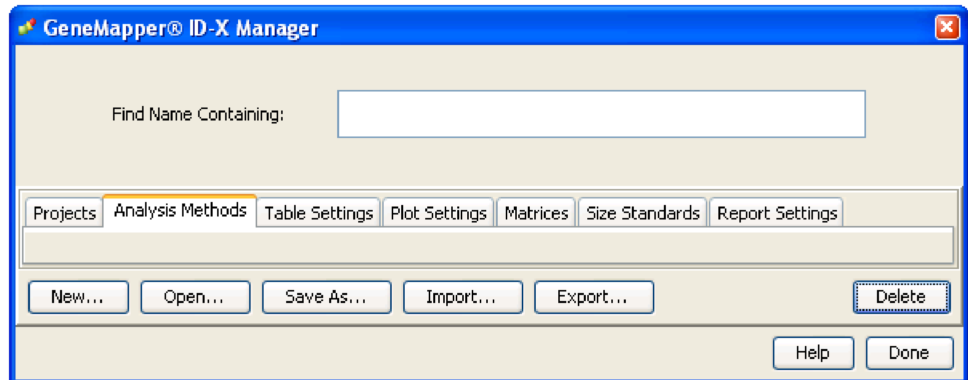
Create an analysis method

Use the following procedure to create an analysis method for the SGM Plus™ 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* 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.



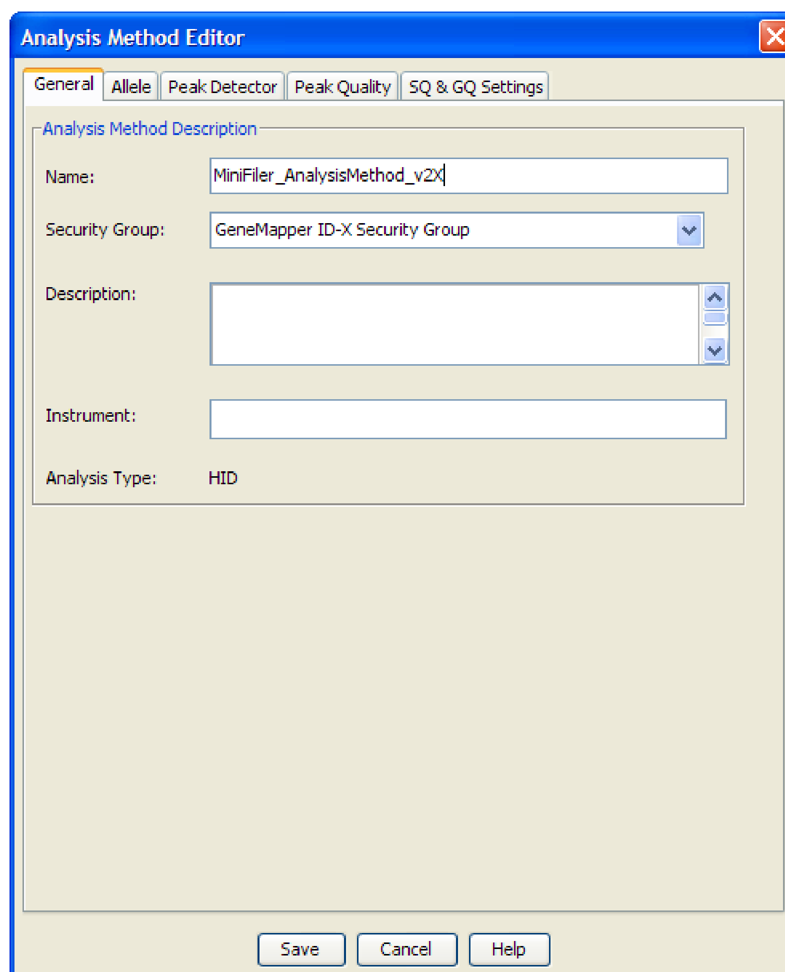
2. Select the **Analysis Methods** tab, then click **New** to open the Analysis Method Editor with the **General** tab selected.

   The figures below show the settings for each tab of the Analysis Method Editor. Configure the Analysis Method Editor tab settings as shown in the figures below, unless the instructions state otherwise.

   Note: The Analysis Method Editor closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.

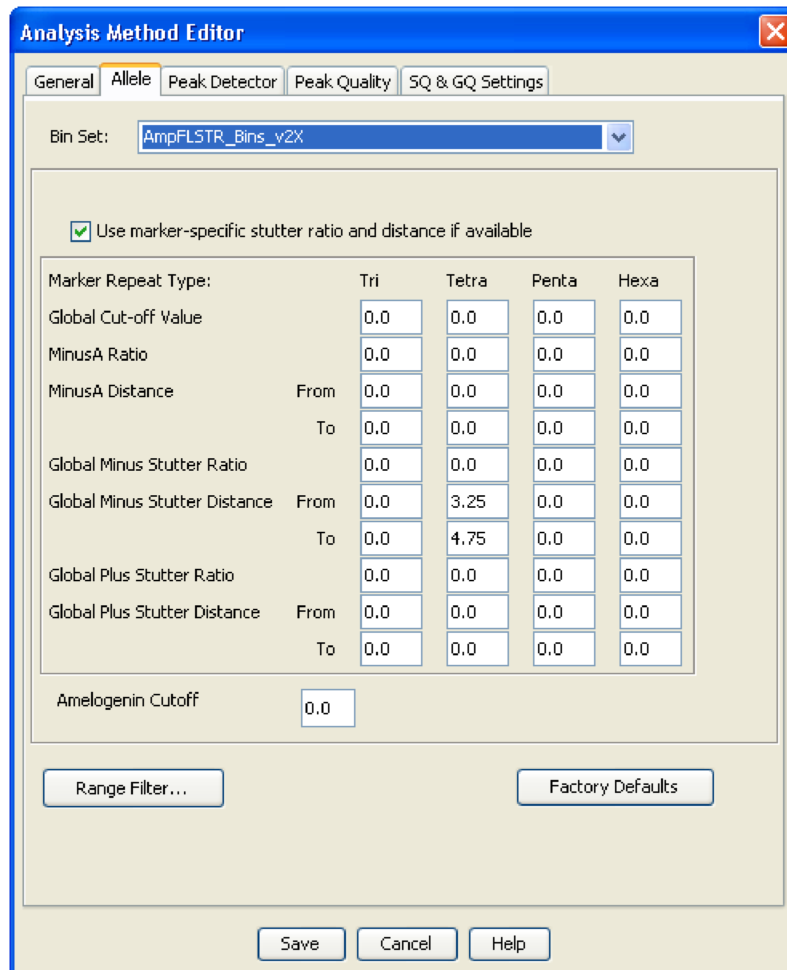
3. After you enter settings in all tabs, click **Save**.

General tab settings



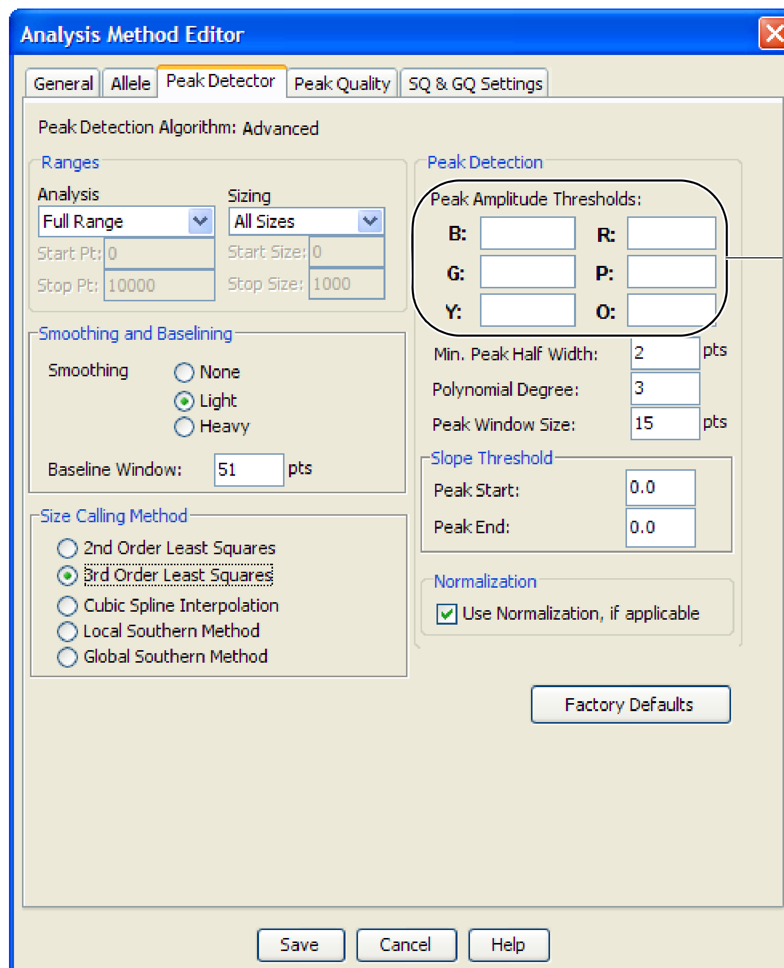
In the Name field, either type the name as shown or enter a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the dropdown list. The Description and Instrument fields are optional.

Allele tab settings



- In the Bin Set field, select the **AmpFLSTR_Bins_v2X** bin set and configure the stutter distance parameters as shown.
- GeneMapper™ *ID-X* Software v1.0.1 or higher allows you to specify 4 types of marker repeat motifs: tri, tetra, penta and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- Specify the stutter ratio:
  - To apply the stutter ratios listed in the Allele tab for single-source data, deselect the “Use marker-specific stutter ratio if available” check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.
    Note: Applying global stutter ratios may reduce the editing required for single-source sample data.
  - To apply the stutter ratios contained in the AmpFLSTR_Stutter_v2X file, select the “Use marker-specific stutter ratio if available” check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.

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 MiniFiler™ 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 MiniFiler™ Kit has been validated using the 3rd Order Least Squares sizing method with the GeneScan™ 500 LIZ™ Size Standard. If you use the GeneScan™ 600 LIZ™ Size Standard v2.0, select the Local Southern Method. Select alternative sizing methods only after performing the appropriate internal validation studies.
- **Normalization** – A Normalization checkbox is available on this tab in GeneMapper™ *ID-X* Software v1.2 for use in conjunction with data run on the Applied Biosystems™ 3500 Series Genetic Analyzers. Normalization cannot be applied to 4-dye data, so this feature is not for use with MiniFiler™ Kit data.

Peak Quality tab settings

Analysis Method Editor

General | Allele | Peak Detector | Peak Quality | SQ & GQ Settings

Min/Max Peak Height (LPH/MPH)
Homozygous min peak height
Heterozygous min peak height
Max Peak Height (MPH)

Peak Height Ratio (PHR)
Min peak height ratio

Broad Peak (BD)
Max peak width (basepairs) 1.5

Allele Number (AN)
Max expected alleles 2

Allelic Ladder Spike
Cut-off Value 0.2

Factory Defaults

Save Cancel Help

Perform internal validation studies to determine settings

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

SQ & GQ tab settings

Analysis Method Editor

General | Allele | Peak Detector | Peak Quality | SQ & GQ Settings

Quality weights are between 0 and 1.

Sample and Control GQ Weighting

Broad Peak (BD)	0.8	Allele Number (AN)	1.0
Out of Bin Allele (BIN)	0.8	Low Peak Height (LPH)	0.3
Overlap (OVL)	0.8	Max Peak Height (MPH)	0.3
Marker Spike (SPK)	0.3	Off-scale (OS)	0.8
		Peak Height Ratio (PHR)	0.3

Control Concordance (CC) Weight = 1.0 (Only applicable to controls)

SQ Weighting

Broad Peak (BD) 0.5

Allelic Ladder GQ Weighting

Spike (SSPK/SPK) 1 Off-scale (OS) 1

SQ & GQ Ranges

	Pass Range:		Low Quality Range:
Sizing Quality:	From 0.75	to 1.0	From 0.0 to 0.25
Genotype Quality:	From 0.75	to 1.0	From 0.0 to 0.25

Reset Defaults

Save As | Save | Cancel | Help

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 size standard (optional)

The CE_G5_GS500 (75–450) size standard definition is installed with the software for use with the MiniFiler™ Kit and contains the following size standard peaks:

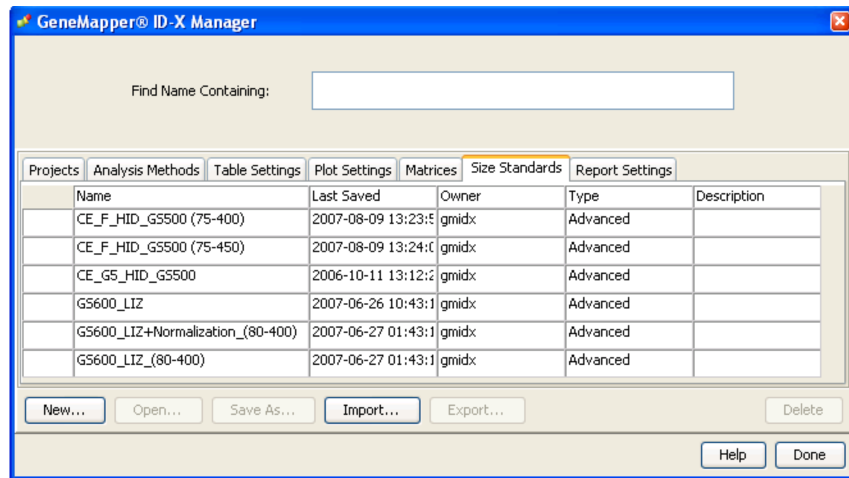
GeneScan™ 500 LIZ™ Size Standard peak sizes	GeneScan™ 600 LIZ™ Size Standard v2.0 peak sizes
75, 100, 139, 150, 160, 200, 300, 350, 400, and 450	80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440 and 460

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

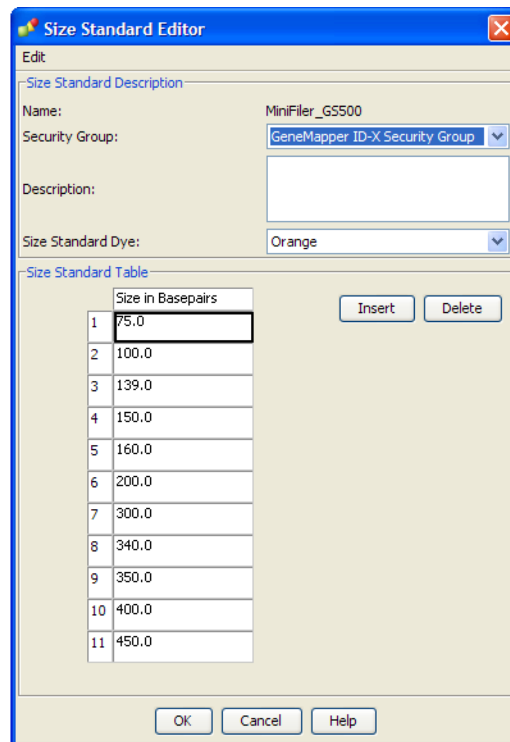
Use the following procedure if you want to create your own size standard:

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

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



3. Enter a name. In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the sizes specified in on page 58. The example below is for the GeneScan™ 500 LIZ™ Size Standard.



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

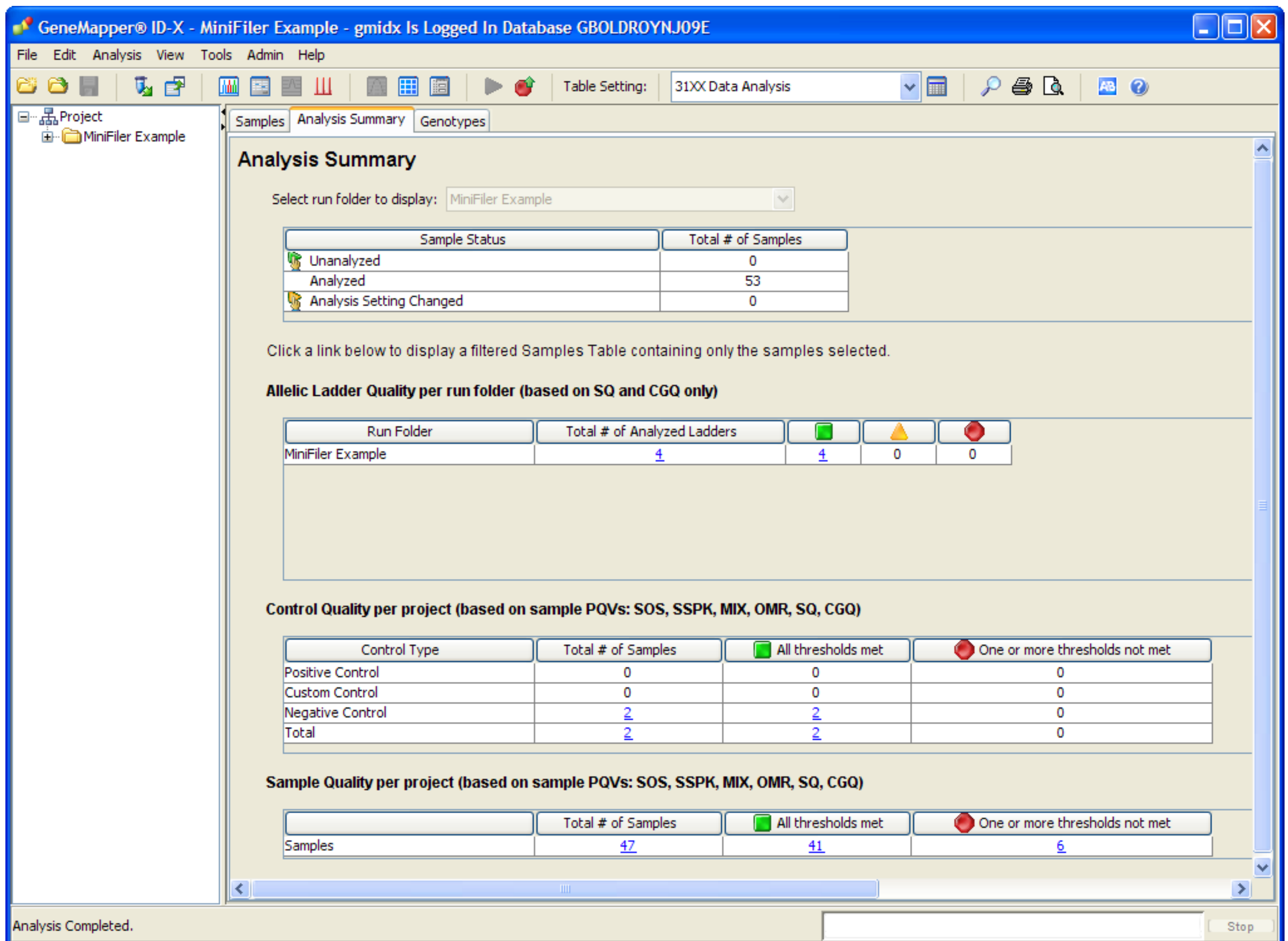
1. In the Project window, select **File ▸ Add Samples to Project**, then navigate to the disk or directory containing the sample files.
2. Apply analysis settings to the samples in the project.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	MiniFiler_AnalysisMethod_v2X (or the name of the analysis method you created)
Panel	AmpFLSTR_Panels_v2X
Size Standard	CE_G5_HID_GS500 (or the name of the size standard you created)

Note: For more information about how the Size Caller works, refer to the *GeneScan™ Analysis Software for the Windows™ NT Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (Pub. no. 4335617).

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 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 and the Genotypes tab becomes available upon completion of the analysis.

   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, 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) Getting Started Guide* (Pub. no. 4396773)
- *GeneMapper™ ID-X Software Version 1.2 Reference Guide* (Pub. no. 4426481)
- *GeneMapper™ ID-X Software Version 1.2 Quick Reference Guide* (Pub. no. 4426482)

5 Experiments and Results

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Section 5.1 Developmental Validation

Overview

Experiments using the MiniFiler™ Kit

This chapter provides results of the developmental validation experiments we performed using the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit.

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 in casework (Sparkes, Kimpton, Watson *et al.*, 1996; Sparkes, Kimpton, Gilbard *et al.*, 1996; Wallin *et al.*, 1998).

Experiment conditions

The experiments to evaluate the performance of the MiniFiler™ Kit were performed according to the DNA Advisory Board (DAB) Quality Assurance Standards, effective October 1, 1998 (DNA Advisory Board, 1998). The DAB standards describe the quality assurance requirements that a laboratory should follow to ensure the quality and integrity of the data and competency of the laboratory.

We performed additional experiments according to the revised guidelines from the Scientific Working Group on DNA Analysis Methods (SWGDAM, July 10, 2003). Based on these guidelines, we conducted experiments that comply with guidelines 1.0 and 2.0 and its associated subsections. This DNA methodology is not novel. (Moretti *et al.*, 2001; Frank *et al.*, 2001; Wallin *et al.*, 2002; and Holt *et al.*, 2000)

This chapter discusses many of the experiments we performed and provides examples of results obtained. We chose conditions that produced optimum PCR product yield and that met reproducible performance standards. It is our opinion that while these experiments are not exhaustive, they are appropriate for a manufacturer of STR kits intended for forensic and/or parentage testing use.

IMPORTANT! Each laboratory using the MiniFiler™ Kit must perform internal validation studies.

Developmental validation

SWGDAM guideline 1.2.1

"Developmental validation is the demonstration of the accuracy, precision, and reproducibility of a procedure by the manufacturer, technical organization, academic institution, government laboratory, or other party." (SWGDAM, July 2003)

SWGDAM guideline 2.10.1

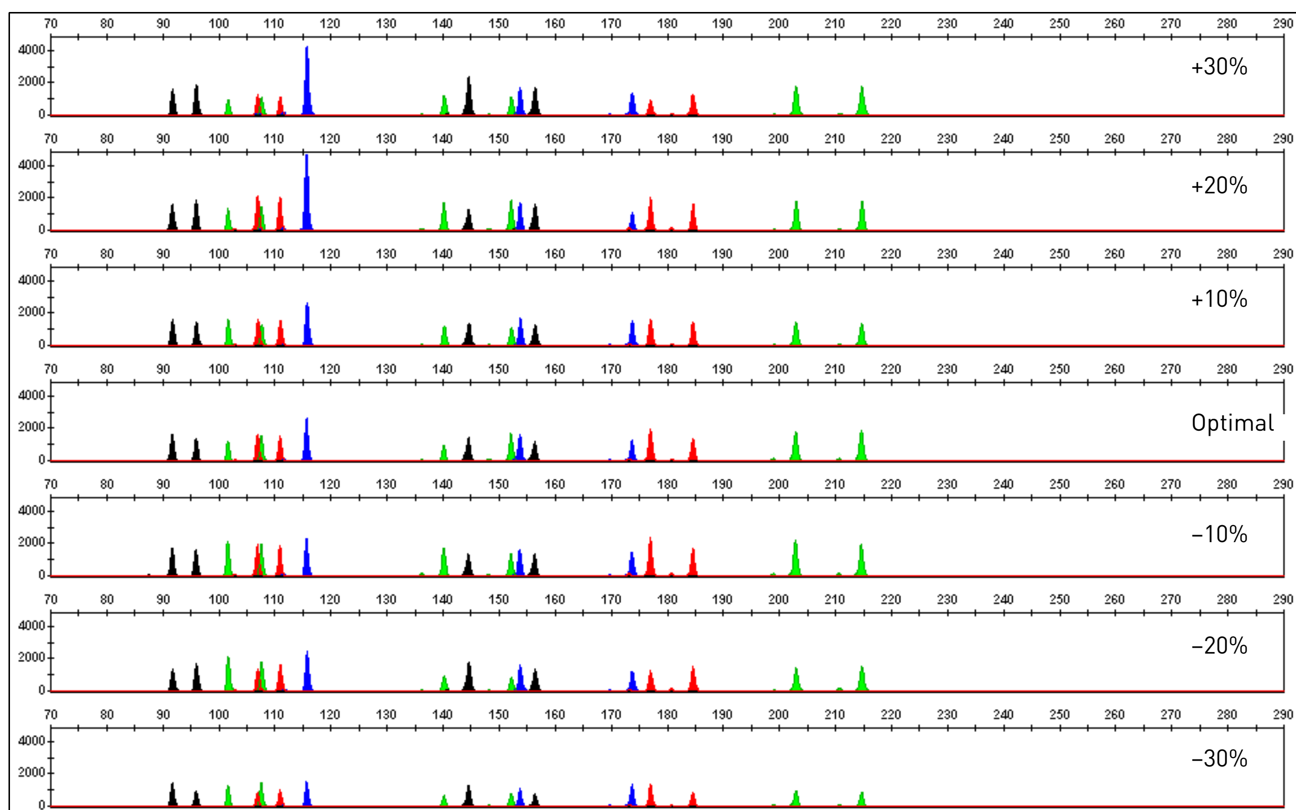
"The reaction conditions needed to provide the required degree of specificity and robustness must be determined. These include thermocycling parameters, the concentration of primers, magnesium chloride, DNA polymerase, and other critical reagents." (SWGDAM, July 2003)

PCR components

We examined the concentration of each component of the MiniFiler™ Kit and established that the concentration of each component was within the range where data indicated that the amplification met the required performance criteria for specificity, sensitivity, and reproducibility. For example, various magnesium chloride concentrations were tested on the Applied Biosystems™ 3130*xl* Genetic Analyzer. The amplification of 0.50 ng of the control DNA 007 is shown in Figure 4 on page 66. We observed that the performance of the multiplex is most robust within a ± 20% window of magnesium chloride.

For example, blood and buccal samples on treated paper substrates or swab sample lysates were amplified in the presence of varying concentrations of magnesium chloride, and the results were analyzed on an Applied Biosystems™ 3130*xl* or 3500xL Genetic Analyzer. Results are shown in Figure 4. The performance of the multiplex is robust within ±20% of the optimal magnesium chloride concentration.

Figure 4 A 0.50 ng sample of Contol DNA 007 amplified with the MiniFiler™ Kit in the presence of varying concentrations of magnesium chloride and analyzed on an Applied Biosystems™ 3130xl Genetic Analyzer (y-axis 5000 RFU).



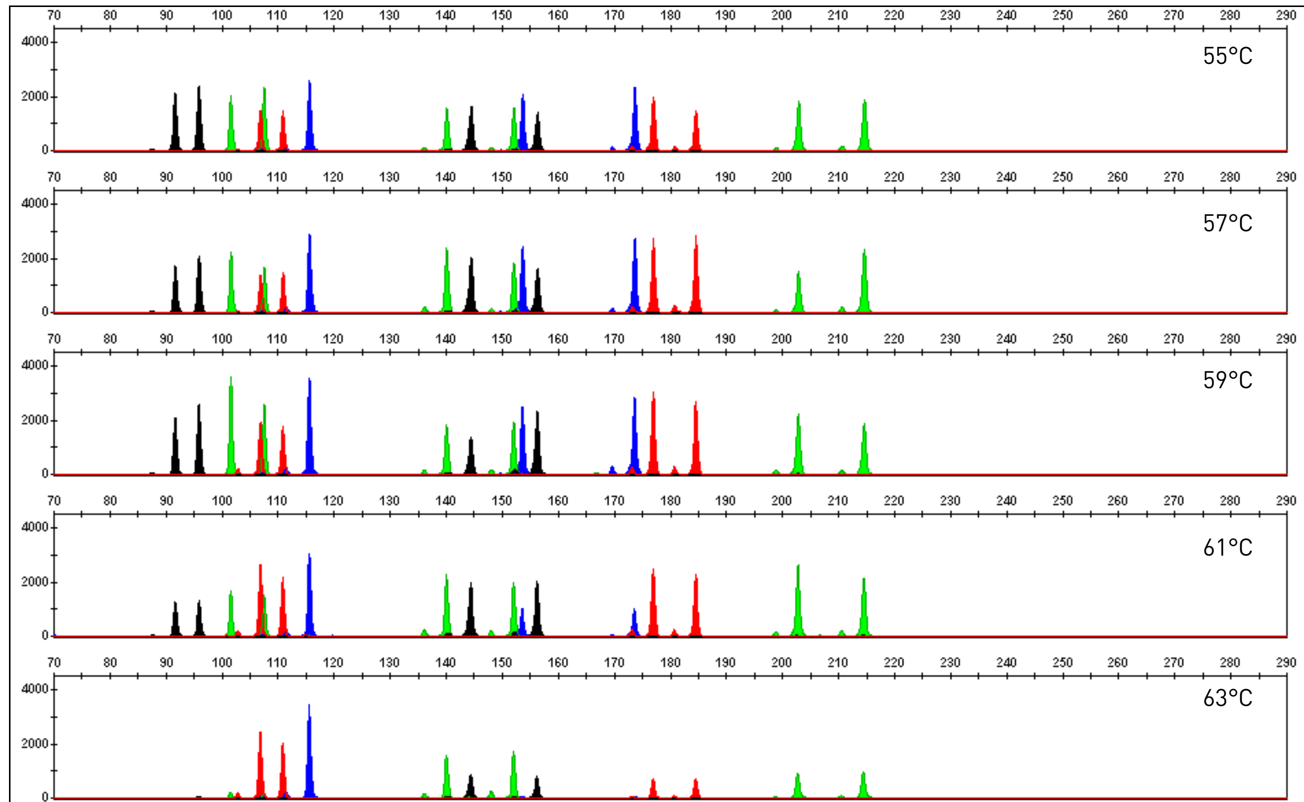
Thermal cycler parameters

Thermal cycling parameters were established for amplification of the MiniFiler™ Kit. Thermal cycling times and temperatures of GeneAmp™ PCR systems were verified. Varying annealing and denaturation temperature windows were tested to verify that a specific PCR product with the desired sensitivity of at least 0.50 ng of Control DNA 007 was produced.

For example, annealing temperatures of 55, 57, 59, 61, and 63°C were tested for two-minute hold times in the Silver 96-Well GeneAmp™ PCR System 9700 (Figure 5). The PCR products were analyzed using an Applied Biosystems™ 3130*xl* Genetic Analyzer.

Of the tested annealing temperatures, 55 to 61°C produced robust profiles. At 63°C the yield of the majority loci was significantly reduced. No preferential amplification was observed at the standard annealing temperature of 59°C. Thermal cycler temperature is critical to assay performance; therefore, routine, regularly scheduled thermal cycler calibration is strongly recommended.

Figure 5 Electropherograms obtained from amplification of 0.50 ng of Control DNA 007 at annealing temperatures of 55, 57, 59, 61, and 63°C, analyzed on an Applied Biosystems™ 3130*xl* Genetic Analyzer (y-axis 4000 RFU).

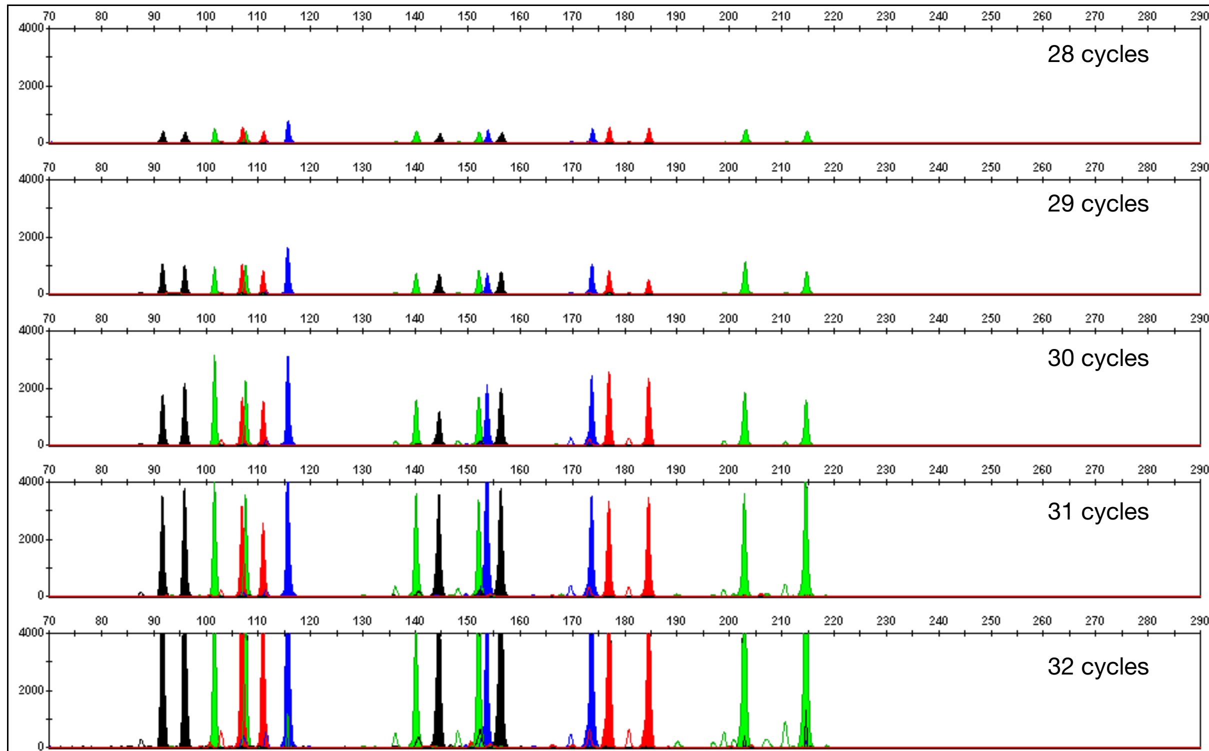


PCR cycle number

MiniFiler™ Kit reactions were amplified for 28, 29, 30, 31 and 32 cycles on the Silver 96-Well GeneAmp™ PCR System 9700 using 0.50 ng from three DNA samples. As expected, the amount of PCR product increased with the number of cycles. A full profile was generated 28 cycles and off-scale data were collected for several allele peaks at 32 cycles (Figure 6).

While none of the cycle numbers tested produced nonspecific peaks, 30 cycles was found to give optimal sensitivity when the amplified products were examined on Applied Biosystems™ 3130*xl* Genetic Analyzers.

Figure 6 Representative MiniFiler™ Kit profiles obtained from amplification of 0.50 ng DNA template using 28, 29, 30, 31, and 32 cycles, analyzed on an Applied Biosystems™ 3130*xl* Genetic Analyzer (y-axis 4000 RFU)



Accuracy, precision, and reproducibility

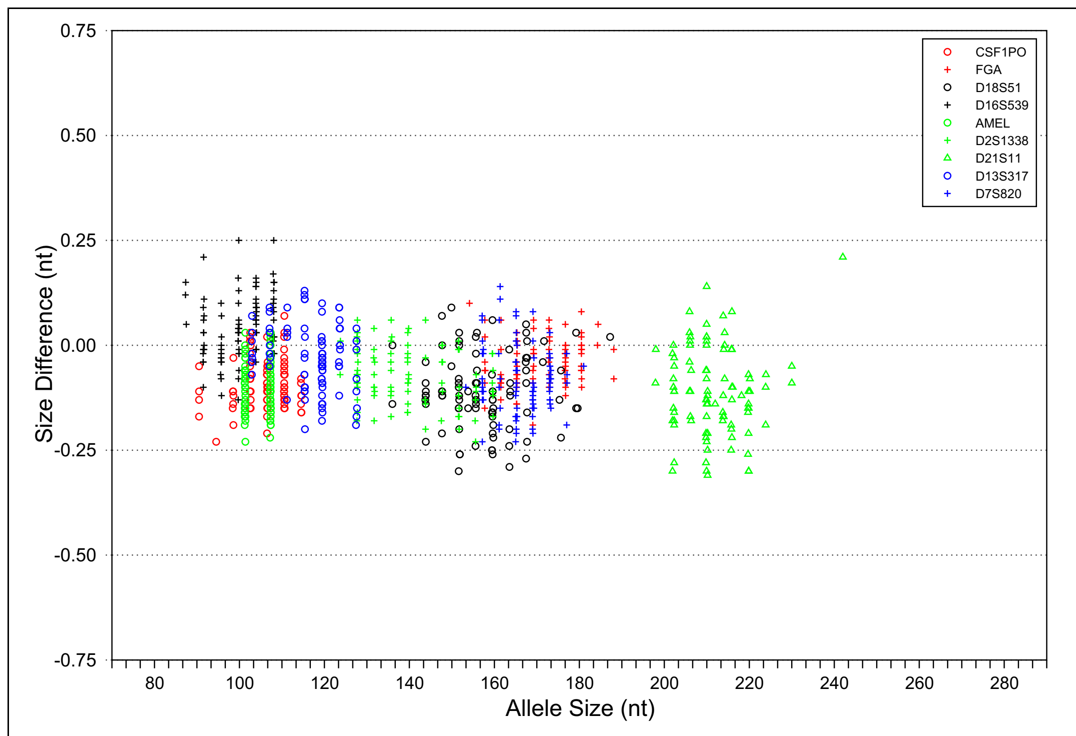
SWGDAM guideline 2.9

"The extent to which a given set of measurements of the same sample agree with their mean and the extent to which these measurements match the actual values being measured should be determined." (SWGDAM, July 2003)

Accuracy

Laser-induced fluorescence detection of length polymorphism at short tandem repeat loci is not a novel methodology (Holt *et al.,* 2000; and Wallin *et al.,* 2002). However, accuracy and reproducibility of MiniFiler™ Kit profiles have been determined from various sample types. Figure 7 shows the size differences that are typically observed between sample alleles and allelic ladder alleles on the Applied Biosystems™ 3130*xl* Genetic Analyzer with POP-4™ polymer. The x axis in Figure 7 represents the nominal nucleotide sizes for the AmpFℓSTR™ MiniFiler™ Allelic Ladder. The dashed lines parallel to the x axis represent ±0.25-nt windows. The y axis represents the deviation of each sample allele size from the corresponding Allelic Ladder allele size. All sample alleles are within ±0.5 nt from a corresponding allele in the Allelic Ladder.

Figure 7 Allele Size vs. Allelic Ladder Sizing for 42 samples analyzed on an Applied Biosystems™ 3130*xl* Genetic Analyzer



Precision and size windows

Sizing precision enables the determination of accurate and reliable genotypes. Sizing precision was measured on an Applied Biosystems™ 3130*xl* Genetic Analyzer. The recommended method for genotyping is to employ a ±0.5-nt "window" around the size obtained for each allele in the AmpFℓSTR™ MiniFiler™ Allelic Ladder. A ±0.5-nt window allows for the detection and correct assignment of alleles. Any sample allele that sizes outside the specified window could be:

- An "off-ladder" allele, that is, an allele of a size that is not represented in the AmpFℓSTR™ MiniFiler™ Allelic Ladder
  or
- An allele that does correspond to an Allelic Ladder allele, but whose size is just outside a window because of measurement error

The measurement error inherent in any sizing method can be defined by the degree of precision in sizing an allele multiple times. Precision is measured by calculating the standard deviation in the size values obtained for an allele that is run in several injections on a capillary instrument.

Table 3 on page 70 shows typical precision results obtained from five runs (16 capillaries/run) of the AmpFℓSTR™ MiniFiler™ Allelic Ladder on an Applied Biosystems™ 3130*xl* Genetic Analyzer (36-cm capillary and POP-4™ polymer), using the GeneScan™ 500 LIZ™ Size Standard. The results were obtained within a set of injections on a single capillary array.

Sample alleles may occasionally size outside of the ±0.5-nt window for a respective Allelic Ladder allele because of measurement error. The frequency of such an occurrence is lowest in detection systems having the smallest standard deviations in sizing. Figure 7 on page 69 illustrates the tight clustering of allele sizes obtained on the

Applied Biosystems™ 3130*xl* Genetic Analyzer, where the standard deviation in sizing is typically less than 0.15 nt. The instance of a sample allele sizing outside the ±0.5-nt window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 nt or less (Smith, 1995).

For sample alleles that do not size within a ±0.5-nt window, the PCR product must be rerun to distinguish between a true off-ladder allele versus measurement error of a sample allele that corresponds with an allele in the Allelic Ladder. Repeat analysis, when necessary, provides an added level of confidence in the final allele assignment.

GeneMapper™ *ID* Software and GeneMapper™ *ID-X* Software automatically flag sample alleles that do not size within the prescribed window around an allelic ladder allele by labelling the allele as OL (off-ladder).

Maximum sizing precision is obtained within the same set of capillary injections. Cross-platform sizing differences occur due to a number of factors including type and concentration of polymer, run temperature, and electrophoresis conditions. Variations in sizing can also occur between runs on the same instrument and between runs on different instruments of the same platform type because of these factors.

We strongly recommend that the allele sizes be compared to the sizes obtained for known alleles in the AmpFℓSTR™ MiniFiler™ Allelic Ladder from the same run and then be converted to genotypes as described in "Before you start" on page 33 (GeneMapper™ *ID* Software) and "Before you start" on page 46 (GeneMapper™ *ID-X* Software). See Table 3 for the results of five runs of the AmpFℓSTR™ MiniFiler™ Allelic Ladder on an Applied Biosystems™ 3130*xl* Genetic Analyzer. For more information on precision and genotyping, see Lazaruk *et al.*, 1998 and Mansfield *et al*., 1998.

In Table 3, the mean sizes for all the alleles in each run (16 capillaries) were calculated. The mean range shown in the table represents the lowest and highest mean size values obtained across all five runs. Similarly, the standard deviation for the allele sizing was calculated for all the alleles in each run. The standard deviation range shown in Table 3 represents the lowest and highest standard deviation values obtained across all five runs.

Table 3 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR™ MiniFiler™ Allelic Ladder

Allele	Mean	Standard Deviation
AMEL		
X	101.54–101.59	0.024–0.037
Y	107.51–107.56	0.029–0.038
CSF1PO		
6	86.65–86.67	0.027–0.038
7	90.70–90.72	0.026–0.038
8	94.72–94.77	0.023–0.036
9	98.76–98.79	0.033–0.041
10	102.79–102.81	0.028–0.038
11	106.80–106.85	0.031–0.044
12	110.82–110.85	0.030–0.043
13	114.83–114.88	0.027–0.045

Allele	Mean	Standard Deviation
14	118.83–118.87	0.023–0.041
15	122.83–122.89	0.031–0.041
D13S317		
8	103.25–103.28	0.029–0.039
9	107.38–107.43	0.028–0.042
10	111.50–111.54	0.035–0.044
11	115.63–115.66	0.031–0.045
12	119.73–119.78	0.037–0.044
13	123.82–123.85	0.038–0.047
14	127.83–127.88	0.038–0.049
15	131.93–131.97	0.035–0.051
D16S539		
5	74.96–75.01	0.033–0.047
8	87.58–87.61	0.030–0.044
9	91.78–91.81	0.021–0.039
10	95.91–95.95	0.038–0.046
11	100.06–100.09	0.038–0.046
12	104.20–104.22	0.041–0.045
13	108.30–108.36	0.031–0.044
14	112.42–112.46	0.037–0.050
15	116.52–116.58	0.037–0.048
D18S51		
7	124.68–124.73	0.035–0.060
9	132.53–132.57	0.044–0.059
10	136.46–136.50	0.040–0.056
10.2	138.37–138.42	0.040–0.056
11	140.38–140.43	0.038–0.055
12	144.33–144.37	0.039–0.059
13	148.27–148.31	0.048–0.054
13.2	150.19–150.22	0.040–0.062
14	152.21–152.24	0.043–0.057
14.2	154.14–154.18	0.035–0.054
15	156.17–156.20	0.042–0.061
16	160.13–160.16	0.047–0.060
17	164.06–164.10	0.046–0.057
18	168.05–168.06	0.039–0.058
19	172.00–172.02	0.041–0.054
20	175.97–175.99	0.035–0.061

Allele	Mean	Standard Deviation
21	179.93–179.96	0.045–0.055
22	183.92–183.95	0.048–0.064
23	187.89–187.94	0.043–0.062
24	191.87–191.91	0.045–0.058
D18S51		
7	124.68–124.73	0.035–0.060
9	132.53–132.57	0.044–0.059
10	136.46–136.50	0.040–0.056
10.2	138.37–138.42	0.040–0.056
11	140.38–140.43	0.038–0.055
12	144.33–144.37	0.039–0.059
13	148.27–148.31	0.048–0.054
13.2	150.19–150.22	0.040–0.062
14	152.21–152.24	0.043–0.057
14.2	154.14–154.18	0.035–0.054
15	156.17–156.20	0.042–0.061
16	160.13–160.16	0.047–0.060
17	164.06–164.10	0.046–0.057
18	168.05–168.06	0.039–0.058
19	172.00–172.02	0.041–0.054
20	175.97–175.99	0.035–0.061
21	179.93–179.96	0.045–0.055
22	183.92–183.95	0.048–0.064
23	187.89–187.94	0.043–0.062
24	191.87–191.91	0.045–0.058
25	195.85–195.87	0.053–0.070
26	199.86–199.89	0.047–0.063
27	203.84–203.88	0.044–0.070
D21S11		
24	186.96–186.99	0.056–0.067
24.2	188.95–188.97	0.047–0.066
25	190.89–190.92	0.051–0.071
26	194.82–194.84	0.052–0.063
27	198.67–198.7	0.053–0.071
28	202.71–202.74	0.055–0.065
28.2	204.63–204.66	0.060–0.070
29	206.73–206.77	0.048–0.072
29.2	208.50–208.55	0.051–0.077

Allele	Mean	Standard Deviation
30	210.59–210.63	0.053–0.075
30.2	212.53–212.59	0.058–0.067
31	214.54–214.59	0.054–0.069
31.2	216.51–216.55	0.054–0.075
32	218.48–218.55	0.051–0.067
32.2	220.48–220.53	0.057–0.073
33	222.46–222.51	0.055–0.073
33.2	224.52–224.57	0.052–0.078
34	226.35–226.39	0.054–0.063
34.2	228.42–228.47	0.049–0.072
35	230.35–230.40	0.047–0.081
35.2	232.38–232.45	0.055–0.083
36	234.42–234.48	0.053–0.080
37	238.31–238.36	0.057–0.074
38	242.41–242.47	0.063–0.077
D2S1338		
15	120.06–120.10	0.032–0.042
16	124.11–124.16	0.028–0.044
17	128.07–128.12	0.031–0.044
18	132.04–132.08	0.036–0.051
19	136.05–136.08	0.028–0.046
20	140.00–140.04	0.028–0.045
21	144.01–144.04	0.034–0.043
22	147.99–148.02	0.029–0.039
23	151.96–151.99	0.034–0.044
24	155.95–155.97	0.029–0.042
25	159.93–159.94	0.038–0.049
26	163.91–163.94	0.032–0.055
27	167.99–168.01	0.033–0.052
28	172.24–172.26	0.038–0.052
D7S820		
6	149.69–149.73	0.032–0.051
7	153.65–153.68	0.036–0.051
8	157.62–157.65	0.031–0.051
9	161.59–161.62	0.032–0.057
10	165.55–165.57	0.035–0.046
11	169.53–169.54	0.037–0.050
12	173.50–173.52	0.034–0.055

Allele	Mean	Standard Deviation
13	177.48–177.50	0.041–0.047
14	181.46–181.49	0.034–0.050
15	185.45–185.47	0.034–0.053
FGA		
17	150.52–150.55	0.031–0.040
18	154.26–154.29	0.031–0.043
19	158.03–158.04	0.029–0.047
20	161.78–161.80	0.033–0.044
21	165.55–165.57	0.030–0.042
22	169.32–169.34	0.031–0.047
23	173.11–173.12	0.032–0.041
24	176.88–176.91	0.034–0.048
25	180.68–180.70	0.025–0.045
26	184.49–184.51	0.031–0.047
26.2	186.29–186.34	0.027–0.049
27	188.34–188.37	0.022–0.047
28	192.20–192.25	0.037–0.047
29	195.97–196.02	0.032–0.046
30	199.69–199.74	0.032–0.047
30.2	202.12–202.17	0.034–0.055
31.2	205.94–205.98	0.034–0.055
32.2	209.74–209.80	0.034–0.051
33.2	213.57–213.64	0.035–0.064
42.2	248.46–248.55	0.042–0.064
43.2	252.35–252.43	0.038–0.067
44.2	256.39–256.46	0.043–0.064
45.2	260.28–260.36	0.043–0.054
46.2	263.89–263.95	0.040–0.055
47.2	267.71–267.77	0.039–0.057
48.2	271.69–271.76	0.040–0.058
50.2	279.48–279.54	0.036–0.062
51.2	283.23–283.28	0.041–0.061

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 (see 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 major STR 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 (n=967) at the loci used in the MiniFiler™ Kit. All data were generated on the Applied Biosystems™ 3130*xl* Genetic Analyzer.

Some conclusions from these measurements and observations are:

- For each MiniFiler™ Kit locus, the stutter percentage generally increases with allele length, as shown in Figure 8 on page 76 through Figure 11 on page 77.
- 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* and GeneMapper™ *ID-X* Software, calculated as the mean stutter for the locus plus three standard deviations (N = 668), are shown in Table 4 on page 78. 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.

Figure 8 Stutter percentages for D13S317 and D7S820 loci

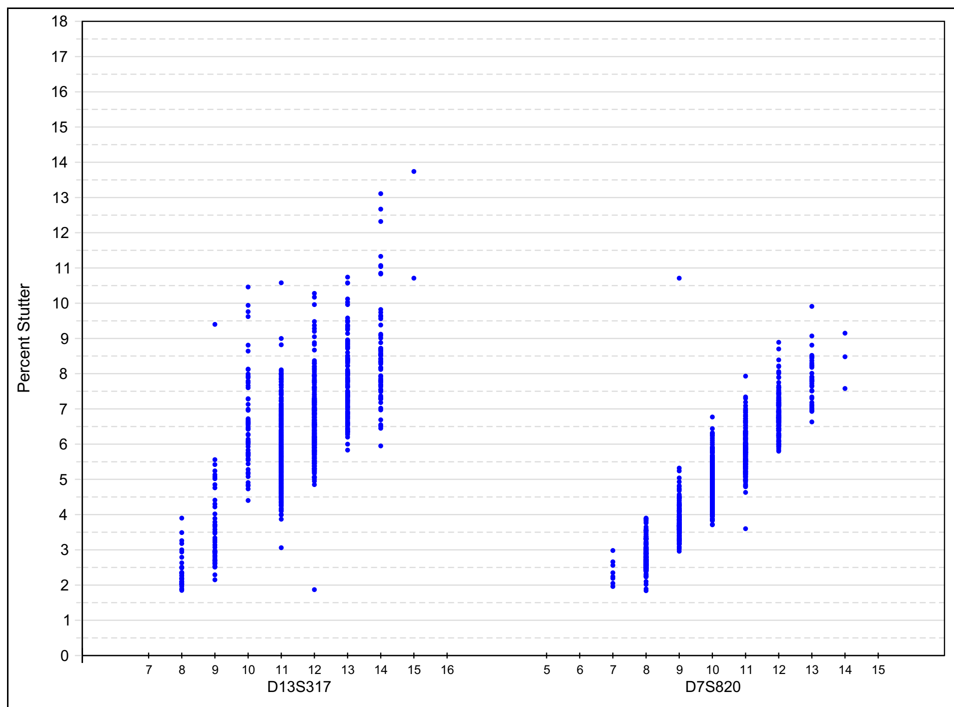


Figure 9 Stutter percentages for the D2S1338 and D21S11 loci

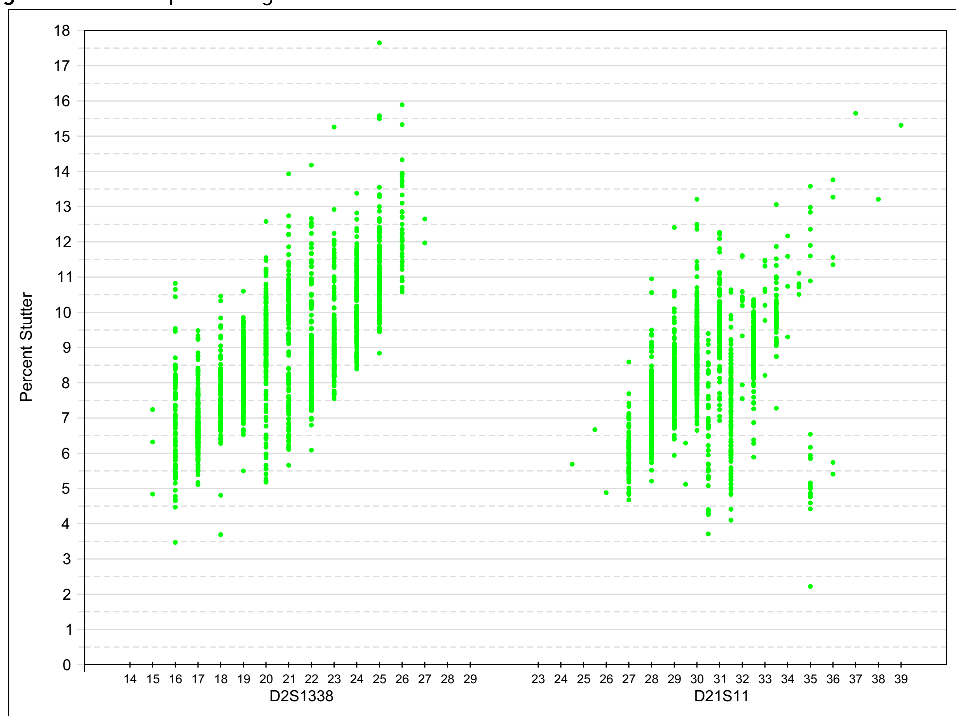


Figure 10 Stutter percentages for the D16S539 and D18S51 loci

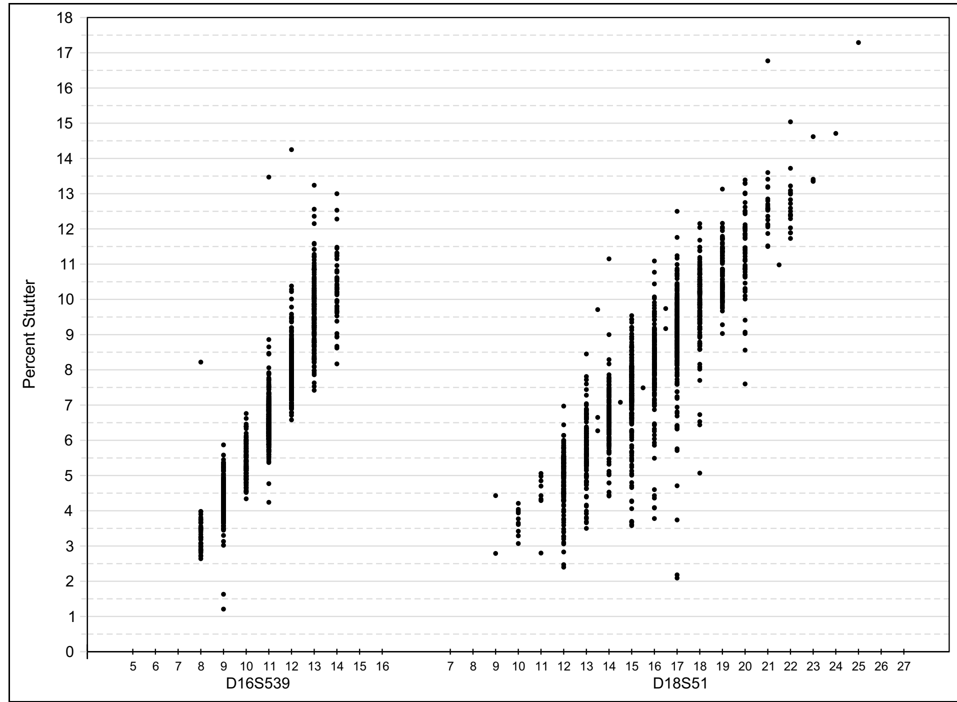


Figure 11 Stutter percentages for the CSF1PO and FGA loci

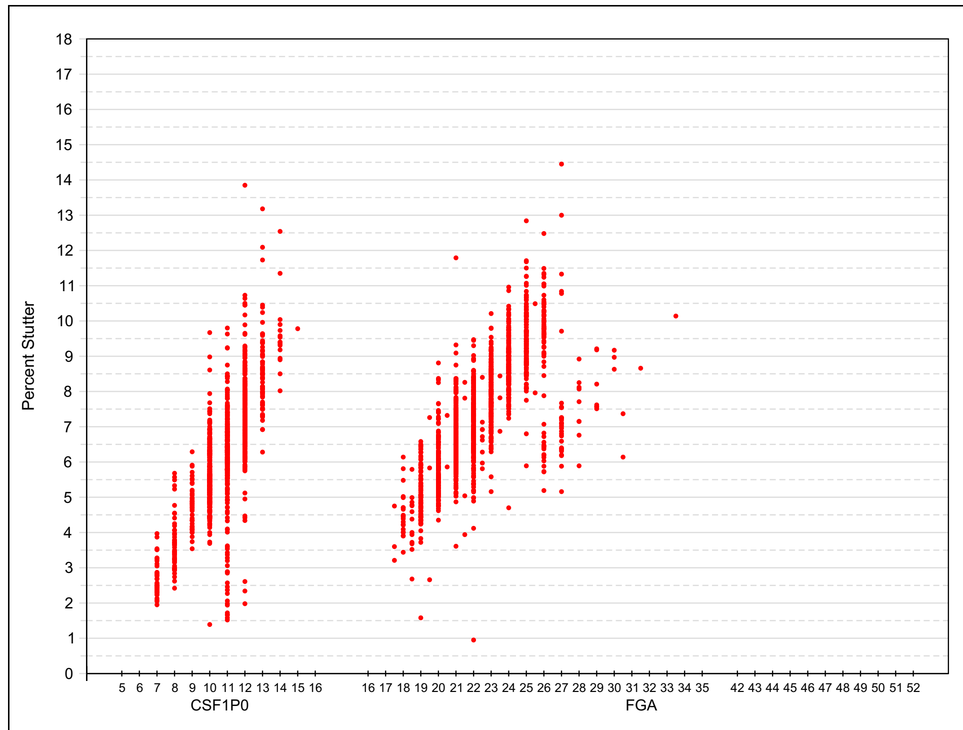


Table 4 Marker-specific stutter filter percentages for MiniFiler™ Kit loci

Locus†	% Stutter
D13S317	14
D7S820	11
D2S1338	18
D21S11	16
D16S539	15
D18S51	18
CSF1PO	14
FGA	15

† These percentages are used as stutter filters in GeneMapper™ *ID* v3.2.1 AmpFLSTR_MiniFiler_Panels_v1.txt and GeneMapper™ *ID-X* AmpFLSTR_Stutter_v2X.

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 nontemplate 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.

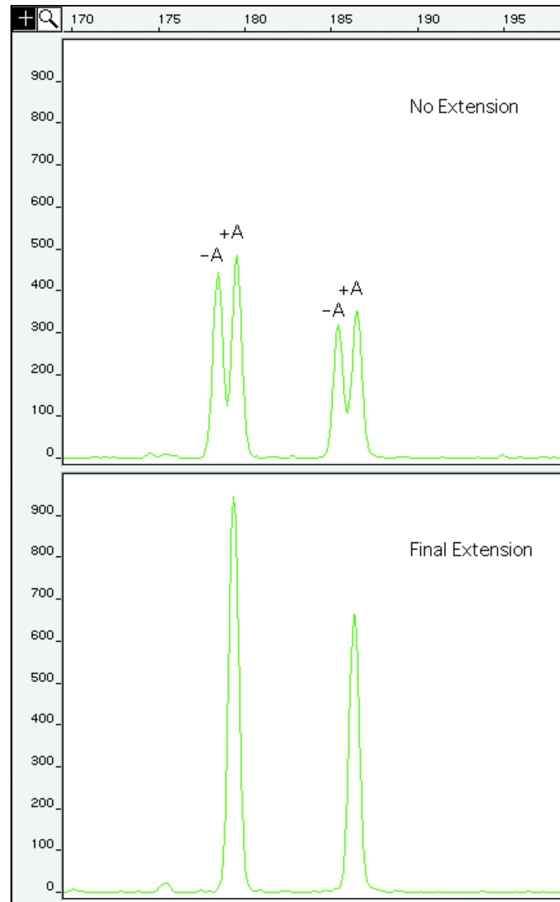
The efficiency of +A addition is related to the particular sequence of the DNA at the 3´ end of the PCR product. The MiniFiler™ Kit includes two main design features that promote maximum +A addition:

- 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 5 minutes.

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products. STR systems, where each allele is represented by two peaks one nucleotide apart, that have not been optimized for +A addition may have "split peaks."

Figure 12 shows examples of incomplete and normal +A addition.

Figure 12 Omission of the final extension step resulted in split peaks due to incomplete A nucleotide addition. These data were generated on the 310 Genetic Analyzer using another AmpFℓSTR™ kit.



Lack of complete +A nucleotide addition may be observed in MiniFiler™ Kit results when the amount of input DNA is greater than the recommended protocols, because more time is needed for the DNA Polymerase to add the +A nucleotide to all molecules as more PCR product is generated. Amplification of too much input DNA may also result in off-scale data.

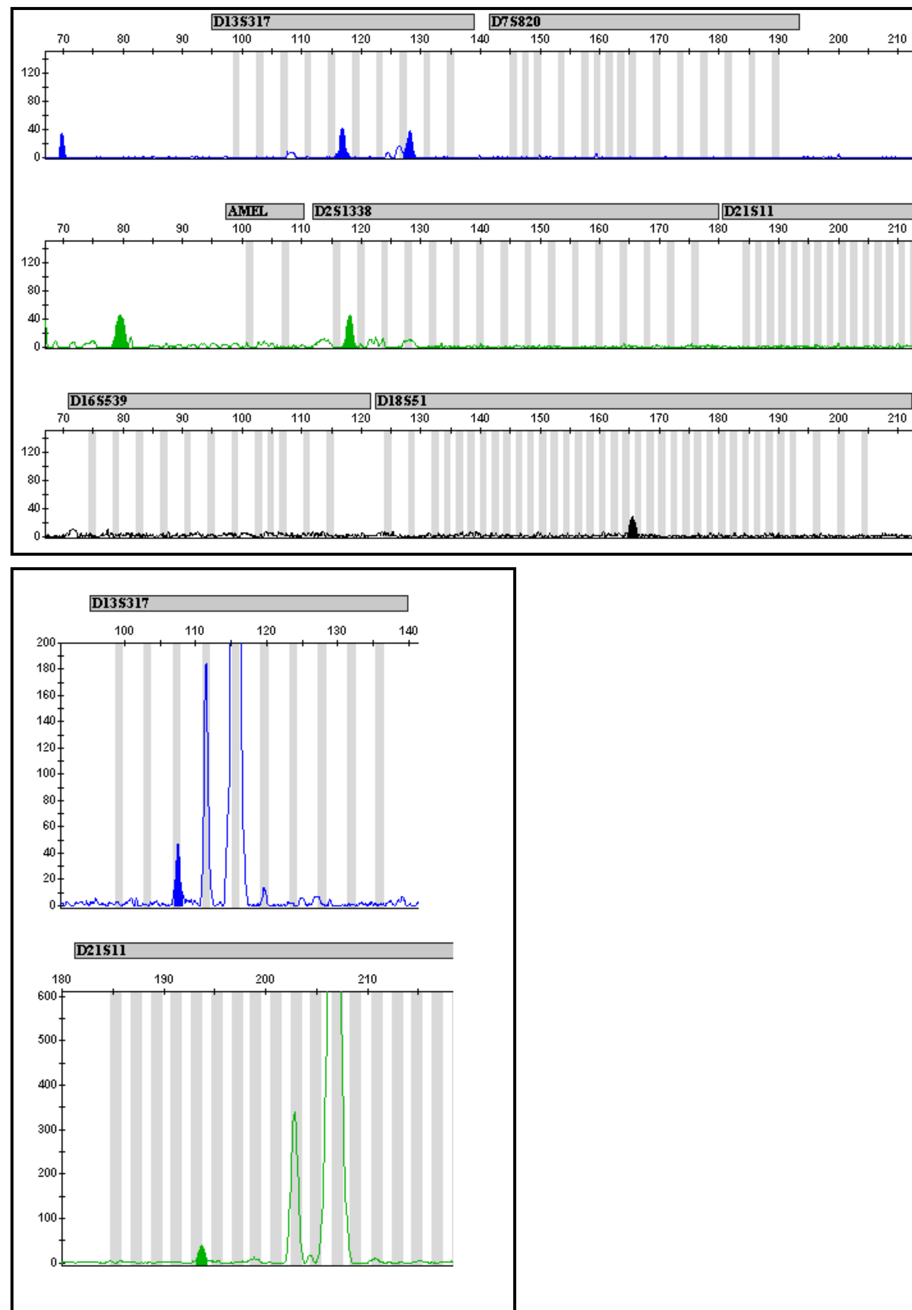
Artifacts

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). Artifacts have been seen in data produced on genetic analyzers when using the MiniFiler™ Kit. In amplified samples, artifacts in the non-calling region may appear in the blue (70 nt) and VIC™ (80 nt) dyes. Low-level artifacts in the calling region may appear in the blue (117 and 127 nt), green (118 nt), and black (166 nt) dyes, depending on the sensitivity of the instrument.

Figure 13 shows examples of baseline noise and artifacts in an electropherogram while using the MiniFiler™ Kit. You should consider possible noise and artifacts when interpreting data from the MiniFiler™ Kit on the Applied Biosystems™ 3130*xl* Genetic Analyzer.

Note: A high degree of magnification (y-axis) is used in Figure 13 to illustrate these artifacts (data produced on capillary electrophoresis instrument platforms).

Figure 13 Examples of baseline noise and reproducible artifacts in data produced on an Applied Biosystems™ 3130*xl* Genetic Analyzer



Another type of PCR artifact occurs when the amount of input DNA exceeds the recommended amount (0.50 to 0.75 ng). These artifacts were characterized as secondary stutter products in D13S317 and D21S11 as shown in the bottom example in Figure 13. Their mobility varies with that of the main amplification product.

Genotyping may result in the detection of these artifacts as off-ladder alleles, or "OL Alleles". This occurs if the recommended amount of input DNA is exceeded and off-scale data is obtained.

Characterization of loci

SWGDAM guideline 2.1

"The basic characteristics of a genetic marker must be determined and documented." (SWGDAM, July 2003)

This section describes basic characteristics of the eight loci and the sex-determining marker, Amelogenin, which are amplified with the MiniFiler™ Kit. Most of these loci have been extensively characterized by other laboratories.

Nature of the polymorphisms

The primers for the Amelogenin locus flank a six-nucleotide deletion within intron 1 of the X homologue. Amplification results in 104-nt and 110-nt products from the X and Y chromosomes, respectively. (Sizes are the actual nucleotide size according to sequencing results, including 3′ A nucleotide addition.) The remaining MiniFiler™ Kit loci are all tetranucleotide short tandem repeat (STR) loci. The length differences among alleles of a particular locus result from differences in the number of 4-nt repeat units.

We have sequenced all the alleles in the AmpFℓSTR™ MiniFiler™ Allelic Ladder. In addition, other groups in the scientific community have sequenced alleles at some of these loci. Among the various sources of sequence data on the MiniFiler™ Kit loci, there is consensus on the repeat patterns and structure of the STRs.

Inheritance

The Centre d'Etude du Polymorphisme Humain (CEPH) has collected DNA from families of Utah Mormon, French Venezuelan, and Amish descent. These DNA sets have been extensively studied all over the world and are routinely used to characterize the mode of inheritance of various DNA loci. Each family set contains three generations, generally including four grandparents, two parents, and several offspring. Consequently, the CEPH family DNA sets are ideal for studying inheritance patterns (Begovich *et al*., 1992).

Three CEPH family DNA sets were examined. 0.50 ng of DNA from each sample was amplified using the MiniFiler™ Kit and Identifiler™ Kit, followed by analysis using an Applied Biosystems™ 3130*xl* Genetic Analyzer. The families examined included #1333 (9 offspring), #1340 (7 offspring), and #1345 (7 offspring), representing 23 meiotic divisions. The results showed concordance between MiniFiler™ Kit and Identifiler™ Kit genotypes and confirmed that the loci are inherited according to Mendelian rules, as expected.

Mapping

The MiniFiler™ Kit loci have been mapped, and the chromosomal locations have been published (Nakahori *et al.*, 1991; Edwards *et al.*, 1992; Kimpton *et al.*, 1992; Mills *et al.*, 1992; Sharma and Litt, 1992; Li *et al.*, 1993; Straub et al., 1993; Barber and Parkin, 1996).

Species specificity

SWGDAM Guideline 2.2

"For techniques designed to type human DNA, the potential to detect DNA from forensically relevant nonhuman species should be evaluated." (SWGDAM, July 2003)

The MiniFiler™ Kit provides the required specificity for primates. Other species do not amplify for the loci tested.

Nonhuman studies

The following species were tested (in the specified amounts) using standard PCR and capillary electrophoresis conditions for the MiniFiler™ Kit.

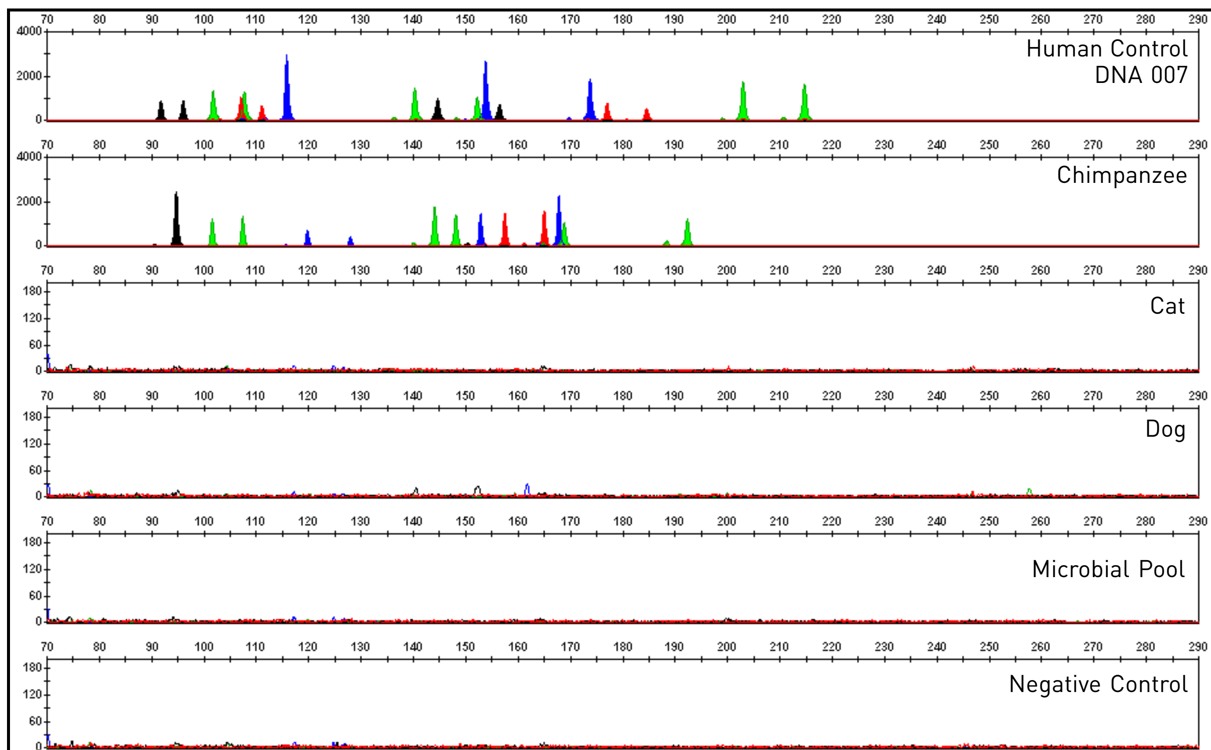
- Primates: gorilla, chimpanzee, orangutan, and macaque (0.50 ng each)
- Non-primates: mouse, dog, pig, cat, horse, hamster, rat, chicken and cow (10 ng each)
- Microorganisms: *Candida alb icans*, *Escherichia coli*, *Lactob acillus casei*, *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Bacillus sub tilis,* and *Lactob acillus rhamnosus* (equivalent to 10^5 copies).

The chimpanzee and gorilla DNA samples produced partial profiles within the 70 to 283 nucleotide region.

The remaining species tested did not yield reproducible detectable products.

Figure 14 on page 82 shows example electropherogram results from the species specificity tests.

Figure 14 Representative electropherograms for some species tested in a species specificity study including positive and non-template controls (NTC)



Sensitivity

SWGDAM guideline 2.3

"When appropriate, the range of DNA quantities able to produce reliable typing results should be determined." (SWGDAM, July 2003)

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 is a problem because:

  – Quantitation (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").

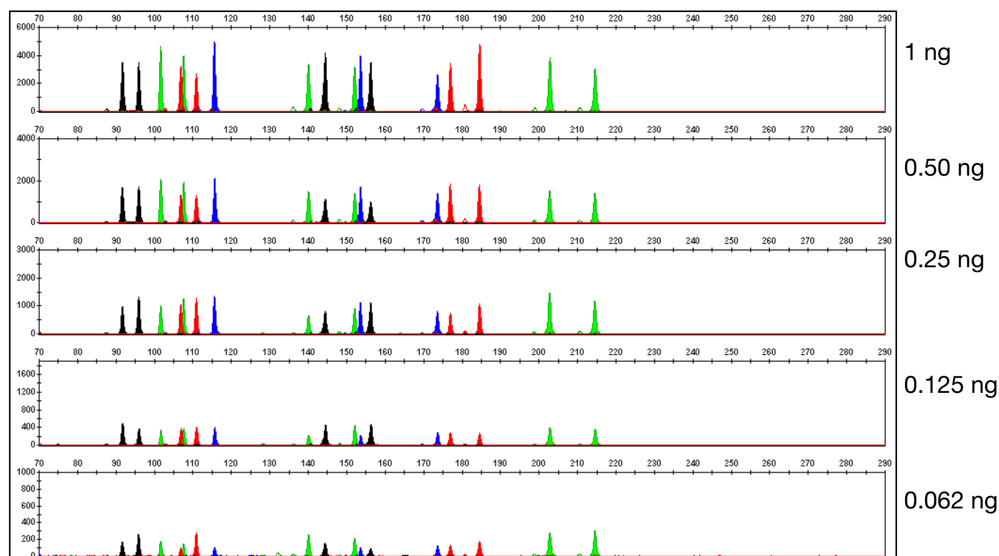
- Incomplete +A nucleotide addition.

  The sample can be re-amplified 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 15 Effect of amplifying varying amounts of Control DNA 007



Note that the y-axis scale is magnified for the lower amounts of DNA, analyzed using the Applied Biosystems™ 3130*xl* Genetic Analyzer.

Stability

SWGDAM guideline 2.4

"The ability to obtain results from DNA recovered from biological samples deposited on various substrates and subjected to various environmental and chemical insults has been extensively documented. In most instances, assessment of the effects of these factors on new forensic DNA procedures is not required. However, if substrates and/or environmental and/or chemical insults could potentially affect the analytical process, then the process should be evaluated using known samples to determine the effects of such factors." (SWGDAM, July 2003)

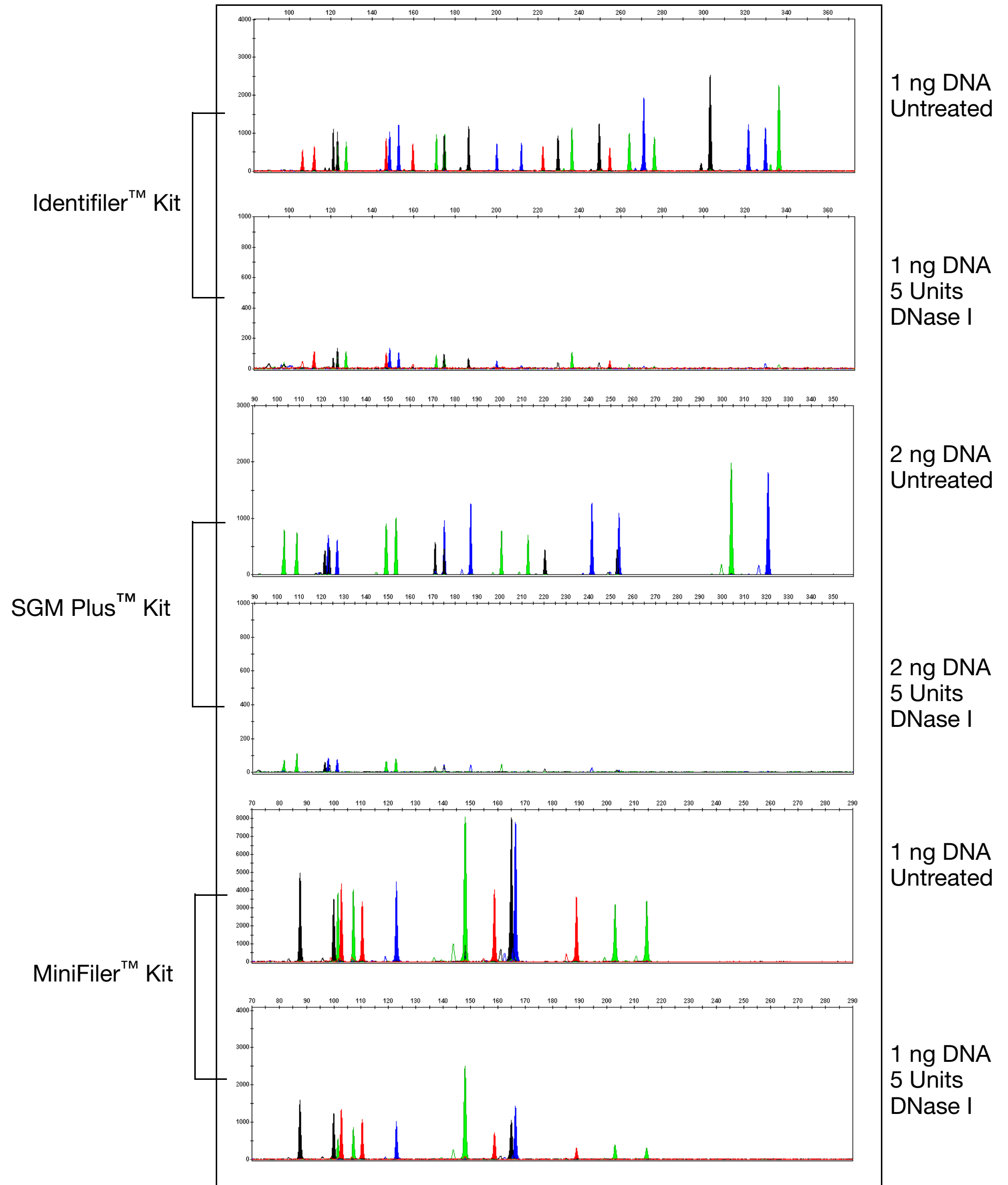
Degraded DNA

As the average size of degraded DNA approaches the size of the target sequence, the amount of PCR product generated is reduced, due to the reduced number of intact templates in the size range necessary for amplification.

Degraded DNA was prepared to examine the potential for preferential amplification of loci. High molecular weight Raji DNA was sonicated and incubated with increasing doses of DNase I (0 to 6 Units) for 20 minutes (Bender *et al*., 2004). The DNA was examined by capillary electrophoresis analysis to determine the average size of the DNA fragments at each time point.

One nanogram of degraded DNA was amplified using the MiniFiler™ Kit and Identifiler™ Kit. Two nanograms of degraded DNA was amplified using the SGM Plus™ PCR Amplification Kit. As the DNA became increasingly degraded, the larger size Identifiler™ Kit and SGM Plus™ Kit loci became undetectable. However, the amplification with the MiniFiler™ Kit resulted in an increased overall typing success rate.

Figure 16 Amplification of Raji DNA samples untreated or sonicated for 5 minutes and incubated with DNase I



The performance of the three kits was compared in a simulated model of DNA degradation (sonication and DNase I treatments). Only those loci (>50 RFUs) represented in the MiniFiler™ Kit were measured in the Identifiler™ Kit and SGM Plus™ Kit (see Table 5). A complete profile with Raji DNA yields 14 peaks using the MiniFiler™ Kit.

Table 5 Comparison of MiniFiler™, Identifiler™, and SGM Plus™ Kit performance in simulated model of DNA degradation (n = 3)

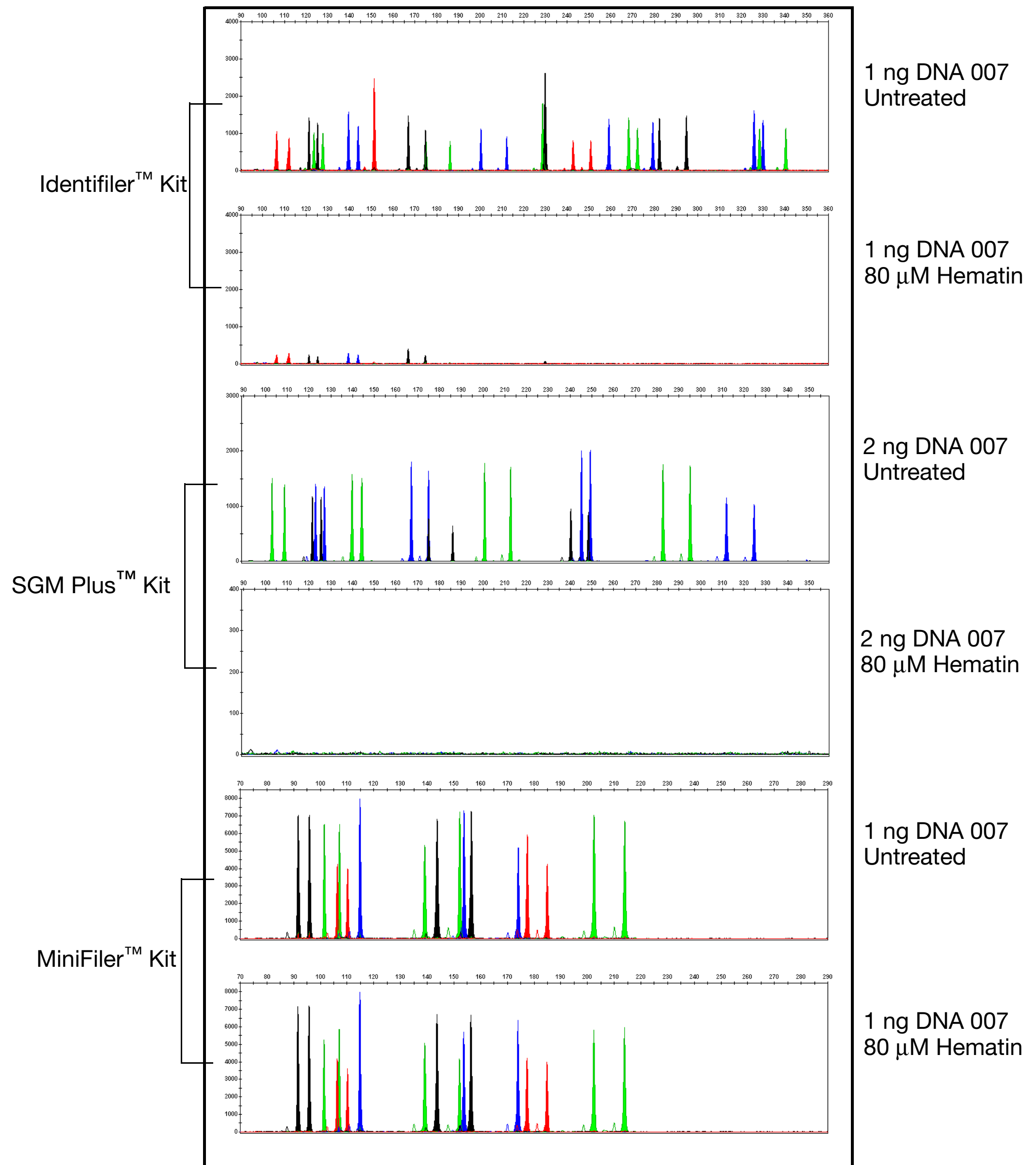
DNase I	MiniFiler™ Kit	Identifiler™ Kit	SGM Plus™ kit
0 units	14/14, 14/14, 14/14	14/14, 14/14, 14/14	10/10, 10/10, 10/10
4 units	14/14, 14/14, 14/14	8/14, 3/14, 4/14	2/10, 4/10, 5/10
5 units	14/14, 14/14, 14/14	3/14, 4/14, 4/14	2/10, 2/10, 2/10
6 units	14/14, 14/14, 13/14	0/14, 0/14, 0/14	0/10, 1/10, 1/10

Effect of inhibitors – Hematin

Heme compounds have been identified as PCR inhibitors in DNA samples extracted from bloodstains (DeFranchis *et al*., 1988; Akane *et al*., 1994). It is believed that the inhibitor is co-extracted and co-purified with the DNA and subsequently interferes with PCR by inhibiting polymerase activity.

To examine the effects of hematin on the amplification results obtained by the MiniFiler™ Kit, male Control DNA 007 (1 ng input DNA for the MiniFiler™ Kit and the Identifiler™ Kit and 2 ng for the SGM Plus™ Kit) was amplified with increasing concentrations of hematin. The concentrations of hematin used were 0 µM, 20 µM, 40 µM, 60 µM, and 80 µM. No preferential amplification was observed in the presence of increasing amounts of hematin (Figure 17 on page 87).

Figure 17 Amplification of Control DNA 007 in the presence of hematin analyzed on the Applied Biosystems™ 3130*xl* Genetic Analyzer



Comparison of performance of the three kits in a simulated model of hematin inhibition. Only those loci (>50 RFU) represented in the MiniFiler™ Kit were measured in the Identifiler™ Kit and SGM Plus™ Kit (see Table 6). A complete profile with Control DNA 007 yields 17 peaks using the MiniFiler™ Kit.

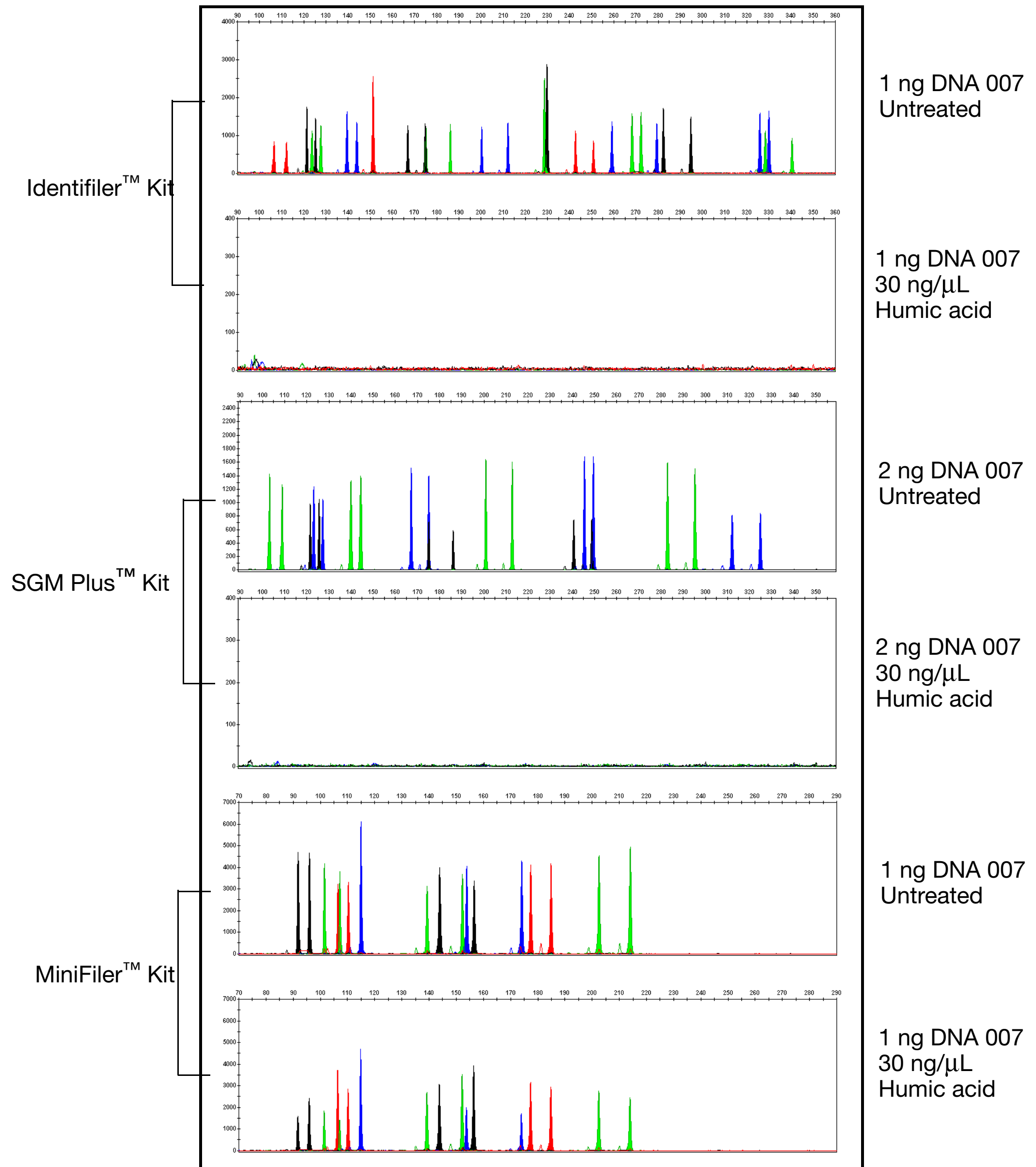
Table 6 Comparison of MiniFiler™, Identifiler™, and SGM Plus™ Kit performance in simulated model of hematin inhibition (n = 3)

Hematin	MiniFiler™ Kit	Identifiler™ Kit	SGM Plus™ kit
20 µM	17/17, 17/17, 17/17	17/17, 17/17, 17/17	14/14, 14/14, 14/14
40 µM	17/17, 17/17, 17/17	17/17, 17/17, 9/17	14/14, 14/14, 14/14
60 µM	17/17, 17/17, 17/17	2/17, 2/17, 0/17	2/14, 1/14, 2/14
80 µM	17/17, 17/17, 17/17	0/17, 0/17, 0/17	0/14, 0/14, 0/14

Effect of inhibitors – Humic Acid

Traces of humic acid may inhibit the PCR amplification of DNA evidence collected from soil. In this study, we tested increasing amounts of humic acid in the PCR amplification of 1 ng of Control DNA 007 with the Identifiler™ Kit and the MiniFiler™ Kit and 2 ng with the SGM Plus™ Kit. As the concentration of humic acid increased in the reaction, the larger Identifiler™ Kit and SGM Plus™ Kit loci failed to amplify. However, the MiniFiler™ Kit loci efficiently amplified the DNA at concentrations of humic acid that inhibited the amplification of DNA with the Identifiler™ Kit and SGM Plus™ Kit (Figure 18 on page 89). The concentrations of humic acid tested were 0, 10, 30, and 50 ng/µL.

Figure 18 Amplification of Control DNA 007 in the presence of humic acid analyzed on the Applied Biosystems™ 3130*xl* Genetic Analyzer



Comparison of performance of the three kits in a simulated model of humic acid inhibition. Only those loci (>50 RFU) represented in the MiniFiler™ Kit were measured in the Identifiler™ Kit and SGM Plus™ Kit (see Table 7). A complete profile with control 007 DNA yields 17 peaks using the MiniFiler™ Kit.

Table 7 Comparison of MiniFiler™, Identifiler™, and SGM Plus™ Kit performance in simulated model of humic acid inhibition (n = 5)

Humic Acid	MiniFiler™ Kit	Identifiler™ Kit	SGM Plus™ kit
10 ng/µL	17/17, 17/17, 17/17, 17/17, 17/17	17/17, 17/17, 17/17, 17/17, 14/17	14/14, 14/14, 14/14, 14/14, 14/14
30 ng/µL	17/17, 17/17, 17/17, 17/17, 17/17	0/17, 0/17, 0/17, 0/17, 0/17	0/14, 0/14, 0/14, 0/14, 0/14
50 ng/µL	17/17, 17/17, 17/17, 17/17, 14/17	0/17, 0/17, 0/17, 0/17, 0/17	0/14, 0/14, 0/14, 0/14, 0/14

Mixture studies

SWGDAM guideline 2.8

"The ability to obtain reliable results from mixed source samples should be determined." (SWGDAM, July 2003)

Evidence samples may contain DNA from more than one individual. The possibility of multiple contributors should be considered when interpreting the results. We recommend that individual laboratories assign a minimum peak height threshold based on validation experiments performed in each laboratory to avoid typing when stochastic effects are likely to interfere with accurate interpretation of mixtures.

Mixture studies

Evidence samples that contain body fluids and/or tissues originating from more than one individual are an integral component of forensic casework. Therefore, it is essential to ensure that the DNA typing system is able to detect DNA mixtures. Mixed samples can be distinguished from single source samples in a variety of ways:

- The presence of greater than two alleles at a locus.
- The presence of a peak at a stutter position that is significantly greater in percentage than what is 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 MiniFiler™ Kit loci in unmixed population database samples are shown in Table 8.

Table 8 Peak height ratios for 0.50 ng input DNA

Allele	Number of Observations (n)	Mean	Median	Minimum	Maximum
CSF1PO	781	87.9	89.3	57.9	100.0
D2S1338	911	87.0	88.8	52.3	100.0
D7S820	820	88.1	89.7	58.4	100.0
D13S317	733	88.4	90.4	50.5	100.0
D16S539	804	87.5	89.1	46.1	100.0
D18S51	906	87.9	89.2	55.1	100.0
D21S11	856	88.2	90.0	47.1	100.0
FGA	904	88.0	89.4	53.9	100.0

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
- SNP 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 be comprised (at a single locus) of any of the seven genotype combinations listed below.

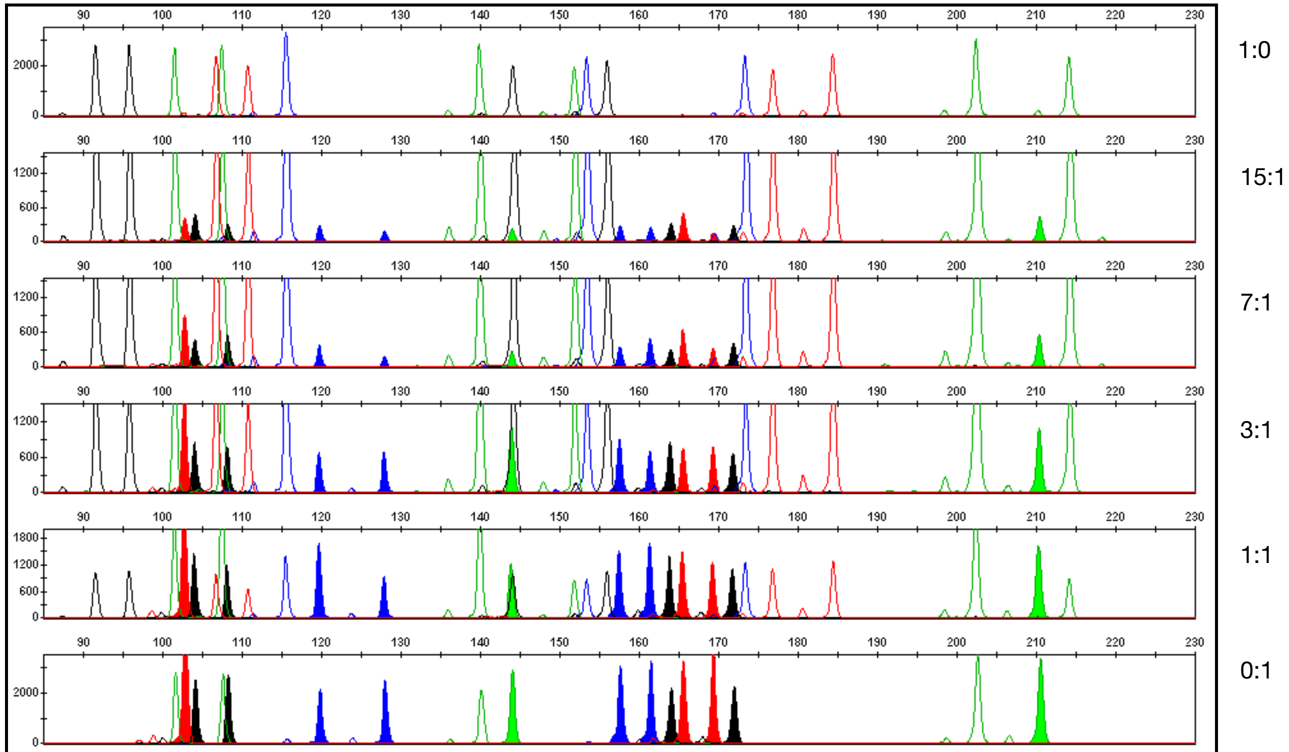
- Heterozygote + heterozygote, no overlapping alleles (four peaks)
- Heterozygote + heterozygote, one overlapping allele (three peaks)
- Heterozygote + heterozygote, two overlapping alleles (two peaks)
- Heterozygote + homozygote, no overlapping alleles (three peaks)
- Heterozygote + homozygote, overlapping 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 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 Life Technologies instruments provides additional valuable data to aid in resolving mixed genotypes. This quantitative value is much less subjective than comparing relative intensities of bands on a stained gel.

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).

Figure 19 Amplification of DNA mixtures at various ratios



Limit of detection of the minor component

Mixtures of two DNA samples were examined at various ratios (0:1, 1:1, 3:1, 7:1, 15:1, 1:0). The total amount of genomic input DNA mixed at each ratio was 1 ng. The samples were amplified in a GeneAmp™ PCR System 9700 and were electrophoresed and detected using an Life Technologies 3130*xl* Genetic analyzer.

The results of the mixed DNA samples are shown in Figure 19 above, where samples A and B were mixed according to the ratios provided. The minor component allele calls at non-overlapping loci are highlighted. The amplification of the minor contributor at 3:1 and 7:1 (0.875:0.125 ng) mixture ratios was readily typeable. 15:1 ratios generally resulted in partial profiles for the minor component.

The profiles of the samples in Figure 19 are shown in Table 9.

Table 9 Genotypes of mixed DNA samples

Allele	Profile Sample A (Control DNA 007)	Profile Sample B
D13S317	11	12, 14
D7S820	7, 12	8, 9
Amelogenin	X, Y	X, Y
D2S1338	20, 23	20, 21
D21S11	28, 31	28, 30
D16S539	9, 10	12, 13
D18S51	12, 15	17, 19
CSF1PO	11, 12	10
FGA	24, 26	21, 22

The MiniFiler kit has been optimized to amplify and type approximately 0.50 to 0.75 ng of single source DNA reliably.

Population data

SWGDAM guideline 2.7

"The distribution of genetic markers in populations should be determined in relevant population groups." (SWGDAM, July 2003)

Overview

To interpret the significance of a match between genetically typed samples, you must know the population distribution of alleles at each locus in question. If the genotype of the relevant evidence sample is:

- Different from the genotype of a suspects reference sample, then the suspect is excluded as the donor of the biological evidence that was tested. An exclusion is independent of the frequency of the two genotypes in the population.
- The same as the genotype of a suspects reference sample, then the suspect is included as a possible source of the evidence sample.

The probability that another, unrelated individual would also match the evidence sample is estimated by the frequency of that genotype in the relevant population(s).

The MiniFiler™ Kit contains loci for which extensive population data are available. For additional information on 11 loci shared between many of the AmpFℓSTR™ kits, see the population data and additional studies section of the *AmpFℓSTR™ Identifiler™ PCR Amplification Kit User Guide* (Part no. 4323291).

Analyzing the four databases

Analysis across the four databases of 2274 total chromosomes per locus revealed the following number of different alleles: 10 CSF1PO alleles, 13 D2S1338 alleles, 9 D7S820 alleles, 8 D13S317 alleles, 8 D16S539 alleles, 20 D18S51 alleles, 26 D21S11 alleles, and 31 FGA alleles.

In addition to the alleles that were observed and recorded in the Life Technologies databases, other alleles have been published or reported to us by other laboratories (see the STRBase at **www.cstl.nist.gov/div831/strbase**).

Low-frequency alleles

Some alleles of the MiniFiler™ Kit loci occur at a low frequency. For these alleles, a minimum frequency (five divided by 2n, where n equals the number of individuals in the database) was assigned for the MiniFiler™ Kit African-American, U.S. Caucasian, U.S. Hispanic and Native American databases, as suggested in the 1996 report of the Committee on DNA Forensic Science (National Research Council, 1996). These databases are summarized in Chapter 5 of the *AmpFℓSTR™ Identifiler™ PCR Amplification Kit User Guide* (Part no. 4323291). The minimum reportable genotype frequency at each locus is as follows:.

- 1.19×10^{-4} for the African-American database
- 1.19×10^{-4} for the U.S. Caucasian database
- 1.70×10^{-4} for the U.S. Hispanic database; and
- 2.97×10^{-4} for the Native American database
  [$p^2 + p(1-p)\,\theta$, where $\theta = 0.01$].

Therefore, the minimum combined multilocus genotype frequency at 8 loci is as follows:

- 4.02×10^{-32} for the African-American database;
- 4.02×10^{-32} for the U.S. Caucasian database;
- 6.98×10^{-31} for the U.S. Hispanic database; and
- 6.05×10^{-29} for the Native American database

Concordance studies

Primer relocation in the MiniFiler™ Kit could unintentionally lead to allele imbalance or allele dropouts that are not found in the Identifiler™ kit. These may be caused by a SNP or a deletion in the primer binding site. Experimental data was used to quantitate allele calling differences between the MiniFiler™ Kit and the Identifiler™ Kit.

We analyzed 1,064 samples (353 Caucasians, 347 African Americans, 207 Hispanics, and 157 Asians) by comparing allele calls between the Identifiler™ Kit and MiniFiler™ Kit. In the majority of samples analyzed, the results were found to be concordant between the kits with minor discordancy found in few exceptions. Discordant data were found in D13S317 (1.73% in African Americans, 0.57% in Caucasians, and 1.45% in Hispanics), D7S820 (0.29% in African Americans), CSF1PO (0.48% in Hispanics), D16S539 (1.73% in African Americans and 0.64% in Asians), and D18S51 (0.48% in Hispanics). The variants leading to discordant genotypes in the D13S317 locus have been characterized previously (Drabek, 2004).

Mutation rate

Estimating germ-line mutations

Estimation of spontaneous or induced germ-line mutation at genetic loci can be achieved by comparing the genotypes of offspring to those of their parents. From such comparisons the number of observed mutations are counted directly.

In previous studies, genotypes of ten STR loci that were amplified by the AmpFℓSTR SGM Plus™ PCR Amplification Kit were determined for a total of 146 parent-offspring allelic transfers (meioses) at the Forensic Science Service, Birmingham, England. One length-based STR mutation was observed at the D18S11 locus; mutations were not detected at any of the other nine STR loci. The D18S11 mutation was represented by an increase of one 4-nt repeat unit, allele 17 was inherited as allele 18 (single-step mutation). The maternal/paternal source of this mutation could not be distinguished.

Additional mutation studies

Additional studies (Edwards *et al.*, 1991; Edwards *et al.*, 1992; Weber and Wong, 1993; Hammond *et al.*, 1994; Brinkmann *et al.*, 1995; Chakraborty *et al.*, 1996; Chakraborty *et al.*, 1997; Brinkmann *et al.*, 1998; Momhinweg *et al.*, 1998; Szibor *et al.*, 1998) of direct mutation rate counts produced:

- Larger sample sizes for some of the MiniFiler™ Kit loci.
- Methods for modifications of these mutation rates (to infer mutation rates indirectly for those loci where the rates are not large enough to be measured directly and/or to account for those events undetectable as Mendelian errors).

Probability of identity

Table 10 shows the Probability of identity (P_I) values of the MiniFiler™ Kit loci individually and combined. The P_I value is the probability that two individuals selected at random will have an identical MiniFiler™ Kit genotype (Sensabaugh, 1982). The P_I values for the populations described in this section are then approximately $1/1.53 \times 10^{10}$ (African-American), $1/1.22 \times 10^{10}$ (U.S. Caucasian), $1/9.57 \times 10^{9}$ (U.S. Hispanic), and $1/4.82 \times 10^{9}$ (Native American).

Table 10 Probability of identity (P_I) values for the MiniFiler™ Kit loci

Locus	African-American	Caucasian	Hispanic	Native American
CSF1PO	0.079	0.132	0.141	0.123
D2S1338	0.023	0.027	0.038	0.043
D7S820	0.085	0.063	0.083	0.081
D13S317	0.132	0.079	0.056	0.056
D16S539	0.077	0.097	0.090	0.082
D18S51	0.033	0.031	0.031	0.046
D21S11	0.037	0.044	0.047	0.074
FGA	0.034	0.035	0.032	0.031
Combined	6.52×10^{-11}	8.21×10^{-11}	1.05×10^{-10}	2.08×10^{-10}

Probability of paternity exclusion

Table 11 shows the Probability of paternity exclusion (P_E) values of the MiniFiler™ Kit STR loci individually and combined.

Table 11 Probability of paternity exclusion (P_E) values for the MiniFiler™ Kit loci

Locus	African-American	Caucasian	Hispanic	Native American
CSF1PO	0.545	0.496	0.450	0.409
D2S1338	0.748	0.725	0.671	0.399
D7S820	0.591	0.582	0.574	0.492
D13S317	0.383	0.487	0.638	0.370
D16S539	0.649	0.566	0.567	0.428
D18S51	0.760	0.731	0.767	0.329
D21S11	0.737	0.708	0.586	0.399
FGA	0.760	0.766	0.739	0.309
Combined	0.99985	0.99976	0.99970	0.98188

The P_E value is the probability, averaged over all possible mother-child pairs, that a random alleged father will be excluded from paternity after DNA typing using the MiniFiler™ Kit STR loci (Chakraborty, Stivers, and Zhong, 1996).

Section 5.2 Performance Validation After Buffer and Enzyme Component Replacement

Overview

As part of an ongoing program to exercise greater control over raw materials used in the AmpFℓSTR™ PCR Amplification Kits, manufacturing of the AmpliTaq Gold™ enzyme and 10X PCR Buffer II (Tris-KCl buffer) components is transitioning from Roche Molecular Systems to Life Technologies. Manufacturing of both components by Life Technologies will be conducted according to the same specifications used previously by Roche. The in-house components are established raw materials in our next generation kits (for example, the NGM™, NGM SElect™ and Identifiler™ Plus Kits).

Experiments

We performed studies to compare the performance of the MiniFiler™ Kit containing the in-house components (updated kit) with the performance of the original kit, focussing on studies most relevant to forensic DNA testing (see SWGDAM Guidelines effective January 1, 2011). These studies, while not exhaustive, are in our opinion appropriate for a manufacturer.

Our studies compared the performance of two Roche-manufactured enzyme and buffer lots (Control mixes) with three new lots of buffer and two new lots of enzyme manufactured by Life Technologies (Test mixes). Studies were performed using Test mixes containing both the enzyme and buffer manufactured by Life Technologies.

Test Material	Control A mix	Control B mix	Test A mix	Test B mix	Test C mix
Buffer	Control Buffer Lot 1	Control Buffer Lot 2	Test Buffer Lot 1	Test Buffer Lot 2	Test Buffer Lot 3
Enzyme	Control Enzyme Lot 1	Control Enzyme Lot 2	Test Enzyme Lot 1	Control Enzyme Lot 2	Test Enzyme Lot 2

Each of the five mixes listed above were used to conduct reproducibility, sensitivity, degraded DNA, and inhibition studies. All amplifications were performed using a GeneAmp™ PCR System 9700 with either silver or gold-plated silver block using the recommended amplification conditions and cycle number for the MiniFiler™ Kit. All data was run on an Applied Biosystems™ 3130*xl* Genetic Analyzer running Data Collection Software v3.0 and analyzed using GeneMapper™ *ID-X* Software. Subsequent data analysis was performed using Minitab™ Statistical Software.

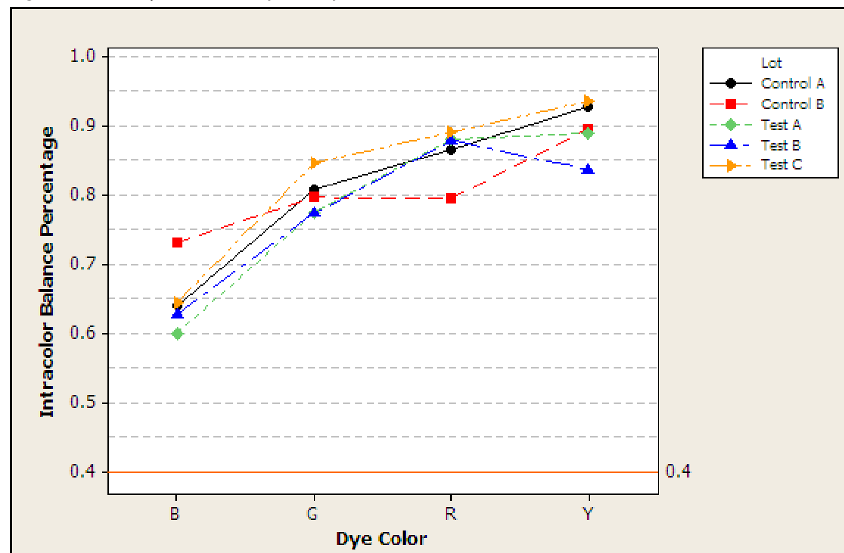
Reproducibility study

For the reproducibility study, 12 replicates of control DNA 007 at 0.5 ng input and three negative control replicates were amplified. The results were evaluated for intracolor balance, stutter percentage, and the presence, signal intensity, and location of artifacts.

Intracolor balance

No significant difference (<10% increase or decrease) in the level of intracolor balance was observed between the Test and Control mixes with the exception of Control B Mix, which showed slightly increased levels of intracolor balance for the FAM™ dye (blue) but decreased intracolor balance results for the PET™ dye (red) (Figure 20). The levels of intracolor balance obtained for all Test and Control mixes fall within the expected range of performance for the MiniFiler™ Kit.

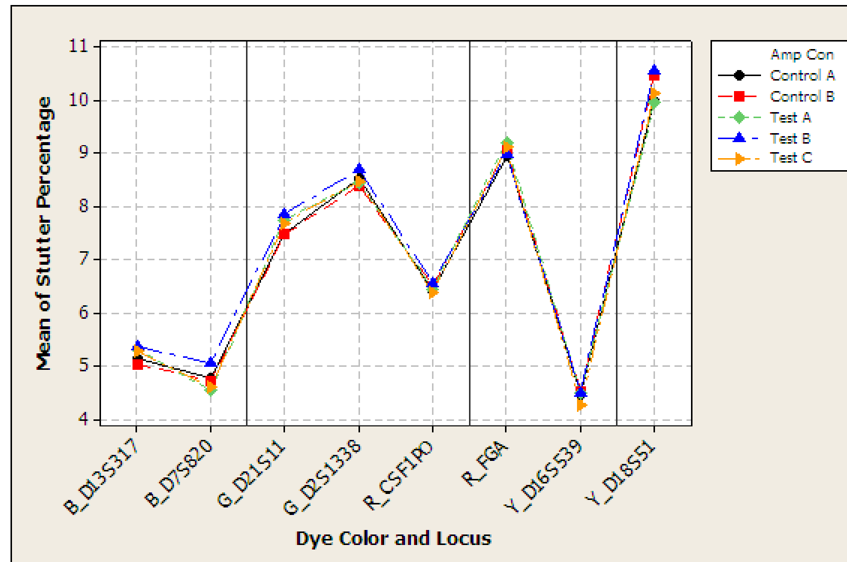
Figure 20 Reproducibility study: intracolor balance



Stutter percentages

Stutter percentage results for each marker were comparable across all Test and Control mixes (Figure 21).

Figure 21 Reproducibility study: mean stutter percentage

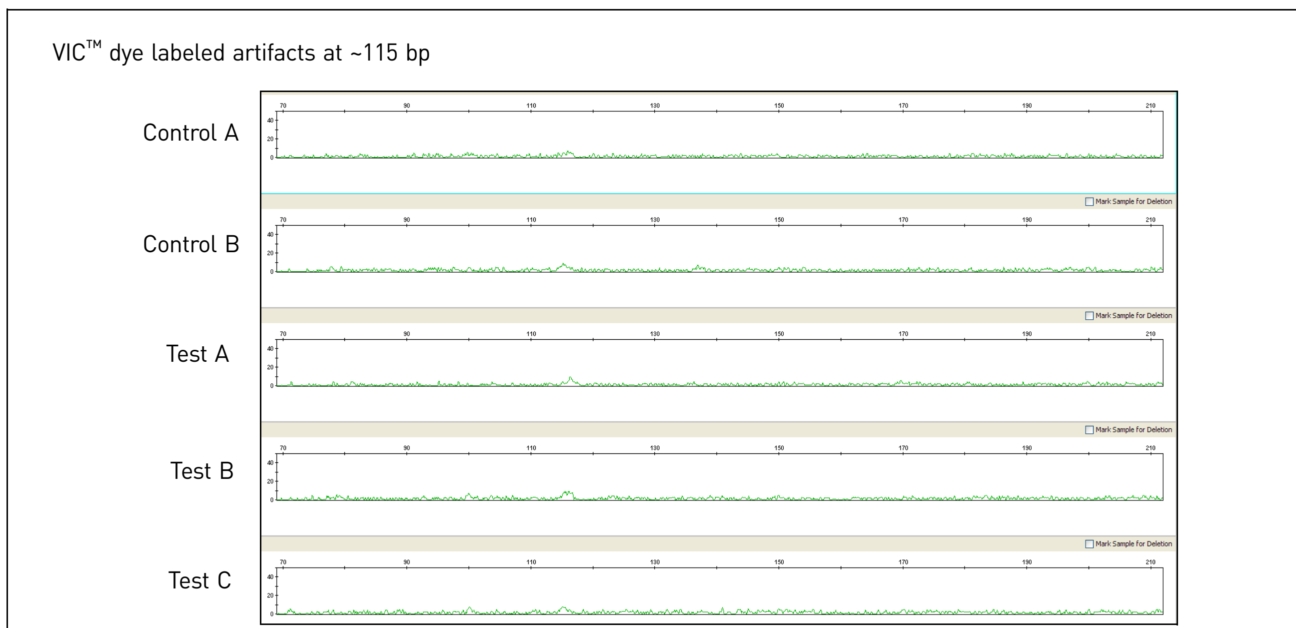


Artifacts

Known artifacts observed showed the same morphology, signal intensity, and location in all Test and Control mixes and do not exceed 50 RFU (Figure 22). No new artifacts were observed in the Test mixes.

No artifacts were observed in the Test and Control mixes for the 6-FAM™ dye (blue), NED™ dye (yellow), and PET™ (red) dye. A very low level artifact was visible in the VIC™ (green) dye at ~115 bp for all Test and Control mixes but did not exceed 50 RFU.

Figure 22 Reproducibility study: known artifact VIC™ dye (Y-scale 50 RFU)



Sensitivity study

For the sensitivity study, dilution series of three genomic DNA samples were amplified: 0.75 ng (three replicates), 0.5 ng, 0.25 ng, and 0.125 ng (four replicates each). The results were evaluated for mean referenced peak height, degree of linearity between input DNA concentration and peak height, level of allelic dropout at 125 pg, and genotype concordance.

Mean referenced peak height

Overall mean referenced peak height observations were consistent between all Test and Control mixes (Figure 23) demonstrating equivalent performance (Figure 24).

Figure 23 Sensitivity study: mean peak heights for three genomic DNA samples

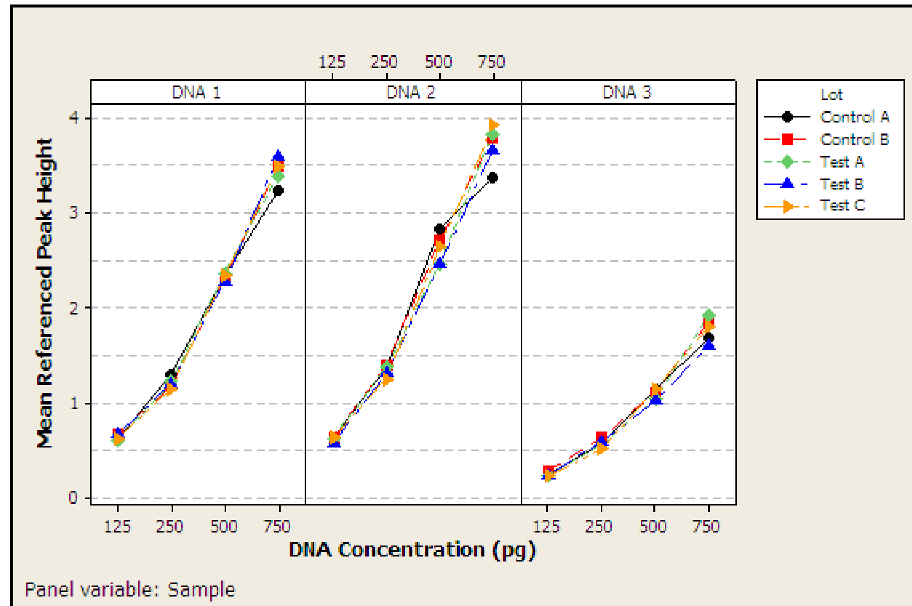
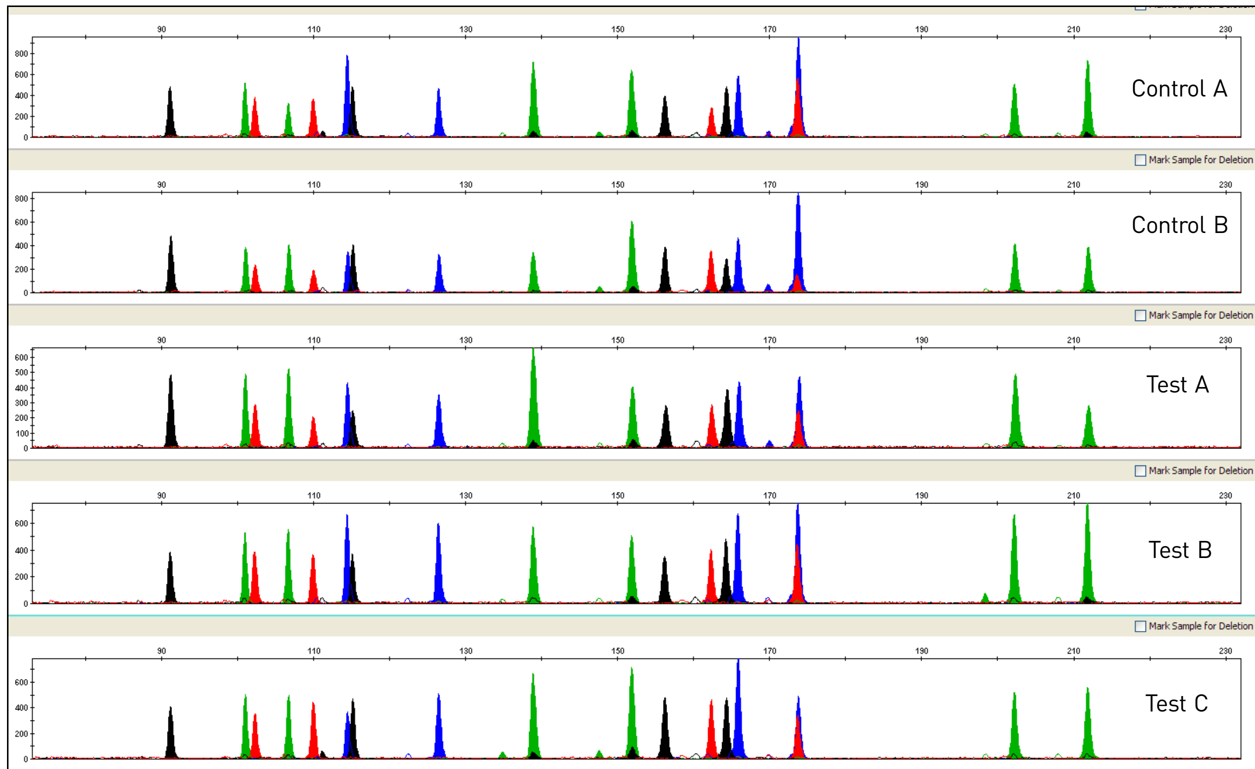


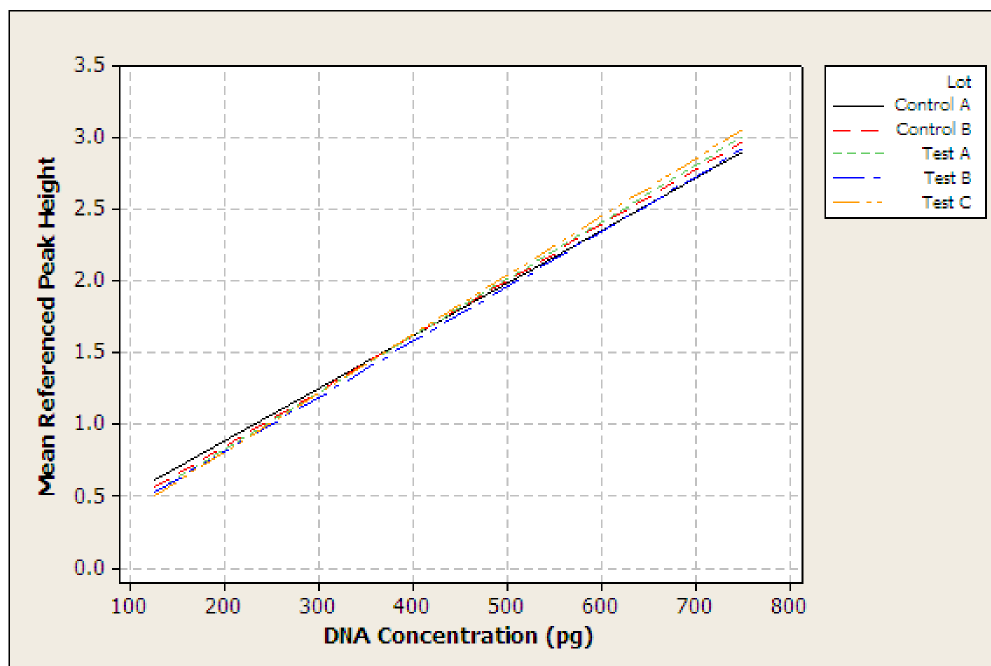
Figure 24 Sensitivity study: representative electropherograms for Sample 2 amplified using 250 pg input DNA (Y-scale 500 RFU)



DNA concentration and peak height

The calculated slope and R^2 values for each of the plotted curves are equivalent, showing comparable relationships between peak height and DNA input amount for the Test and Control mixes (Figure 25).

Figure 25 Sensitivity study: linear regression plot of combined mean peak height for three genomic DNA samples



Allelic dropout

Allelic dropout was only visible at 125 pg input DNA concentration. Levels of allelic dropout at 125 pg were comparable across all Test and Control mixes and are compared in Table 12. Examples of allelic dropout are shown in Figure 26 and Figure 27.

Table 12 Sensitivity study: summary of allelic dropout observed at 125 pg input DNA concentration

Reagent Mix	Number of samples	Number of alleles expected	Number of alleles dropped	Percent of alleles dropped
Test A	3	51	2	4%
Test B	4	68	4	6%
Test C	4	68	2	3%
Control A	4	68	2	3%
Control B	4	68	4	6%

Figure 26 Sensitivity study: electropherogram of 125 ng Sample 3 amplified with Control B Mix. Two alleles are below the analysis threshold of 50 RFU: at the D18S51 locus in the NED™ dye (yellow); at the FGA locus in the PET™ dye (red) (Y-scale 400 RFU)

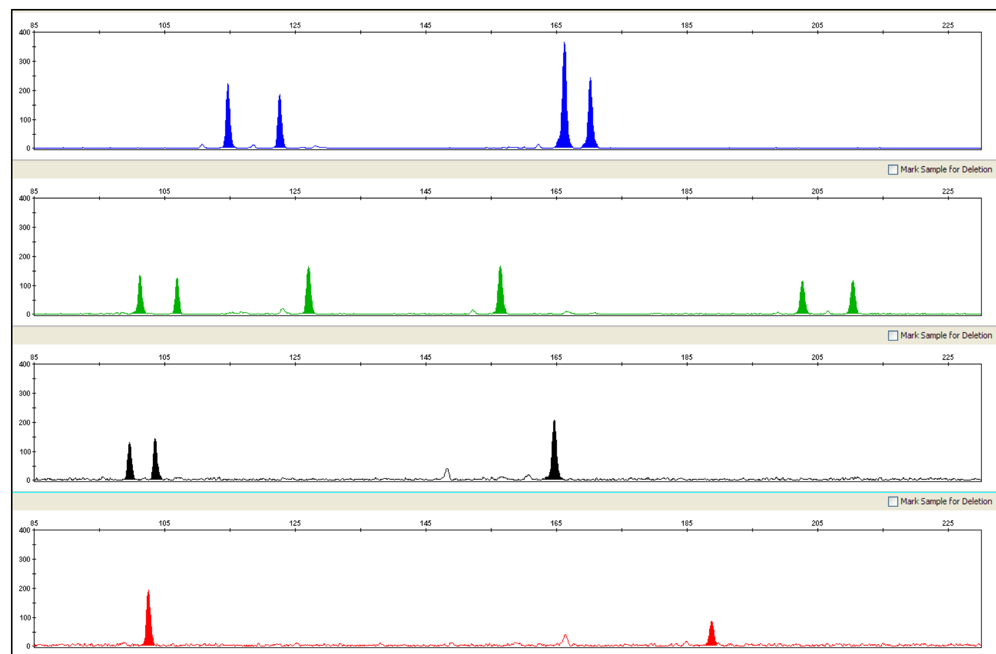
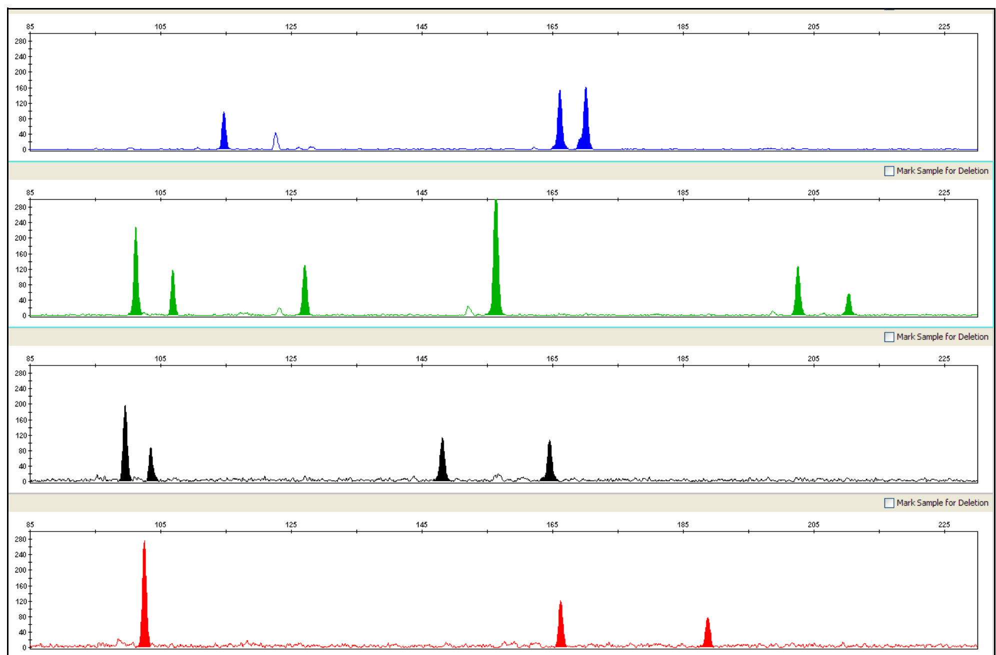


Figure 27 Sensitivity study: electropherogram of 125 ng Sample 3 amplified with Test B Mix. One allele at the D7S820 locus in the FAM™ dye (blue) is below the analysis threshold of 50 RFU: (Y-scale 300 RFU)



Genotype concordance

Genotypes for Test and Control mixes were 100% concordant (Table 13).

Table 13 Sensitivity study: genotype concordance

DNA Input Amount	Reagent Mix	Genotype Concordance
125 pg	Test A	100%
	Test B	100%
	Test C	100%
	Control A	100%
	Control B	100%
250 pg	Test A	100%
	Test B	100%
	Test C	100%
	Control A	100%
	Control B	100%
500 pg	Test A	100%
	Test B	100%
	Test C	100%
	Control A	100%
	Control B	100%
750 pg	Test A	100%
	Test B	100%
	Test C	100%
	Control A	100%
	Control B	100%

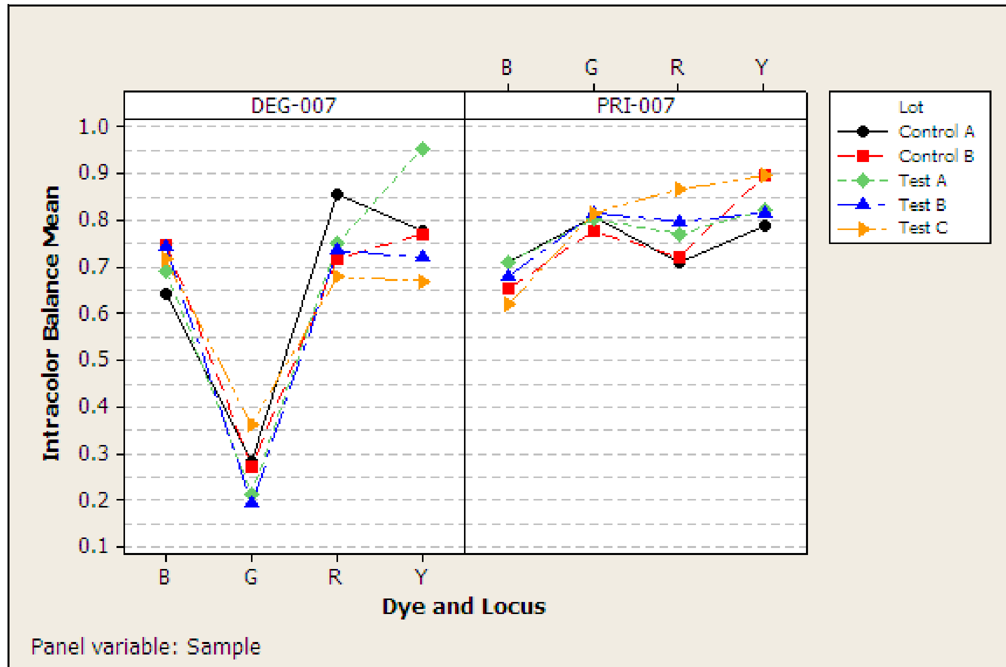
Degraded DNA study

To reflect the specific design of the MiniFiler™ Kit for degraded samples, 5 replicates of 0.5 ng degraded Control DNA 007 DNA and 5 replicates of 0.5 ng pristine Control DNA 007 were amplified. Results were evaluated for intracolor balance, mean referenced peak height, and levels of allelic dropout (degraded DNA replicates only). Degraded DNA was prepared by first sonicating the DNA then treating with 1 U DNAse I enzyme for increasing time increments to simulate increasing levels of degradation. A final input DNA concentration of 500 pg was used for all amplifications.

Intracolor balance

No significant difference (<10% increase of decrease) in the level of intracolor balance was observed between the Test and Control mixes on either degraded or pristine DNA with the exception of Test A Mix, which showed higher levels of intracolor balance for the NED™ (yellow) dye in degraded samples. The levels of intracolor balance obtained for all Test and Control mixes fall within the expected range of performance for the MiniFiler™ Kit.

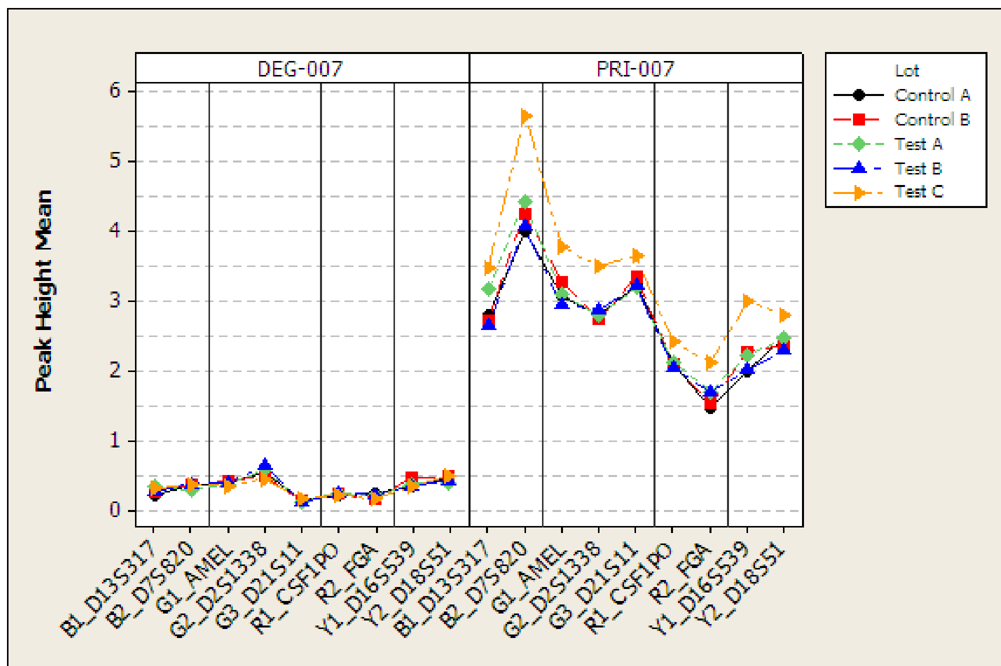
Figure 28 Degraded DNA study: intracolor balance 0.5 ng input DNA amount



Mean referenced peak height

Overall mean referenced peak height observations were consistent between all Test and Control mixes with the exception of Test C Mix on pristine DNA, which showed slightly higher referenced peak heights overall. The mean referenced peak height results for all Test and Control mixes fall within the expected range of performance for the MiniFiler™ Kit.

Figure 29 Degraded DNA study: intracolor balance 0.5 ng input DNA amount



Allelic dropout

Simulated degraded samples showed an overall drop in peak height compared to pristine DNA samples. Peak height of the higher molecular weight loci showed a greater drop in peak height compared to lower molecular weight loci, in some cases leading to allelic dropout. This is representative of the typical pattern observed in partially degraded samples. Profile morphology and levels of allelic dropout were comparable across all Test and Control mixes demonstrating equivalent performance.

Degraded DNA study: summary of Allelic Dropout observed at 125 pg input DNA concentration

Reagent Mix	Number of samples	Number of alleles expected	Number of alleles dropped	Percent of alleles dropped
Control A	5	85	3	4%
Control B	5	85	9	11%
Test A	5	85	12	14%
Test B	5	85	5	6%
Test C	5	85	13	15%

Figure 30 Degraded DNA study: representative electropherograms from 500 pg input DNA amplifications of simulated degraded DNA samples (Y-scale 1000 RFU)

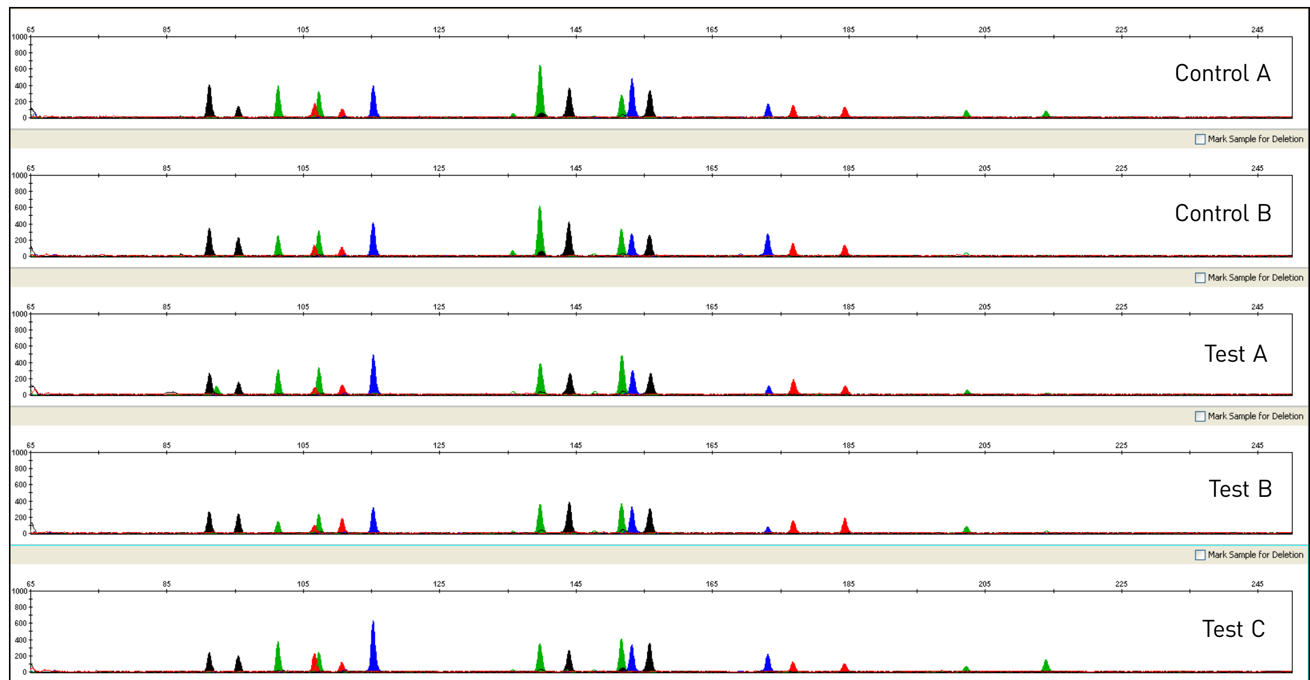
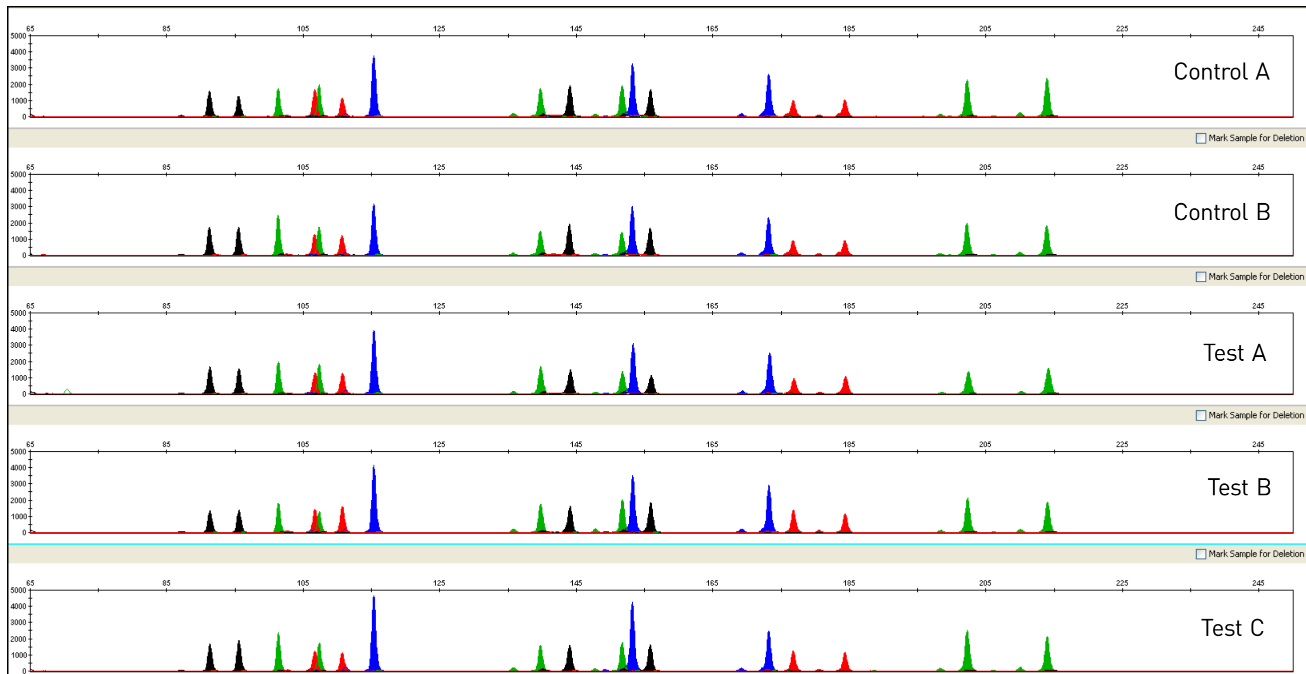


Figure 31 Degraded DNA study: representative electropherograms from 500 pg input DNA amplifications of pristine DNA samples for comparison to simulated degraded samples (Y-scale 5000 RFU)



Inhibition study

An inhibition series of 0.5 ng control DNA 007 consisting of uninhibited control, humic acid at a final concentration of 50 ng/µL, and hematin at a final concentration of 45 µM in replicates of five were amplified using each of Test and Control mixes. The amount of each inhibitor tested was titrated to cause an approximate 50% reduction in overall peak height of the samples. Results were evaluated for mean peak height, minimum peak height, intracolor balance, and levels of allelic dropout.

Mean peak height, minimum mean peak height, and intracolor balance

No significant difference in mean peak height or mean minimum peak height was observed for any Test or Control mixes tested on Control DNA 007 inhibited with hematin or humic acid. A significant increase in intracolor balance was observed only for Control A Mix on Control DNA 007 inhibited with Hematin (Figure 32, 33, and 34).

More variation was seen in mean peak height, mean minimum peak height, and intracolor balance on uninhibited DNA. This is not unexpected because the MiniFiler™ Kit was designed and developed for use on inhibited or degraded samples and is optimized for performance on such sample types. All results obtained for all Test and Control mixes fall within the expected range of performance for the MiniFiler™ Kit.

Figure 32 Inhibition study: mean referenced peak height

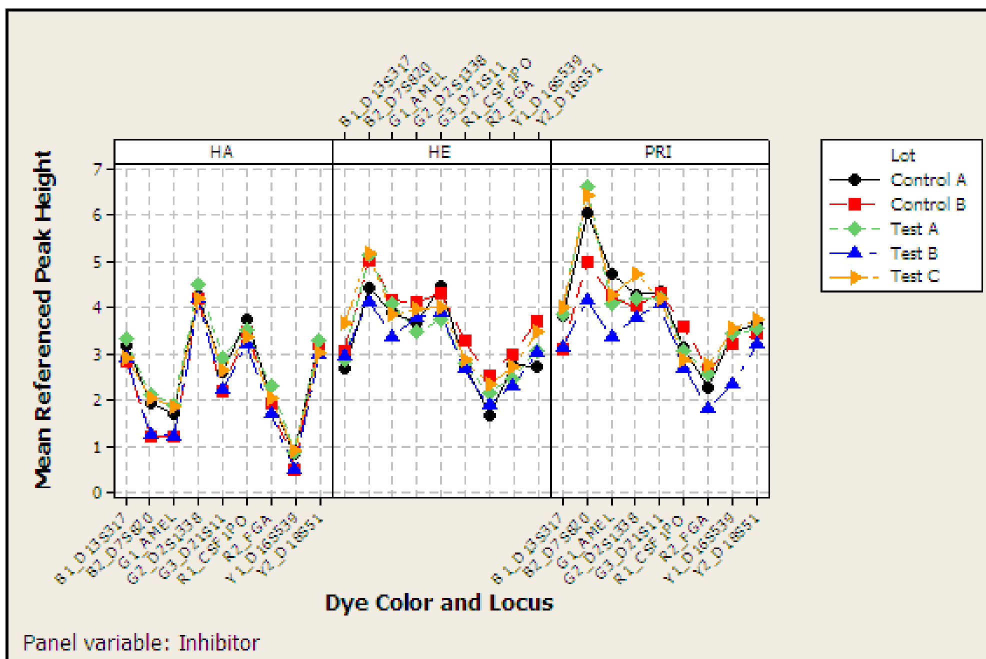


Figure 33 Inhibition study: minimum referenced peak height

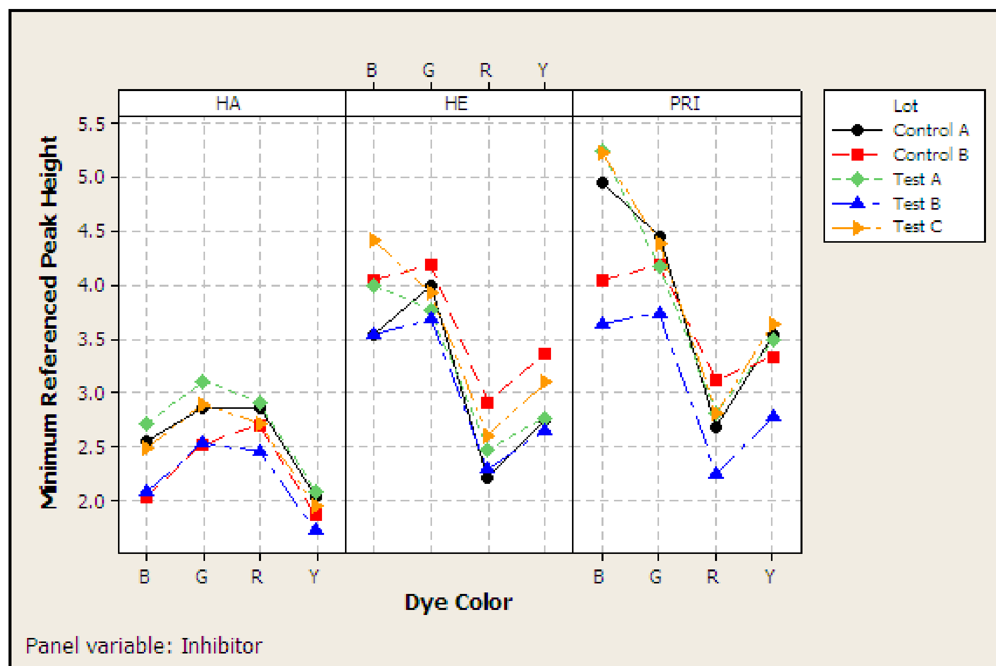
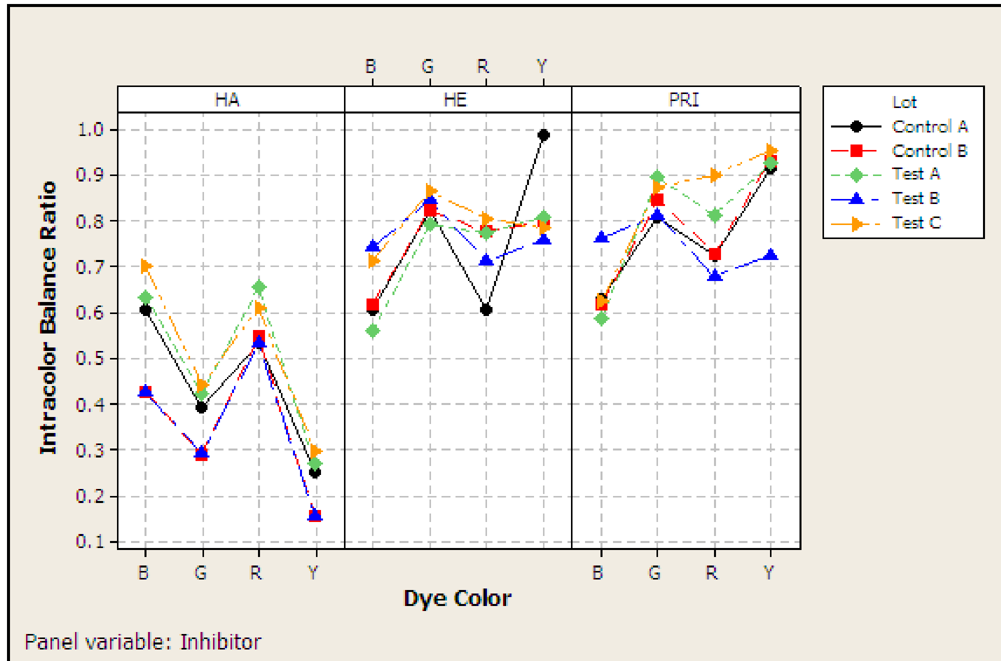


Figure 34 Inhibition study: intracolor balance



Representative electropherograms from the inhibition study are shown in Figure 35, Figure 36, and Figure 37.

Figure 35 Inhibition study: representative electropherograms using uninhibited Control DNA 007 (Y-scale 3000 RFU)

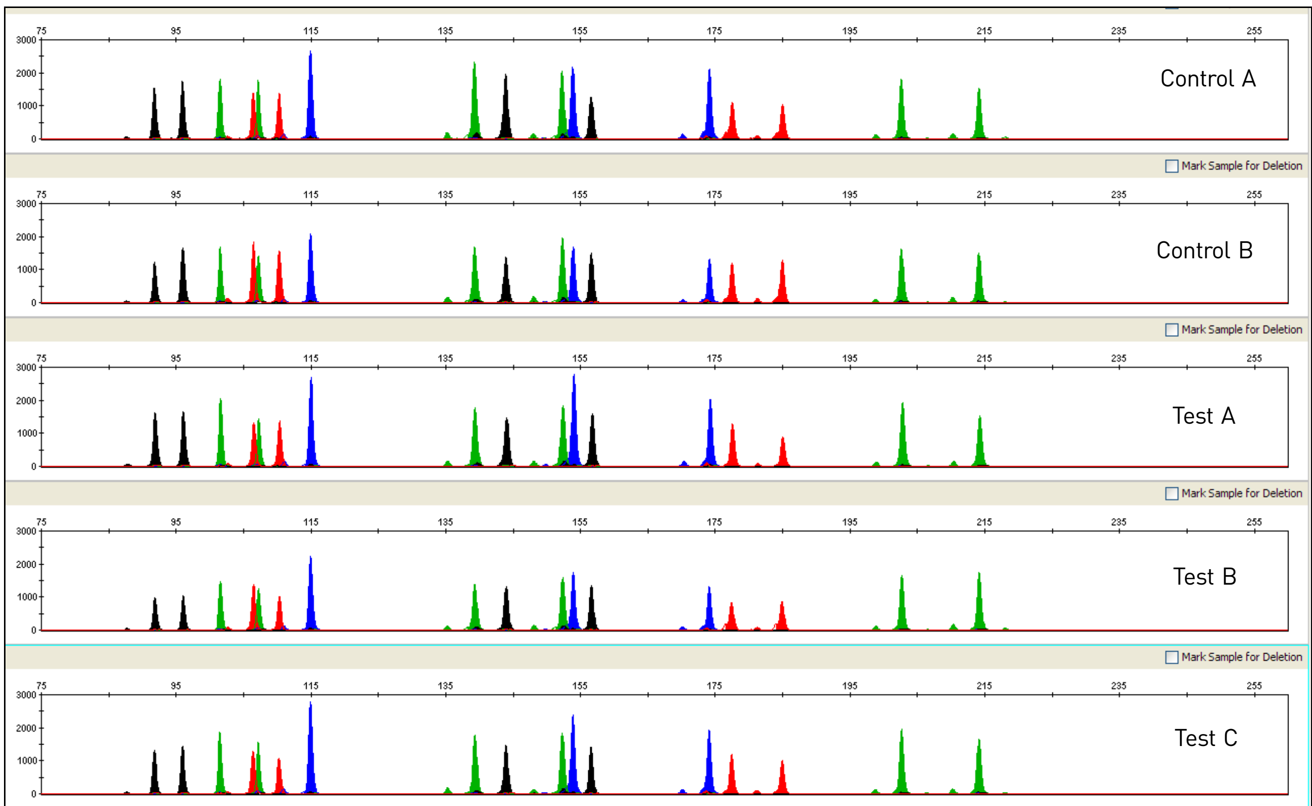


Figure 36 Inhibition study: representative electropherograms using Control DNA 007 inhibited with 45 μM Hematin (Y-scale 3000 RFU)

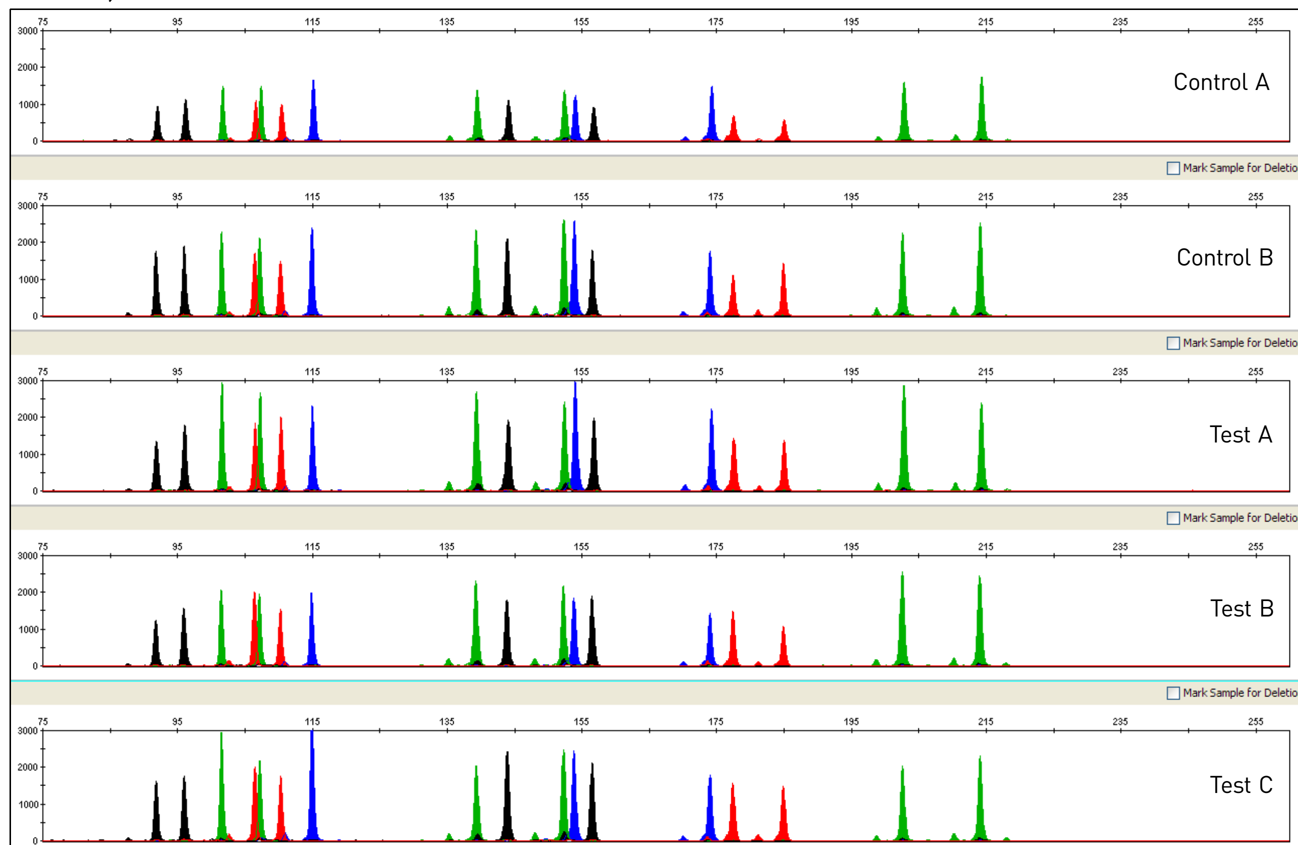
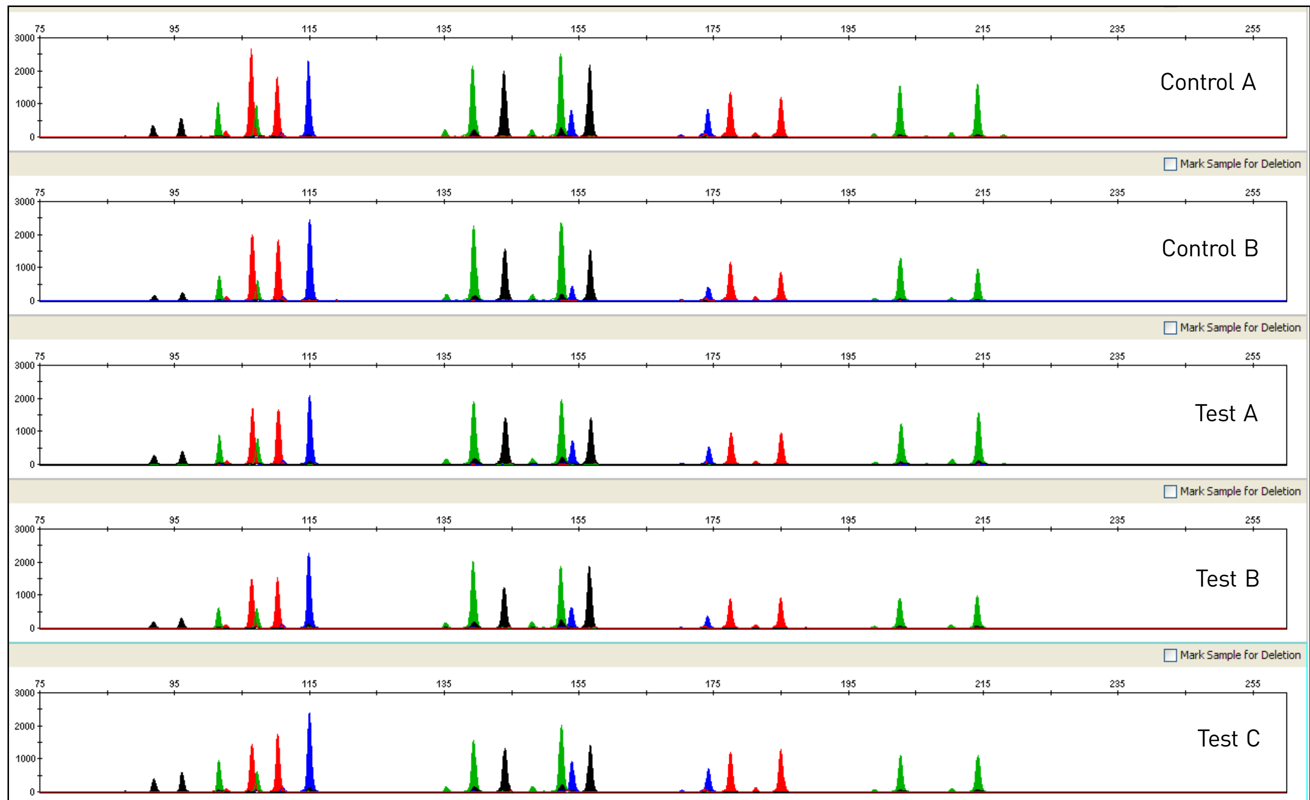


Figure 37 Inhibition study: representative electropherograms using Control DNA 007 inhibited with 50 ng/µL Humic Acid (Y-scale 4000 RFU)



Allelic dropout

No allelic dropout events were seen for any Test or Control mixes tested on uninhibited Control DNA 007 and Control DNA 007 inhibited with hematin or humic acid.

Conclusions

Laboratories can expect to obtain equivalent quality profiles across a wide range of forensic samples when using the MiniFiler™ Kit containing the AmpliTaq Gold™ enzyme and 10X PCR Buffer II manufactured by Life Technologies as compared to the original MiniFiler™ Kit containing AmpliTaq Gold™ enzyme and 10X PCR Buffer II manufactured by Roche Molecular Systems.

Troubleshooting

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

Table 14 Troubleshooting

Observation	Possible causes	Recommended actions
Faint or no signal from both the Control DNA 007 and the DNA test samples at all loci	Incorrect volume or absence of Master Mix or Primer Set	Repeat amplification.
	No activation of DNA Polymerase	Repeat amplification, making sure to hold reactions initially at 95°C for 1 minute.
	Master Mix not vortexed thoroughly before aliquoting	Vortex the Master Mix thoroughly.
	Primer Set exposed to too much light	Store the Primer Set protected from light.
	PCR System malfunction	Refer to the thermal cycler user's manual and check instrument calibration.
	Use of incorrect thermal cycling parameters	Check the protocol for correct thermal cycling parameters.
	MicroAmp™ Base used with tray/ retainer set and tubes in GeneAmp™ 9700	Remove MicroAmp Base from tray/retainer set and repeat test.
	Insufficient PCR product electrokinetically injected	Prepare PCR product as described in Chapter 3, "Perform Electrophoresis" on page 25.
	Degraded formamide	Check the storage of formamide; do not thaw and refreeze multiple times. Try Hi-Di™ Formamide.
Positive signal from Control DNA 007 but partial or no signal from DNA test samples	Quantity of test DNA sample is below assay sensitivity	Quantitate DNA and add 0.5 to 0.75 ng of DNA. Repeat test.
	Test sample contains high concentration of PCR inhibitor (e.g., heme compounds, certain dyes)	Quantitate DNA and add minimum necessary volume. Repeat test.
		Wash the sample in a Centricon™-100. 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, re-amplify with an increased amount of DNA.
	Dilution of test sample DNA in water or wrong buffer (for example, wrong EDTA concentration)	Redilute DNA using TE Buffer (with 0.1-mM EDTA).

Observation	Possible causes	Recommended actions
More than two alleles present at a locus	Presence of exogenous DNA	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Too much DNA in reaction	Use recommended amount of template DNA (0.5 to 0.75 ng).
	Mixed sample	See "Stutter products" on page 75.
	Amplification of stutter product (1 repeat unit position)	
	Incomplete 3´ A base addition (n–1 nt position)	See "Experiments and Results" on page 63. Be sure to include the final extension step of 60°C for 5 minutes in the PCR.
	Signal exceeds dynamic range of instrument (off-scale data)	Ensure cycle number is optimized according to instructions on page 19. 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.
Some but not all loci visible on electropherogram of DNA Test Samples	Test sample DNA is severely degraded	If possible, evaluate the quality of DNA sample by running an agarose gel. If DNA is degraded, re-amplify with an increased amount of DNA.
	Test sample contains high concentrations of a PCR inhibitor for example, heme compounds, certain dyes)	Quantitate DNA and add minimum necessary volume. Repeat test.
		Wash the sample in a Centricon-100.

B The 3rd Order Least Squares Sizing Method

You can use the 3rd Order Least Squares Sizing Method as an alternative to the Local Southern method when analyzing MiniFiler™ Kit data.

When to use

We recommend using the 3rd Order Least Squares method to size data obtained when analyzing MiniFiler™ Kit data using the GeneScan™ 500 LIZ™ Size Standard.

The Least Squares methods (both 2nd Order and 3rd Order) use regression analysis to build a best-fit size-calling curve. This method is used to extrapolate sizes that extend beyond the physical range of the size standard. Small (< 75 nt) fragments generated by the MiniFiler™ Kit can be sized using the GeneScan™ 500 LIZ™ Size Standard.

About the Local Southern algorithm

For the highest level of accuracy, the Local Southern method requires two size-standard fragments below the smallest unknown fragment and two size-standard fragments above the largest unknown fragment. All AmpFℓSTR™ kits except the MiniFiler™ Kit, have an allele size range between 100 to 360 nt. Optimal genotyping accuracy of all kits other than MiniFiler, require detection of all of the GeneScan™ 500 LIZ™ Size Standard (or ROX™) fragments between 75 and 450 nt (or 75 and 400 nt depending on the kit).

In the MiniFiler™ Kit, the amplicon sizes for the large AmpFℓSTR™ loci have been reduced to improve genotyping performance with degraded and inhibited DNA samples. The allele range for the MiniFiler™ Kit is 70 to 283 nt. In order to use the Local Southern algorithm, at least one peak <70 nt would have to be detected.

Although the GeneScan™ 500 LIZ™ Size Standard does contain 50-nt and 35-nt size-standard peaks, they are often difficult or impossible to detect. The fragments are obscured by the primer front associated with the MiniFiler™ Kit amplifications.

Because fragment sizes cannot be extrapolated when using the Local Southern algorithm, we recommend the 3rd Order Least Squares algorithm as an alternative sizing method for the MiniFiler™ Kit.

For a full description of the Least Squares Method refer to the *GeneMapper™ ID Software Version 3.1 Human Identification Analysis: User Guide* (Part no. 4338775).

Comparing genotyping accuracy

We compared the Local Southern and 3rd Order Least Squares methods for genotyping accuracy, using a data set of 1,156 Identifiler™ Kit amplifications. The amplified samples were from a single source, electrophoresed on an Applied Biosystems™ 3100 or 3130*xl* instruments, and sized with the GeneScan™ 500 LIZ™ Size Standard. The samples were analyzed using both sizing methods and their allele calls were compared. The size standard definitions for both methods include all the peaks from 75 to 450 nt, with the exception of the 250-nt peak.

The genotyping results (n=36,000 alleles) for the two methods were compared for concordance. The genotyping accuracy rates for the Local Southern and 3rd Order Least Squares algorithms were equivalent. No alleles were labeled with an incorrect genotype and only a very small percentage (Local Southern, 0.05%; 3rd Order Least Squares, 0.008%) of the alleles were designated as off-ladder when they did not represent a true micovariant allele. All of the discordant off-ladder allele calls were within 0.08 nt of the ±0.5 nt off set for the bin sizing window.

Ordering Information

Equipment and materials not included

Table 15 Equipment

Equipment	Source
Applied Biosystems™ 3100/3100-*Avant* Genetic Analyzer	Contact your local Life Technologies sales representative
Applied Biosystems™ 3500/3500xL Genetic Analyzer	
Applied Biosystems™ 3130/3130*xl* Genetic Analyzer	
Veriti™ 96-Well Thermal Cycler	4375786
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
Tabletop centrifuge with 96-Well Plate Adapters (optional)	MLS (major laboratory supplier)

Table 16 User-supplied materials

Item†	Source
AmpFℓSTR™ MiniFiler™ PCR Amplification Kit	4373872
3100 Analyzer materials	
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3130*xl* Genetic Analyzer Capillary Array, 36-cm	4315931
POP-4™ Polymer for 3100/3100-*Avant* Genetic Analyzers	4316355
3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96-well	4316471
GeneScan™ 500 LIZ™ Size Standard *OR* GeneScan™ 600 LIZ™ Size Standard v2.0	4322682 *OR* 4408399
Running Buffer, 10X	402824
Hi-Di™ Formamide	4311320
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp™ Optical 96-Well Reaction Plate	N8010560
250-μL Glass Syringe (array-fill syringe)	4304470

Item†	Source
5.0-mL Glass Syringe (polymer-reserve syringe)	628-3731
For a complete list of parts and accessories for the 3100 instrument, refer to Appendix B of the *3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide* (Part no. 4335393).	
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
3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96-well	4316471
GeneScan™ 500 LIZ™ Size Standard *OR* GeneScan™ 600 LIZ™ Size Standard v2.0	4322682 *OR* 4408399
Running Buffer, 10✕	402824
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp™ Optical 96-Well Reaction Plate	N8010560
Hi-Di™ Formamide	4311320
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* (Part no. 4352716).	
3500/3500xL Analyzer materials	
Anode buffer container (ABC)	4393927
Cathode buffer container (CBC)	4408256
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
POP-4™ polymer (960 samples) for 3500/3500xL Genetic Analyzers	4393710
POP-4™ polymer (384 samples) for 3500/3500xL Genetic Analyzers	4393715
GeneScan™ 600 LIZ™ Size Standard v2.0	4408399
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
For a complete list of parts and accessories for the 3500/3500xL instrument, refer to the Applied Biosystems™ *3500/3500xL Genetic Analyzer User Guide* (PN 4401661).	

Item†	Source
310 Analyzer materials	
310 DNA Analyzer capillary array, 47-cm	402839
0.5 mL sample tray	5572
96-well tray adaptor (for 9700 thermal cycler trays)	4305051
GeneScan™ 500 LIZ™ Size Standard *OR* GeneScan™ 600 LIZ™ Size Standard v2.0	4322682 *OR* 4408399
Running Buffer, 10✕	4335643
Genetic analyzer septa retainer clips for 96-tube sample tray	402866
Genetic analysis sample tubes (0.5-mL)	401957
Septa for 0.5-mL sample tubes	401956
DS-33 Matrix Standard Set (6-FAM™, VIC™, NED™, PET™, and LIZ™ dyes) for 310/377 systems	4318159
MicroAmp™ 8-tube strip, 0.2-mL	N8010580
MicroAmp™ 96-well base (holds 0.2-mL reaction tubes)	N8010531
MicroAmp™ 96-well full plate cover	N8010550
MicroAmp™ 96-well tray/retainer set	403081
POP-4™ polymer for the 310 Genetic Analyzer	402838
For a complete list of parts and accessories for the 310 instrument, refer to Appendix B of the *310 Genetic Analyzer User Guide* (Pub. no. 4317588).	
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

Item[†]	Source
Vortex	MLS

† For the Safety Data Sheet (SDS) of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

PCR Work Areas

- Work area setup and lab design 121
- PCR setup work area 121
- Amplified DNA work area 122

Work area setup and lab design

Many resources are available for the appropriate design of a PCR laboratory. If you are using an AmpFℓSTR™ Kit for:

- Forensic DNA testing, refer to "Forensic Laboratories: Handbook for Facility Planning, Design, Construction and Moving," National Institute of Justice, 1998
- Parentage DNA testing, refer to the "Guidance for Standards for Parentage Relationship Testing Laboratories," American Association of Blood Banks, 7th edition, 2004

The sensitivity of AmpFℓSTR™ Kits (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:

- Veriti™ 96-Well Thermal Cycler (Part no. 4375786)
- GeneAmp™ PCR System 9700 with the Silver 96-Well Block
- GeneAmp™ PCR System 9700 with the Gold-plated Silver 96-Well Block

IMPORTANT! The MiniFiler™ Kit is validated for use with the Veriti™ 96-well Thermal Cycler (Part no. 4375786) and the GeneAmp™ PCR System 9700 with the silver block (Part no. N8050251) or the gold-plated silver block (Part no. 4314443).

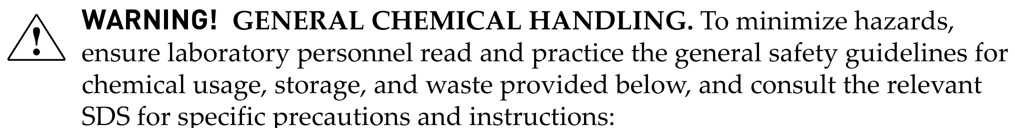
It is not verified for use with the Veriti™ 96-Well Fast Thermal Cycler (Part no. 4375305) or the GeneAmp™ PCR System 9700 with the aluminum block (Part no. 4314879).

E 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/**

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Documentation and Support

Related documentation

Document title	Part number
3100/3100-Avant Data Collection v2.0 User Guide	4347102
3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin	4350218
3100 Genetic Analyzer User Manual (Data Collection v1.1)	4315834
3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR™ *PCR Amplification Kit PCR Products User Bulletin*	4332345
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Life Technologies 3500/3500xL Genetic Analyzer User Bulletin: Solutions to issues related to software, data, hardware, and consumables **Note:** Additional user bulletins may be available at **www.lifetechnologies.com**	4445098
Life Technologies 3730/3730xl Genetic Analyzer Getting Started Guide	4359476
GeneAmp™ PCR System 9700 Base Module User's Manual	N805-0200
Quantifiler™ Kits: Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User's Manual	4344790
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