AmpFℓSTR™ MiniFiler™ PCR Amplification Kit

USER GUIDE

Catalog Numbers 4373872

Publication Number MAN0029851

Revision A

ThermoFisher SCIENTIFIC

Life Technologies Ltd | 7 Kingsland Grange | Woolston, Warrington WA1 4SR | United Kingdom
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0029851 A (English)

Revision	Date	Description
A	2 May 2025	New document for the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit; replaces Pub. No. 4374618. The following changes were made: • Storage conditions were updated (see "Contents and storage" on page 14). • Compatible instruments, compatible software, and materials required were updated (throughout the document). • Copy edits and formatting changes were made to align with current documentation style (throughout the document).
G	24 August 2018	Branding and trademarks were updated. No technical changes.
F	31 August 2012	• 3500/3500xL Genetic Analyzer and GeneMapper™ *ID-X* Software information was added. • Validation experiments and results for buffer and enzyme kit component changes were added.
E	31 March 2012	A change to limited licensing information was made.
D	30 April 2011	A change to limited licensing information was made.
C	31 December 2010	A change to limited licensing information was made.
B	31 March 2007	A new chapter was added (see Chapter 5, "Experiments and results").
A	31 October 2006	New document for the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit.

The information in this guide is subject to change without notice.

Contents

1 Product information

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

Product description

The AmpFℓSTR™ MiniFiler™ PCR Amplification Kit is a 5-dye, short tandem repeat (STR) multiplex assay for the direct amplification of human genomic DNA (single-source samples). The kit is optimized for genotyping degraded and/or inhibited DNA samples.

The kit amplifies the following:

- 8 autosomal STR loci: D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO and FGA
- Amelogenin (sex determining marker)

The loci span a range of 70–283 nucleotides with the aid of non-nucleotide linkers to achieve appropriate spacing between loci.

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™ PCR Amplification Kit and AmpFℓSTR™ SGM Plus™ PCR Amplification Kit. The comparison is shown in Table 1. 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 5-dye fluorescent system and the use of non-nucleotide linkers allows simultaneous

amplification and efficient separation of the 8 STR loci and Amelogenin during automated DNA fragment analysis.

Table 1 MiniFiler™ kit amplicon length reduction vs. the Identifiler™ and SGM Plus™ kits

Locus	Amplicon length reduction
D7S820	–129 nt
D13S317	–99 nt
D21S11	–33 nt
D2S1338	–183 nt
Amelogenin	0 nt
D18S51	–168 nt
D16S539	–157 nt
FGA	–87 nt
CSF1PO	–201 nt

Dyes used in the kit

Dye	Color	Label
6-FAM™	Blue	Samples, allelic ladders, and controls
VIC™	Green	
NED™	Yellow	
PET™	Red	
LIZ™	Orange	One of the following: • GeneScan™ 500 LIZ™ Size Standard • GeneScan™ 600 LIZ™ Size Standard v2.0

Loci amplified by the kit

Locus designation	Chromosome location	Alleles in the allelic ladder	Dye label	Alleles in DNA Control 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

Standards and controls that are required

The MiniFiler™ kit requires the following standards and controls for PCR amplification, PCR product sizing, and genotyping:

Item	Description	Included in the kit
DNA Control 007	Positive control. Used to evaluate amplification efficiency and to evaluate STR genotyping using the kit allelic ladder. See “DNA Control 007 profile” on page 12.	Yes
MiniFiler™ Allelic Ladder	Developed for accurate characterization of the alleles amplified in the kit. The allelic ladder allows automatic genotyping of most of the reported alleles for the loci in the kit. See “Loci amplified by the kit” on page 10 and “Allelic ladder profile” on page 13.	Yes

(continued)

Item	Description	Included in the kit
One of the following: • GeneScan™ 500 LIZ™ Size Standard (Cat. No. 4322682) • GeneScan™ 600 LIZ™ Size Standard v2.0 (Cat. No. 4408399)	Used for obtaining sizing results. This standard, which has been evaluated as an internal size standard, yields precise sizing results for PCR products.	No (order separately)

DNA Control 007 profile

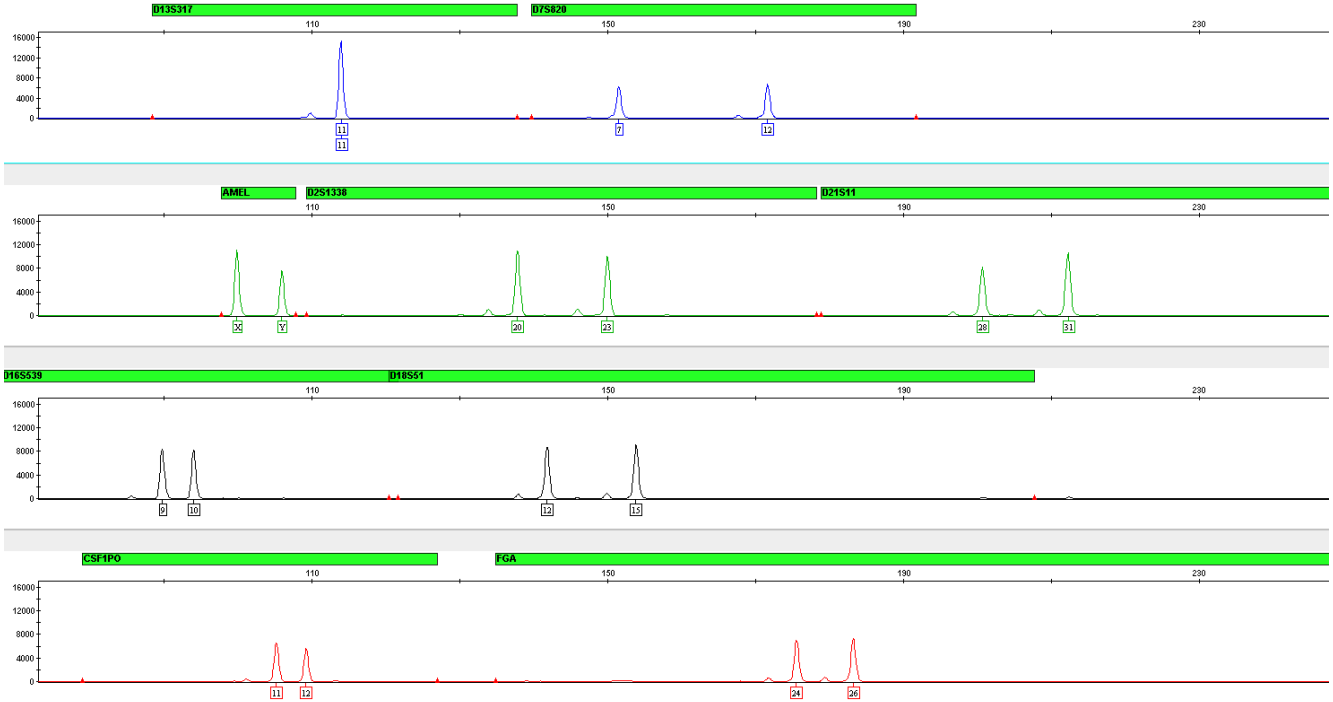


Figure 1 DNA Control 007 (1 ng) amplified with the MiniFiler™ kit and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0–16,000 RFU).

Allelic ladder profile

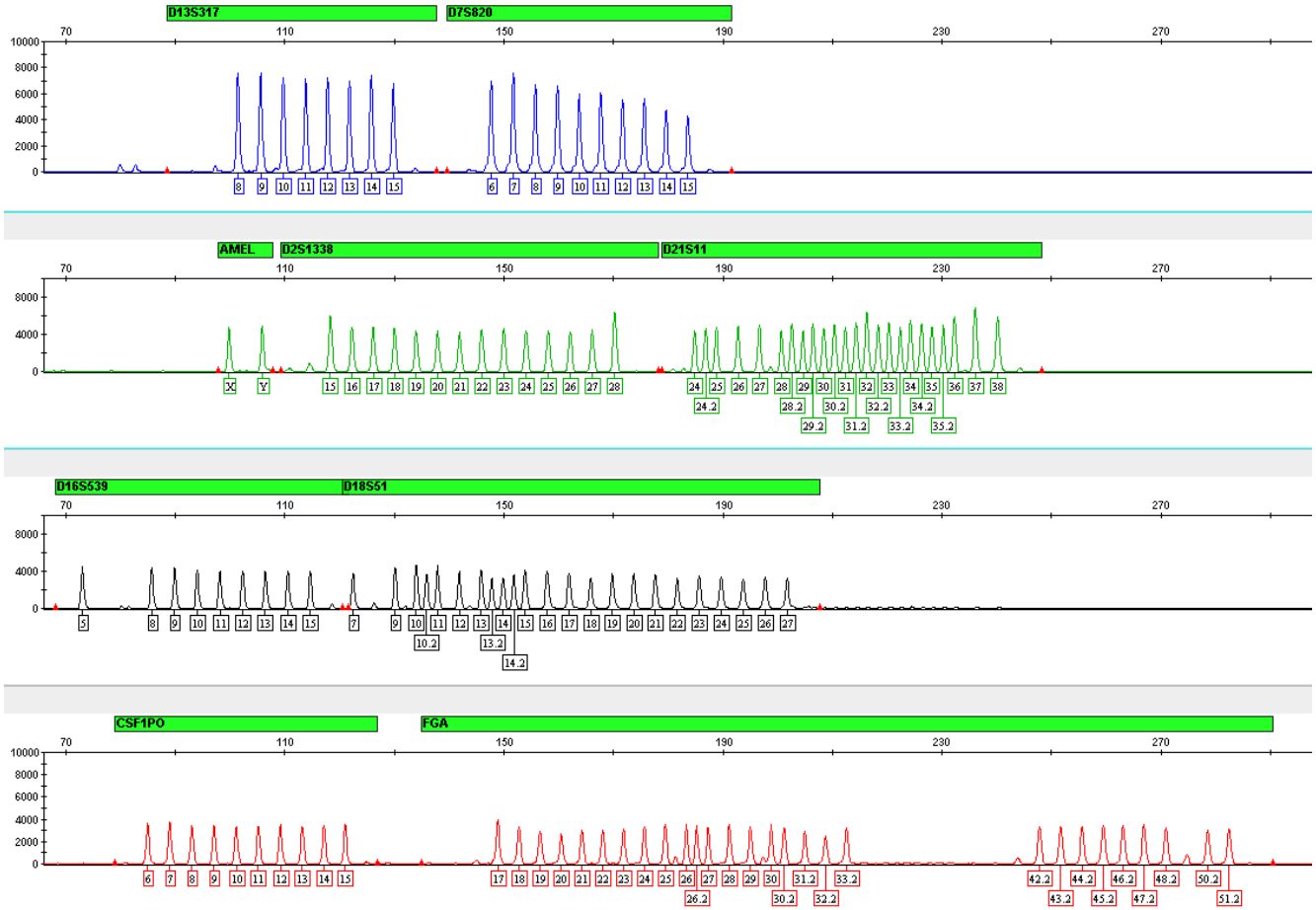


Figure 2 GeneMapper™ *ID-X* Software plot of the MiniFiler™ Allelic Ladder (Y-axis scale 0–8,000 RFU or 0–10,000 RFU).

Contents and storage

The MiniFiler™ kit contains sufficient quantities of the reagents for 100 (Cat. No. 4373872) amplification reactions at 25 µL/reaction.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set and allelic ladder from light when not in use.

IMPORTANT! The allelic ladder contains PCR products that should not be amplified. To avoid contamination, store the allelic ladder separately from the other kit components and unamplified DNA.

Note: If there is more than one tube or bottle for a single reagent, thaw only the number of tubes or bottles required for the current number of reactions.

IMPORTANT! Do not refreeze kit components after thawing.

Note: For recommendations on kit handling, go to **thermofisher.com**, then search for *Technical Note: Handling STR Kits and Ladder Decontamination*, or contact your local Human Identification representative.

Item	Description	Amount	Storage[1]
		100 reactions (Cat. No. 4373872)	
MiniFiler™ Master Mix	Contains enzyme, salts, dNTPs, bovine serum albumin, and 0.05% sodium azide in buffer and salt.	2 × 0.5 mL	–25°C to –15°C on receipt. 2–8°C after first use, up to the expiration date stated on the kit.
DNA Control 007	Contains 0.1 ng/µL human male genomic DNA in 0.05% sodium azide and buffer.[2] See "DNA Control 007 profile" on page 12.	1 × 0.3 mL	
MiniFiler™ Primer Set	Contains forward and reverse primers to amplify human DNA targets.	1 × 0.5 mL	–25°C to –15°C on receipt. 2–8°C after first use, up to the expiration date stated on the kit. Store protected from light.
MiniFiler™ Allelic Ladder	Contains amplified alleles. See "Allelic ladder profile" on page 13.	1 × 0.05 mL	

[1] See packaging for expiration date. Do not use expired product.

[2] DNA Control 007 is included at a concentration that is appropriate for use as an amplification control (that is, to provide confirmation that the kit reagents can generate a profile of expected genotype). It is not designed for use as a DNA quantification control. If you quantify aliquots of DNA Control 007, the concentration may differ from the labeled concentration.

Required materials not supplied

See Appendix C, “Materials required but not supplied”.

Instruments and software compatibility

Note: Compatible instruments and software that have been discontinued are not listed in this user guide.

Thermal cyclers

- HID VeritiPro™ Thermal Cycler, 96-well
- ProFlex™ 96-well PCR System
- ProFlex™ 2 × 96-well PCR System
- ProFlex™ 3 × 32-Well PCR System

Genetic analyzers and data collection software

Genetic analyzer	Data collection software
SeqStudio™ Flex Series Genetic Analyzer for Human Identification	SeqStudio™ Flex Series Instrument Software v1.1.1
SeqStudio™ Genetic Analyzer for HID	SeqStudio™ Data Collection Software v1.2.5
	SeqStudio™ Data Collection Software v1.2.4
	SeqStudio™ Data Collection Software v1.2.1
3500 Series Genetic Analyzer for Human Identification	3500 Series HID Data Collection Software v4.0.1
	3500 Series Data Collection Software 4 (Windows™ 10 operating system)

Analysis software

Genetic analyzer	Analysis software
SeqStudio™ Flex Series Genetic Analyzer for Human Identification	GeneMapper™ *ID-X* Software v1.7.2 or later
SeqStudio™ Genetic Analyzer for HID	GeneMapper™ *ID-X* Software v1.6 or later
3500 Series Genetic Analyzer for Human Identification	GeneMapper™ *ID-X* Software v1.5 or later

For more information

- For the instruments and software used during the kit validation, see Chapter 5, “Experiments and results”.
- For testing information on specific platforms, see the instrument or software user documentation.
- For ordering information, see Appendix C, “Materials required but not supplied”.

Workflow

AmpFℓSTR™ MiniFiler™ PCR Amplification Kit

Extract and quantify DNA

1. Extract DNA—Go to: www.thermofisher.com/hid-sampleprep
2. Quantify DNA—See “DNA quantification” on page 17

Perform PCR

1. “Prepare the amplification kit reactions” on page 19
2. “Perform PCR” on page 20

Perform capillary electrophoresis

1. “(Before first use of the kit) Set up the capillary electrophoresis instrument” on page 24
2. “Prepare samples for electrophoresis and start the run” on page 27

Analyze data

1. “Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)” on page 31
2. “Create an analysis method” on page 37
3. “(If needed) Create a size standard definition file” on page 45
4. “Analyze and edit sample files with GeneMapper™ ID-X Software” on page 48
5. “Examine or edit a project” on page 48

2 Perform PCR

Validated DNA amounts and PCR cycles

The kit is validated for use with 0.5–0.75 ng/µL of DNA for 30 PCR cycles. The DNA input volume is 10 µL, for a total reaction volume of 25 µL.

DNA quantification

Importance of DNA quantification before STR analysis

DNA quantification can be used to determine the following:

- If the sample contains sufficient human DNA and/or human male DNA to proceed with short tandem repeat (STR) amplification.
- (When using the Quantifiler™ Trio DNA Quantification Kit) The relative quantities of human male and female DNA in a sample. Relative quantities can help you select the appropriate STR chemistry.
- The amount of sample to use in STR analysis applications.
- If PCR inhibitors are present in a sample. If inhibitors are present, the sample may require additional purification before proceeding to STR analysis.
- The DNA quality, in regards to the inhibition level and the DNA degradation level. DNA quality can help you determine the likelihood of recovery of STR loci with larger amplicon sizes.

Note: Highly degraded samples that cannot be recovered by STR analysis with capillary electrophoresis can be analyzed with the Precision ID NGS System and Panels. Optimized for degraded samples, the Precision ID Identity Panel enables discrimination of individuals similar to STR genotype match probabilities. The Precision ID Ancestry Panel infers biogeographical ancestry for investigative leads.

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 the following:

- Fluorescence intensity that exceeds the linear dynamic range for detection by the capillary electrophoresis instrument ("off-scale" data). Off-scale data are a problem because:
  - Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause a corresponding stutter peak to appear higher in relative intensity, therefore 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.

To address these problems, rerun the amplification reaction using less DNA.

If too little DNA is added to the PCR reaction, the total number of allele copies added to the PCR reaction could be extremely low. Unbalanced amplification of the alleles can occur because of stochastic fluctuation.

Methods of quantifying DNA

Kit	Detects	How it works
Quantifiler™ HP DNA Quantification Kit (Cat. No. 4482911)	• Total human DNA (two targets—one small amplicon and one larger amplicon) • Degraded DNA	• Uses 5′ nuclease assays with multiple-copy target loci, for improved detection sensitivity:[1] – The human-specific target loci are multiple-copy, and dispersed on various autosomal chromosomes – The primary quantification targets have relatively short amplicons (75–80 bases), to improve the detection of degraded DNA samples • Uses features that maximize consistency of quantification: – Genomic targets have conserved primer- and probe-binding sites – Minimal copy number variation between different individuals and population groups • Contains a Large Autosomal target with a longer amplicon (>200 bases) to help determine if a DNA sample is degraded
Quantifiler™ Trio DNA Quantification Kit (Cat. No. 4482910)	• Total human DNA (two targets—one small amplicon and one larger amplicon) • Human male DNA • Degraded DNA	

[1] The detection sensitivity of the Quantifiler™ HP Kit and the Quantifiler™ Trio kit is improved over the Quantifiler™ Duo Kit.

Note: For information on the Quantifiler™ kits, see the *Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide* (Pub. No. 4485354).

Before you begin

(Optional) Prepare low-TE buffer

For optimal results, we recommend using low-TE buffer for sample preparation. Prepare the low-TE buffer as described in this procedure or purchase TE Buffer (Cat. No. 12090015).

1. Mix the buffer components 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 proportionally for specific needs.

2. Aliquot, then autoclave the solutions.

3. Store the aliquots at room temperature.

(Before first use of the kit) Thaw reagents

Thaw the master mix and primer set.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set and allelic ladder from light when not in use.

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

Prepare the amplification kit reactions

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set and allelic ladder from light when not in use.

1. Vortex the master mix and primer set for 3 seconds. Before opening the tubes or bottles, remove droplets from the caps by briefly centrifuging the tubes or tapping the bottles on the bench.

2. Pipet the required component volumes into an appropriately sized polypropylene tube.

Component	Amount per reaction
Master mix	10.0 µL
Primer set	5.0 µL

   Note: Include volume for extra reactions to provide excess volume for the loss that occurs during reagent transfers.

3. Vortex the reaction mix for 3 seconds, then briefly centrifuge.

4. Pipet 15 µL of the reaction mix into each well of a MicroAmp™ Optical 96-Well Reaction Plate or each MicroAmp™ tube.

5. *(If needed)* Adjust the sample input amount and volume.
   - If the total sample input amount is >0.75 ng, dilute with low-TE buffer to obtain a 10-µL input volume.
   - If the total sample input volume is <10 µL, bring to volume with low-TE buffer to obtain a 10-µL input volume.

6. Prepare the samples and controls as shown in the following table, then add to the appropriate wells of a MicroAmp™ Optical 96-Well Reaction Plate or to each MicroAmp™ tube.

Component	Amount per reaction
	30-cycle protocol
Negative control	10 µL of low-TE buffer
Test sample	10 µL of sample with a total of 0.5–0.75 ng DNA
Positive control	Combine, then add to the reaction well or tube: • 5 µL of DNA Control 007 (0.1 ng/µL) • 5 µL of low-TE buffer (For a total amount of 0.5 ng of DNA Control 007)

   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.

   IMPORTANT! We recommend adhesive film for plate sealing to provide a consistent seal across all wells and prevent evaporation. Do not use caps for the plate, which may not provide a consistent seal across all wells.

8. Vortex the plate or tubes at medium speed for 3 seconds.

9. Centrifuge the tubes or plate at 3,000 × *g* for ~20 seconds in a tabletop centrifuge (with plate holders, if using 96-well plates).

Proceed to “Perform PCR” on page 20.

Perform PCR

IMPORTANT! The kit is optimized for use with the thermal cyclers that are listed in “Instruments and software compatibility” on page 15.

1. Program the thermal cycler.
   a. Set the ramping mode to **9600 Simulation**.

b. Set the thermal cycling conditions as shown in the following table.

Initial incubation step	Cycle (30 cycles)[1]			Final extension	Final hold
	Denature	Anneal	Extend		
HOLD	CYCLE			HOLD	HOLD
95°C 11 minutes	94°C 20 seconds	59°C 2 minutes	72°C 1 minute	60°C 45 minutes	4°C ≤24 hours[2]

[1] See "Validated DNA amounts and PCR cycles" on page 17.
[2] The infinity (∞) setting allows an unlimited hold time.

2. Load the plate or tubes into the thermal cycler, close the heated cover, then start the run.

3. When the run is complete, store the amplified DNA.

Storage time	Temperature
<2 weeks	2–8°C
>2 weeks	–25°C to –15°C

IMPORTANT! Protect the amplified DNA from light.

Direct amplification

FTA™ cards or NUCLEIC-CARD™ devices are useful for the collection, storage, and processing of biological samples. A small punch disk of the card containing the sample can be placed directly into an amplification tube or plate, purified, then amplified, without transferring the disk.

Our studies indicate that a 1.2-mm bloodstained disk contains ~5–20 ng of DNA. Because of the high quantity of DNA, a lower cycle number is required to produce on-scale data. In our testing, an appropriate cycle number for this high quantity of DNA was 24 cycles. We recommend performing internal validation studies to determine the optimum cycle number for your laboratory.

A 1.2-mm disk of a bloodstained FTA™ card was purified using three washes with FTA™ Purification Reagent and two washes with 1X low-TE buffer. The disk was then amplified in a MicroAmp™ tube for 24 cycles. See Figure 3.

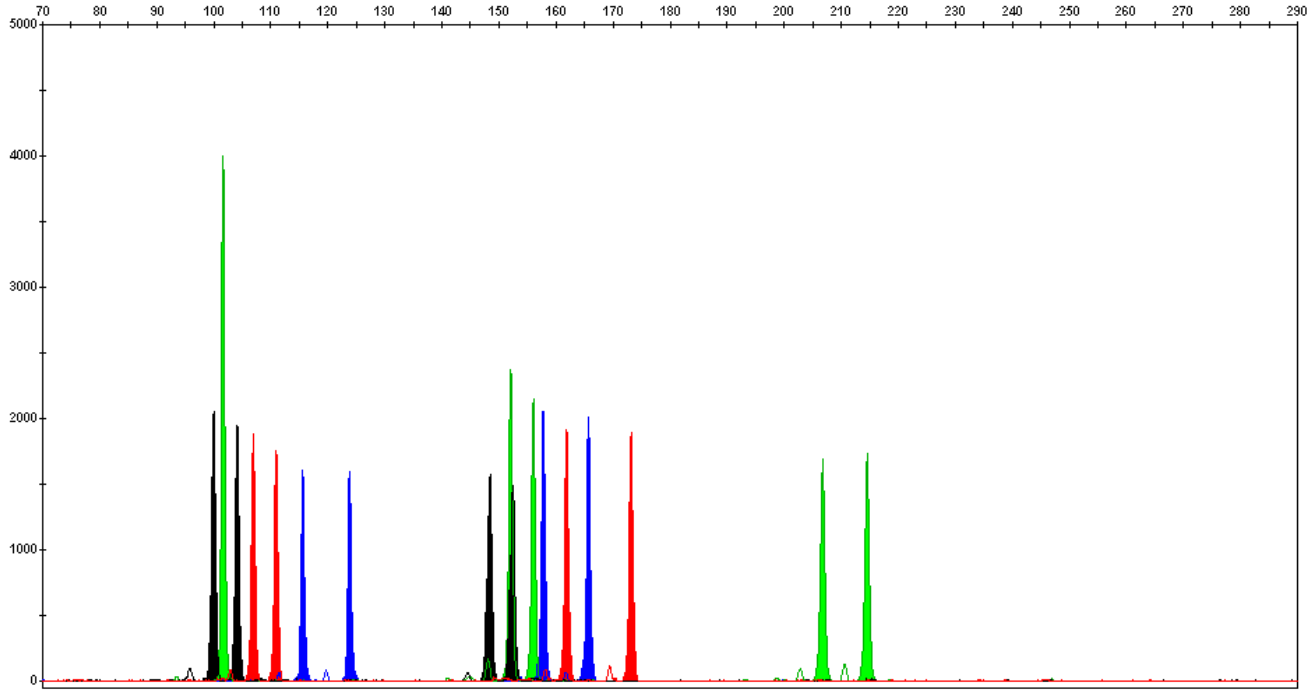


Figure 3 Combined dyes electropherogram of a 1.2-mm disk of a bloodstained FTA™ card amplified for 24 cycles with the MiniFiler™ kit on a 3130*xl* Genetic Analyzer (Y-axis scale 0–5,000 RFU).

3 Perform electrophoresis

Allelic ladder requirements for electrophoresis

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

Instrument	Number of allelic ladders to run	One injection equals	Number of samples per allelic ladder
SeqStudio™ 24 Flex Genetic Analyzer	1 per injection	24 samples	23 samples + 1 allelic ladder
SeqStudio™ 8 Flex Genetic Analyzer	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
SeqStudio™ Genetic Analyzer	1 per 6 injections	4 samples	23 samples + 1 allelic ladder
3500xL Genetic Analyzer	1 per injection	24 samples	23 samples + 1 allelic ladder
3500 Genetic Analyzer	1 per 3 injections	8 samples	23 samples + 1 allelic ladder

IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between runs. Follow the guidelines in the preceding table, which should account for normal variation in run speed. To facilitate accurate genotyping of all samples in your laboratory environment, perform internal validation studies to verify the required allelic ladder injection frequency.

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

(Before first use of the kit) Set up the capillary electrophoresis instrument

Data collection software setup

To analyze PCR products generated by the kit, you can use the data collection software and run parameters provided in this section. See the appropriate table for your instrument.

Note: With 0.5–0.75 ng of input DNA, our studies indicate that the injection conditions provided in this section produce well-balanced profiles with no instances of allelic dropout and minimal occurrence of off-scale allele peaks. However, individual CE instrument signal intensities can vary; therefore, changes to injection parameters may need to be explored and validated to deliver the best results on your system. Large deviations from the recommended injection parameters can affect the performance of the size standard and allelic ladder, therefore validation is recommended.

Note: For detailed procedures, see the appropriate user documentation for your instrument.

Table 2 Software setup: SeqStudio™ Flex Series Genetic Analyzer for Human Identification

SeqStudio™ Flex Data Collection Software	*(Optional)* Additional software	Run parameters
v1.1.1	• SAE Administrator Console v2.1 • SeqStudio™ Plate Manager Software v2.1, v2.1.1 • SeqStudio™ Flex Remote Monitoring Software	**Injection protocol:** HID_Protocol_G5_36_POP4(xl)
		Size standard: GS600 LIZ (60–460)
		Dye set: G5 (DS-33)
		Run module: HID_G5_36_POP4(xl)
		Injection conditions: • 1.2 kV/15 seconds (8 Flex) • 1.2 kV/24 seconds (24 Flex)
		Run conditions: • 15 kV/1,210 seconds (8 Flex) • 15 kV/1,210 seconds (24 Flex)

Table 3 Software setup: SeqStudio™ Genetic Analyzer for HID

SeqStudio™ Data Collection Software	*(Optional)* Additional software	Run parameters	Plate setup
v1.2.1, v1.2.4, v1.2.5	• SAE Administrator Console v2.0, v2.1 • SeqStudio™ Plate Manager Software v1.2, v1.3	**Run Module:** HID Analysis	**Kit:** MiniFiler™ kit
		Injection conditions: 1.2 kV/10 seconds	**Dye set:** G5 (DS-33)
		Run conditions: 11 kV/1,120 seconds	**Size standard:** GS600 LIZ (60–460)

Table 4 Software setup: 3500 Series Genetic Analyzer for Human Identification

Operating system	3500 Data Collection Software	Run parameters
Windows™ 10	v4, v4.0.1	**Assay:** AB_G5_LS_POP4(xl)
		Instrument protocol: AB_HID36_POP4(xl)_G5
		Run module: HID36_POP4 (xl)
		Injection conditions: 1.2 kV/15 seconds (xl: 24 seconds)
		Run conditions: 15 kV/1,210 seconds
		Dye set: G5

Perform spectral calibration

Perform a spectral calibration using the DS-33 Matrix Standard Kit (Dye Set G5) (Cat. No. 4345833).

Examples of spectral calibrations are shown in this section. See the appropriate figure for your instrument.

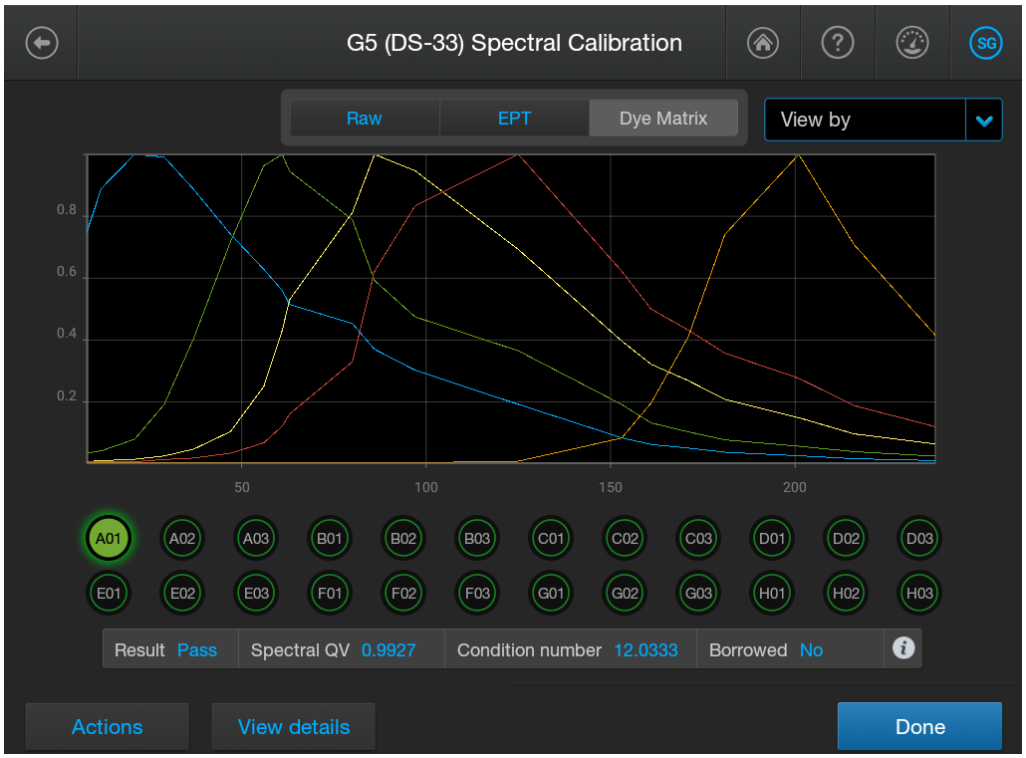


Figure 4 Example spectral calibration: SeqStudio™ Flex Series Genetic Analyzer for Human Identification

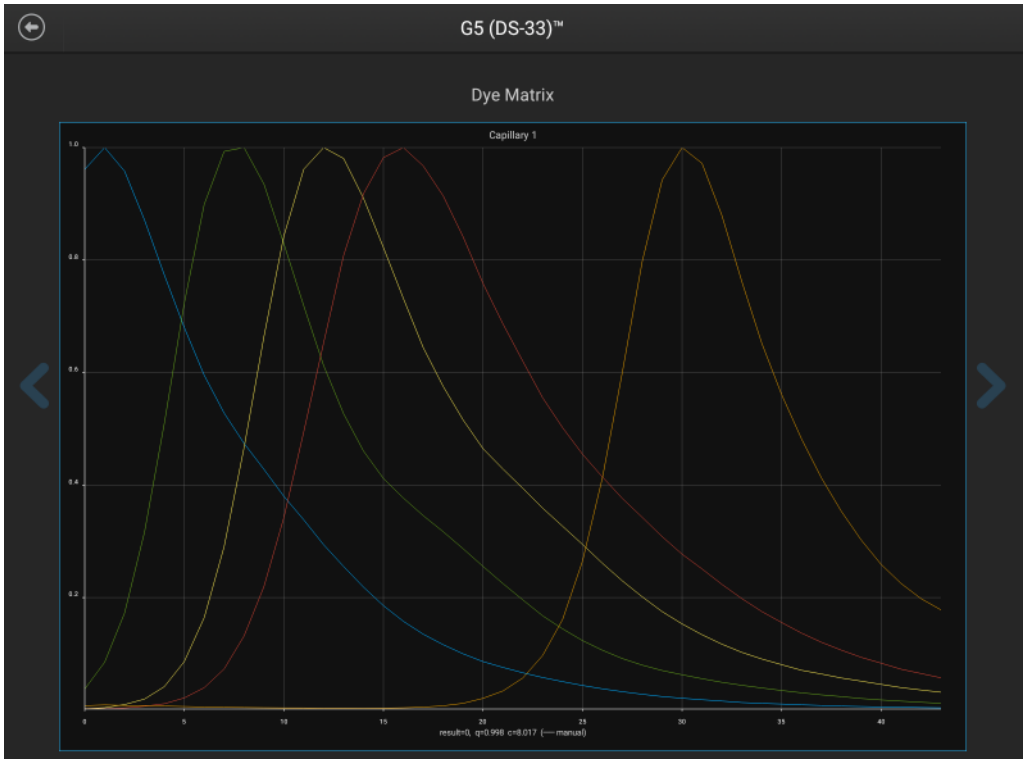


Figure 5 Factory-provided spectral calibration: SeqStudio™ Genetic Analyzer for HID

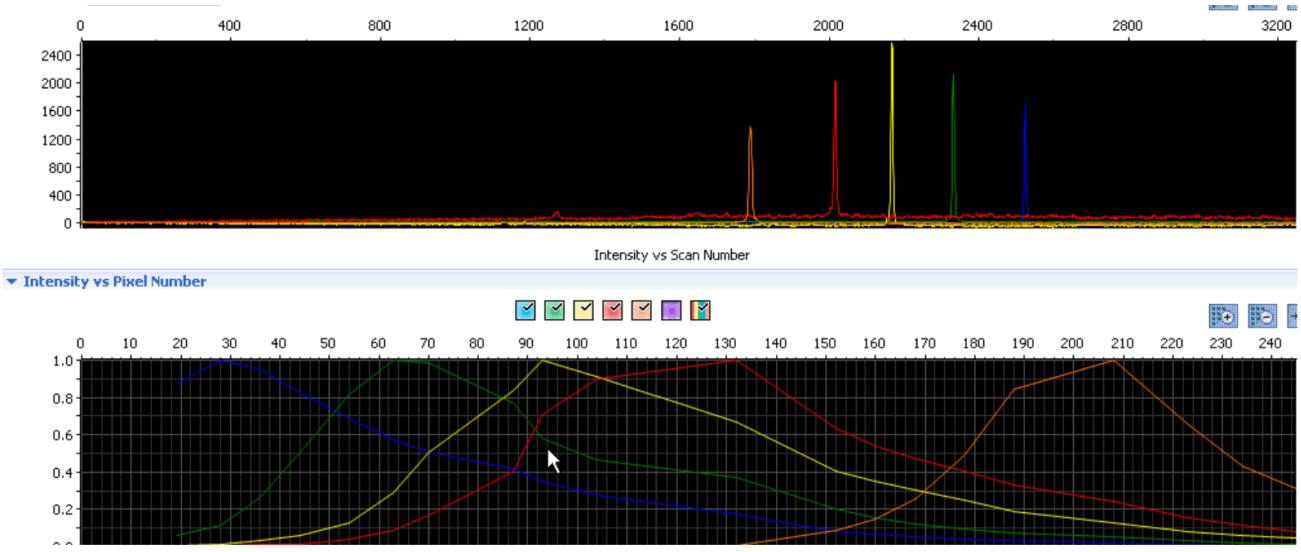


Figure 6 Example spectral calibration: 3500 Series Genetic Analyzer for Human Identification

Prepare samples for electrophoresis and start the run

Prepare the samples for electrophoresis immediately before loading.

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.

1. Pipet the required component amounts into an appropriately sized polypropylene tube.

   IMPORTANT! The component amounts vary, depending on the size standard that you are using.

   Table 5 For GeneScan™ 500 LIZ™ Size Standard

Component	Amount per reaction
GeneScan™ 500 LIZ™ Size Standard	0.3 µL
Hi-Di™ Formamide	8.7 µL

   Table 6 For GeneScan™ 600 LIZ™ Size Standard v2.0

Component	Amount 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 account for the loss that occurs during reagent transfers.

   IMPORTANT! The amount 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. Vortex the tube, then briefly centrifuge.

3. Pipet the required component amounts into each well of a MicroAmp™ Optical 96-Well Reaction Plate.

Component	Amount per reaction
Formamide/size standard mixture	9 µL
PCR product or allelic ladder	1 µL

   Note: For blank wells, add 10 µL of Hi-Di™ Formamide.

4. 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.

5. Heat the reaction plate in a thermal cycler for 3 minutes at 95°C.

6. Immediately place the plate on ice for 3 minutes.
7. Place the sample tray on the autosampler, then start the electrophoresis run.

4 Analyze data with GeneMapper™ *ID-X* Software

Overview of GeneMapper™ *ID-X* Software

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

After capillary electrophoresis, the data collection software stores information for each sample in a FSA or HID file. The GeneMapper™ *ID-X* Software allows you to analyze and interpret the data from the FSA or HID files.

Note: For a list of GeneMapper™ *ID-X* Software versions that are compatible with your kit and capillary electrophoresis instrument, see “Instruments and software compatibility” on page 15.

Allelic ladder requirements for data analysis

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

  For multiple allelic ladder samples, the GeneMapper™ *ID-X* Software calculates allelic bin offsets by using an average of all allelic ladders that use the same panel in 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 ladders in 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. Analysis will fail if the **Allelic Ladder Sample Type** is not specified.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples, to help ensure proper allele calling.
- Alleles that are not in the allelic ladders do exist. Off-ladder (OL) alleles can contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the bin window of any known allelic ladder allele or virtual bin.

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

File names and versions used in this section

The file names and version numbers of panel, bin, and stutter files that are shown in this section may differ from the file names that you see when you download or import files.

If you need help to determine the correct files to use, contact your local Human Identification representative, or go to **thermofisher.com/support**.

Set up the GeneMapper™ *ID-X* Software for analysis (before first use of the kit)

Workflow

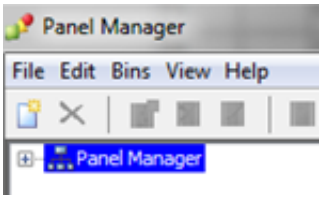
Before you use GeneMapper™ *ID-X* Software to analyze data for the first time, you must do the following:

Set up GeneMapper™ *ID-X* Software

- **Check panel, bin, and stutter file versions on your computer**
- **(If needed) Download newer versions of panel, bin, and stutter files**
- **(If needed) Import panels, bins, and marker stutter**
- **(Optional) Define custom table or plot settings**

Check panel, bin, and stutter file versions on your computer

1. Start the GeneMapper™ *ID-X* Software, then sign in with the appropriate user name and password.
2. Select **Tools** ▸ **Panel Manager**.
3. Check the version of files that are currently available in the **Panel Manager**.
   a. Select **Panel Manager** in the navigation pane.
   b. Expand the **Panel Manager folder** and any subfolders to identify the analysis file version that is 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 the **Panels** folder, then check the version of panel, bin, and stutter files installed.

GeneMapper™ *ID-X* Software v1.7.x contains the latest panel, bin, and stutter files for the STR kits.

- If the latest files are not installed on your copy of the GeneMapper™ *ID-X* Software, proceed to "(If needed) Download newer versions of panel, bin, and stutter files" on page 32.
- If the latest files are already installed on your copy of the GeneMapper™ *ID-X* Software, skip to "Create an analysis method" on page 37.

(If needed) Download newer versions of panel, bin, and stutter files

1. Go to **www.thermofisher.com/GMIDXsoftware**.
   The page provides a list of kit-specific analysis files. The analysis files for each kit can be downloaded in a single ZIP file.
2. If the analysis file versions listed for your kit are newer than the versions on your computer, download the ZIP file.

   Note: When downloading new versions of analysis files, see the associated **Read Me** file for details of changes between software file versions. Perform the appropriate internal validation studies before using new file versions for analysis.
3. Unzip the file.

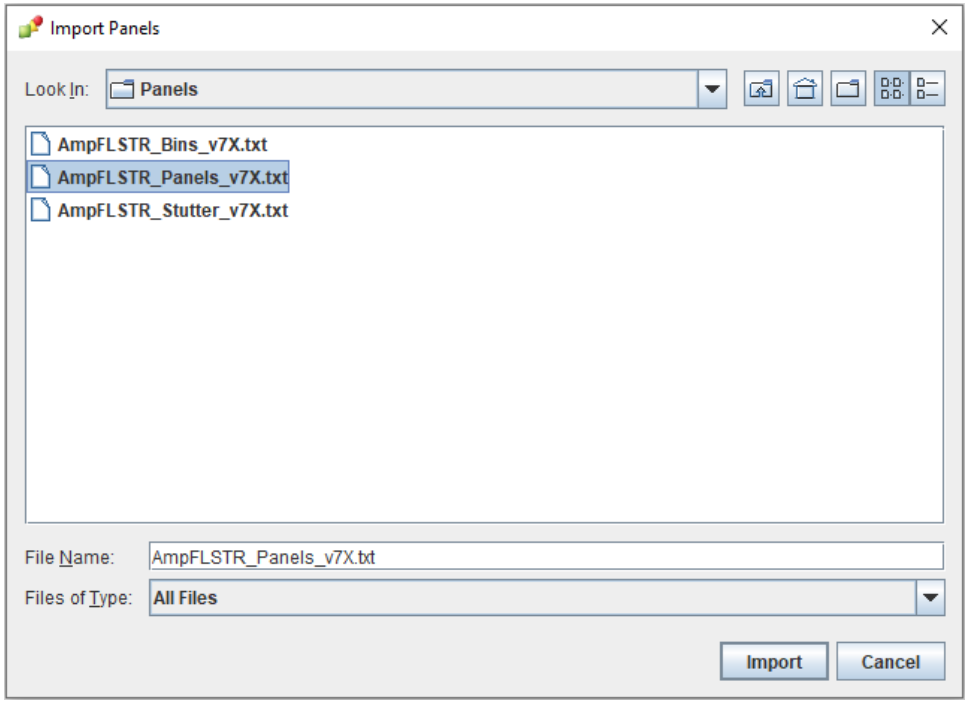
(If needed) Import panels, bins, and marker stutter

Import the latest panel, bin set, and marker stutter from the website into the GeneMapper™ *ID-X* Software database.

Note: The file names specified in this procedure are examples only. The files that you import may have different file names.

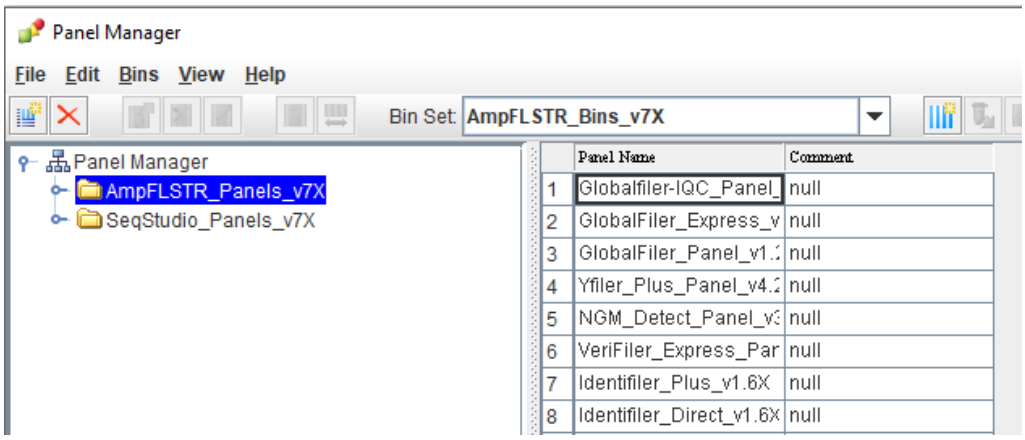
1. Start the GeneMapper™ *ID-X* Software, then sign in with the appropriate user name and password.
2. Select **Tools** ▸ **Panel Manager**.
3. Open the folder that contains the panels, bins, and marker stutter.
   a. Select **Panel Manager**, then select **File** ▸ **Import Panels** to open the **Import Panels** dialog box.
   b. Navigate to the analysis files folder that you unzipped in "(If needed) Download newer versions of panel, bin, and stutter files" on page 32.

4. Select the panels TXT file for your kit, then click **Import**.



Importing the panels TXT file creates a new folder in the navigation pane of the **Panel Manager**. This folder contains the panels and associated markers.

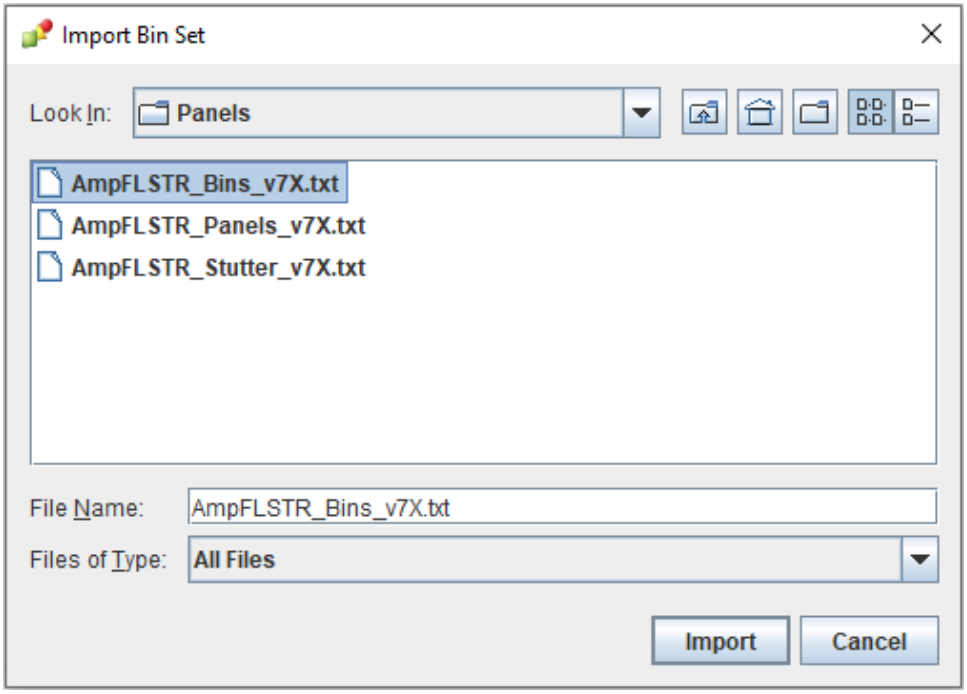
5. Import the bins file.
   a. In the navigation pane, select the panel folder created in step 4.



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

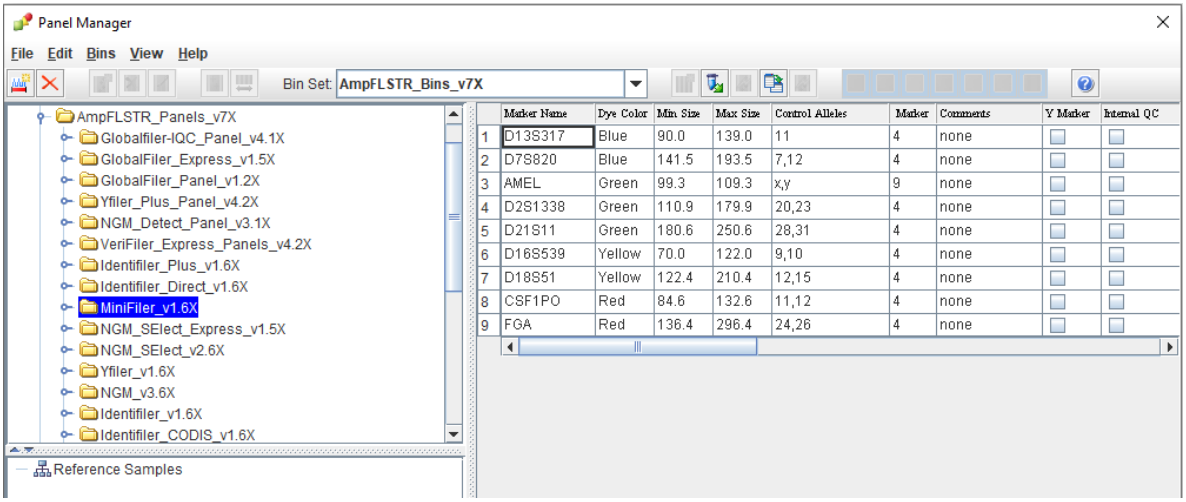
   c. Navigate to the analysis files folder for your kit (from step 3).

d. Select the bins TXT file for your kit, then click **Import**.



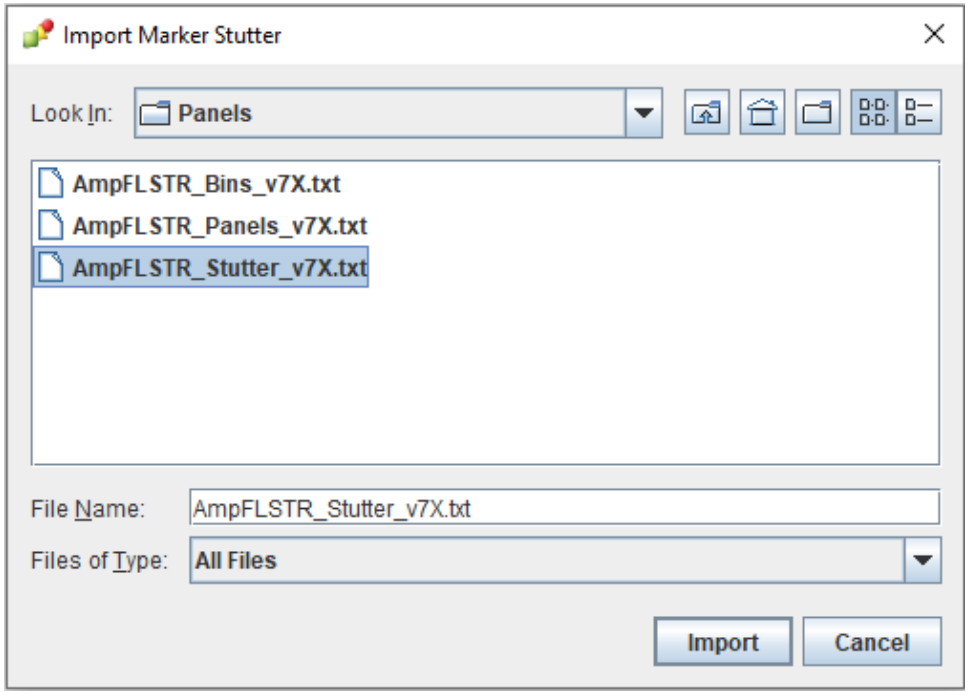
Importing the bins TXT file associates the bin set with the panels imported in step 4.

6. *(Optional)* View marker and panel information: In the navigation pane, select the panel folder for your kit.
   The panel information is displayed in the right pane and the markers are displayed below it.



7. Import the stutter file.
   a. In the navigation pane, select the panel folder for your kit.
   b. Select **File** ▸ **Import Marker Stutter** to open the **Import Marker Stutter** dialog box.
   c. Navigate to the analysis files folder for your kit (from step 3).

d. Select the stutter TXT file for your kit, then click **Import**.

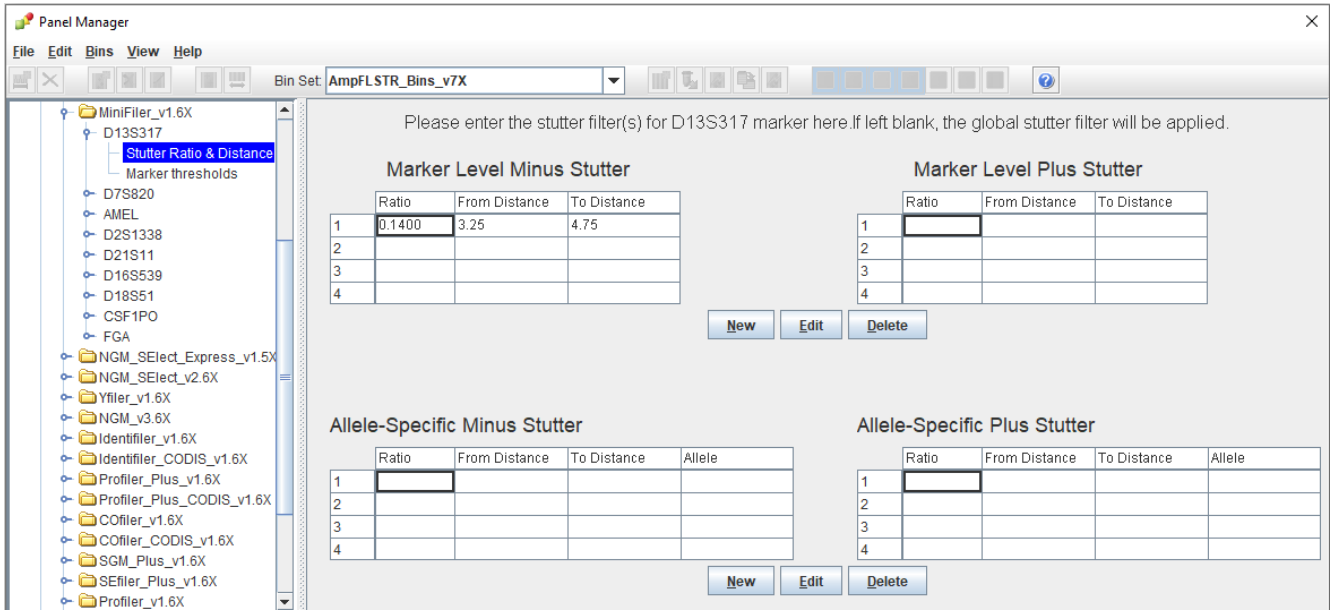


Importing the stutter TXT file associates the marker stutter ratio with the bin set in the panel folder for your kit (step 4) and overwrites any existing stutter ratios associated with the panels and bins in that folder.

8. *(Optional)* View the imported marker stutters.
   a. In the navigation pane, click the panel folder for your kit to expand it.
   The markers are displayed in the navigation pane.

b. Double-click a marker, then select the **Stutter Ratio & Distance** view for the marker in the right pane.

Note: The allele-specific stutter fields shown in the image are not implemented in GeneMapper™ *ID-X* Software v1.6 and earlier.



9. Click **Apply**, then click **OK** to add the 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 are not imported into the GeneMapper™ *ID-X* Software database.

(Optional) Define custom table or plot settings

Default views for table and plot settings are provided with the software.

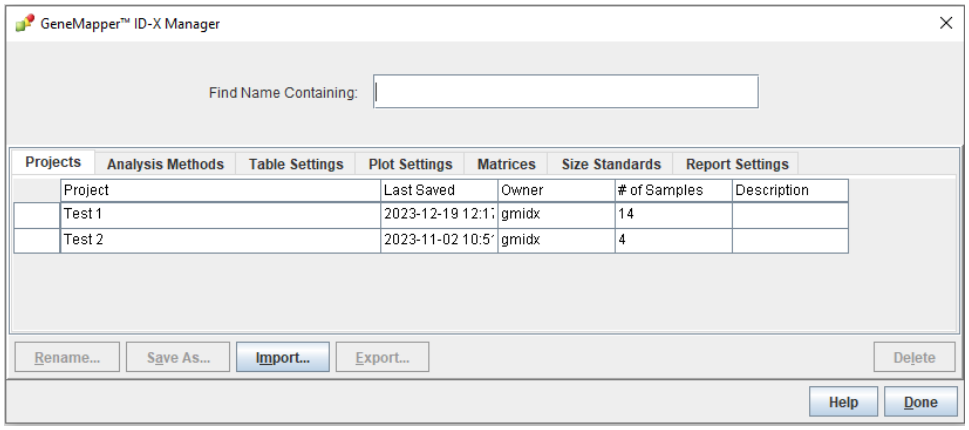
For information on defining custom views, see *GeneMapper™ ID-X Software v1.5 Getting Started Guide — Basic Features*.

Create an analysis method

Create an analysis method

IMPORTANT! Because analysis methods are version-specific, you need to create an analysis method for each version of the software. For example, an analysis method that is created in GeneMapper™ *ID-X* Software v1.6 is not compatible with analysis methods that are created in v1.5 or v1.7.x.

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



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

3. Enter the settings as described in the following pages.

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

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

Enter Analysis Method settings

Enter General tab settings

1. Enter an analysis method name.

2. Select the security group appropriate for your software configuration.

3. *(Optional)* Enter a description and an instrument.

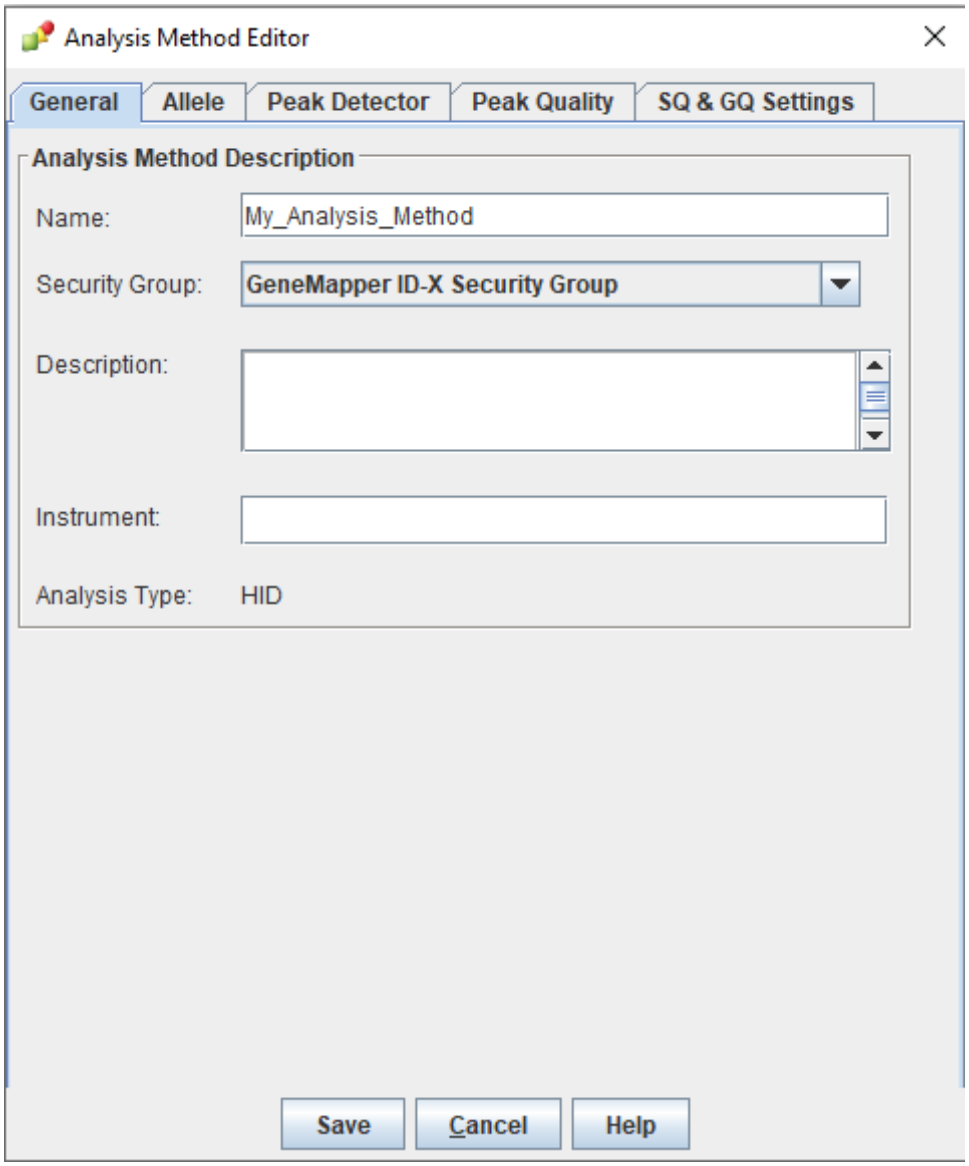


Figure 7 General tab settings

Enter Allele tab settings

IMPORTANT! Perform internal validation studies to determine the appropriate settings for your laboratory.

1. Select the appropriate bin set.
2. *(Optional)* Select stutter options.

Option	Action	Additional information
Use marker-specific stutter ratio and distance if available	Select or deselect the checkbox, as needed.	To apply the stutter ratios that are contained in the Panel Manager, select the checkbox.
Use allele-specific stutter ratios and distances if available The checkbox is available only for GeneMapper™ *ID-X* Software v1.7 or later.	Select or deselect the checkbox, as needed.	To use allele-specific stutter filtering, select the checkbox.
Consider additive stutters (forward and back) The checkbox is available only for GeneMapper™ *ID-X* Software v1.7 or later.	Select or deselect the checkbox, as needed.	To take additive stutter into consideration, select the checkbox.

Note: For more information on the GeneMapper™ *ID-X* Software v1.7 options, see the *GeneMapper™ ID-X Software v1.7 New Features and Software Verification and Validation User Bulletin* (Pub. No. MAN0029209).

3. In the **Marker Repeat Type** pane, enter values for the Tri, Tetra, Penta, and Hexa loci.

Note: For paternity and database applications: In the **Global Cut-off Value** field, we recommended using a cut-off value of 20% for the Tri, Tetra, and Penta loci.

4. Enter the appropriate filter settings.

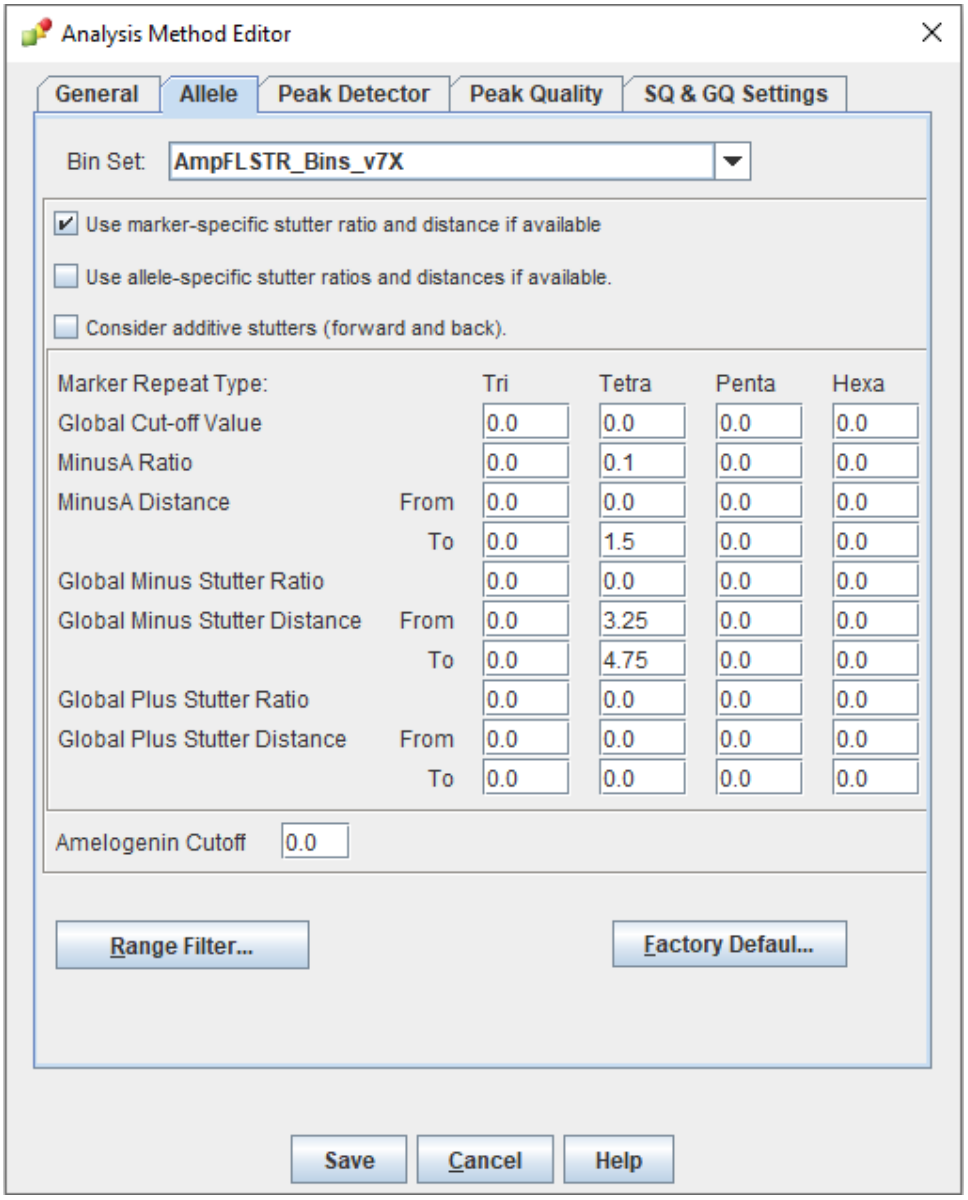


Figure 8 Allele tab settings

Enter Peak Detector tab settings

Enter or select the appropriate values.

Option	Action	Additional information
Use marker-specific thresholds (if available) The checkbox is available only for GeneMapper™ *ID-X* Software v1.7 or later.	Select or deselect the checkbox, as needed.	To use the marker-specific thresholds defined in the Panel Manager, select the checkbox.
Ranges	**Analysis**—Select **Full Range** from the dropdown list.	*(if needed)* The analysis range can be narrowed.
	Sizing—Select **All Sizes** from the dropdown list.	—
Smoothing and Baselining	**Smoothing**—Select **Light**.	The MiniFiler™ kit was validated with the values listed. If your laboratory uses an unvalidated polymer, you may need to adjust these values.
	Baseline Window—Enter **51** pts.	
Size Calling Method	Select **3rd Order Least Squares**.	The MiniFiler™ kit was validated using the 3rd Order Least Squares method. Do not select another method unless you perform internal validation studies to determine the appropriate method for your laboratory.
Peak Detection	**Peak Amplitude Thresholds**—User-defined.	The default value is 50 RFU for all dyes. Perform internal validation studies to determine the appropriate peak amplitude thresholds for your laboratory.
	Min. Peak Half Width—Enter **2** pts.	The MiniFiler™ kit was validated with the values listed. Do not enter other values unless you perform internal validation studies to determine the appropriate values for your laboratory.
	Polynomial Degree—Enter **3**.	
	Peak Window Size—Enter **15** pts.	
Slope Threshold	**Peak Start**—Enter **0.0**.	
	Peak End—Enter **0.0**.	

(continued)

Option	Action	Additional information
Use Normalization, if applicable The checkbox is available for use with data run on the following instruments: • SeqStudio™ Flex Series Genetic Analyzer for Human Identification • 3500 Series Genetic Analyzer for Human Identification	Select or deselect the checkbox, as needed.	To apply size standard normalization data to the analysis, select the checkbox. The size standard normalization data are collected on the capillary electrophoresis instrument. To see if normalization data have been collected for a specific data file, see **SS Normalization Factor** in the GeneMapper™ *ID-X* Software.

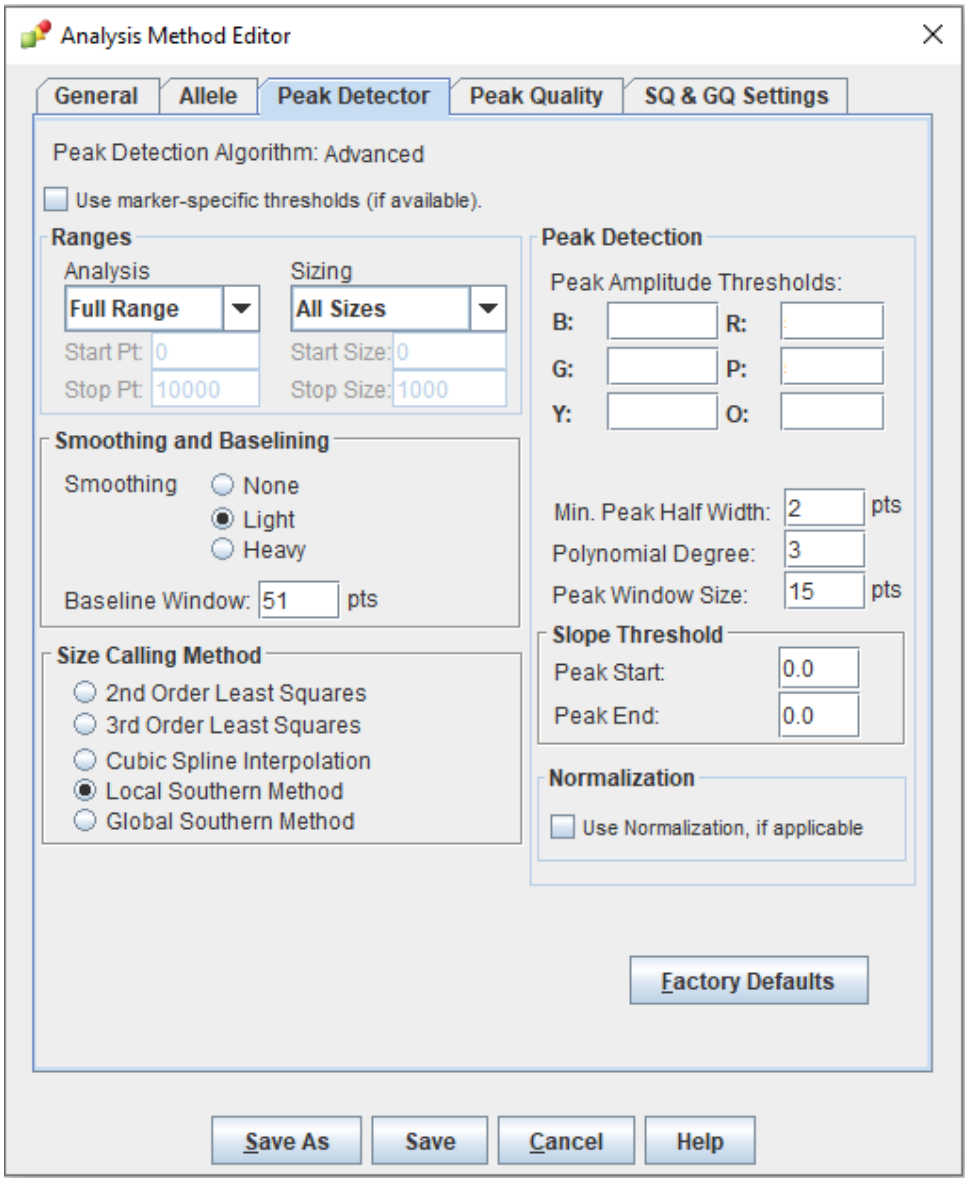


Figure 9 Peak Detector tab settings

Enter Peak Quality tab settings

1. Perform internal validation studies to determine the heterozygous and homozygous minimum peak height thresholds, maximum peak height threshold, and the minimum peak height ratio threshold for interpretation of data.

2. For the remaining fields, enter the values shown in Figure 10.

Note: The **Pull-Up Ratio (PU)** settings shown in the figure are implemented only in GeneMapper™ *ID-X* Software v1.7 or later. For more information on these settings, see the *GeneMapper™ ID-X Software v1.7 New Features and Software Verification and Validation User Bulletin* (Pub. No. MAN0029209).

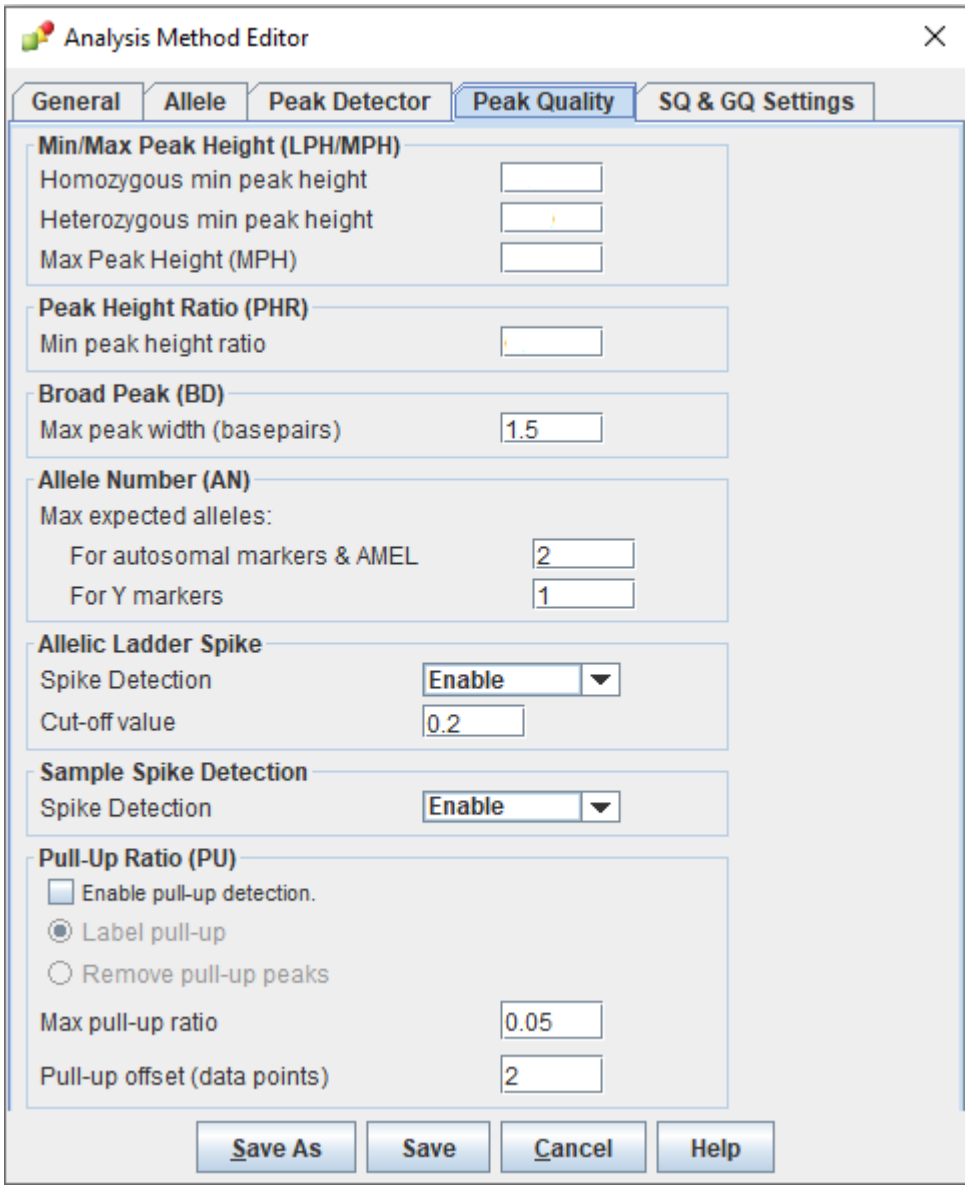


Figure 10 Peak Quality tab settings

Enter SQ & GQ tab settings

Enter the appropriate values.

IMPORTANT! The software default values are shown in Figure 11. We used the software default values during developmental validation. We recommend that you perform internal validation studies to determine the appropriate values for your laboratory.

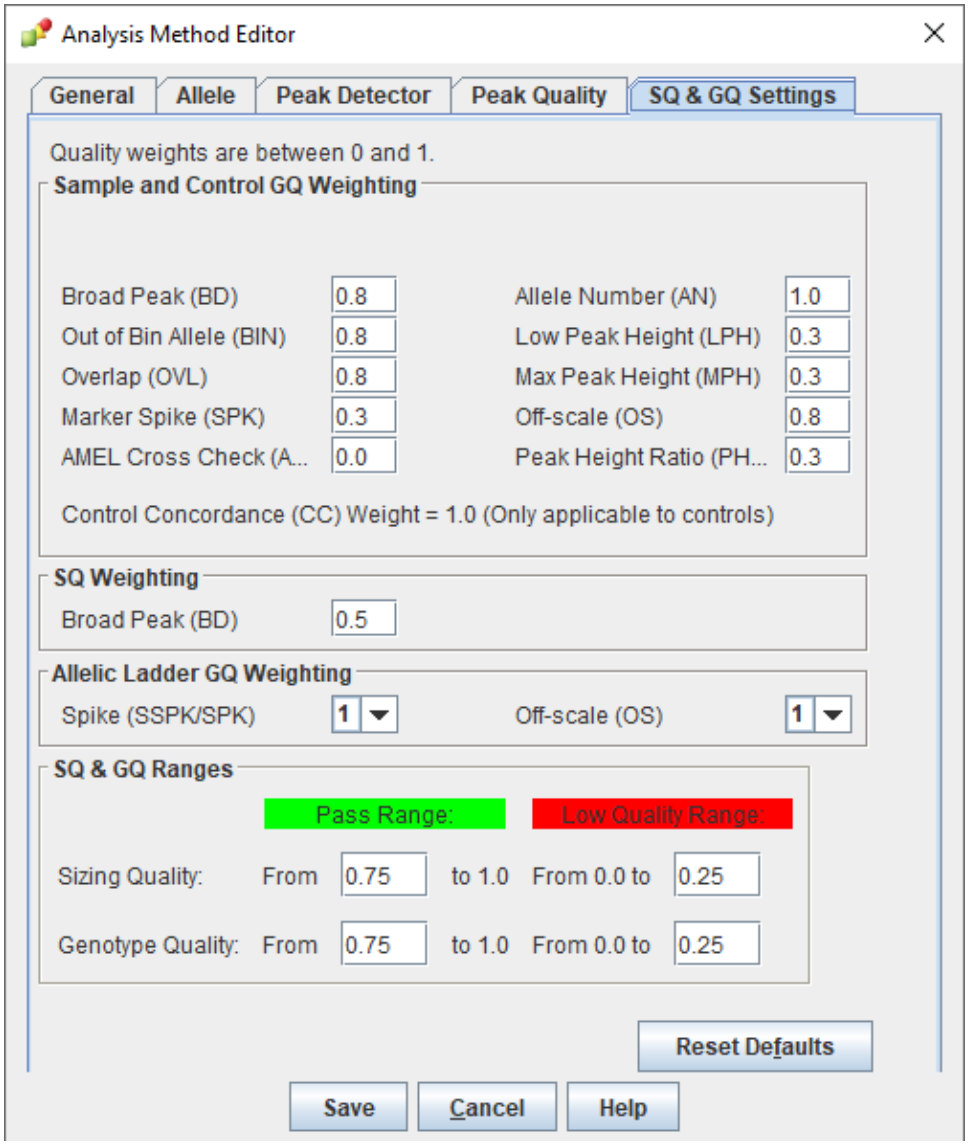


Figure 11 SQ & GQ tab settings

(If needed) Create a size standard definition file

If you cannot use the default settings that are provided, create a new size standard definition file.

About the size standard definition file

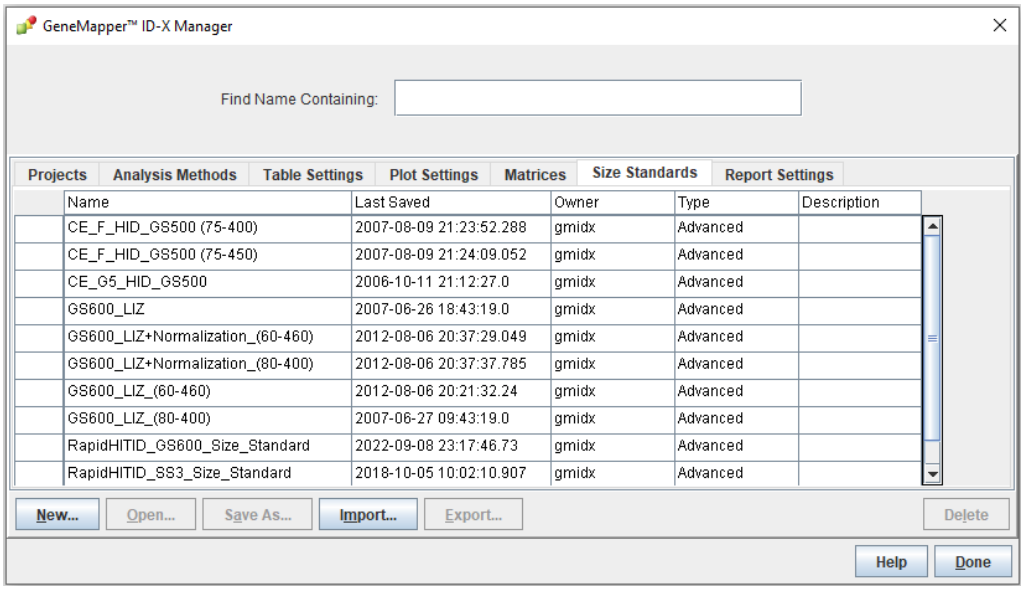
Two size standard definition files are provided with the GeneMapper™ *ID-X* Software. Select the appropriate file depending on the size standard that was used in the capillary electrophoresis run (“Prepare samples for electrophoresis and start the run” on page 27).

- **CE_G5_GS500 (75-400)**—For use with the GeneScan™ 500 LIZ™ Size Standard. The file contains the following peaks: 75, 100, 139, 150, 160, 200, 300, 350, 400, 450. (The 250 and 340 peaks are not included in the definition. These can be used as an indicator of precision in the run.)
- **GS600_LIZ_(60–460)**—For use with the GeneScan™ 600 LIZ™ Size Standard v2.0. The file contains the following peaks: 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.

Both size standard definitions are supported for use with this kit on the genetic analyzers listed in “Instruments and software compatibility” on page 15. If you need to create your own size standard definition, see “Create a size standard definition file” on page 46.

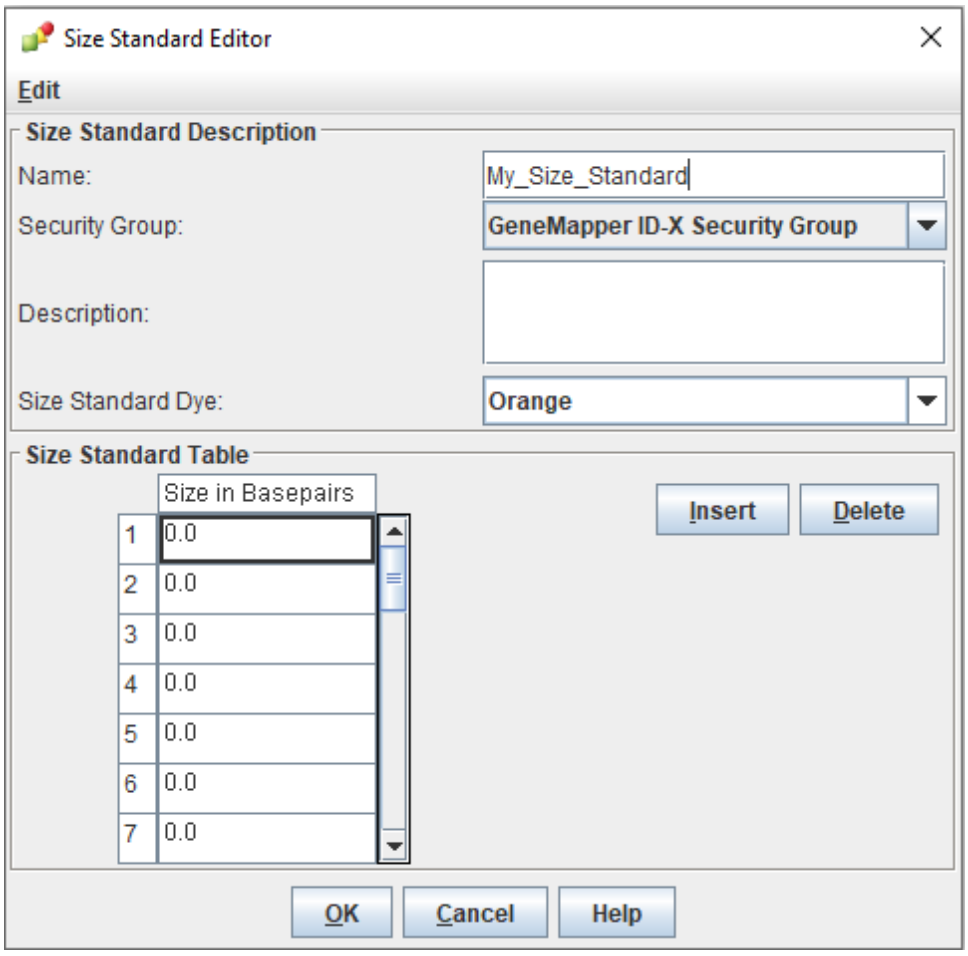
Create a size standard definition file

1. Select **Tools ▸ GeneMapper ID-X Manager** to open the **GeneMapper ID-X Manager**.
2. Click the **Size Standards** tab, then click **New**.



3. Specify settings in the **Size Standard Editor**.
   a. Enter a name.
   b. In the **Security Group** field, select the security group appropriate for your software configuration.
   c. In the **Size Standard Dye** field, select **Orange**.

d. In the **Size Standard Table**, enter the peak sizes that correspond to your size standard. *(If needed)* Click **Insert** to add rows or click **Delete** to remove rows.



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

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

Option	Action
Sample Type	Select the sample type for each sample, control, and allelic ladder in the project.
Analysis Method	Select the analysis method that you created in "Create an analysis method" on page 37.
Panel	Select the current kit panel. If needed, see "Check panel, bin, and stutter file versions on your computer" on page 31.
Size Standard	Select the **GS600_LIZ_(60-460) size standard definition**, or select another validated size standard definition, as described in "(If needed) Create a size standard definition file" on page 45.

3. Click **Analyze**.
4. In the **Save Project** dialog box, enter a name for the project, then click **OK** to start analysis.
   - The status bar displays the progress of analysis.
   - 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 is available when the analysis is complete.

Examine or edit a project

Display electropherogram plots from the Samples and Genotypes tabs of the Project window to examine the data.

For more information on using the GeneMapper™ *ID-X* Software

See "Related documentation" on page 122 for a list of available documents.

5 Experiments and results

Importance of validation

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

Experiment conditions

The experiments described in this chapter were performed according to the DNA Advisory Board (DAB) Quality Assurance Standards, effective October 1, 1998 (DAB, 1998). The DAB standards describe the quality assurance requirements that a laboratory should follow to maintain the quality and integrity of the data and competency of the laboratory.

Additional validation was performed 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 that we performed and provides examples of the results we obtained. We chose conditions that produced optimum PCR product yield and that met reproducible performance standards. While these experiments are not exhaustive, it is our opinion that the experiments are appropriate for a manufacturer of STR kits intended for forensic and/or parentage testing use.

Laboratory requirements for internal validation

Each laboratory using this kit must perform internal validation studies. Performance of this kit is supported when used according to the following developmentally validated parameters. Modifications to the protocol should be accompanied by appropriate validation studies performed by the laboratory.

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 thermal cycling 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 in the kit. We 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 3130*xl* Genetic Analyzer. The amplification of 0.50 ng of DNA Control 007 is shown in Figure 12. The performance of the multiplex is most robust within ±20% of the optimal magnesium chloride concentration.

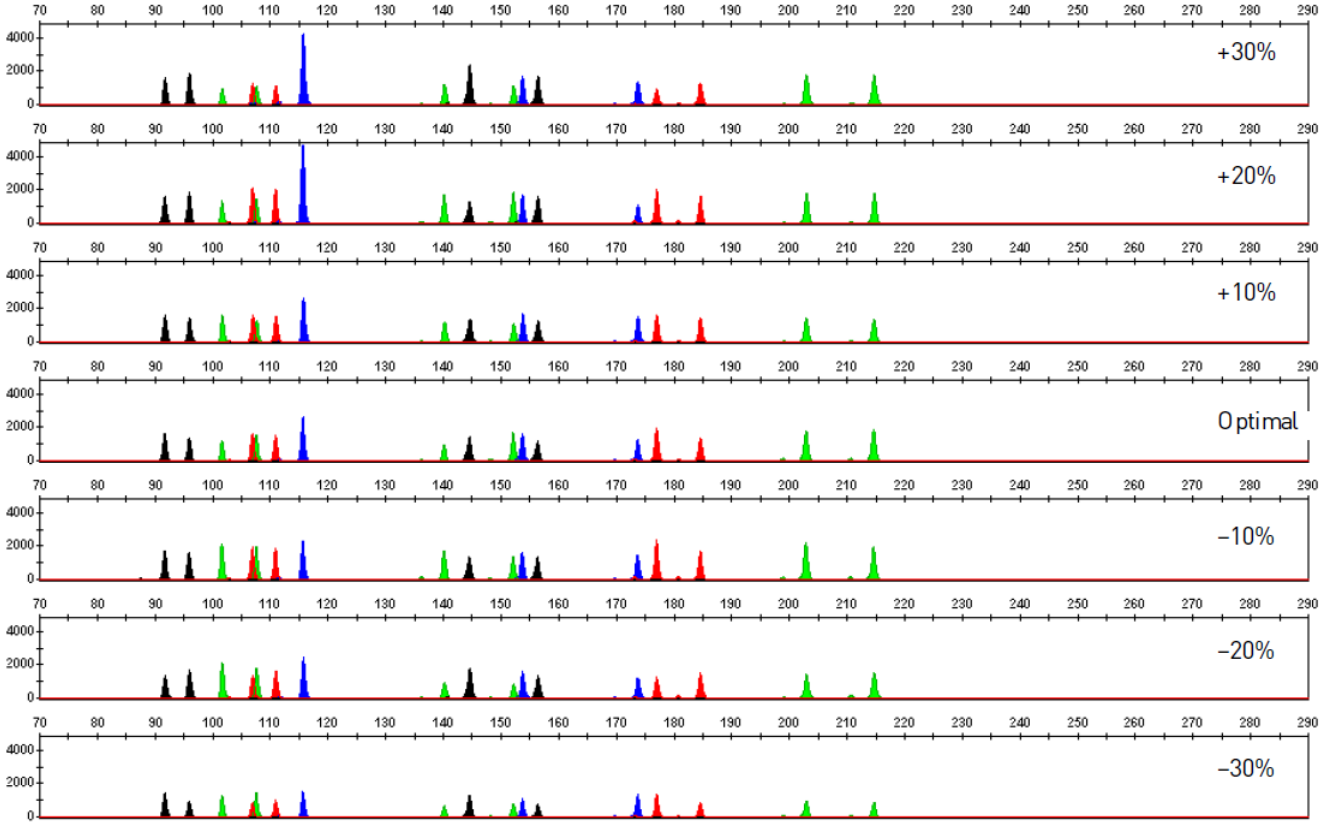


Figure 12 A 0.50 ng sample of DNA Control 007 amplified with the MiniFiler™ kit in the presence of varying concentrations of magnesium chloride and analyzed on a 3130*xl* Genetic Analyzer (Y-axis scale 0–5,000 RFU).

Thermal cycling temperatures

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 ≥0.50 ng of DNA Control 007 was produced.

For example, annealing temperatures of 55, 57, 59, 61, and 63°C were tested for 2-minute hold times in the Silver 96-Well GeneAmp™ PCR System 9700 (see Figure 13). The PCR products were analyzed using a 3130*xl* Genetic Analyzer.

Of the tested annealing temperatures, 55–61°C produced robust profiles. At 63°C, the yield of the majority of 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, we strongly recommend regularly scheduled thermal cycler calibration.

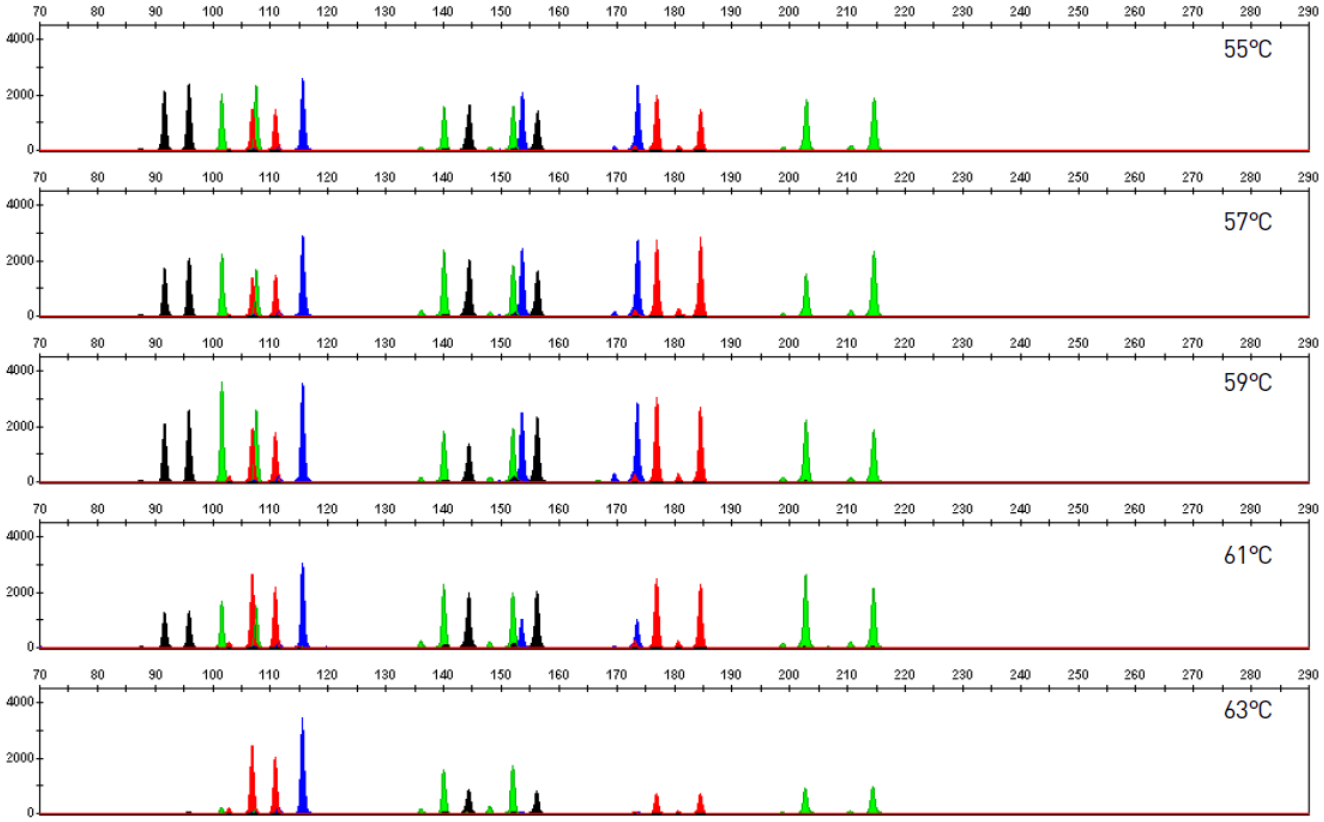


Figure 13 Electropherograms obtained from amplification of 0.50 ng of DNA Control 007 at annealing temperatures of 55, 57, 59, 61, and 63°C, analyzed on a 3130*xl* Genetic Analyzer (Y-axis scale 0–4,000 RFU).

PCR cycle number

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 at 28 cycles and off-scale data were collected for several allele peaks at 32 cycles (see Figure 14).

While none of the cycle numbers tested produced nonspecific peaks, 30 cycles were found to give optimal sensitivity when the amplified products were analyzed on a 3130*xl* Genetic Analyzer.

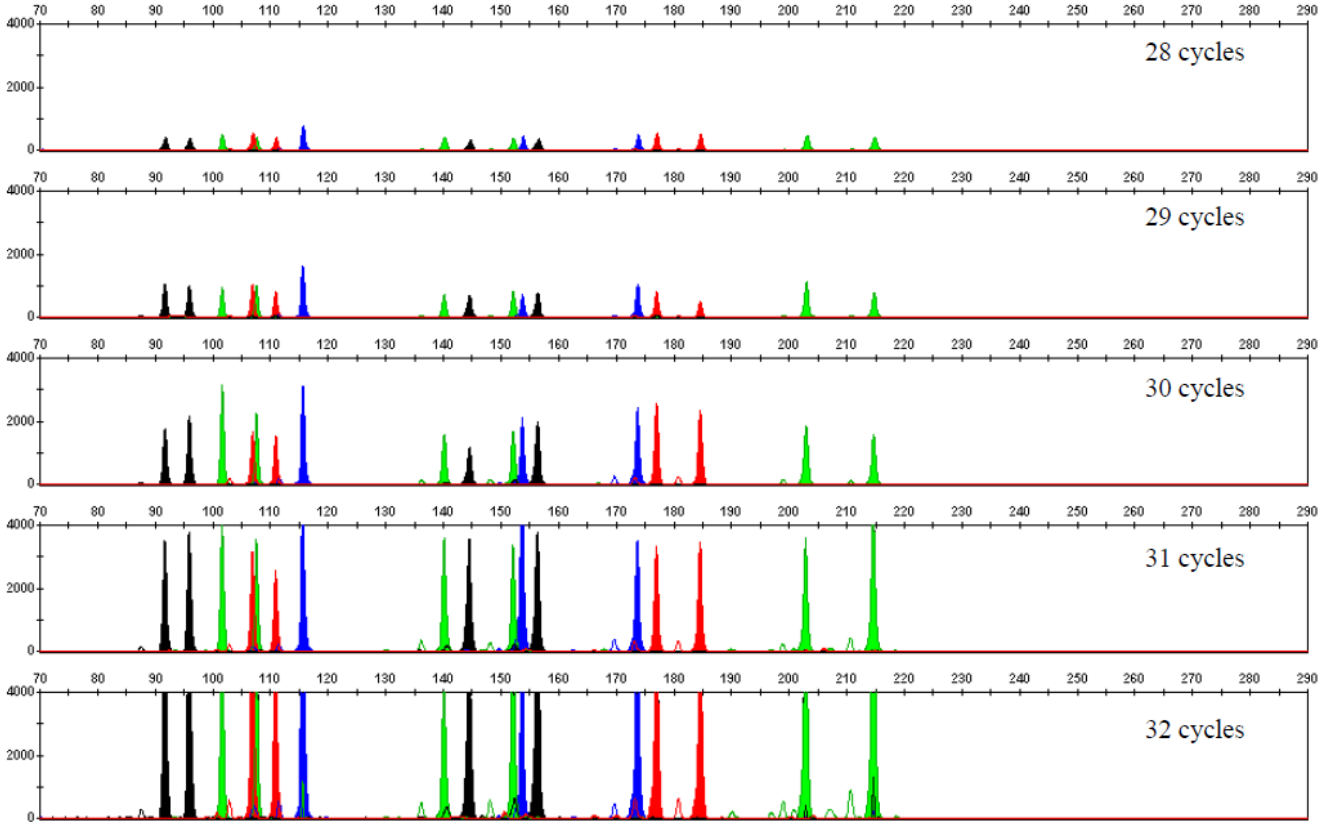


Figure 14 Representative MiniFiler™ kit profiles obtained from amplification of 0.50 ng of DNA template using 28, 29, 30, 31, and 32 cycles, analyzed on a 3130*xl* Genetic Analyzers (Y-axis scale 0–4,000 RFU).

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 observation

The size differences that are typically observed between sample alleles and the MiniFiler™ Allelic Ladder alleles on the 3130*xl* Genetic Analyzer with POP-4™ Polymer are shown in Figure 15. The X-axis represents the nominal base pair sizes for the allelic ladder. The dashed lines parallel to the X-axis represent the ±0.25-bp 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 bp from a corresponding allele in the allelic ladder.

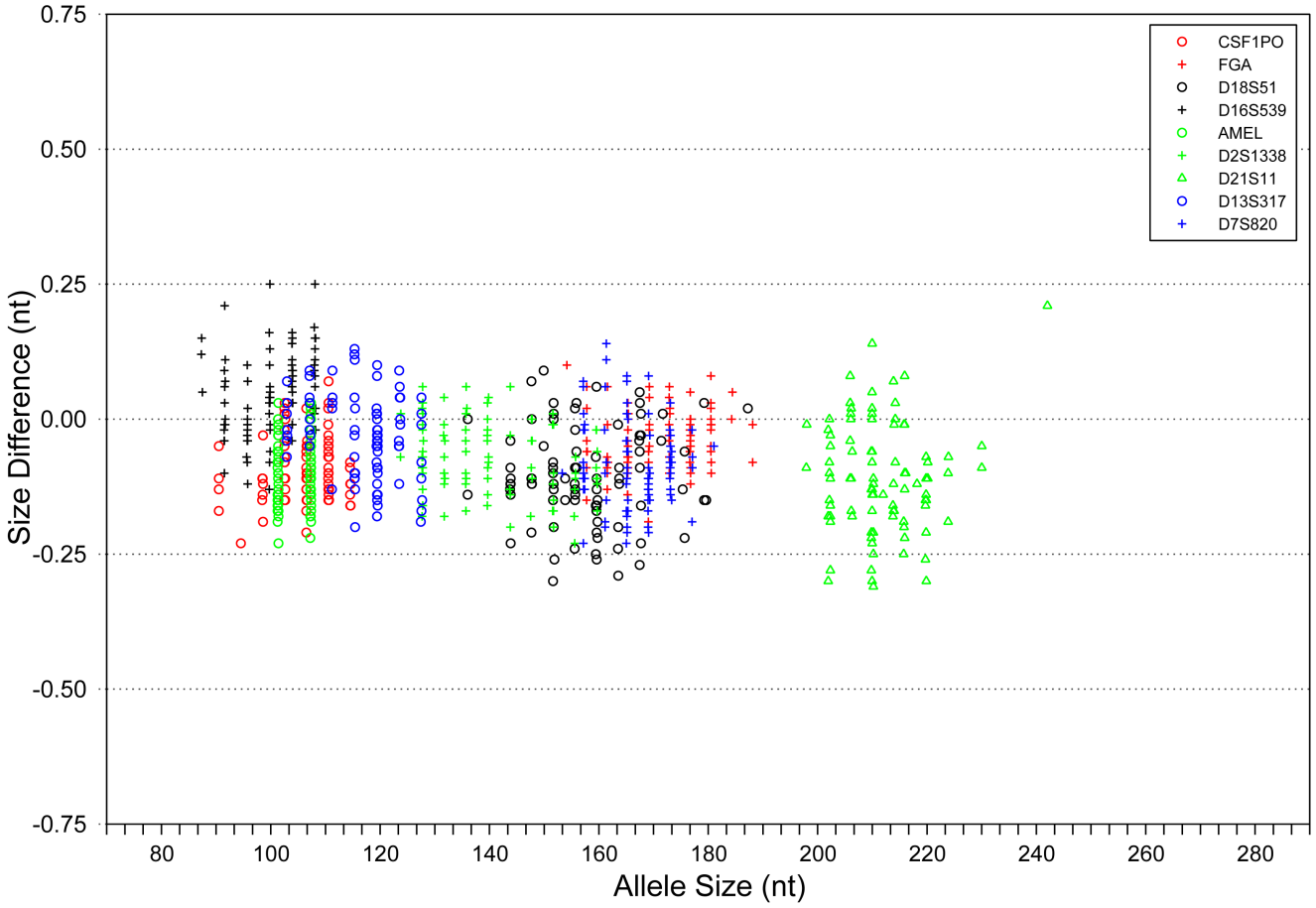


Figure 15 Allele size vs. allelic ladder sizing for 42 samples analyzed on a 3130*xl* Genetic Analyzer

Precision and size window description

Sizing precision enables the determination of accurate and reliable genotypes. The recommended method for genotyping is to use a ±0.5-bp "window" around the size obtained for each allele in the allelic ladder. A ±0.5-bp window allows for the detection and correct assignment of alleles. Any sample allele that sizes outside the specified window could be one of the following:

- An "off-ladder" allele; that is, an allele of a size that is not represented in the allelic ladder
- An allele that does correspond to an allele in the allelic ladder, 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 electrophoresis instrument.

Precision and size window observation

Typical precision results obtained from 5 runs (16 capillaries/run) of the MiniFiler™ Allelic Ladder on a 3130*xl* Genetic Analyzer (36-cm capillary and POP-4™ polymer) using GeneScan™ 500 LIZ™ Size Standard are shown in Table 7. The results were obtained within a set of injections on a single capillary array.

Sample alleles can occasionally size outside the ±0.5-bp window for a respective allelic ladder allele because of measurement error. The frequency of such an occurrence is lowest in detection systems that have the smallest standard deviations in sizing. The tight clustering of allele sizes obtained on the 3130*xl* Genetic Analyzer, where the standard deviation in sizing is typically <0.15 bp, is illustrated in Figure 15. The instance of a sample allele sizing outside the ±0.5-bp window because of measurement error is relatively rare when the standard deviation in sizing is approximately ≤0.15 bp (Smith, 1995).

For sample alleles that do not size within a ±0.5-bp 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-X* Software automatically flags 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 you compare the allele sizes to the sizes obtained for known alleles in the MiniFiler™ Allelic Ladder from the same run, then convert to genotypes as described in “Allelic ladder requirements for data analysis” on page 30. The results of five runs of the MiniFiler™ Allelic Ladder on a 3130*xl* Genetic Analyzer are shown in Table 7. The table shows the following:

- **Mean**—The mean sizes calculated for all alleles in each run (16 capillaries). The mean range represents the lowest and highest mean size values obtained across all five runs.
- **Standard deviation**—The standard deviation for the allele sizing calculated for all alleles in each run. The standard deviation range represents the lowest and highest standard deviation values obtained across all five runs.

Note: For more information on precision and genotyping, see Lazaruk *et al*., 1998 and Mansfield *et al*., 1998.

Table 7 Precision results of five runs (16 capillaries/run) of the MiniFiler™ Allelic Ladder on a 3130*xl* Genetic Analyzer

Allele	Mean	Standard deviation
AMEL		
X	101.54–101.59	0.024–0.037
Y	107.51–107.56	0.029–0.038

Table 7 Precision results of five runs (16 capillaries/run) of the MiniFiler Allelic Ladder on a 3130xl Genetic Analyzer *(continued)*

Allele	Mean	Standard deviation
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
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

Table 7 Precision results of five runs (16 capillaries/run) of the MiniFiler Allelic Ladder on a 3130xl Genetic Analyzer *(continued)*

Allele	Mean	Standard deviation
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
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

Table 7 Precision results of five runs (16 capillaries/run) of the MiniFiler Allelic Ladder on a 3130xl Genetic Analyzer *(continued)*

Allele	Mean	Standard deviation
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

Table 7 Precision results of five runs (16 capillaries/run) of the MiniFiler Allelic Ladder on a 3130xl Genetic Analyzer *(continued)*

Allele	Mean	Standard deviation
29.2	208.50–208.55	0.051–0.077
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

Table 7 Precision results of five runs (16 capillaries/run) of the MiniFiler Allelic Ladder on a 3130xl Genetic Analyzer *(continued)*

Allele	Mean	Standard deviation
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
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

Table 7 Precision results of five runs (16 capillaries/run) of the MiniFiler Allelic Ladder on a 3130xl Genetic Analyzer *(continued)*

Allele	Mean	Standard deviation
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.

Extra peaks: Stutter

Stutter definition

Stutter is a well-characterized PCR artifact that refers to the appearance of a minor peak one repeat unit smaller than the target STR allele product (minus stutter), or less frequently, one repeat larger (plus stutter) (Butler, 2005; Mulero *et al*., 2006). Sequence analysis of stutter products at tetranucleotide STR loci has revealed that the minus stutter product is missing a single tetranucleotide core repeat unit relative to the main allele (Walsh *et al.*, 1996). Although plus stutter is normally much less significant than minus stutter in STR loci with tetranucleotide repeats, the incidence of plus stutter may be more significant in trinucleotide repeat-containing loci.

Contact HID Support for more information on plus stutter.

The proportion of the stutter product relative to the main allele (percent stutter) is measured by dividing the height of the stutter peak by the height of the main allele peak.

Stutter observation

Peak heights were measured for amplified samples at the loci that are used in the kit. All data were generated on the 3130*x*l Genetic Analyzer. Some conclusions from these measurements and observations are:

- For each locus, the stutter percentage generally increases with allele length.
- Smaller alleles typically show a lower level of stutter relative to the longer alleles in each locus.
- Each allele in a locus displays a consistent stutter percentage.
- Peaks in the stutter position that are above the stutter filter percentage specified in the software are not filtered. (Stutter filter percentage is calculated as the mean stutter for the locus plus 3 standard deviations.) Peaks in the stutter position that have not been filtered and remain labeled can be further evaluated.
- The measurement of stutter percentage for allele peaks that are off-scale may be unusually high due to artificial truncation of the main allele peak.
- Stutter can be elevated when minus stutter and plus stutter overlap. This is typically observed when a given allele flanks another allele that is 2 repeat units away.
- The magnitude and/or variability of stutter may increase with low DNA input amounts.

Marker-specific plus stutter observed in the population study with the MiniFiler™ kit is shown in Figure 16 through Figure 19.

The stutter filter settings that are derived from these data are listed in “Stutter filter settings provided with the GeneMapper™ ID-X Software” on page 66.

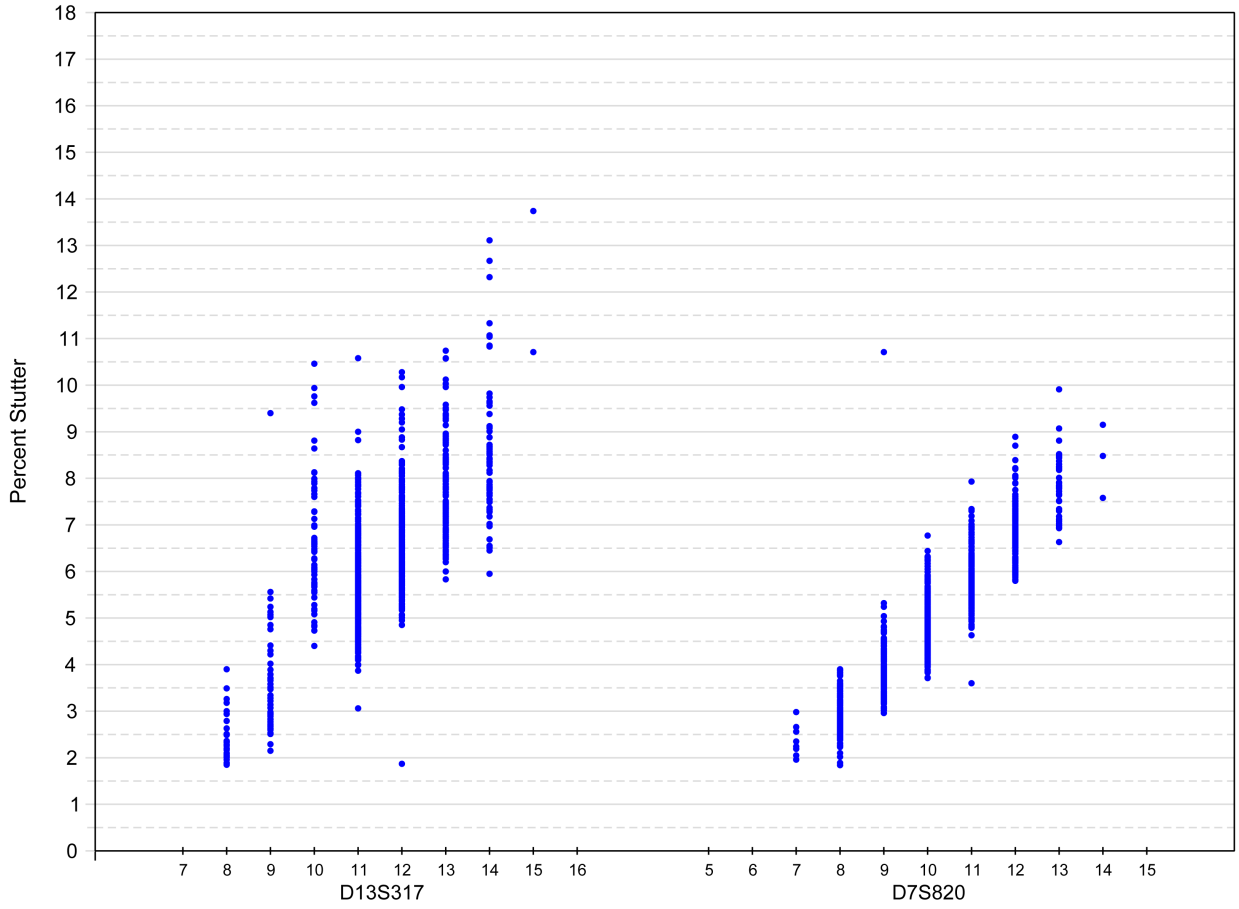


Figure 16 Stutter percentages for D13S317 and D7S820 loci (blue=6-FAM™ dye).

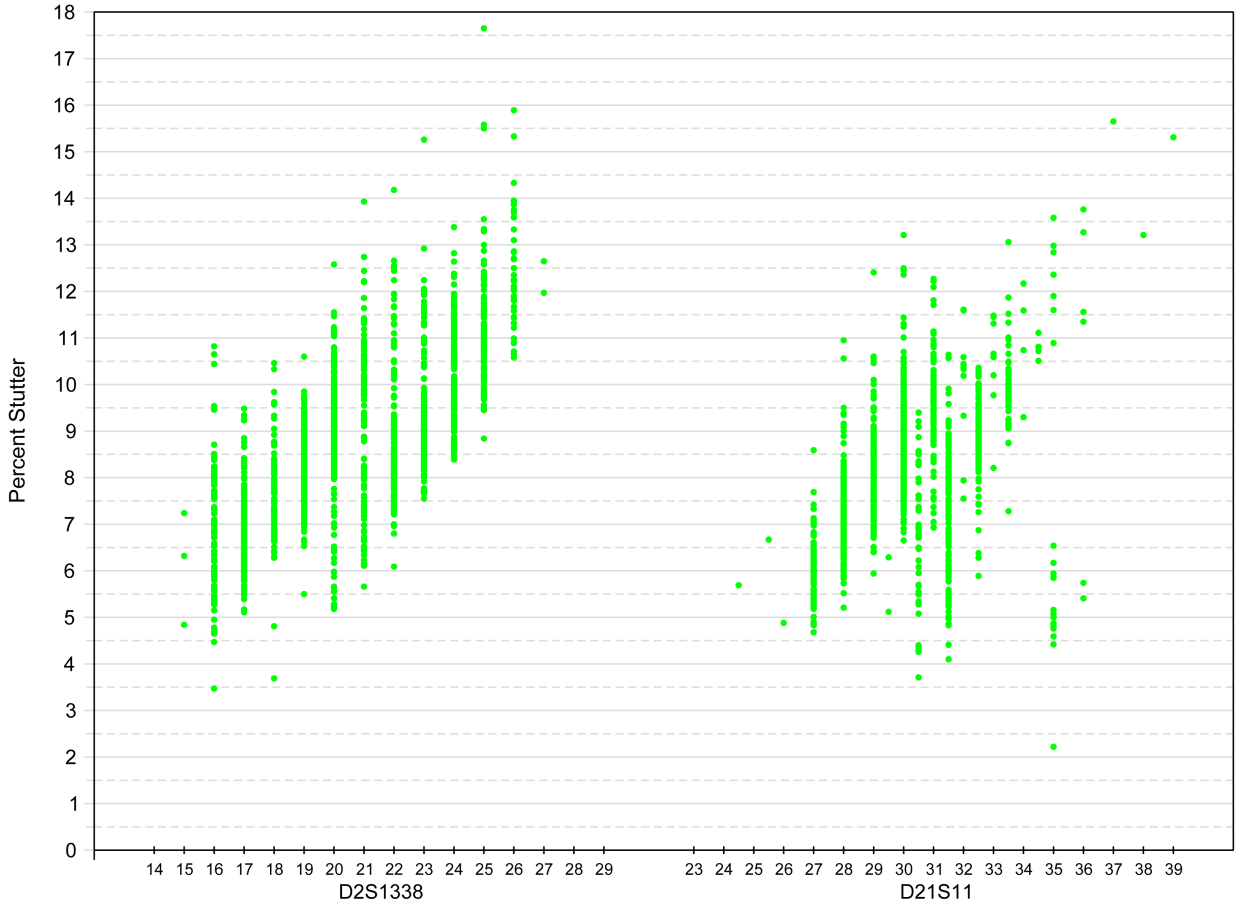


Figure 17 Stutter percentages for the D2S1338 and D21S11 loci (green=VIC™ dye).

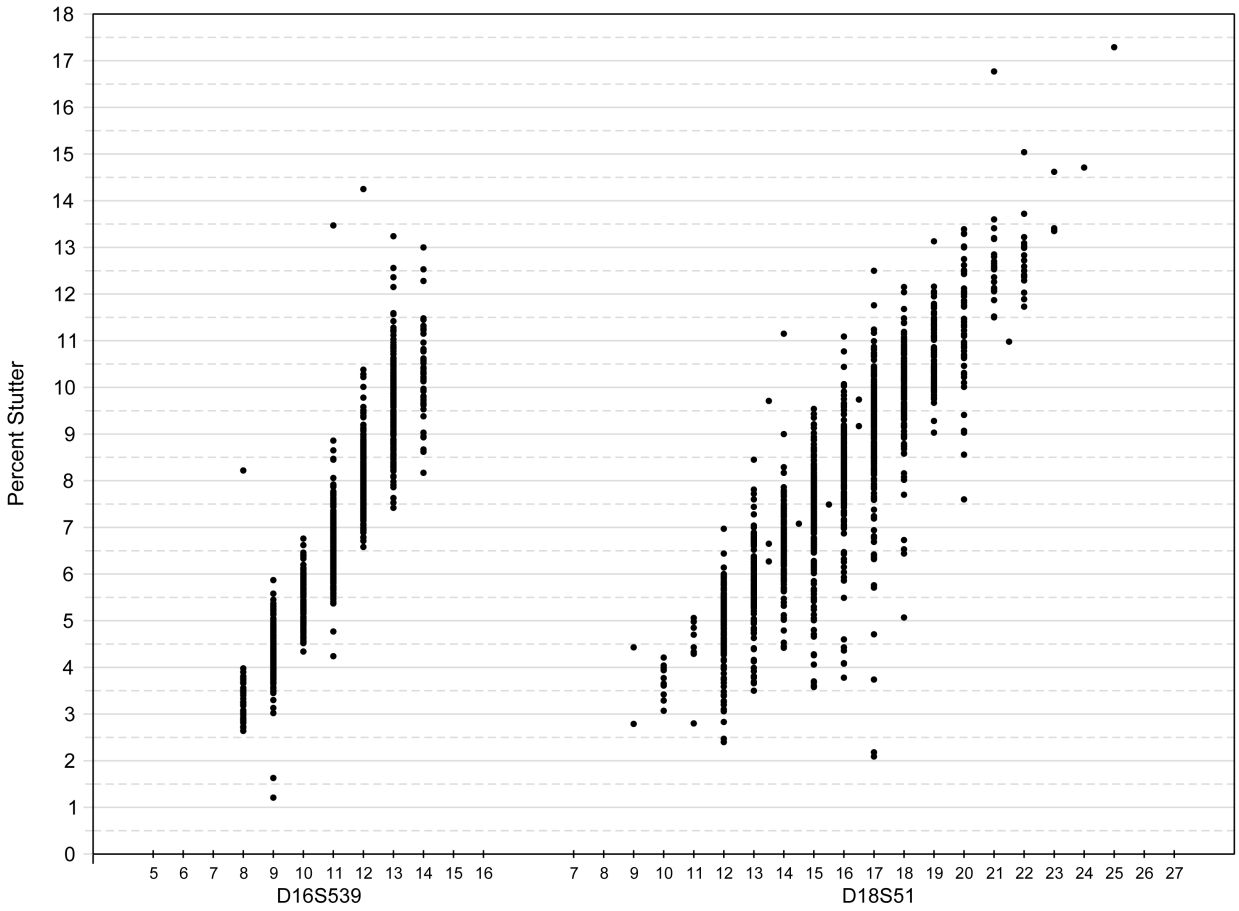


Figure 18 Stutter percentages for the D16S539 and D18S51 loci (black=NED™ dye).

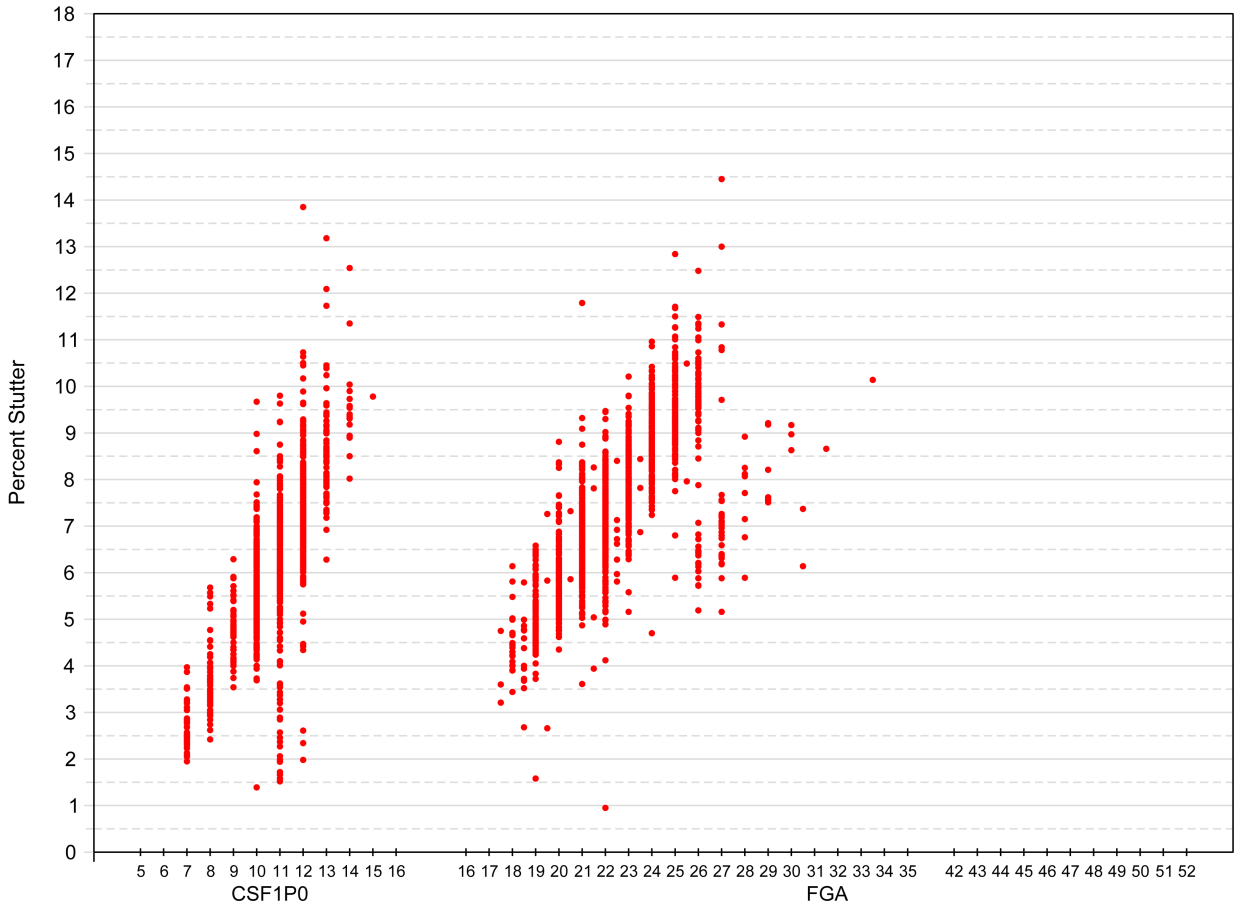


Figure 19 Stutter percentages for the CSF1PO and FGA loci (red=PET™ dye).

Stutter filter settings provided with the GeneMapper™ *ID-X* Software

The stutter filter settings are shown in Table 8. The data used to derive the settings are shown in Figure 16 through Figure 19. 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.

IMPORTANT! The values that are shown in the table are the values that were determined during developmental validation studies using specific data sets. To determine the appropriate values to use for your applications, always perform internal validation studies.

Table 8 Percentages used in the stutter filters included with the GeneMapper™ *ID-X* Software

Locus	% Stutter
D13S317	14
D7S820	11
D2S1338	18
D21S11	16

Table 8 Percentages used in the stutter filters included with the GeneMapper ID-X Software *(continued)*

Locus	% Stutter
D16S539	15
D18S51	18
CSF1PO	14
FGA	15

Extra peaks: Addition of 3' A nucleotide

3′ A nucleotide addition definition

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.

3′ A observation

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

- The primer sequences have been optimized to encourage +A addition.
- The PCR chemistry allows complete +A addition with a short final incubation for 5 minutes at 60°C.

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products. STR systems that have not been optimized for maximum +A addition may have "split peaks", where each allele is represented by 2 peaks that are 1 bp apart. Examples of incomplete and normal +A addition are shown in Figure 20.

Lack of complete +A nucleotide addition may be observed in the 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.

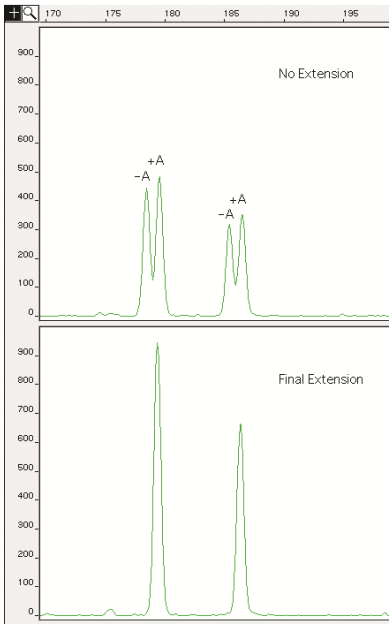


Figure 20 Omitting the final extension step resulted in split peaks due to incomplete A nucleotide addition. These data were generated on the 310 Genetic Analyzer using another AmpFℓSTR™ kit.

Extra peaks: Artifacts

Artifact definition

Artifacts and anomalies are seen in all molecular biological systems. Artifacts are typically reproducible. Anomalies are non-reproducible, intermittent occurrences that are not observed consistently in a system (for example, spikes and baseline noise).

Artifact observation

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 6-FAM™ (70 bp) and VIC™ (80 bp) dyes. Low-level artifacts in the calling region may appear in the 6-FAM™ (117 bp and 127 bp), VIC™ (118 bp), and NED™ (166 bp) dyes, depending on the sensitivity of the instrument.

Examples of baseline noise and artifacts in an electropherogram while using the MiniFiler™ kit are shown in Figure 21. You should consider possible noise and artifacts when interpreting data from the MiniFiler™ kit on the 3130*xl* Genetic Analyzer.

Note: To illustrate these artifacts, a high degree of magnification (Y-axis) is used in Figure 21.

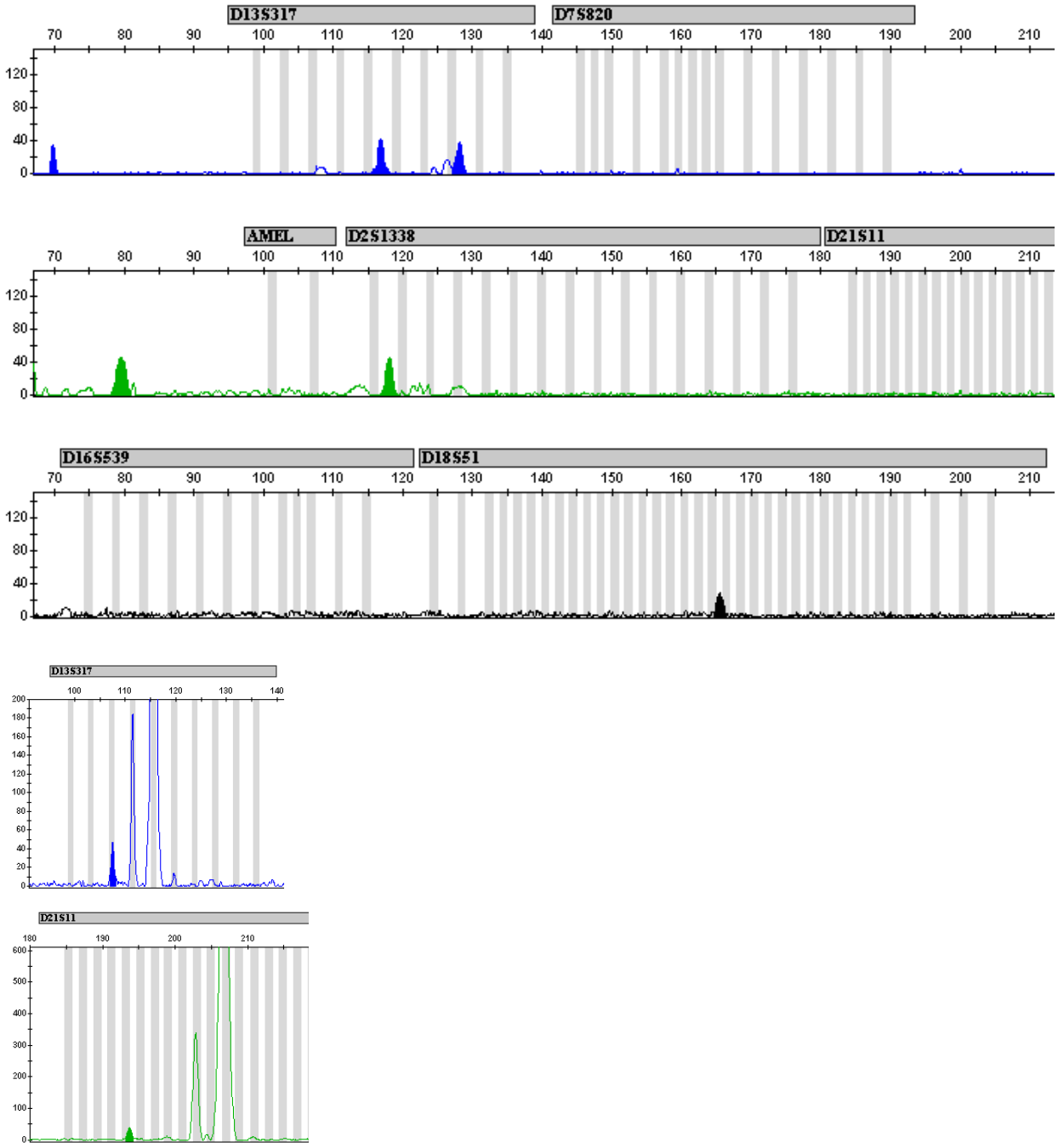


Figure 21 Examples of baseline noise and reproducible artifacts in data produced on a 3130*xl* Genetic Analyzer

Another type of PCR artifact occurs when the amount of input DNA exceeds the recommended amount (0.50–0.75 ng). These artifacts were characterized as secondary stutter products in D13S317 and D21S11 as shown in the bottom example in Figure 21. Their mobility varies with that of the main amplification product.

Genotyping may result in the detection of these artifacts as off-ladder (**OL**) alleles.

Characterization of loci

SWGDAM guideline 2.1

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

Loci in this kit

This section describes basic characteristics of the 8 loci and sex-determining marker (Amelogenin) that are amplified with the MiniFiler™ kit. Most of these loci have been extensively characterized by other laboratories.

Nature of polymorphisms

The primers for the Amelogenin locus flank a 6-nucleotide deletion within intron 1 of the X homologue. Amplification results in 104-bp and 110-bp 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-bp repeat units.

We have sequenced all the alleles in the 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).

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; and Lareu, *et al.*, 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)

Nonhuman study observation

The MiniFiler™ kit provides the required specificity for detecting human alleles. Species specificity testing was performed to show that there is no cross-reactivity with nonhuman DNA that may be present in forensic casework samples.

The following species were tested (in the specified amounts) using standard PCR and capillary electrophoresis conditions for the 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 albicans, Escherichia coli, Lactobacillus casei, Staphylococcus aureus, Neisseria gonorrhoeae, Bacillus subtilis, and Lactobacillus rhamnosus* (equivalent to 10^5 copies)

Results were evaluated for the presence of any amplified peaks that would indicate cross-reactivity of the kit with any of these non-human species.

The chimpanzee and gorilla DNA samples produced partial profiles within the 70–283 bp region. The remaining species tested did not yield reproducible detectable products.

Example electropherogram results from the species specificity tests are shown in Figure 22.

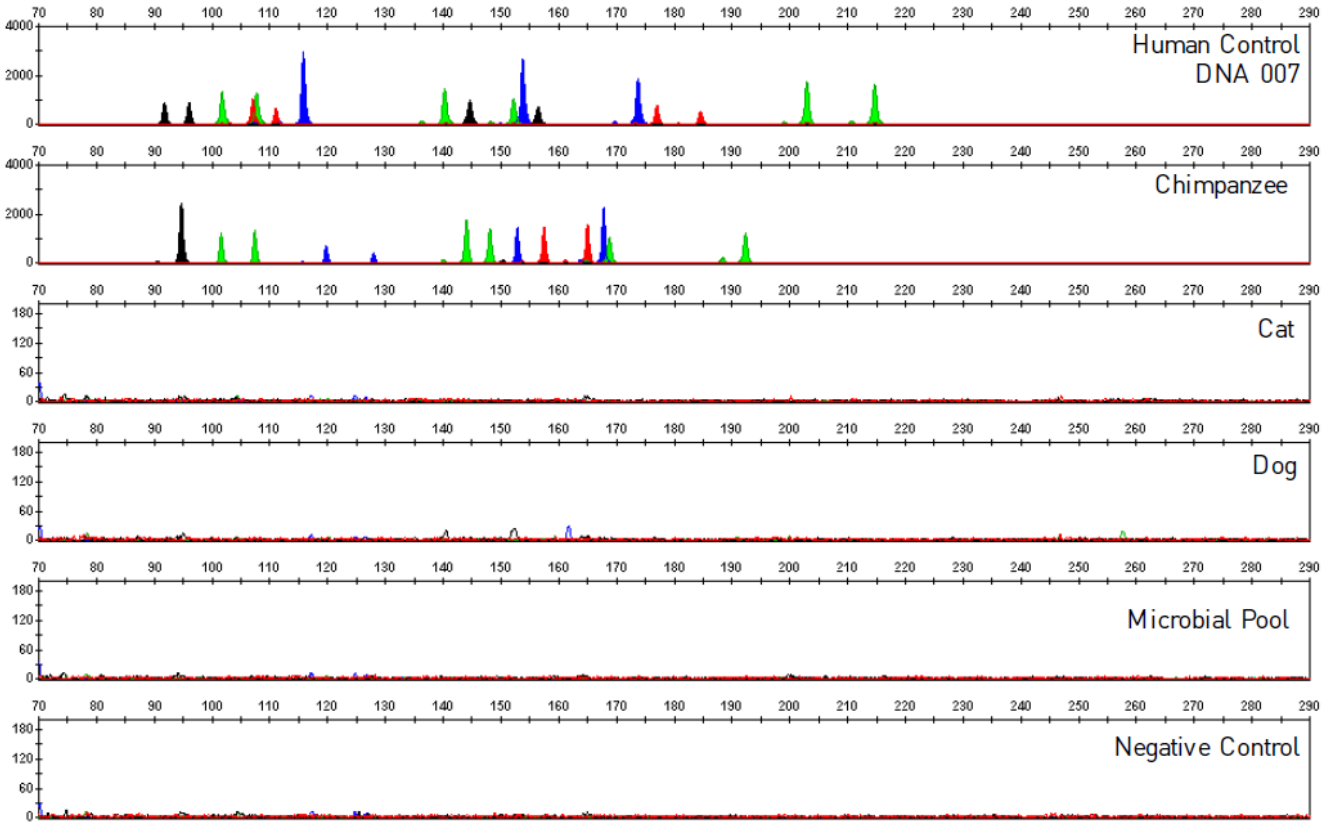


Figure 22 Representative electropherograms from a species specificity study including positive and negative controls (Y-axis scale 0–180 or 0–4,000 RFU).

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 the following:

- Fluorescence intensity that exceeds the linear dynamic range for detection by the capillary electrophoresis instrument ("off-scale" data). Off-scale data are a problem because:
  - Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause a corresponding stutter peak to appear higher in relative intensity, therefore 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.

To address these problems, rerun the amplification reaction using less DNA.

If too little DNA is added to the PCR reaction, the total number of allele copies added to the PCR reaction could be extremely low. Unbalanced amplification of the alleles can occur because of stochastic fluctuation.

Sensitivity observation

The amplification results of different input DNA amounts are shown in Figure 23.

To determine an appropriate minimum peak height threshold for your instruments and data, perform internal validation studies.

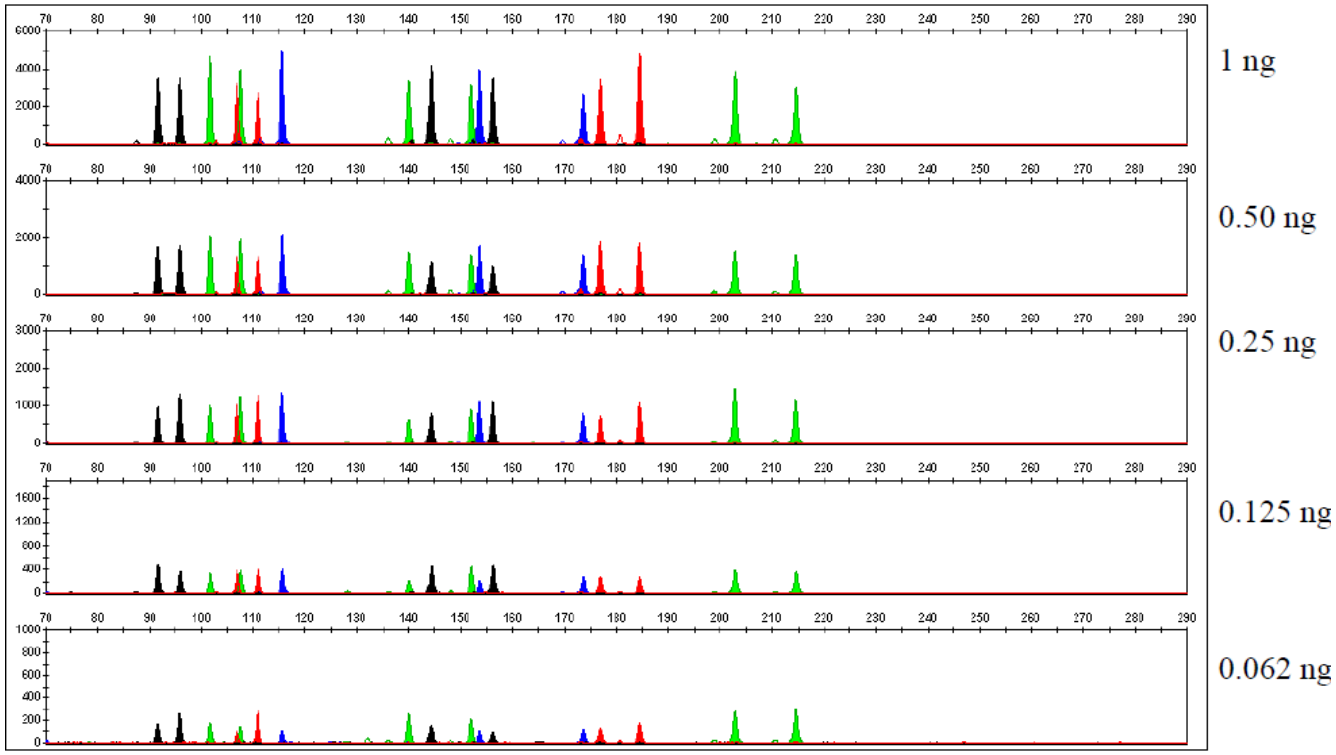


Figure 23 Effect of amplifying 1 ng, 0.5 ng, 0.25 ng, 0.125 ng, and 0.62 ng of DNA Control 007. Data analyzed using the 3130*xl* Genetic Analyzer (Y-axis scale 0–1,000, 0–1,600, 0–3,000, 0–4,000, or 0–6,000 RFU; the Y-axis scale is magnified for the lower amounts of DNA).

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)

Lack of amplification of some loci

As with any multi-locus system, the possibility exists that not every locus amplifies. This possibility is most often observed when the DNA sample contains PCR inhibitors or when the DNA sample has been severely degraded. Valuable information can be obtained from partial profiles.

Degraded DNA

As the average size of degraded DNA approaches the size of the target sequence, the amount of PCR product generated is reduced. This is 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 (0–6 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.

1 ng of degraded DNA was amplified using the MiniFiler™ kit and AmpFℓSTR™ Identifiler™ PCR Amplification Kit. 2 ng of degraded DNA was amplified using the AmpFℓSTR™ SGM Plus™ PCR Amplification Kit. As the DNA became increasingly degraded, the larger size Identifiler™ kit and SGM Plus™ kit loci became undetectable. However, amplification with the MiniFiler™ kit resulted in an increased overall typing success rate.

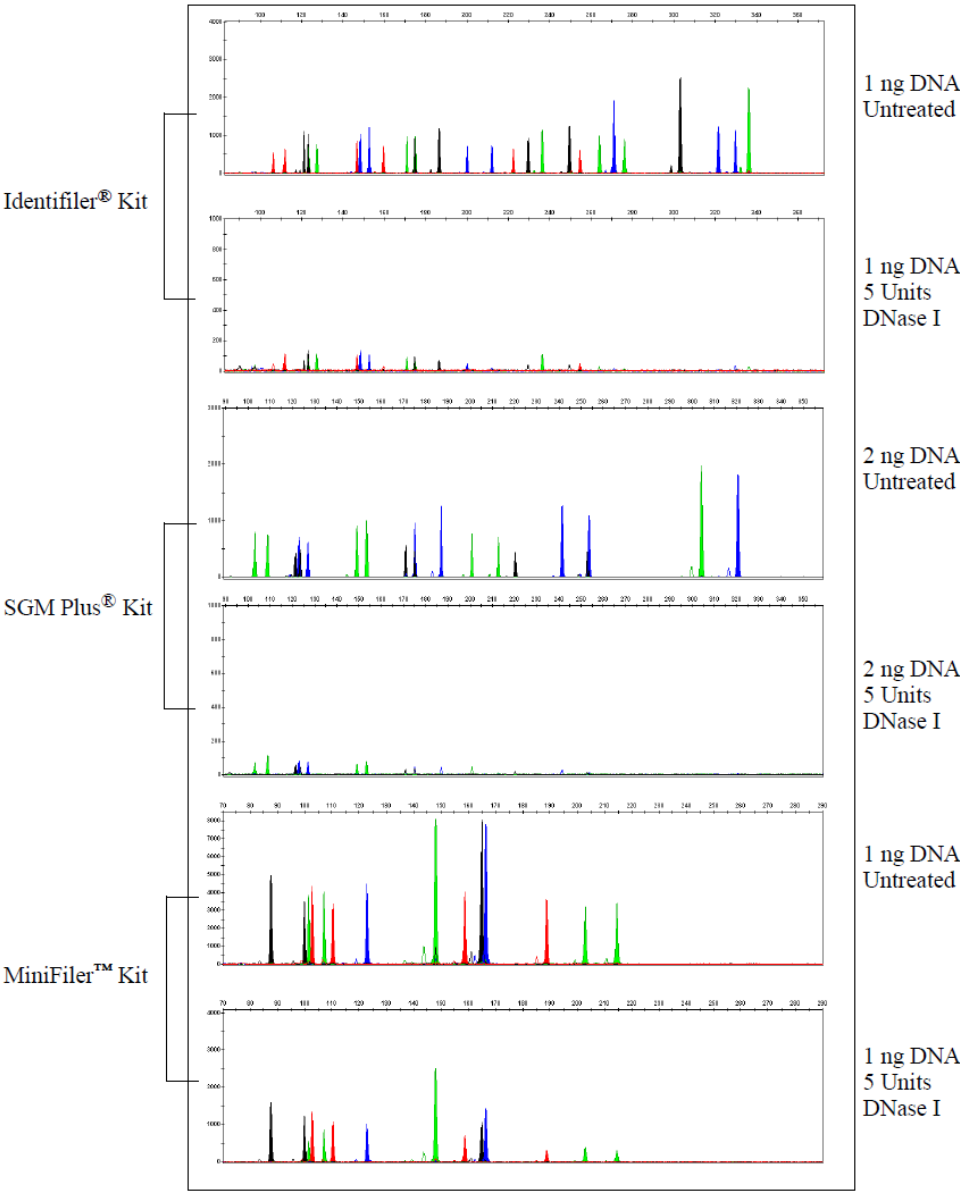


Figure 24 Amplification of Raji DNA samples untreated or sonicated for 5 minutes and incubated with DNase I (Y-axis scale 0–1,000, 0–3,000, 0–4,000, or 0–8,000).

The performance of the three kits was compared in a simulated model of DNA degradation (sonication and DNase I treatments). Only those loci (>50 RFU) represented in the MiniFiler™ kit were measured in the Identifiler™ and SGM Plus™ kits. A complete profile with Raji DNA yields 14 peaks using the MiniFiler™ kit. See Table 9.

Table 9 Comparison of MiniFiler™, Identifiler™, and SGM Plus™ kit performance in simulated DNA degradation (n=3)

DNase I	Number of alleles detected		
	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 performance of the MiniFiler™ kit, DNA Control 007 (1 ng input DNA for the MiniFiler™ kit and 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 25).

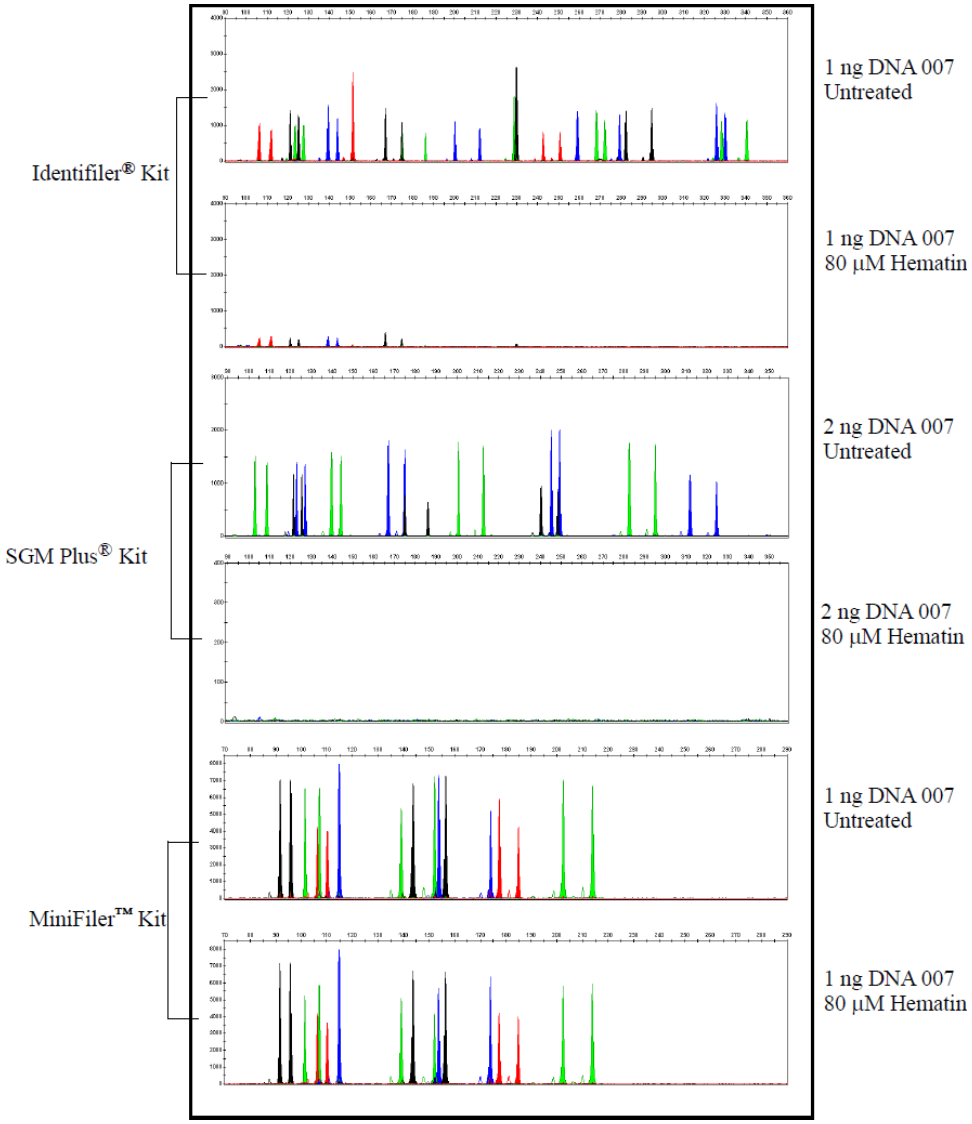


Figure 25 Amplification of DNA Control 007 in the presence of hematin analyzed on a 3130*xl* Genetic Analyzer (Y-axis scale 0–400, 0–3,000, 0–4,000, or 0–8,000 RFU).

The performance of the three kits was compared in a simulated model of hematin inhibition. Only those loci (>50 RFU) represented in the MiniFiler™ kit were measured in the Identifiler™ and SGM Plus™ kits. A complete profile with DNA Control 007 yields 17 peaks using the MiniFiler™ kit. See Table 10.

Table 10 Comparison of MiniFiler™, Identifiler™, and SGM Plus™ kit performance in simulated hematin inhibition (n=3)

Hematin concentration	Number of alleles detected		
	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 can 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 DNA Control 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 26). The concentrations of humic acid tested were 0, 10, 30, and 50 ng/µL.

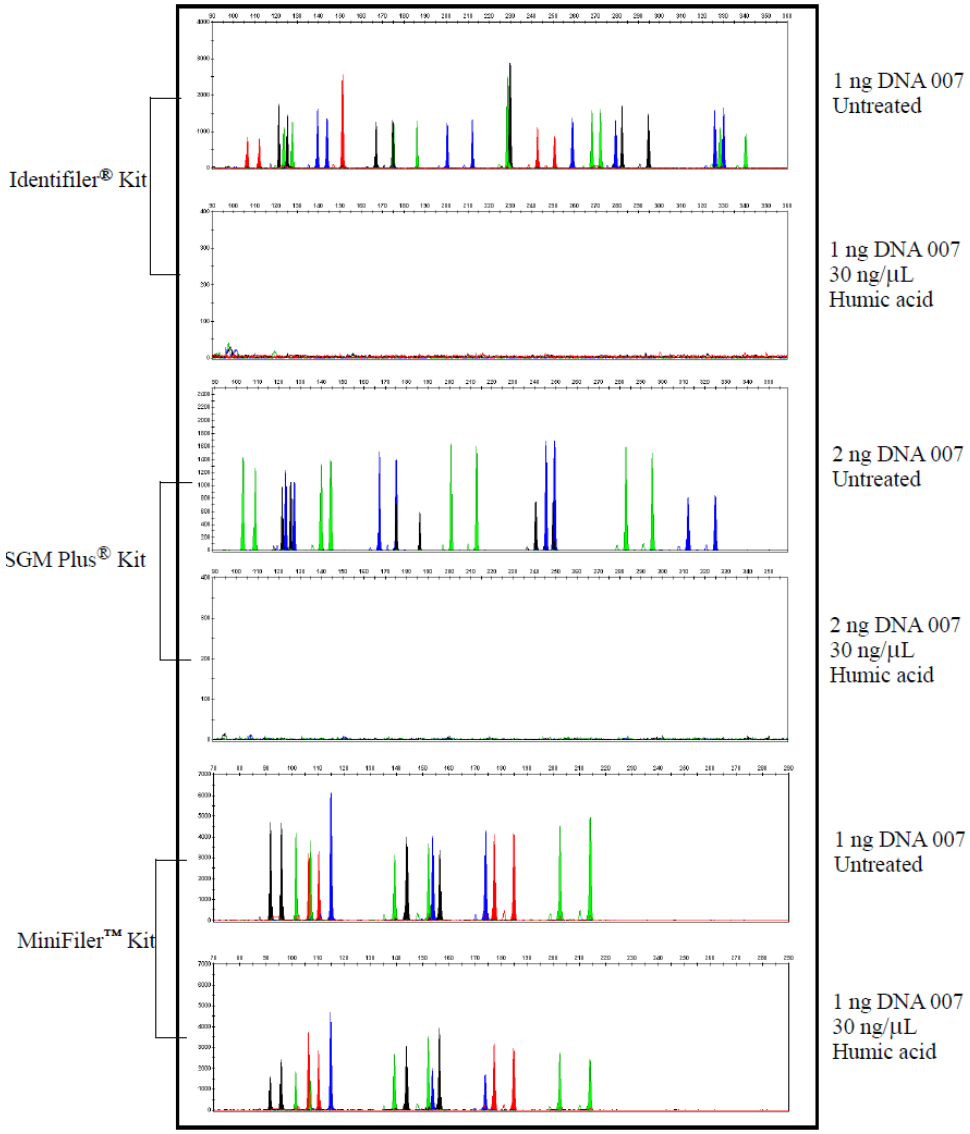


Figure 26 Amplification of DNA Control 007 in the presence of humic acid analyzed on a 3130*xl* Genetic Analyzer (Y-axis scales 0–400, 0–2,400, 0–4,000, or 0–7,000)

The performance of the three kits was compared in a simulated model of humic acid inhibition. Only those loci (>50 RFU) represented in the MiniFiler™ kit were measured in the Identifiler™ and SGM Plus™ kits. A complete profile with DNA Control 007 yields 17 peaks using the MiniFiler™ kit. See Table 11.

Table 11 Comparison of MiniFiler™, Identifiler™, and SGM Plus™ kit performance in simulated humic acid inhibition (n=5)

Humic acid concentration	Number of alleles detected		
	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)

Mixture study overview

Evidence samples that contain body fluids and/or tissues originating from more than one individual are an important category of forensic casework.

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

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

The possibility of multiple contributors should be considered when interpreting the results. Perform studies to determine a minimum peak height threshold to avoid typing when stochastic effects are likely to interfere with accurate interpretation of mixtures.

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.

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

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

Mixture study observation

Mean, median, minimum, and maximum peak height ratios observed for alleles in the MiniFiler™ kit loci in unmixed human population database samples are shown in Table 12.

Table 12 Peak height ratios for 0.5 ng input DNA

Locus	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

Resolution of genotypes in mixed samples

A sample that contains DNA from two sources can comprise (at a single locus) any of the following seven genotype combinations:

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

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

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

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

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, then electrophoresed and detected using a 3130*xl* Genetic Analyzer.

The results of the mixed DNA samples are shown in Figure 27, where samples A and B were mixed according to the ratios indicated. The minor component allele calls at non-overlapping loci are highlighted. Detection of full profiles for the minor contributor was possible at ratios of 3:1 (0.750:0.250 ng) and 7:1 (0.875:0.125 ng). Generally, 15:1 ratios resulted in partial profiles for the minor component. The profiles of these samples are described in Table 13.

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

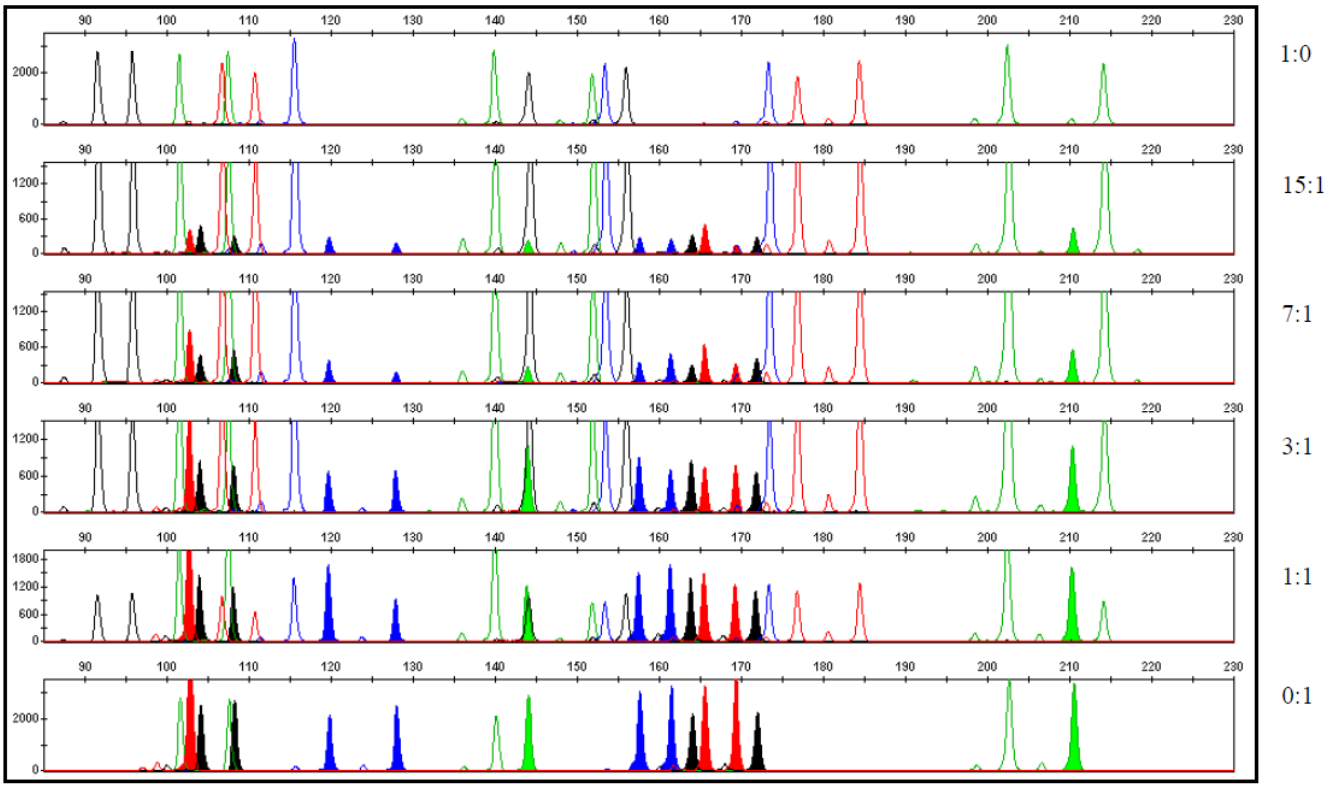


Figure 27 Amplification of DNA mixtures at various ratios. Panels show electropherograms for (top to bottom) 1:0 (major contributor only), 15:1 mixture, 7:1 mixture, 3:1 mixture, 1:1 mixture, and 0:1 (minor contributor only).

Table 13 Genotypes of mixed DNA samples

Locus	Sample A genotype	Sample B genotype
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

Population data

SWGDAM guideline 2.7

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

Loci in the kit

The MiniFiler™ kit contains loci for which extensive population data are available. For additional information on 8 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* (Pub. No. 4323291).

Population distribution

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 the reference sample for a suspect, 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 the reference sample for a suspect, 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 populations.

Analysis across Thermo Fisher Scientific databases

Analysis across four Thermo Fisher Scientific databases of 2,274 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 Thermo Fisher Scientific 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 (5 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 the *AmpFℓSTR™ Identifiler™ PCR Amplification Kit User Guide* (Pub. No. 4323291). The minimum reportable genotype frequency at each locus is as follows:

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

Therefore, the minimum combined multi-locus genotype frequency at 8 loci is as follows:

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

Concordance studies

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

We analyzed 1,064 samples from four different populations 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 some minor discordance, as shown in Table 14.

Table 14 Discordant genotypes between the MiniFiler™ and Identifiler™ kits across four different populations

Locus	African-American (n=347)	Caucasian (n=353)	Hispanic (n=207)	Asian (n=157)
D13S317 [1]	1.73%	0.57%	1.45%	—
D7S820	0.29%	—	—	—
CSF1PO	—	—	0.48%	—
D16S539	1.73%	—	—	0.64%
D18S51	—	—	0.48%	—

[1] 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.

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 a 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.

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-bp 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

Probability of identity definition

The probability of identity (P_I) value is the probability that two individuals selected at random will have an identical genotype (Sensabaugh, 1982).

Probability of identity observation

The probability of identity (P_I) values of the MiniFiler™ kit loci (individually and combined) are shown in Table 15.

The P_I values for the populations described in this section are approximately:

- African-American—$1/1.53 \times 10^{10}$
- U.S. Caucasian—$1/1.22 \times 10^{10}$
- U.S. Hispanic—$1/9.57 \times 10^{9}$
- Native American—$1/4.82 \times 10^{9}$

Table 15 Probability of identity (P_I) values for the MiniFiler™ kit loci

Locus	African-American	U.S. Caucasian	U.S. 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

Probability of paternity exclusion definition

The probability of paternity exclusion (PE) 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 STR loci in the kit (Chakraborty, Stivers, and Zhong, 1996).

Probability of paternity exclusion observation

The PE values of the MiniFiler™ kit STR loci (individually and combined) are shown in Table 16.

Table 16 Probability of paternity exclusion values for the MiniFiler™ kit STR loci

Locus	African-American	U.S. Caucasian	U.S. 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

6 Performance validation after buffer and enzyme replacement

Overview

As part of a 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 was transitioned from Roche Molecular Systems to Thermo Fisher Scientific.

Manufacturing of both components by Thermo Fisher Scientific is conducted according to the same specifications previously used by Roche. The in-house components are established raw materials in our other PCR amplification kits (for example, the NGM™, NGM SElect™ and Identifiler™ PLUS™ kits).

Experiments

We performed studies to compare the performance of the MiniFiler™ kit that contains the in-house components (updated kit) with the performance of the original kit, focusing on studies most relevant to forensic DNA testing (see SWGDAM Guidelines effective January 1, 2011). Each laboratory using the kit should assess their own requirements for evaluation of kits.

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 Thermo Fisher Scientific (Test mixes). Studies were performed using Test mixes containing both the enzyme and buffer manufactured by Thermo Fisher Scientific.

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 was used to perform reproducibility, sensitivity, degraded DNA, and inhibition studies. All amplifications were performed using a GeneAmp™ PCR System 9700 with a silver block or gold-plated silver block using the recommended amplification conditions and cycle number for the MiniFiler™ kit. All data were run on a 3130*xl* Genetic Analyzer with Data Collection Software v3.0 and analyzed using GeneMapper™ *ID-X* Software. Subsequent data analysis was performed using Minitab™ Statistical Software. To minimize the effect of injection-to-injection variation on result interpretation, peaks heights for all studies were normalized using an in-house, multicolor reference standard.

Reproducibility study

For the reproducibility study, 12 replicates of DNA Control 007 at 0.5 ng input and 3 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 28). The levels of intracolor balance obtained for all Test and Control mixes fall within the expected range of performance for the MiniFiler™ kit.

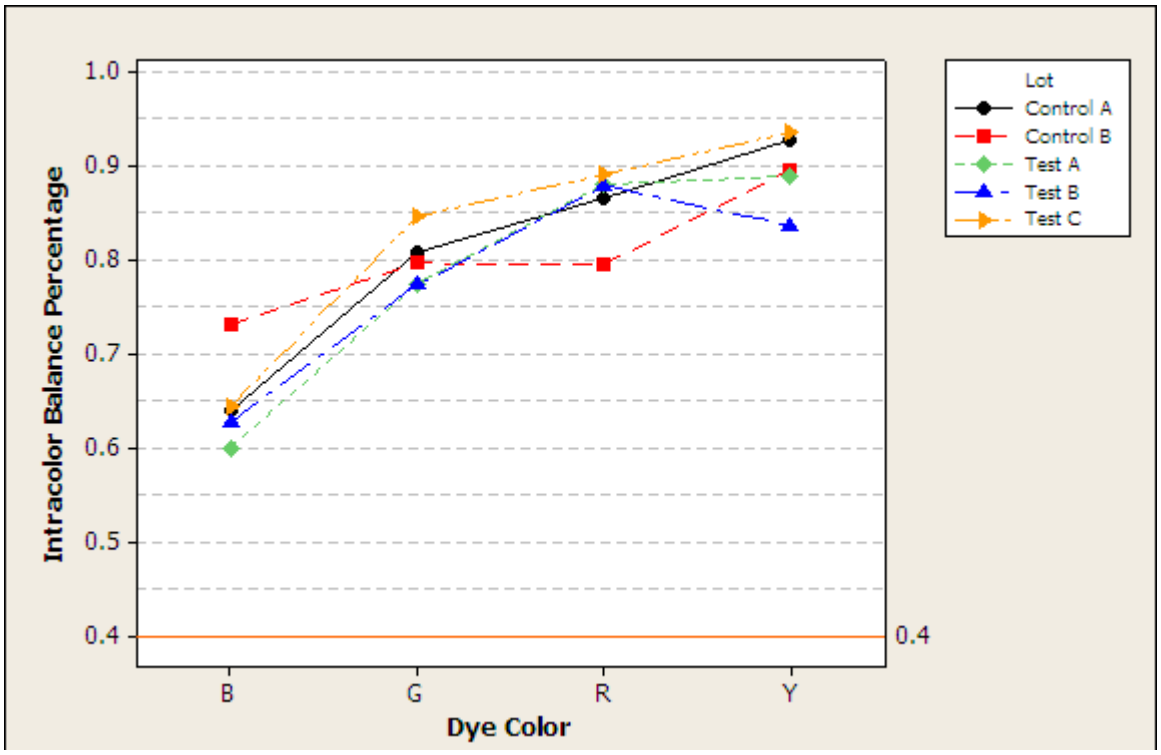


Figure 28 Reproducibility study: intracolor balance

Stutter percentages

Stutter percentage results for each marker were comparable across all Test and Control mixes. See Figure 29.

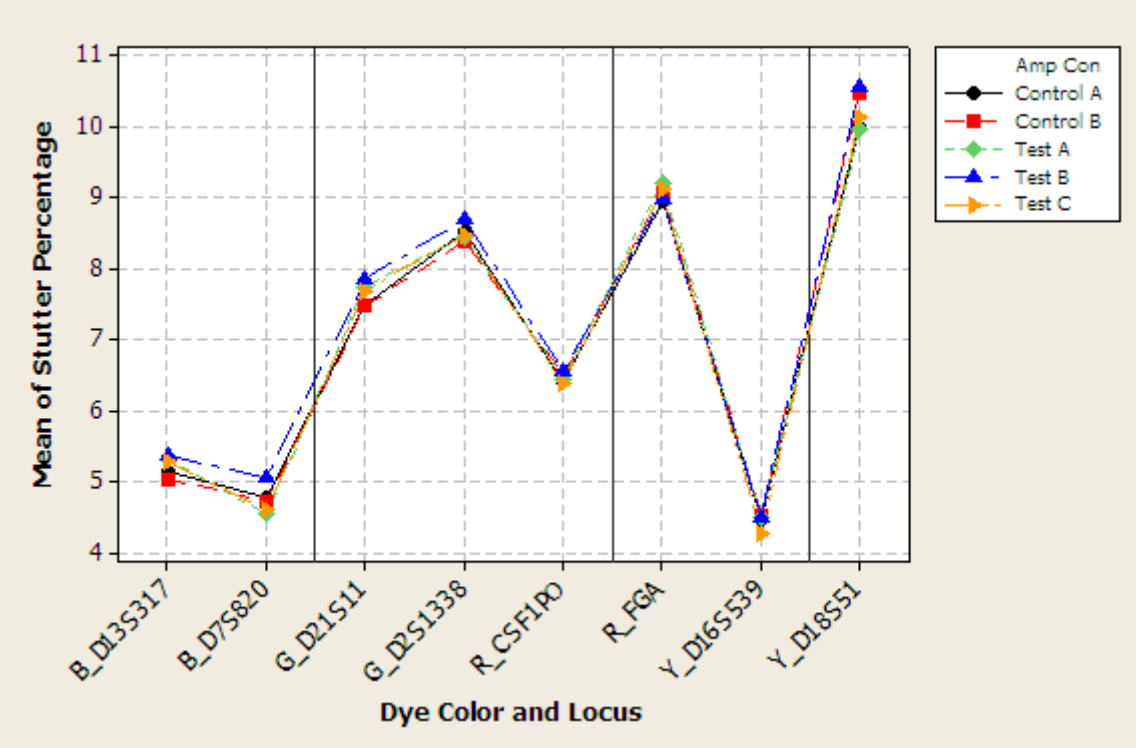


Figure 29 Reproducibility study: mean stutter percentage

Artifacts

Known artifacts observed showed the same morphology, signal intensity, and location in all Test and Control mixes and did not exceed 50 RFU. 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™ dye (green) at ~115 bp for all Test and Control mixes but did not exceed 50 RFU. See Figure 30.

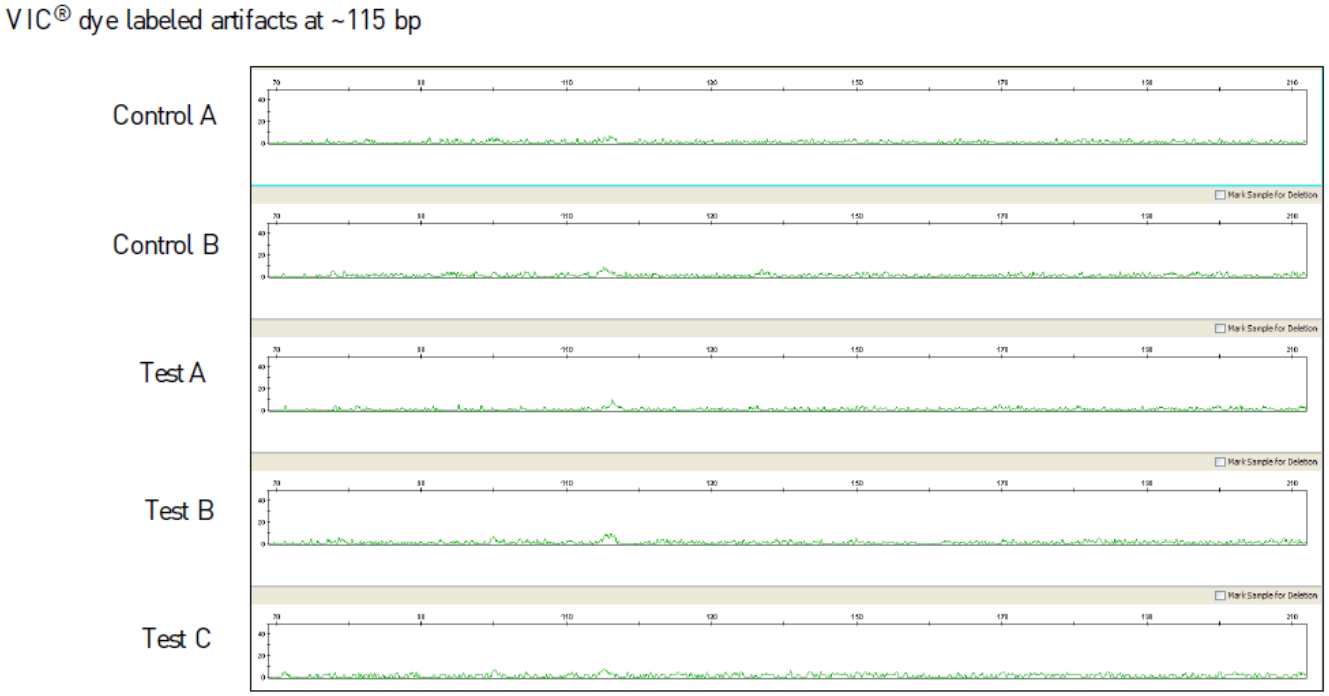


Figure 30 Reproducibility study: known artifacts VIC™ dye (Y-axis scale 0–50 RFU)

Sensitivity study

For the sensitivity study, dilution series of 3 genomic DNA samples were amplified: 0.75 ng (3 replicates), 0.5 ng, 0.25 ng, and 0.125 ng (4 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, demonstrating equivalent performance. See Figure 31 and Figure 32.

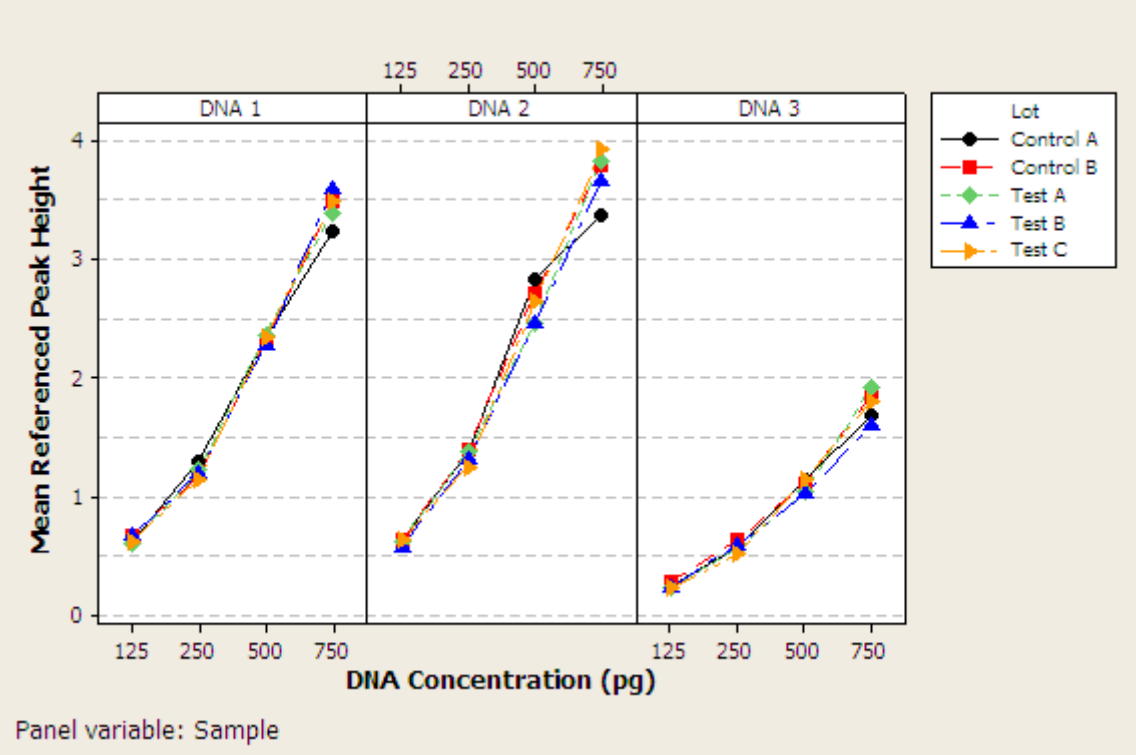


Figure 31 Sensitivity study: mean referenced peak heights for 3 genomic DNA samples

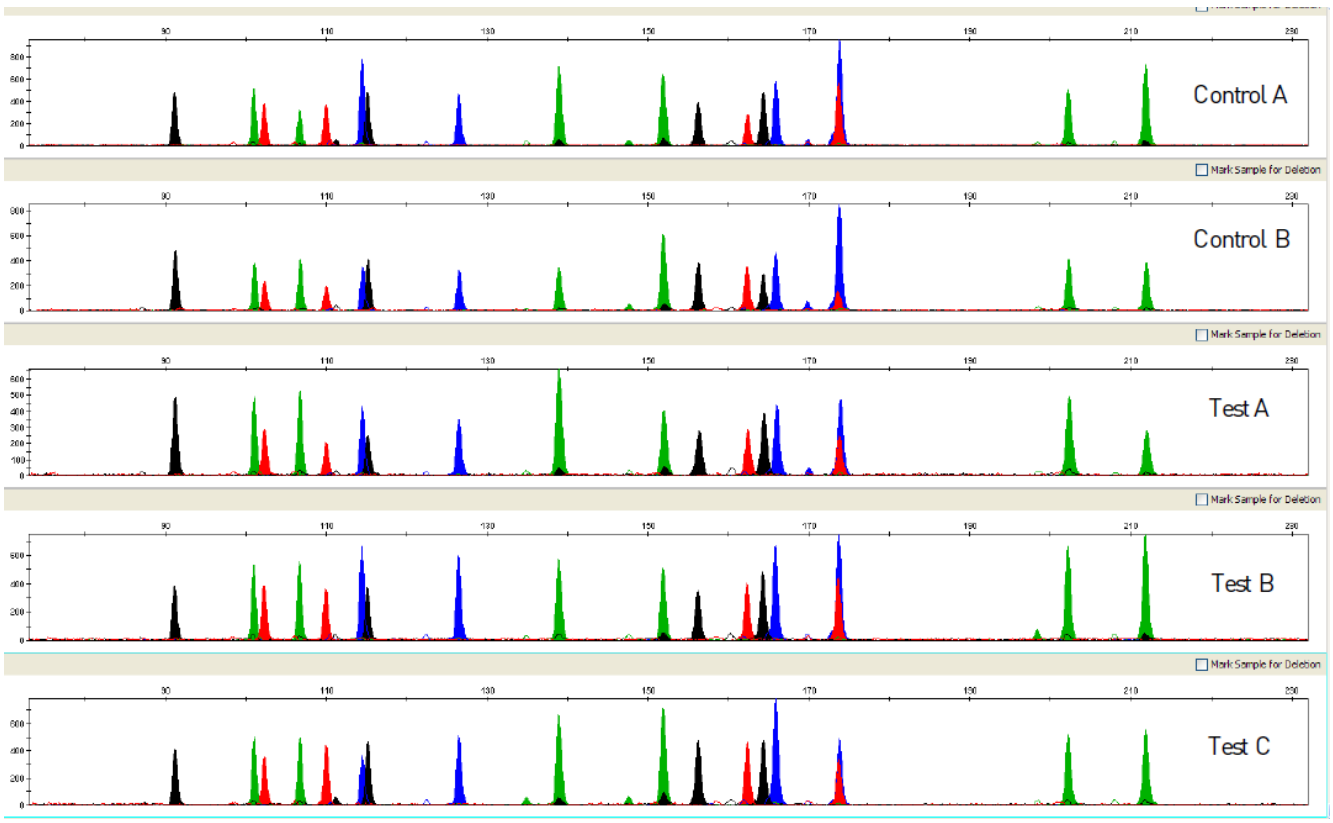


Figure 32 Sensitivity study: representative electropherograms for Sample 2 amplified using 250 pg input DNA (Y-axis scale 0–500 RFU)

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. See Figure 33.

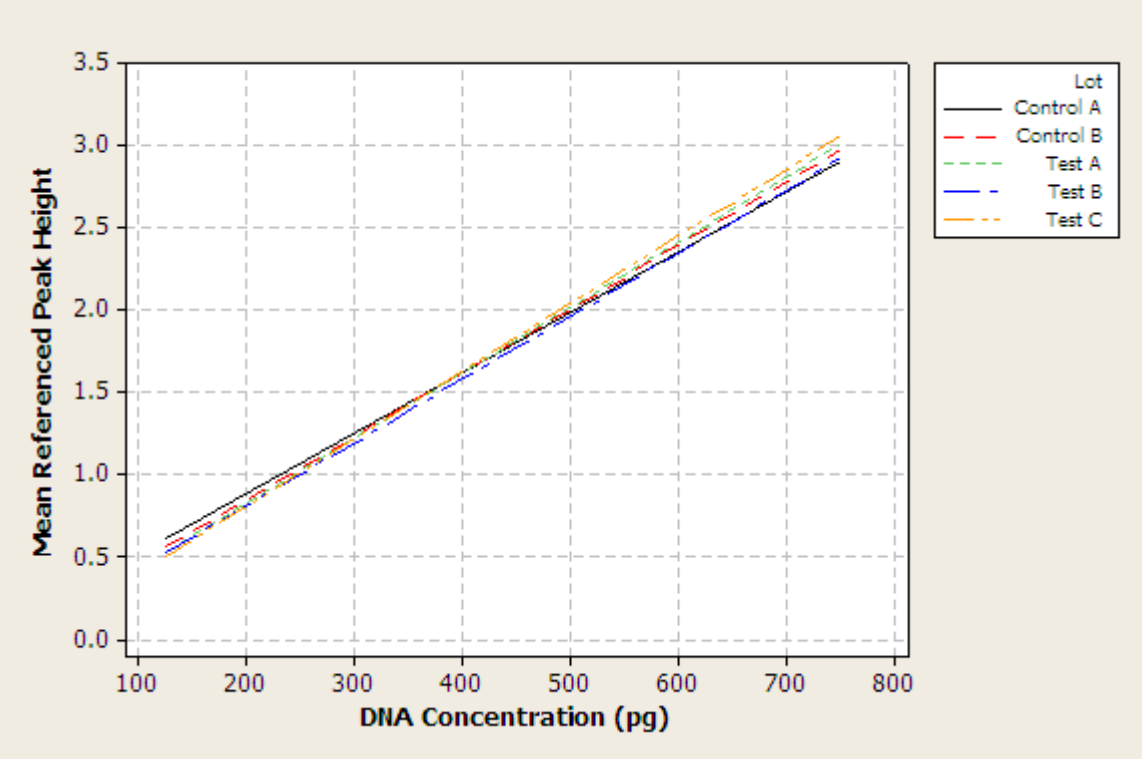


Figure 33 Sensitivity study: linear regression plot of combined mean referenced peak height for 3 genomic DNA samples

Allelic dropout

Allelic dropout was observed only 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 17. Examples of allelic dropout are shown in Figure 34 and Figure 35.

Table 17 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%

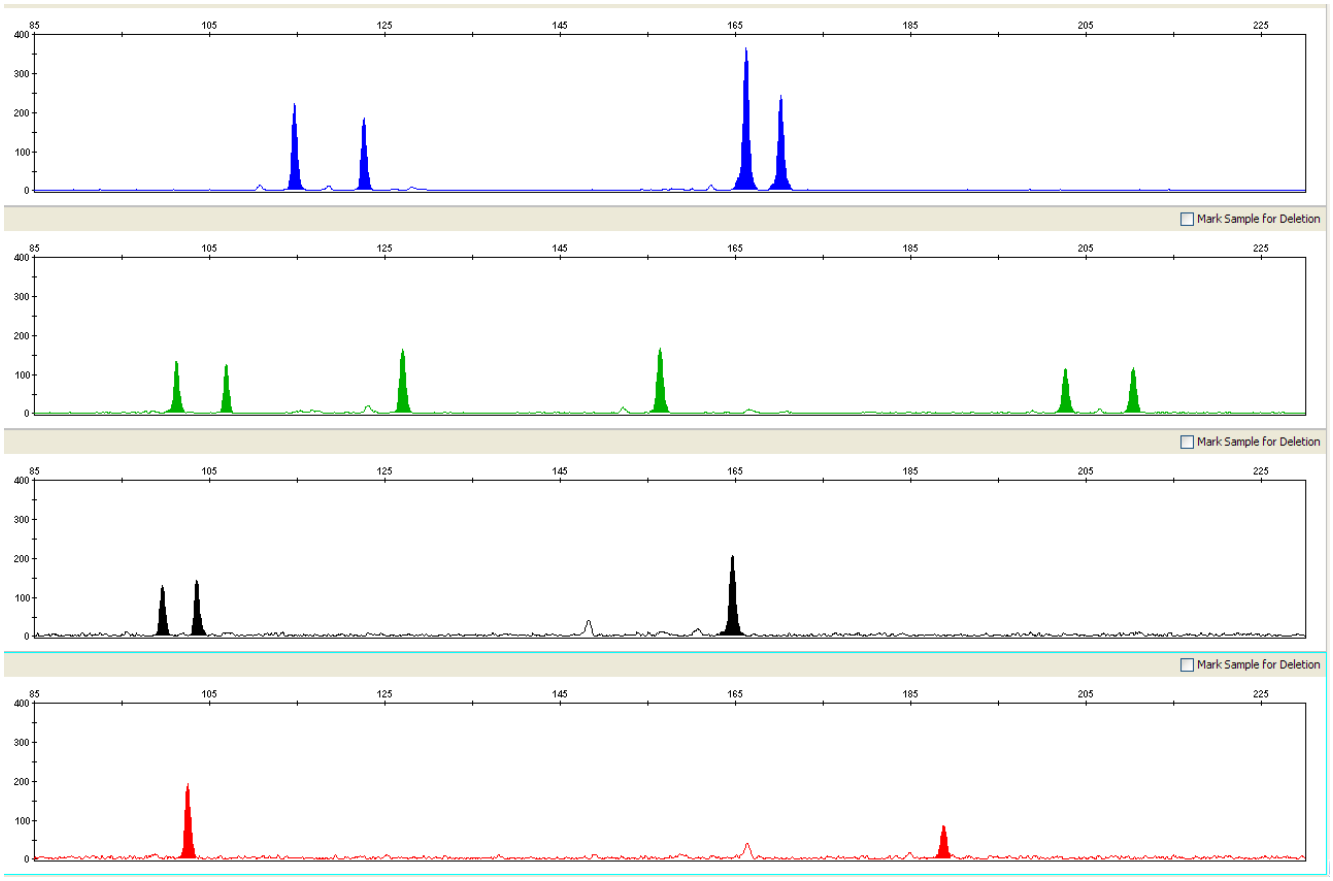


Figure 34 Sensitivity study: electropherogram of 125 pg Sample 3 amplified with Control B mix. Two alleles are below the analysis threshold of 50 RFU: at the D18S51 locus in the NED™ dye (yellow); at the FGA locus in the PET™ dye (red) (Y-axis scale 0–400 RFU)

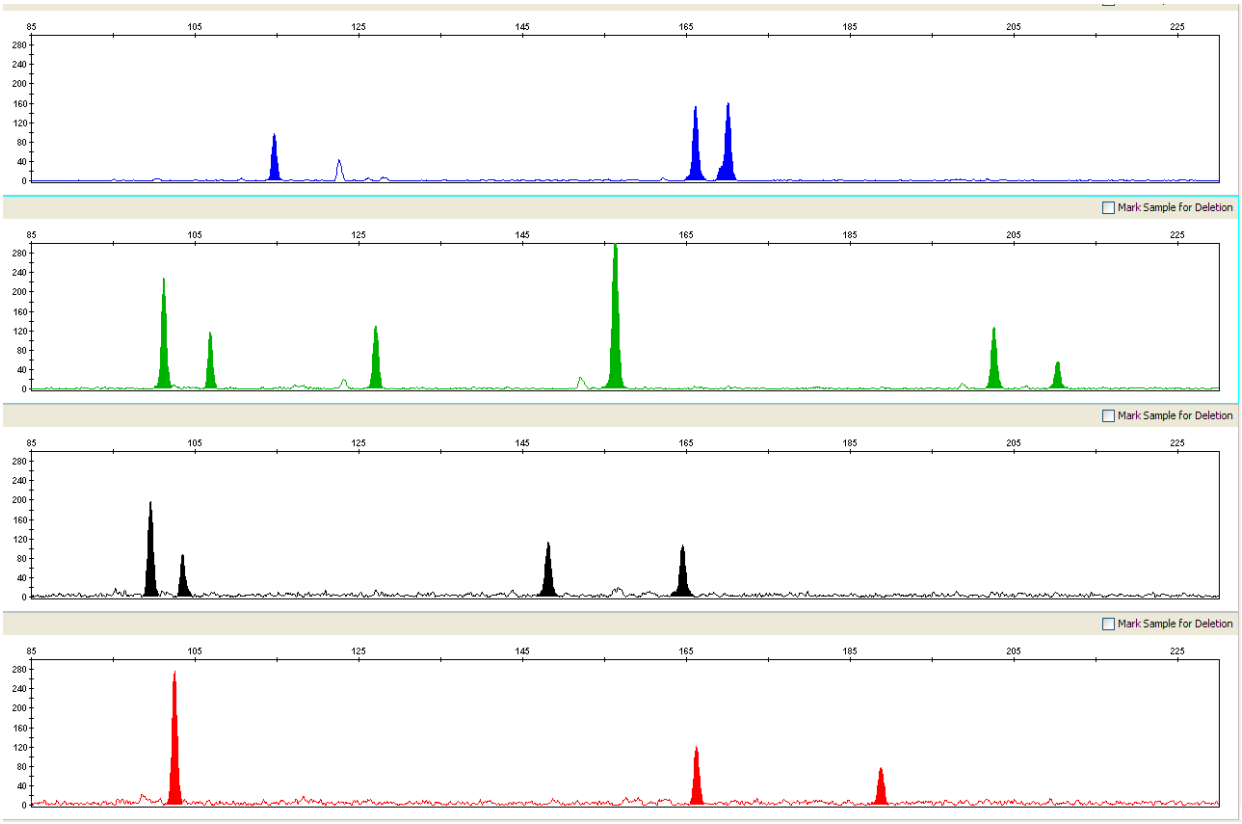


Figure 35 Sensitivity study: electropherogram of 125 pg Sample 3 amplified with Test B mix. One allele at the D7S820 locus in the FAM™ dye (blue) is below the analysis threshold of 50 RFU (Y-axis scale 0–300 RFU)

Genotype concordance

Genotypes for Test and Control mixes were 100% concordant. See Table 18.

Table 18 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%

Table 18 Sensitivity study: genotype concordance *(continued)*

DNA input amount	Reagent mix	Genotype concordance
250 pg	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 DNA Control 007 and 5 replicates of 0.5 ng pristine DNA Control 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 or decrease) in the level of intracolor balance was observed between the Test and Control mixes on degraded or pristine DNA, with the exception of Test A Mix. Test A Mix 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.

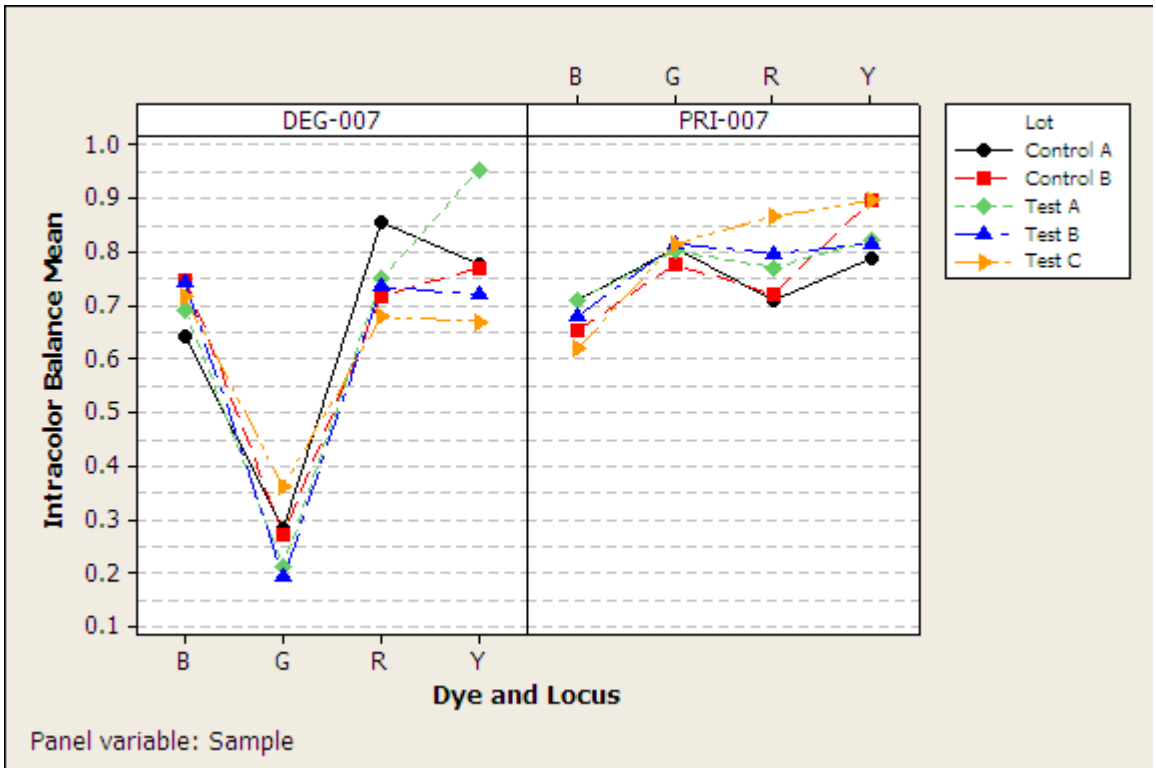


Figure 36 Degraded DNA study: intracolor balance 0.5 ng input DNA amount

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. Test C Mix 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.

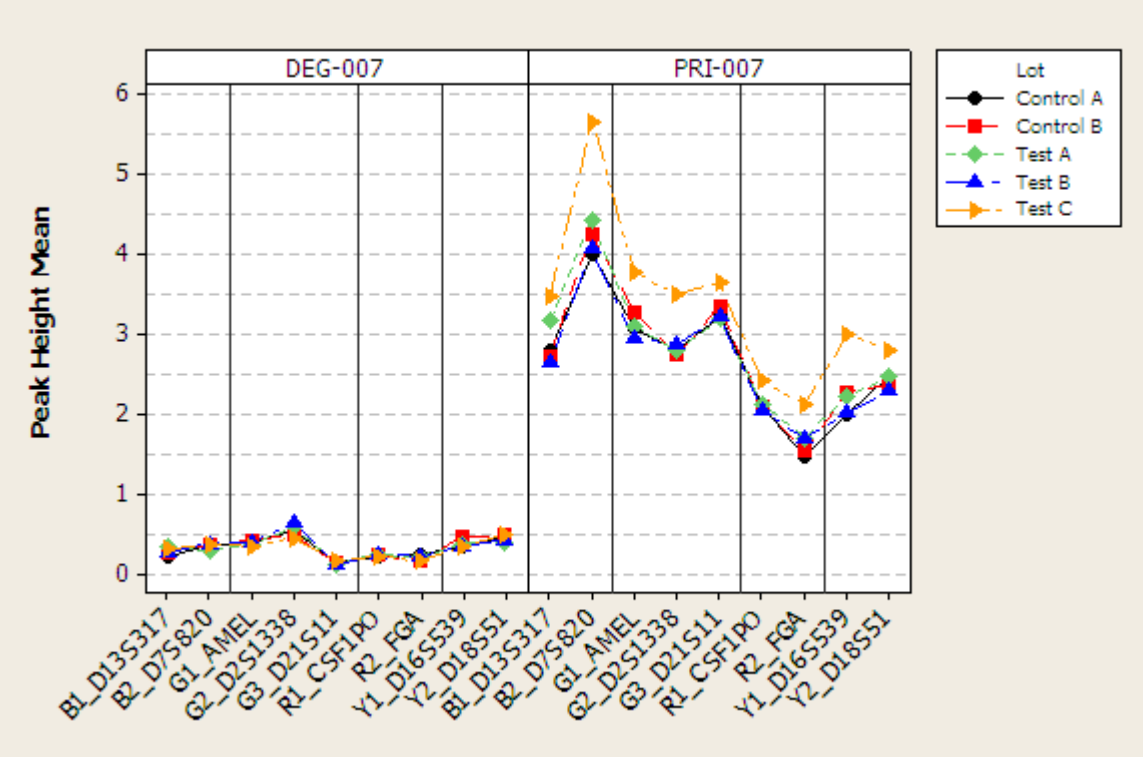


Figure 37 Degraded DNA study: intracolor balance 0.5 ng input DNA amount

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.

Table 19 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%

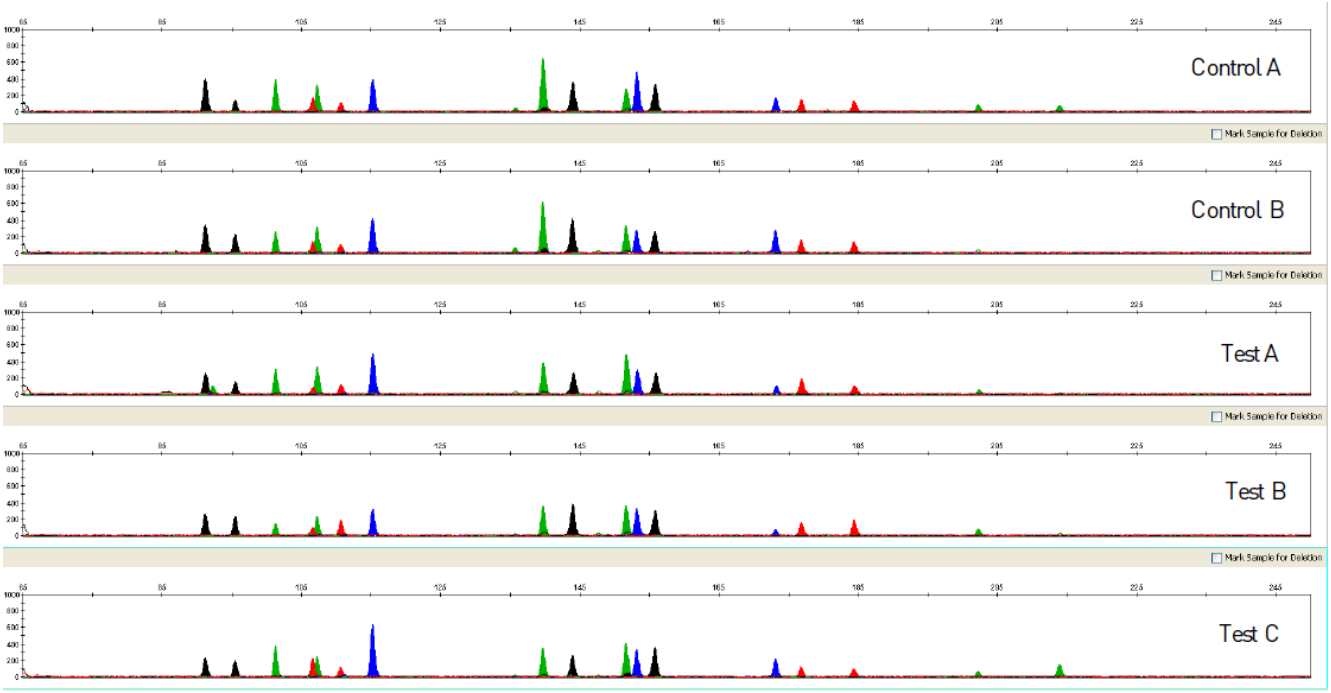


Figure 38 Degraded DNA study: representative electropherograms from 500 pg input DNA amplifications of simulated degraded DNA samples (Y-axis scale 0–1,000 RFU)

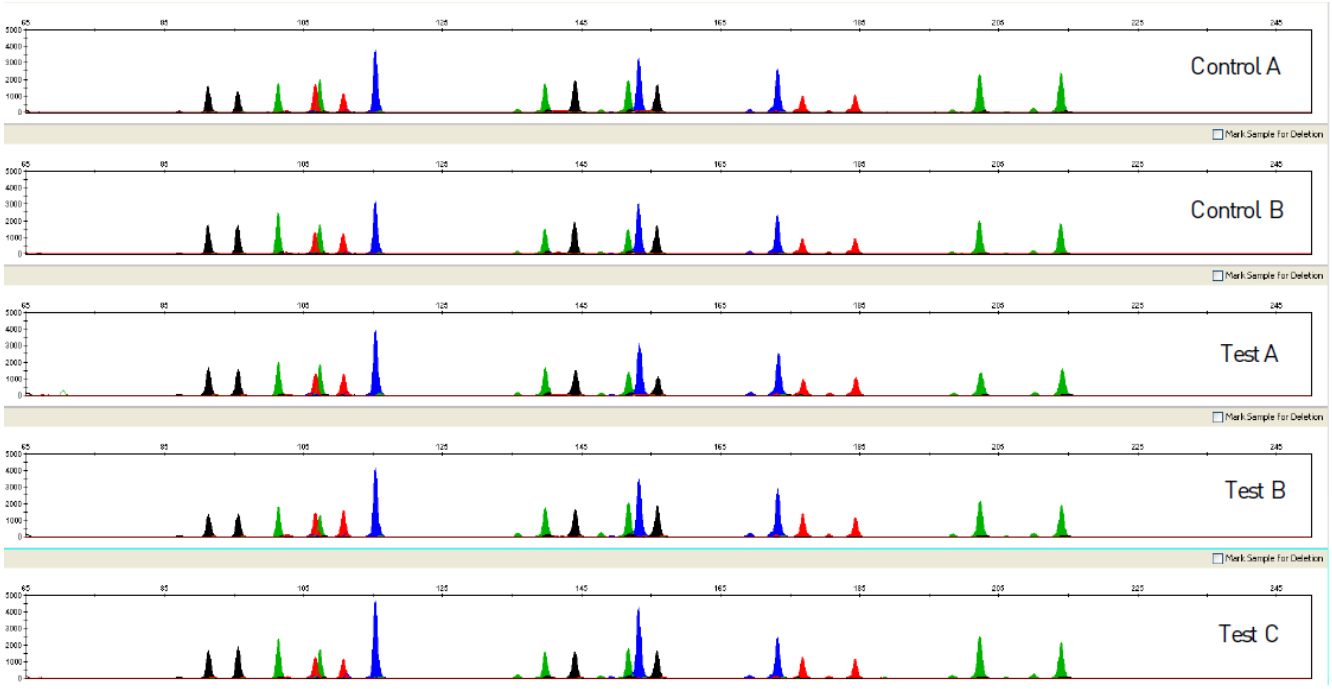


Figure 39 Degraded DNA study: representative electropherograms from 500 pg input DNA amplifications of pristine DNA samples for comparison to simulated degraded samples (Y-axis scale 0–5,000 RFU)

Inhibition study

An inhibition series of 0.5 ng DNA Control 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 5) was amplified using each Test and Control mix. The amount of each inhibitor tested was titrated to cause an ~50% reduction in overall peak height of the samples. Results were evaluated for mean referenced peak height, minimum referenced 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 DNA Control 007 inhibited with hematin or humic acid. A significant increase in intracolor balance was observed only for Control A mix on DNA Control 007 inhibited with hematin (Figure 40, Figure 41, and Figure 42).

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.

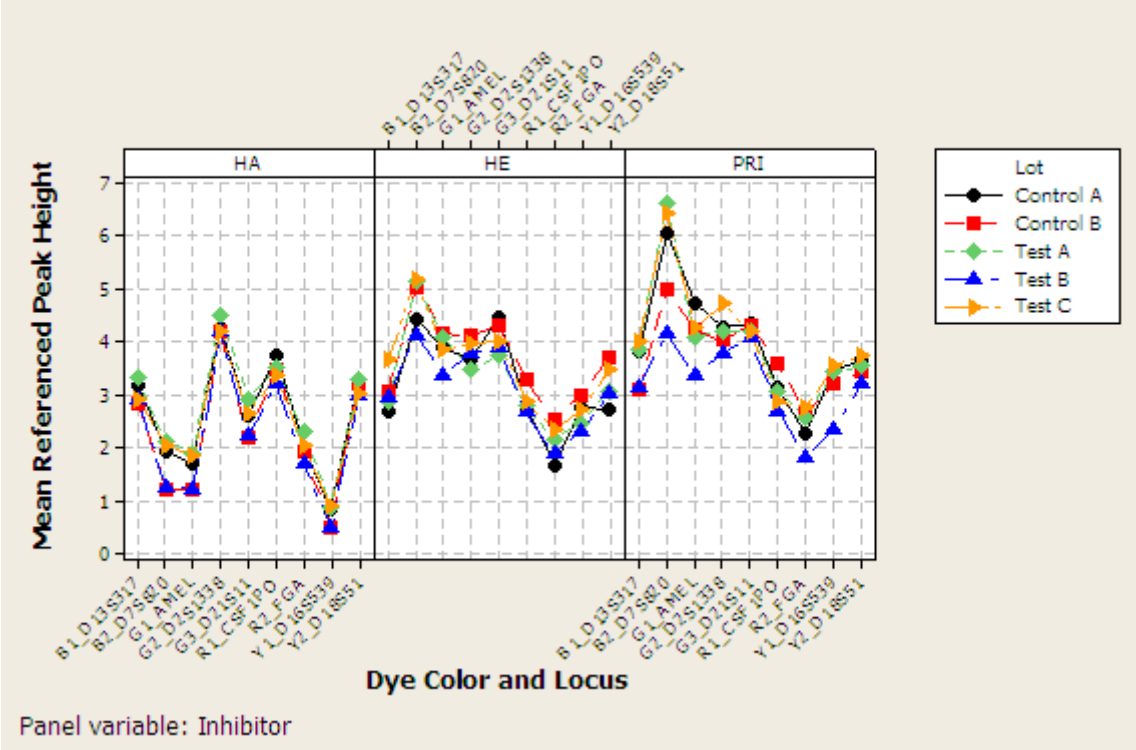


Figure 40 Inhibition study: mean referenced peak height

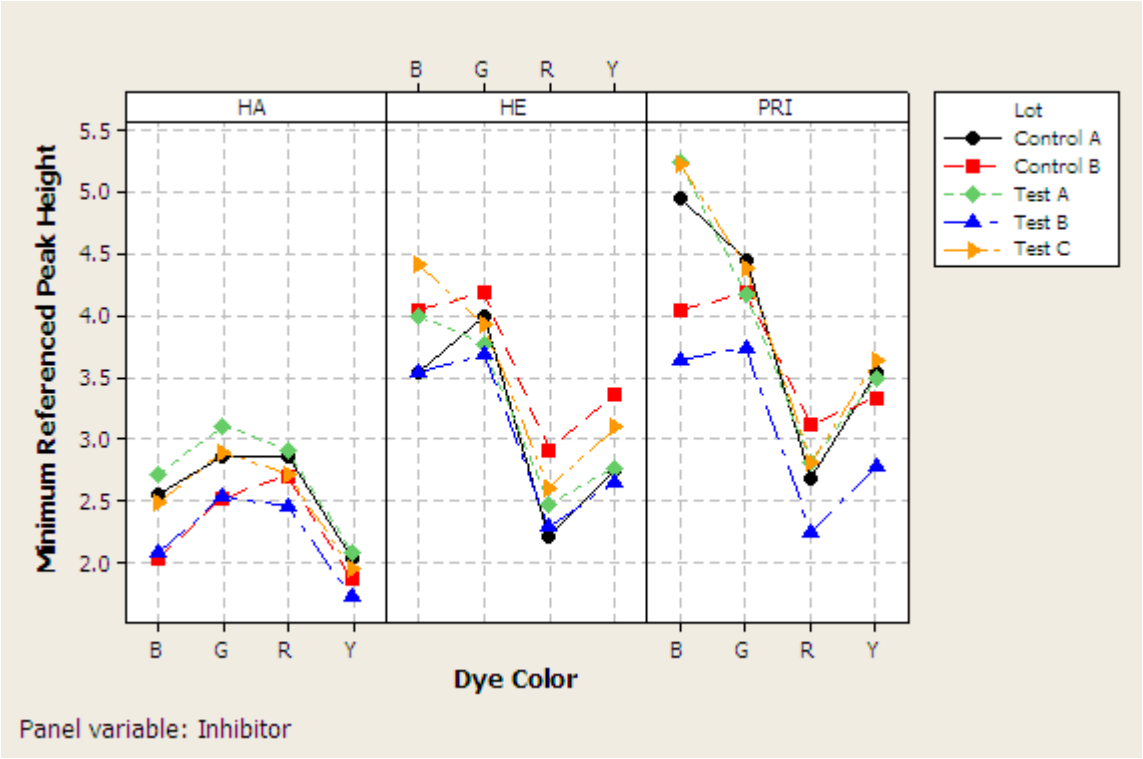


Figure 41 Inhibition study: minimum referenced peak height

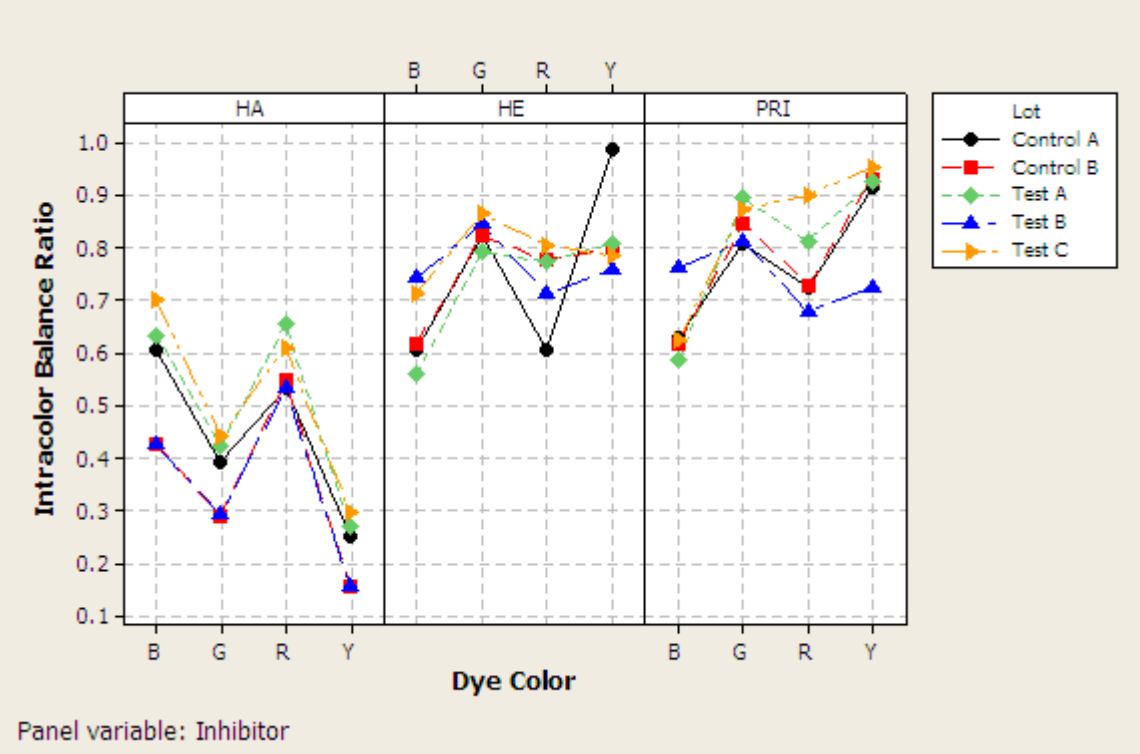


Figure 42 Inhibition study: intracolor balance

Representative electropherograms from the inhibition study are shown in Figure 43, Figure 44, and Figure 45.

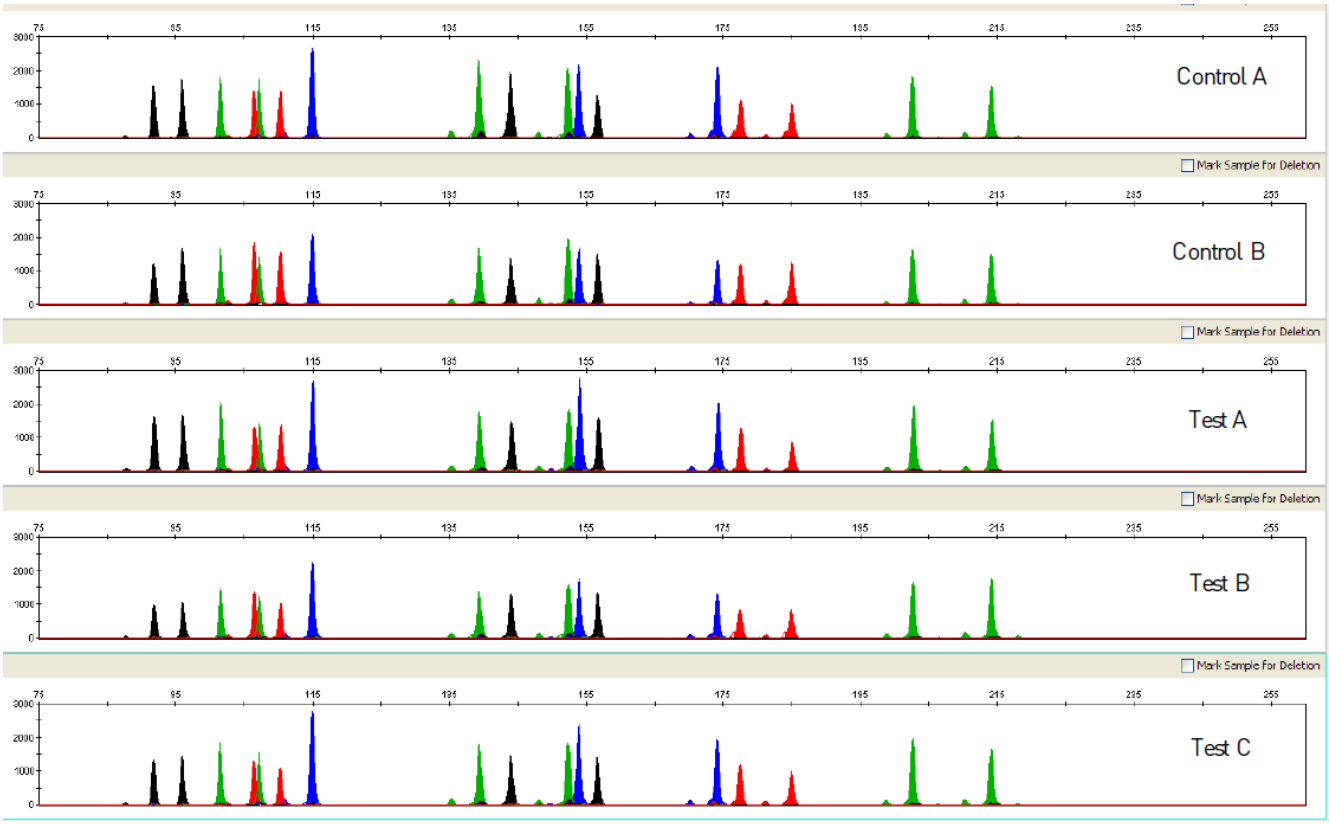


Figure 43 Inhibition study: representative electropherograms using uninhibited DNA Control 007 (Y-axis scale 0–3,000 RFU)

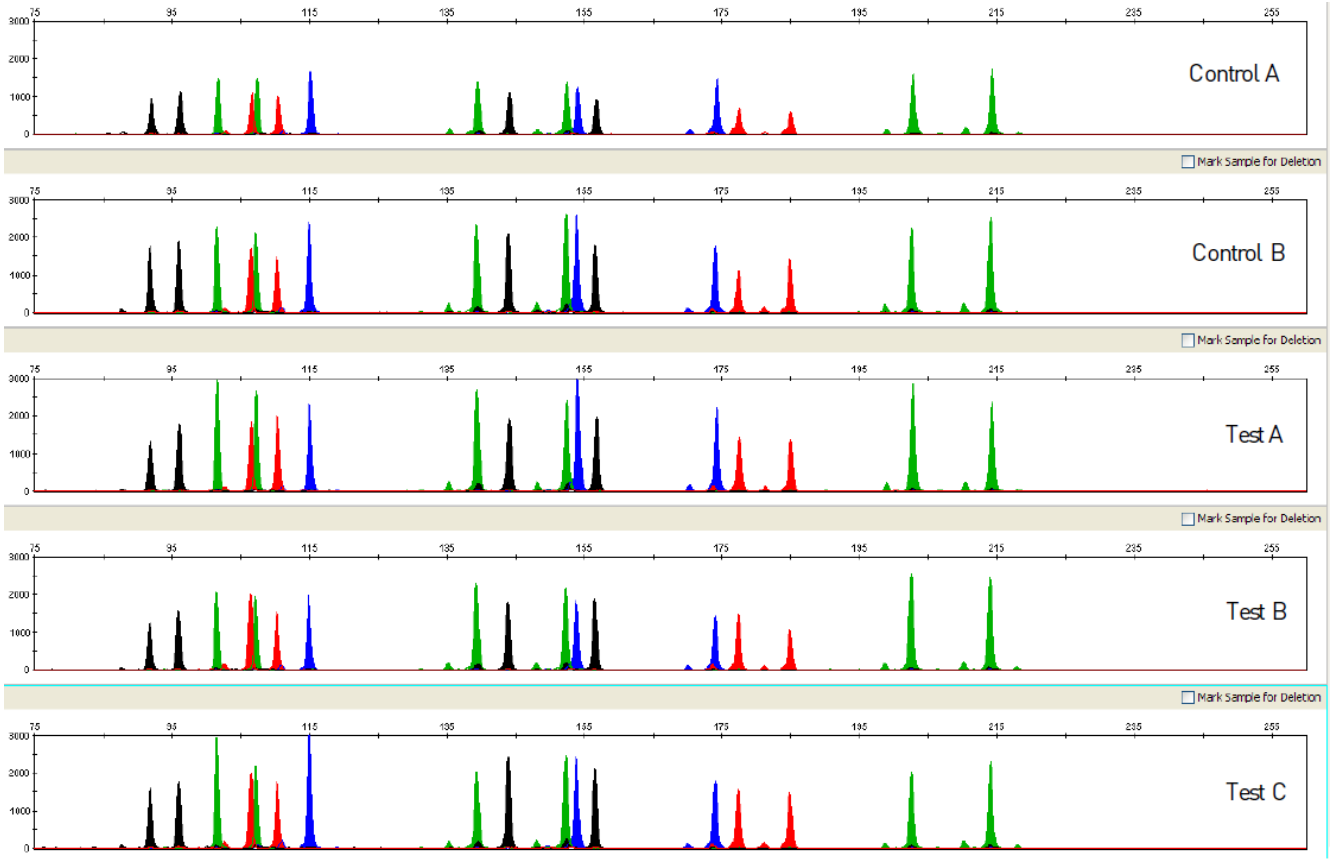


Figure 44 Inhibition study: representative electropherograms using DNA Control 007 inhibited with 45 µM of hematin (Y-axis scale 0–3,000 RFU)

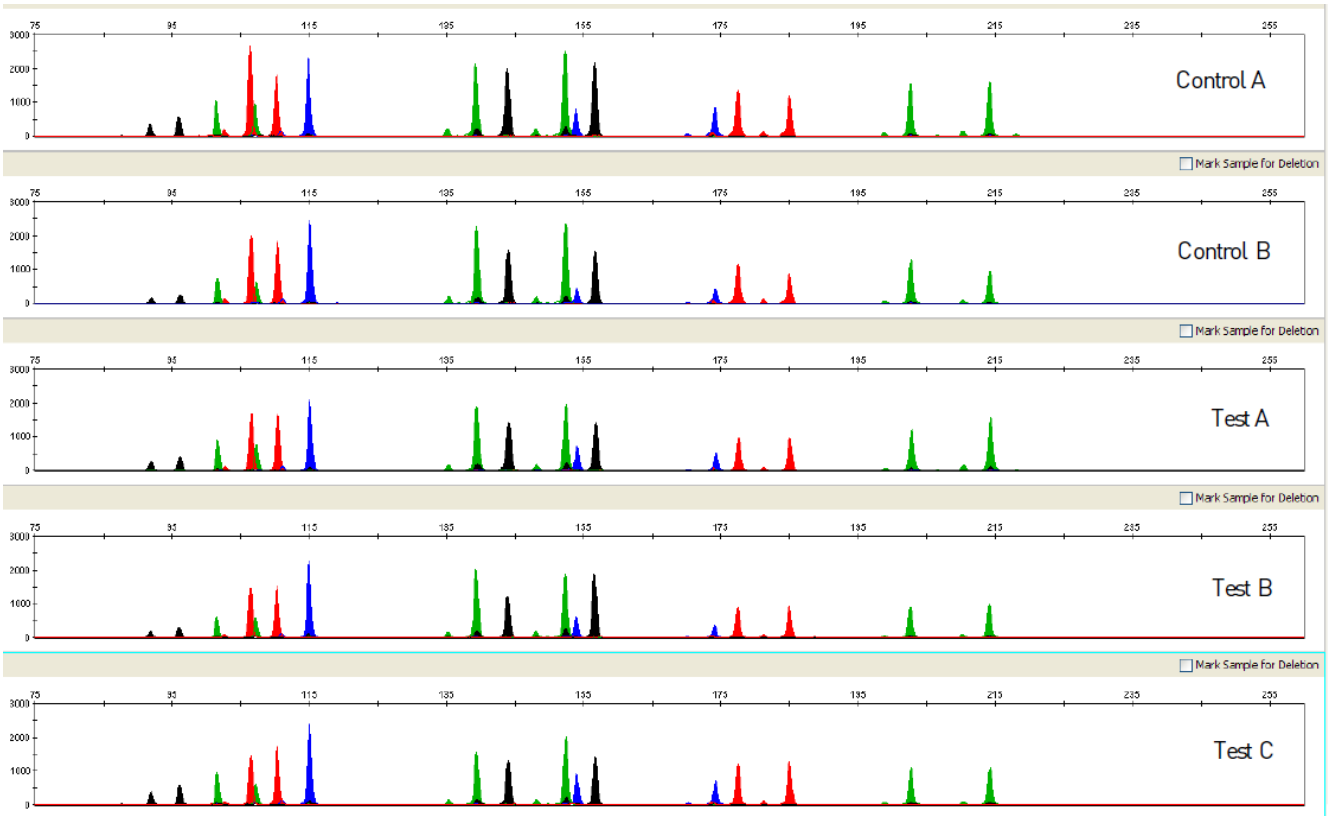


Figure 45 Inhibition study: representative electropherograms using DNA Control 007 inhibited with 50 ng/µL of humic acid (Y-axis scale 0–3,000 RFU)

Allelic dropout

No allelic dropout events were seen for any Test or Control mixes tested on uninhibited DNA Control 007 and DNA Control 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 that contains the AmpliTaq Gold™ enzyme and 10X PCR Buffer II manufactured by Thermo Fisher Scientific as compared to the original MiniFiler™ kit that contains the AmpliTaq Gold™ enzyme and 10X PCR Buffer II manufactured by Roche Molecular Systems.

A

Troubleshooting

Observation	Possible cause	Recommended action
Faint or no signal from DNA Control 007 and the test samples at all loci	The incorrect volume of master mix or primer set was used.	Use the correct volume of master mix or primer set.
	The DNA polymerase was not activated.	Repeat the amplification with an initial hold for 11 minutes at 95°C.
	The master mix was not vortexed thoroughly before aliquoting.	Vortex the master mix thoroughly.
	The primer set was exposed to too much light.	Replace the primer set and store it protected from light.
	The thermal cycler malfunctioned.	See the thermal cycler user guide and check the instrument calibration.
	Incorrect thermal cycler conditions were used.	Use the correct thermal cycler conditions.
	Insufficient PCR product was electrokinetically injected.	Use the correct settings for the capillary electrophoresis instrument.
	Degraded formamide was used.	Ensure that the formamide is correctly stored. Do not thaw and refreeze the formamide multiple times. Try using Hi-Di™ Formamide.
Positive signal from DNA Control 007 but partial or no signal from the test samples	The quantity of test DNA sample is below the assay sensitivity.	Quantify DNA and (when possible) add 0.5–0.75 ng of DNA.
	The test sample contains a high concentration of PCR inhibitor (for example, heme compounds, certain dyes).	Quantify the DNA, then use the minimum required volume of test sample DNA.
		Wash the sample in a Centricon™-100 centrifugal filter unit.
	The test sample DNA is severely degraded.	Use the Quantifiler™ HP DNA Quantification Kit or the Quantifiler™ Trio DNA Quantification Kit to evaluate sample quality during the quantification step. If DNA is degraded, re-amplify with an increased amount of DNA.
	The test sample was diluted in the wrong buffer (for example, a TE buffer with an incorrect EDTA concentration).	Redilute DNA using low-TE buffer (with 0.1 mM EDTA).

Observation	Possible cause	Recommended action
More than two alleles present at a locus	Exogenous DNA is present in the sample.	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Too much DNA was present in the reaction.	Use the recommended amount of template DNA: 0.5–0.75 ng.
	The test sample contained mixed DNA.	See "Extra peaks: Stutter" on page 61.
	Stutter product (1 repeat unit position) was amplified.	See "Extra peaks: Stutter" on page 61.
	Incomplete 3′ A base addition (n–1 bp position) occurred.	In the PCR, include the final extension step: 45 minutes 60°C. See "Extra peaks: Addition of 3' A nucleotide" on page 67.
	The signal exceeds the dynamic range of the instrument and is causing "off-scale" data.	Ensure that the cycle number is optimized. Use fewer PCR cycles or interpret the off-scale data according to your laboratory procedure.
	Poor spectral separation occurred.	Perform a spectral calibration.
		Confirm that Filter Set G5 modules are installed and used for analysis.
Some, but not all, loci are visible on test sample electropherograms	The test sample DNA is severely degraded.	Use the Quantifiler™ HP DNA Quantification Kit or the Quantifiler™ Trio DNA Quantification Kit to evaluate sample quality during the quantification step. If DNA is degraded, re-amplify with an increased amount of DNA.
	The test sample contains a high concentration of PCR inhibitor (for example, heme compounds, certain dyes).	Quantify the DNA, then use the minimum required volume of test sample DNA.
		Wash the sample in a Centricon™-100 centrifugal filter unit.

B

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 sizing 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 bp) 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 100–360 bp. Optimal genotyping accuracy of all kits other than the MiniFiler™ kit requires detection of all GeneScan™ 500 LIZ™ Size Standard (or ROX™ dye) fragments between 75–450 bp (or 75–400 bp depending on the kit).

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

Although the GeneScan™ 500 LIZ™ Size Standard does contain 50-bp and 35-bp 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, see the *GeneMapper™ ID-X Software v1.5 Reference Guide* (Pub. No. 100031707).

Comparing genotyping accuracy

We compared the Local Southern and 3rd Order Least Squares methods for genotyping accuracy, using a dataset of 1,156 Identifiler™ kit amplifications. The amplified samples were from a single source, electrophoresed on a 3100 or 3130*xl* Genetic Analyzer, 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 75–450 bp, except for the 250-bp 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 microvariant allele. All the discordant off-ladder allele calls were within 0.08 bp of the ±0.5 bp offset for the bin sizing window.

C Materials required but not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Sample preparation (PCR or CE) required materials

Item		Amount	Cat. No.
Size standard (only one is required) **IMPORTANT!** Do not use GeneScan™ 350 ROX™ or GeneScan™ 500 ROX™ Size Standards with this kit.	GeneScan™ 500 LIZ™ Size Standard	2 × 200 µL	4322682
	GeneScan™ 600 LIZ™ Size Standard v2.0	2 × 200 µL	4408399
TE Buffer [low-TE buffer; 10 mM Tris-HCl (pH 8.0) and 0.1 mM EDTA]		100 mL	12090015 or see "(Optional) Prepare low-TE buffer" on page 19
Hi-Di™ Formamide		25 mL	4311320

Thermal cycler required materials

ProFlex™ PCR System

Item	Source
ProFlex™ 96-well PCR System	4484075
ProFlex™ 2 × 96-well PCR System	4484076
ProFlex™ 3 × 32-Well PCR System	4484073

VeritiPro™ Thermal Cycler

Item	Source
HID VeritiPro™ Thermal Cycler, 96-well	A52127

Capillary electrophoresis instrument required materials

SeqStudio™ Flex Series Genetic Analyzer for Human Identification

Item	Source
SeqStudio™ 8 Flex Genetic Analyzer for Human Identification with SeqStudio™ Flex Series Instrument Software v1.1.1	A56532
SeqStudio™ 24 Flex Genetic Analyzer for Human Identification with SeqStudio™ Flex Series Instrument Software v1.1.1	A56534
Anode Buffer Container 3500/Flex Series	4393927
Cathode Buffer Container 3500/Flex Series	4408256
Septa Cathode Buffer Container 3500/Flex Series	4410715
Capillary array 36-cm SeqStudio™ 8 Flex	A49104
Capillary array 36-cm SeqStudio™ 24 Flex	A49105
96-Well Standard Retainer & Base Set SeqStudio™ Flex Series	A49316
8-Tube Standard Retainer & Base Set SeqStudio™ Flex Series	A49296
8-Strip Septa 3500/Flex Series	4410701
Septa for 96-Well Plates, for 3500/SeqStudio™ Flex	4412614
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
POP-4™ (960) Performance Optimized Polymer	4393710
POP-4™ (384) Performance Optimized Polymer	4393715
Conditioning Reagent Kit 3500/Flex Series	4393718

SeqStudio™ Genetic Analyzer for HID

Item	Source
SeqStudio™ Genetic Analyzer for HID with SeqStudio™ Data Collection Software v1.2.1, v1.2.4, or v1.2.5	A46227
(Optional) SAE Administrator Console v2.0 or v2.1	A46170 or A53717
(Optional) SeqStudio™ Plate Manager v1.2 or v1.3	Available on **apps.thermofisher.com** or for download at **thermofisher.com**
SeqStudio™ Genetic Analyzer Cartridge v2	A41331
SeqStudio™ Genetic Analyzer Cathode Buffer Container	A33401
Reservoir Septa (for Cathode Buffer Container)	A35640
SeqStudio™ Integrated Capillary Protector	A31923
Septa for SeqStudio™ Genetic Analyzer, 96 well	A35641
Septa for SeqStudio™ Genetic Analyzer, 8 strip	A35643
DS-33 Matrix Standard Kit (Dye Set G5)	4345833

3500 Series Genetic Analyzer for Human Identification

Item	Source
3500 Genetic Analyzer for Human Identification with 3500 Series Data Collection Software 4	4406017
3500xL Genetic Analyzer for Human Identification with 3500 Series Data Collection Software 4	4406016
(Software upgrade package) 3500 Series HID Data Collection Software v4.0.1	A46085
Anode Buffer Container 3500/Flex Series	4393927
Cathode Buffer Container 3500/Flex Series	4408256
Septa Cathode Buffer Container 3500/Flex Series	4410715
3500 Genetic Analyzer 8-Capillary Array, 36 cm	4404683
3500xL Genetic Analyzer 24-Capillary Array, 36 cm	4404687
Retainer & Base Set (Standard) for3500/3500xL Genetic Analyzer, 96 well	4410228
Retainer & Base Set (Standard) for3500/3500xL Genetic Analyzer, 8 tube	4410231
8-Strip Septa 3500/Flex Series	4410701
Septa for 96-Well Plates, for 3500/SeqStudio™ Flex	4412614
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
POP-4™ (960) Performance Optimized Polymer	4393710

(continued)

Item	Source
POP-4™ (384) Performance Optimized Polymer	4393715
Conditioning Reagent Kit 3500/Flex Series	4393718

Analysis software required materials

GeneMapper™ *ID-X* Software

Item	Source
GeneMapper™ *ID-X* Software v1.7.2 patch[1]	Thermo Fisher Scientific[2]
GeneMapper™ *ID-X* Software v1.7 Full Installation	A71700
GeneMapper™ *ID-X* Software v1.7 Client Installation	A71701
GeneMapper™ *ID-X* Software v1.6 Full Installation	A39975
GeneMapper™ *ID-X* Software v1.6 Client Installation	A39976
GeneMapper™ *ID-X* Software v1.5 Full Installation	A27884
GeneMapper™ *ID-X* Software v1.5 Client Installation	A27886

[1] The patch addresses known issues and provides new user functionality since the v1.7 release.

[2] Available for free download at **www.thermofisher.com/GMIDXsoftware**.

Note: For a list of GeneMapper™ *ID-X* Software versions that are compatible with your kit and capillary electrophoresis instrument, see “Instruments and software compatibility” on page 15.

Miscellaneous required materials

Plates and tubes

Item	Source
MicroAmp™ 96-Well Tray	N8010541
MicroAmp™ Reaction Tube with Cap, 0.2 mL	N8010540
MicroAmp™ 8-Tube Strip, 0.2 mL	N8010580
MicroAmp™ Optical 8-Tube Strip, 0.2 mL	4316567
MicroAmp™ Optical 8-Cap Strips	4323032

(continued)

Item	Source
MicroAmp™ 96-Well Tray/Retainer Set (Adapter for 8-Tube Strip)	403081
MicroAmp™ 96-Well Base	N8010531
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Optical 96-Well Reaction Plate	N8010560
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4326659

Laboratory supplies

Item	Source
Various procedures	
Aerosol resistant pipette tips	MLS[1]
Microcentrifuge tubes	MLS
Pipettors	MLS
Tape, labeling	MLS
Tube, 50-mL Falcon™	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Vortex	MLS
(*Optional*) Tabletop centrifuge with 96-Well Plate Adapters	MLS
(Optional) Handheld Barcode Scanner	4488442

[1] Major laboratory supplier

D PCR work areas

Work area setup and lab design

Many resources are available for the appropriate design of a PCR laboratory. If you are using this kit for:

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

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

Process samples carefully 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 materials

IMPORTANT! Do not remove these items from the PCR Setup Work Area.

- Calculator
- Gloves, disposable
- Marker pen, permanent
- Microcentrifuge
- Microcentrifuge tubes, 1.5-mL, or 2.0-mL, or other appropriate nuclease-free tube (for master mix preparation)
- Microcentrifuge tube rack
- Pipette tips, sterile, disposable hydrophobic filter-plugged
- Pipettes

- Tube decapper that can be autoclaved
- Vortex

Amplified DNA work area

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

Use only the validated thermal cyclers listed in “Instruments and software compatibility” on page 15.

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, and so on). To obtain SDSs, visit **thermofisher.com/support**.

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. 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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Table 20 STR and quantification kits

Document title	Pub. No.
STR kits	
AmpFℓSTR™ MiniFiler™ PCR Amplification Kit User Guide	MAN0029851
AmpFℓSTR™ Identifiler™ PCR Amplification Kit User Guide	4323291
Technical Note: Handling STR Kits and Ladder Decontamination	Go to **thermofisher.com**, then search for the technical note by title, or contact your local Human Identification representative.
Quantification kits	
Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide	4485354

Table 21 Thermal cyclers

Document title	Pub. No.
ProFlex™ PCR System	
ProFlex™ PCR System User Guide	MAN0007697
ProFlex™ PCR System Kit Validation User Bulletin	100031595[1]
VeritiPro™ Thermal Cycler	
VeritiPro™ Thermal Cycler User Guide	MAN0019157
HID VeritiPro™ Thermal Cycler, 96-well, User Bulletin—Applied Biosystems™ PCR Amplification Kit Validation	MAN0025561

[1] Archived document. To access, go to **https://assets.thermofisher.com/TFS-Assets/LSG/manuals/100031595_ProFlexKit_Validation_UB.pdf**

Table 22 Capillary electrophoresis instruments

Document title	Pub. No.
SeqStudio™ Flex Series Genetic Analyzer for Human Identification	
SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide	100104689
SeqStudio™ Flex Series Genetic Analyzer for HID Validation User Bulletin	MAN0028463
SeqStudio™ Flex Series Instrument Software v1.1.1 User Bulletin	MAN0029757
SeqStudio™ Genetic Analyzer for HID	
SeqStudio™ Genetic Analyzer Instrument and Software User Guide (v1.2 and later)	MAN0018646
SeqStudio™ Genetic Analyzer for HID User Bulletin—New Software Features and Verification/Validation Studies (v1.2 and later)	MAN1001221
3500 Series Genetic Analyzer for Human Identification	
3500 Series Data Collection Software 4 User Bulletin: New Features and Developmental Validation	100075298
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v3.3 User Guide	100079380

Table 23 Analysis software

Document title	Pub. No.
GeneMapper™ *ID-X* Software all versions	
GeneMapper™ ID-X Software Bin Overlap User Bulletin	100029546
Technical Note: Customizing GeneMapper™ ID-X Software Panel and Bin Sets to Include or Exclude Internal Quality Control Markers in the Quality Value System (v1.5.2 and later)	Thermo Fisher Scientific[1]
Technical Note: Compendium of GeneMapper™ ID-X Software version changes from version 1.0.1 through version 1.7.2	
GeneMapper™ *ID-X* Software v1.7	
GeneMapper™ ID-X Software v1.7 Administration Guide	MAN0029245
GeneMapper™ ID-X Software v1.7 Installation Guide	MAN0029246
GeneMapper™ ID-X Software v1.7 New Features and Software Verification and Validation User Bulletin	MAN0029209
GeneMapper™ *ID-X* Software v1.6	
GeneMapper™ ID-X Software v1.6 New Features and Software Verification User Bulletin	100073905
GeneMapper™ *ID-X* Software v1.5	
GeneMapper™ ID-X Software v1.5 New Features and Verification User Bulletin	100031708
GeneMapper™ ID-X Software v1.5 Getting Started Guide— Basic Features	100031701

Table 23 Analysis software *(continued)*

Document title	Pub. No.
GeneMapper™ ID-X Software v1.5 Quick Reference— Basic Features	100031702
GeneMapper™ ID-X Software v1.5 Getting Started Guide— Mixture Analysis Tool	100031704
GeneMapper™ ID-X Software v1.5 Quick Reference— Mixture Analysis Tool	100031705
GeneMapper™ ID-X Software v1.5 Installation Guide	100031706
GeneMapper™ ID-X Software v1.5 Administrator Guide	100031703
GeneMapper™ ID-X Software v1.5 Reference Guide	100031707

[1] Go to thermofisher.com, then search for the technical note by title, or contact your local Human Identification representative.

Customer and technical support

For support, use one of the contact methods listed below, depending on your location.

Location	Contact method
In North America	Send an email to: **HIDTechSupport@thermofisher.com**
	Call **888-821-4443**; select option **2**, say "Application Support", then say "HID" or "Human Identification".
Outside North America	Contact your local support office.

For the latest services and support information for all locations, go to **thermofisher.com/support** to obtain the following information.

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

References

Ballantyne, K.N., Goedbloed, M. Fang, R., et al. 2010. Mutability of Y chromosomal microsatellites: rates, characteristics, molecular bases, and forensic implications. Am. J. Hum. Genet. 87:341–353.

Ballantyne, K.N., Keerl, V., Wollstein, A., Choi, Y., Zuniga, S.B., Ralf, A., Vermeulen, M., de Knijff, P., Kayser, M. 2012. A new future of forensic Y-chromosome analysis: rapidly mutating Y-STRs for differentiating male relatives and paternal lineages. Forensic Sci. Int. Genet. 6(2):208–18.

Ballantyne, K.N., Ralf, A., Aboukhalid, R., et al. 2014. Towards male individualization with rapidly mutating Y-chromosomal STRs. Hum. Mutat. doi: 10.1002/humu.22599. PubMed PMID: 24917567.

Begovich A.B., McClure G.R., Suraj V.C., Helmuth R.C., Fildes N., Bugawan T.L., Erlich H.A., Klitz W. 1992. Polymorphism, recombination, and linkage disequilibrium within the HLA class II region. *J. Immunol.* 148:249–258.

Butler, J.M. 2005. Forensic DNA Typing. Burlington, MA:Elsevier Academic Press.

Clark J.M. 1988. Novel non-templated nucleotide addition reactions catalyzed by procaryotic and eucaryotic DNA polymerases. *Nucleic Acids Res.* 16:9677–9686.

Edwards, A., Hammond, H.A., Lin, J., Caskey, C.T., and Chakraborty, R. 1992. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. *Genomics* 12:241–253.

Frank, W., Llewellyn, B., Fish, P., et al. 2001. Validation of the AmpFlSTR Profiler Plus PCR Amplification Kit for use in forensic casework. *J. Forensic Sci.* 46:642–646.

Furedi, S., Woller, J., Padar, Z., Angyal, M. 1999. Y-STR haplotyping in two Hungarian populations. Int. J. Legal Med. 113:38-42.

Grossman, P.D., Bloch, W., Brinson, E., Chang, C.C., Eggerding, F.A., Fung, S., Iovannisci, D.M., Woo, S., Winn-Deen, E.S. 1994. High-density multiplex detection of nucleic acid sequences: oligonucleotide ligation assay and sequence-coded separation. Nucleic Acids Res. 22:4527–4534.

Gusmao, L., Butler, J.M., Carracedo, A., Gill, P., Kayser, M., Mayr, W.R. et al., 2006. DNA Commission of the International Society of Forensic Genetics (ISFG): An update of the recommendations on the use of Y-STRs in forensic analysis. Forensic Sci. Int. 157:187–97.

Holt, C., Stauffer, C., Wallin, J., et al. 2000. Practical applications of genotypic Surveys for forensic STR testing. *Forensic Sci. Int.* 112:91–109.

Johnson, C.L., Warren, J.H., Giles, R.C., Staub, R.W. 2003. Validation and uses of a Ychromosome STR 10-plex for forensic and paternity laboratories. J. Forensic Sci. 2003. 48(6):1260–8.

Kwok, S., and Higuchi, R. 1989. Avoiding false positives with PCR. Nature 339:237– 238.

Lazaruk, K., Walsh, P.S., Oaks, F., Gilbert, D., Rosenblum, B.B., Menchen, S., Scheibler, D., Wenz, H.M., Holt, C., Wallin, J. 1998. Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. *Electrophoresis* 19:86–93.

Li, H. Schmidt, L., Wei, M-H., Hustad, T. Leman, M.I., Zbar, B. and Tory, K. 1993. Three tetranucleotide polymorphisms for loci:D3S1352; D3S1358; D3S1359. *Hum. Mol. Genet.* 2:1327.

Magnuson, V.L., Ally, D.S., Nylund, S.J., Karanjawala, Z.E., Rayman, J.B., Knapp, J.I., Lowe, A.L., Ghosh, S., Collins, F.S. 1996. Substrate nucleotide-determined nontemplated addition of adenine by Taq DNA polymerase: implications for PCR-based genotyping and cloning. *Biotechniques* 21:700–709.

Mansfield, E.S., Robertson, J.M., Vainer, M., Isenberg, A.R., Frazier, R.R., Ferguson, K., Chow, S., Harris, D.W., Barker, D.L., Gill, P.D., Budowle, B., McCord, B.R. 1998. Analysis of multiplexed short tandem repeat (STR) systems using capillary array electrophoresis. *Electrophoresis* 19:101–107.

Mills, K.A., Even, D., and Murrau, J.C. 1992. Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). Hum. Mol. Genet. 1:779. Möller, A. and Brinkmann, B. 1994. Locus ACTBP2 (SE33): Sequencing data reveal considerable polymorphism. *Int. J. Leg. Med.* 106:262–267.

Moretti, T., Baumstark, A., Defenbaugh, D., Keys, K., Smerick, J., and Budowle, B. 2001. Validation of short tandem repeats (STRs) for forensic usage: Performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. *J. Forensic Sci.* 46(3):647–660.

Mulero, J., Ballantyne, J., Ballantyne, K., Budowle, B., Coble, M., Gusmão, L., Roewer, L., Kayser, M. Nomenclature update and allele repeat structure for the markers DYS518 and DYS449. 2014. Forensic Sci. Int. Genet. doi: 10.1016/j.fsigen.2014.04.009. PubMed PMID: 24854343.

Mulero, J.J., Chang, C.W., and Hennessy, L.K. 2006. Characterization of N+3 stutter product in the trinucleotide repeat locus DYS392. *J. Forensic Sci.* 51:826–830.

Nakahori, Y., Takenaka, O., and Nakagome, Y. 1991. A human X-Y homologous region encodes amelogenin. *Genomics* 9:264–269.

Redd, A.J., Agellon, A.B., Kearney, V.A., Contreras, V.A., Karafet, T., Park, H., de Knijff, P., Butler, J.M., Hammer, M.F. 2002. Forensic value of 14 novel STRs on the human Y chromosome. Forensic Sci. Int. 130:97–111.

Schoske, R., Vallone, P.M., Kline, M.C., Redman, J.W., Butler, J.M. 2004. Highthroughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays. Forensic Sci. Int. 139:107–21.

Scientific Working Group on DNA Analysis Methods (SWGDAM). 2012. Validation Guidelines for DNA Analysis Methods. Available at **media.wix.com/ugd/4344b0_cbc27d16dcb64fd88cb36ab2a2a25e4c.pdf**

Scientific Working Group on DNA Analysis Methods (SWGDAM). 2014. Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories. Available at **media.wix.com/ugd/4344b0_da25419ba2dd4363bc4e5e8fe7025882.pdf**

Sharma, V. and Litt, M. 1992. Tetranucleotide repeat polymorphism at the D21S11 locus. *Hum Mol. Genet.* 1:67.

Smith, R.N. 1995. Accurate size comparison of short tandem repeat alleles amplified by PCR. *Biotechniques* 18:122–128.

Sparkes, R., Kimpton, C., Watson, S., Oldroyd, N., Clayton, T., Barnett, L., Arnold, J., Thompson, C., Hale, R., Chapman, J., Urquhart, A., and Gill, P. 1996a. The validation of a 7-locus multiplex STR test for use in forensic casework. (I). Mixtures, ageing, degradation and species studies. Int. J. Legal Med. 109:186–194.

Sparkes, R., Kimpton, C., Gilbard, S., Carne, P., Andersen, J., Oldroyd, N., Thomas, D., Urquhart, A., and Gill, P. 1996b. The validation of a 7-locus multiplex STR test for use in forensic casework. (II), Artifacts, casework studies and success rates. *Int. J. Legal Med.* 109:195–204.

Straub, R.E., Speer, M.C., Luo, Y., Rojas, K., Overhauser, J., Ott, J., and Gilliam, T.C. 1993. A microsatellite genetic linkage map of human chromosome 18. *Genomics* 15:48– 56.

Wallin, J.M., Buoncristiani, M.R., Lazaruk, K.D., Fildes, N., Holt, C.L., Walsh, P.S. 1998. SWGDAM validation of the AmpFlSTR blue PCR amplification kit for forensic casework analysis. *J. Forensic Sci.* 43:854–870.

Wallin, J.M., Holt, C.L., Lazaruk, K.D., Nguyen, T.H., Walsh, P.S. 2002. Constructing universal multiplex PCR systems for comparative genotyping. *J. Forensic Sci.* 47:52–65.

Walsh, P.S., Fildes, N.J., Reynolds, R. 1996. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Res.* 24:2807– 2812.