GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits

USER GUIDE

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Revision A

For Research, Forensic, or Paternity Use Only. Not for use in diagnostic procedures.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0029969 A (English)

Revision	Date	Description
A	22 April 2025	New document for the GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits; replaces Pub. No. 4477604. The following changes were made: • Storage conditions were updated (see “Contents and storage” on page 17). • Compatible instruments, compatible software, and materials required were updated (throughout the document). • The image was corrected for the thermal cycling temperatures (see “Thermal cycling temperatures” on page 61). • The loci were corrected in the non-standard stutter peak example (“Example of non-standard stutter peaks observed at the D22S1045, D1S1656, and SE33 loci” on page 88). • Copy edits and formatting changes were made to align with current documentation style (throughout the document).
F	3 October 2019	Information was added for the GlobalFiler™ IQC PCR Amplification Kit: pages 9–12, 14, 16, 18–19, 26, 30, 36–40, 43–45, 50, 52–56, 149–170, 174. The 3500 software information was updated: pages 19, 28, 175, 184. The GeneMapper™ *ID-X* Software information was updated: pages 176, 186.
E	7 July 2016	Information on 30 cycles with 500-ng sample concentration was added to Chapter 1, Chapter 2, and Chapter 5. References to 3500 Series Data Collection 3 and GeneMapper ID-X v1.5 were added. A section on amplification artifacts was added to Chapter 5. Non-technical changes: Chapter 1 and Chapter 5 were reorganized.
D	30 September 2015	The master mix additive was removed. The ProFlex™ PCR Systems were added. Information about artifacts identified at the TH01 locus and TPOX locus was added. Section 5.2 “Performance verification after BSA component replacement” was added. Branding was updated.
C	30 September 2014	The storage conditions were changed from 2 months to 6 months. Veriti™ Thermal Cycler verification information was added.
B	30 April 2014	Data were added to Chapter 5 concerning the evaluation of Hardy-Weinberg equilibrium. Information about the Quantifiler™ HP and Trio DNA Quantification Kits was added. The troubleshooting table was updated.
A	31 August 2013	New document for the GlobalFiler™ PCR Amplification Kit.

Contents

References

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Product information

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

Product description

The GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits are 6-dye, short tandem repeat (STR) multiplex assays for the amplification of human genomic DNA.

- Both kits amplify the following:
  - 21 autosomal STR loci:
    - 14 CODIS loci: CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, and D21S11
    - 6 loci from the expanded European Standard Set of Loci (ESSL): D2S441, D22S1045, D10S1248, D1S1656, D12S391, D2S1338
    - SE33, a highly discriminating locus
  - 1 Y-STR: DYS391
  - 1 insertion/deletion polymorphic marker on the Y chromosome: Y indel
  - Amelogenin (sex determining marker)
- In addition to the loci listed above, the GlobalFiler™ IQC PCR Amplification Kit amplifies 2 Internal Quality Control (IQC) markers; see “Internal Quality Control System for PCR” on page 9

Both kits offer a 24-locus multiplex, high discrimination power, high sensitivity, and tolerance to inhibitors. The concentration of 10 mini-STR loci that are entirely below 220 bp maximizes performance on degraded samples. The highly optimized buffer formulation contains an enzyme that allows completion of amplification in ~80 minutes.

Note: The primer set and the allelic ladder in the GlobalFiler™ kit are different from the primer set and the allelic ladder in the GlobalFiler™ IQC kit. Do not interchange.

Internal Quality Control System for PCR

The GlobalFiler™ kit includes an Internal Quality Control (IQC) System for PCR. The IQC System has two synthetic targets that are amplified with the sample:

- IQCS (small), a low molecular weight amplicon
- IQCL (large), a high molecular weight amplicon

The behavior of the IQC target peaks can be used to evaluate the success of the PCR reaction and to indicate sample quality. For more information, see Chapter 5, “Assess the PCR reaction with the Internal Quality Control System”.

About the primers

The GlobalFiler™ and GlobalFiler™ IQC kit primers are manufactured using the same synthesis and purification improvements as the primers in the NGM SElect™ and the Identifiler™ Plus kits. These improvements enhance the assay signal-to-noise ratio and simplify the interpretation of results.

The primers used in the kits are:

- For all loci except AMEL—The same primer sequences as the NGM SElect™ kit and the Identifiler™ Plus kit including SNP-specific primers for the vWA, D16S539, AMEL, D2S441, D22S1045, and D8S1179 loci.
- For AMEL—The same primer sequences as the NGM SElect™ kit (which are different from the Identifiler™ Plus kit).

The kits also include the following primer additions and modifications:

- Addition of DYS391 and a novel Y indel.
- The TPOX reverse primer has been redesigned to relocate the amplicon into the higher size range of the multiplex and optimize marker spacing.
- Addition of 8 new SNP-specific primers for the D3S1358, vWA, D18S51, D19S433, TH01, FGA, D5S818, and SE33 loci. The second degenerate primer was added to the vWA locus to address two different SNPs in the primer binding site.

The GlobalFiler™ IQC kit contains additional PCR primers, which are the same IQC primers contained in the NGM Detect™ PCR Amplification Kit. The IQCS (small) and IQCL (large) markers are amplified from a synthetic, plasmid-borne DNA template that is present in low copy number in the GlobalFiler™ IQC Primer Mix. The primers amplify the Internal Quality Control ("IQC") markers in the blue FAM™ dye channel. For more information, see Chapter 5, “Assess the PCR reaction with the Internal Quality Control System”.

Non-nucleotide linkers are used in primer synthesis for the following loci: D19S433, vWA, CSF1PO, D2S441, TH01, FGA, and D12S391. For these primers, non-nucleotide linkers are placed between the primers and the fluorescent dye during oligonucleotide synthesis (Butler 2005, Grossman *et al*., 1994). Non-nucleotide linkers enable reproducible positioning of the alleles to facilitate interlocus spacing. The combination of a 6-dye fluorescent system and the use of non-nucleotide linkers allows simultaneous amplification and efficient separation of all 24 markers during automated DNA fragment analysis.

Dyes used in the kit

Dye	Color	Label
6-FAM™	Blue	Samples, allelic ladders, and controls
VIC™	Green	
NED™	Yellow	
TAZ™	Red	
SID™	Purple	
LIZ™	Orange	GeneScan™ 600 LIZ™ Size Standard v2.0

Loci amplified by the kits

Note: The GlobalFiler™ IQC kit IQCS and IQCL markers are not included in the following table.

Locus designation	Chromosome location	Alleles in the allelic ladder	Dye label	Alleles in DNA Control 007
D3S1358	3p21.31	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	6-FAM™	15, 16
vWA	12p13.31	11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		14, 16
D16S539	16q24.1	5, 8, 9, 10, 11, 12,13, 14, 15		9, 10
CSF1PO	5q33.3–34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		11, 12
TPOX	2p23–2per	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15		8, 8
Y indel	Yq11.221	1, 2	VIC™	2
Amelogenin	X: p22.1–22.3 Y: p11.2	X, Y		X, Y
D8S1179	8q24.13	5, 6, 7, 8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19		12, 13
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
D18S51	18q21.33	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
DYS391	Yq11.21	7, 8, 9, 10, 11, 12, 13		11
D2S441	2p14	8, 9, 10, 11, 11.3, 12, 13, 14, 15, 16, 17	NED™	14, 15
D19S433	19q12	6, 7, 8, 9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2		14, 15

(continued)

Locus designation	Chromosome location	Alleles in the allelic ladder	Dye label	Alleles in DNA Control 007
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3	NED™	7, 9.3
FGA	4q28	13, 14, 15, 16, 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
D22S1045	22q12.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	TAZ™	11, 16
D5S818	5q21–31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18		11, 11
D13S317	13q22–31	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11, 11
D7S820	7q11.21–22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		7, 12
SE33	6q14	4.2, 6.3, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35, 35.2, 36, 37		17, 25.2
D10S1248	10q26.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	SID™	12, 15
D1S1656	1q42.2	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3		13, 16
D12S391	12p13.2	14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27		18, 19
D2S1338	2q35	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		20, 23

Standards and controls that are required

The GlobalFiler™ and GlobalFiler™ IQC kits require the following standards and controls for PCR amplification, PCR product sizing, and genotyping:

Item	Description	Included in the kits
DNA Control 007	Positive control. Used to evaluate amplification efficiency and to evaluate STR genotyping using the kit allelic ladder. See "DNA Control 007 profiles" on page 13.	Yes
GlobalFiler™ Allelic Ladder or GlobalFiler™ IQC 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 kits" on page 10 and "Allelic ladder profiles" on page 15.	Yes
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)

Note: The primer set and the allelic ladder in the GlobalFiler™ kit are different from the primer set and the allelic ladder in the GlobalFiler™ IQC kit. Do not interchange.

DNA Control 007 profiles

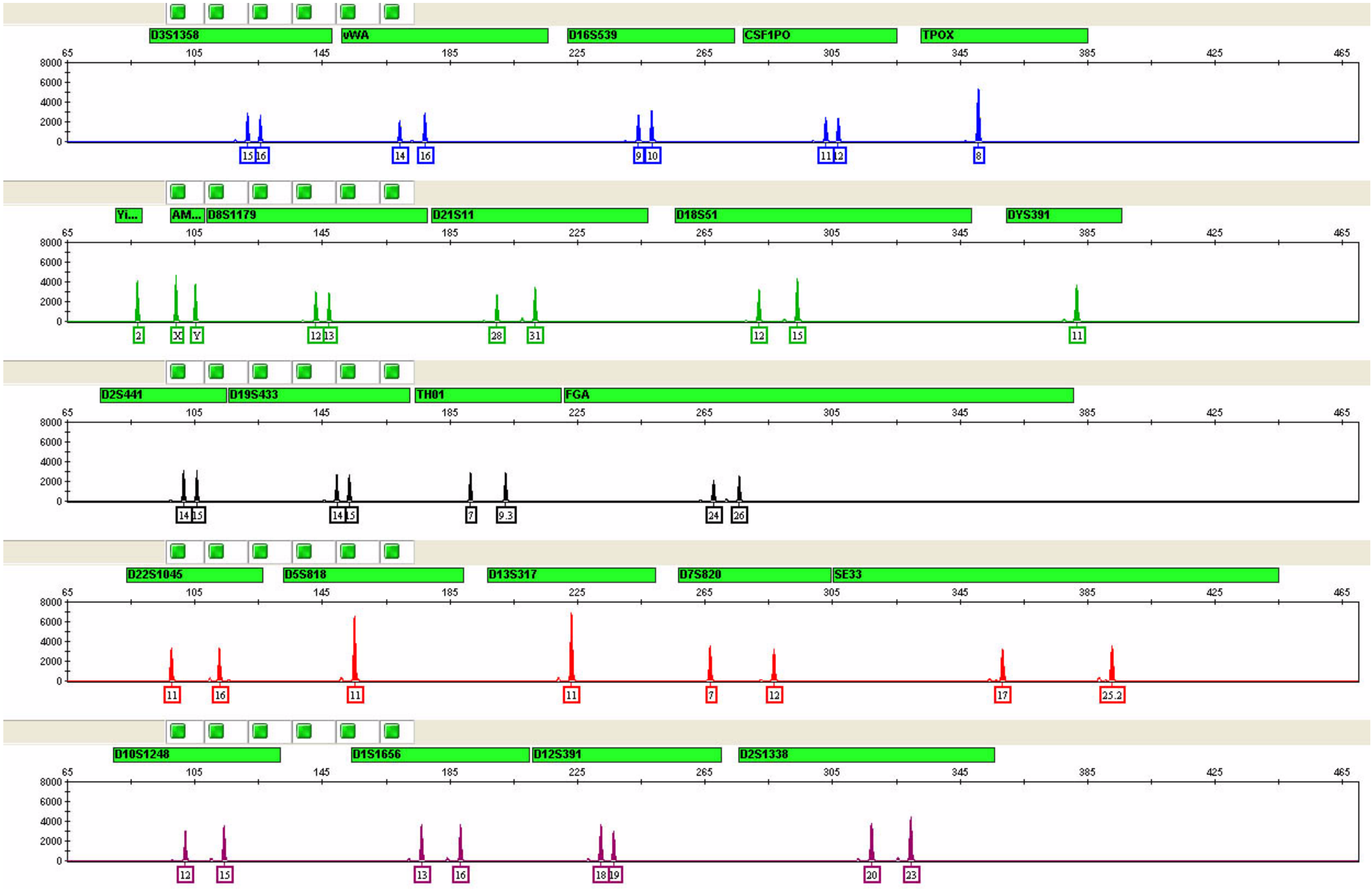

Figure 1 DNA Control 007 (1 ng) amplified with the GlobalFiler™ kit and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0–8,000 RFU)

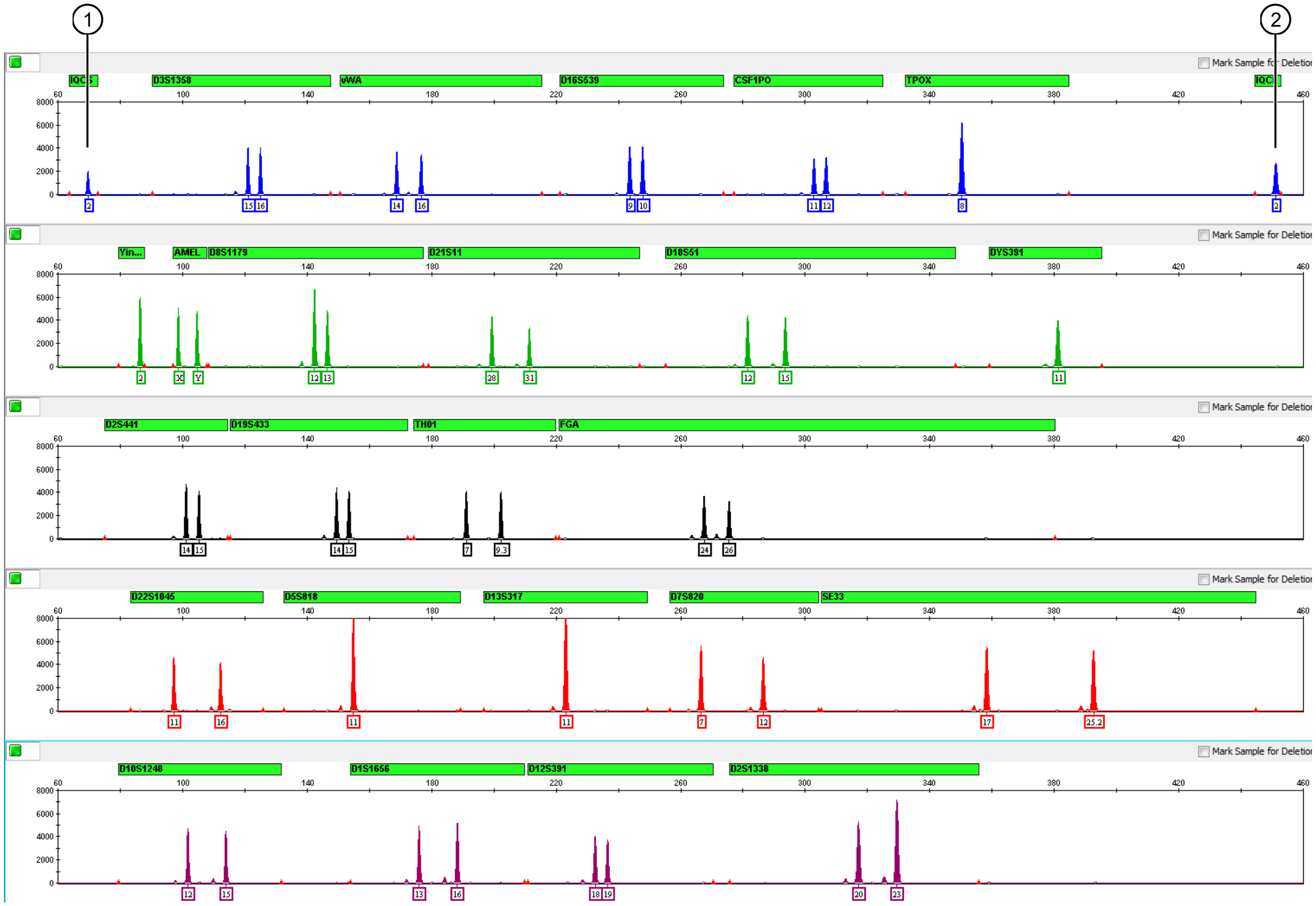

Figure 2 DNA Control 007 (1 ng) amplified with the GlobalFiler™ IQC kit and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0–8,000 RFU)

① IQCS peak
② IQCL peak

Allelic ladder profiles

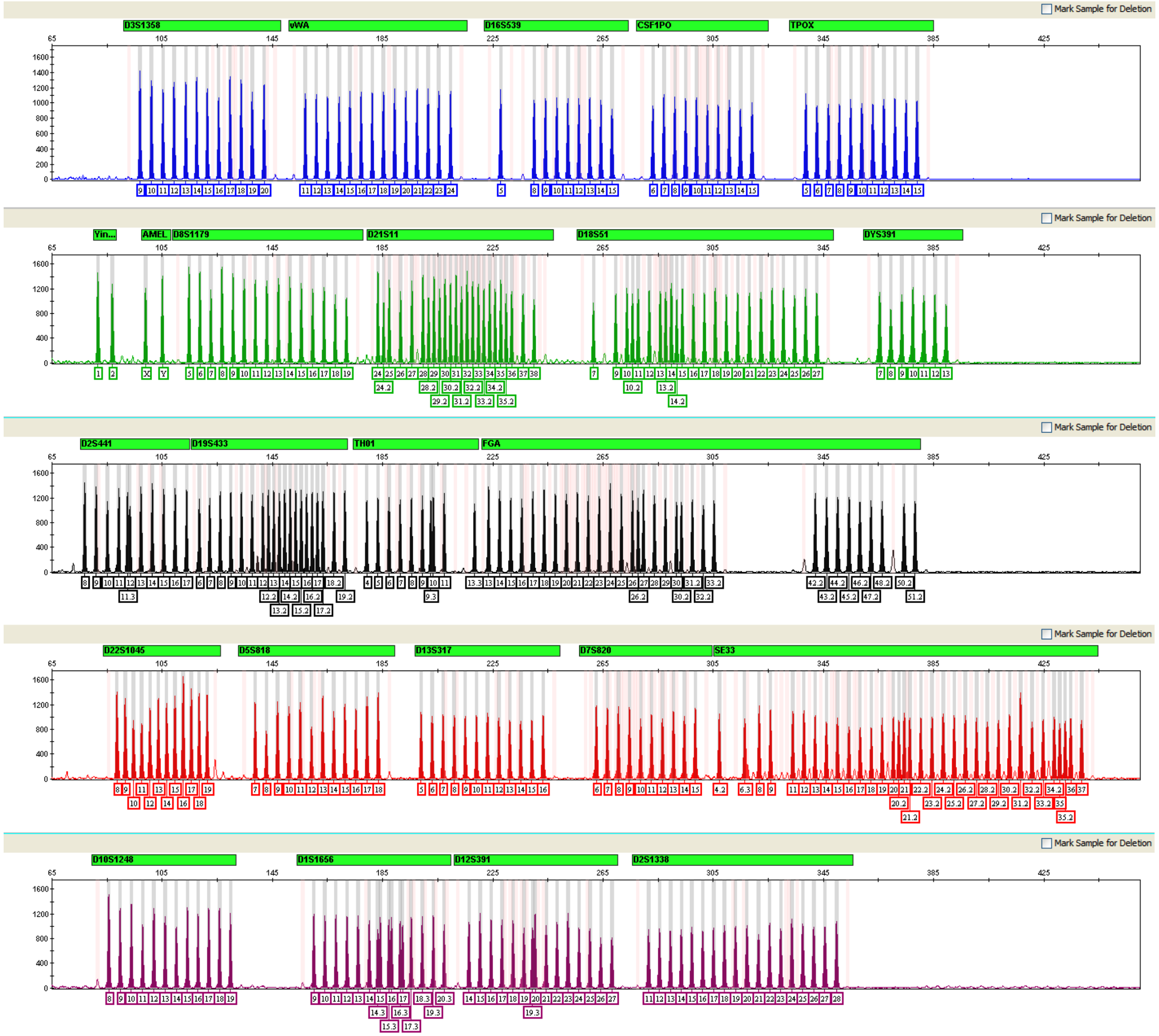

Figure 3 GeneMapper™ *ID-X* Software plot of the GlobalFiler™ Allelic Ladder (Y-axis scale 0–1,600 RFU)

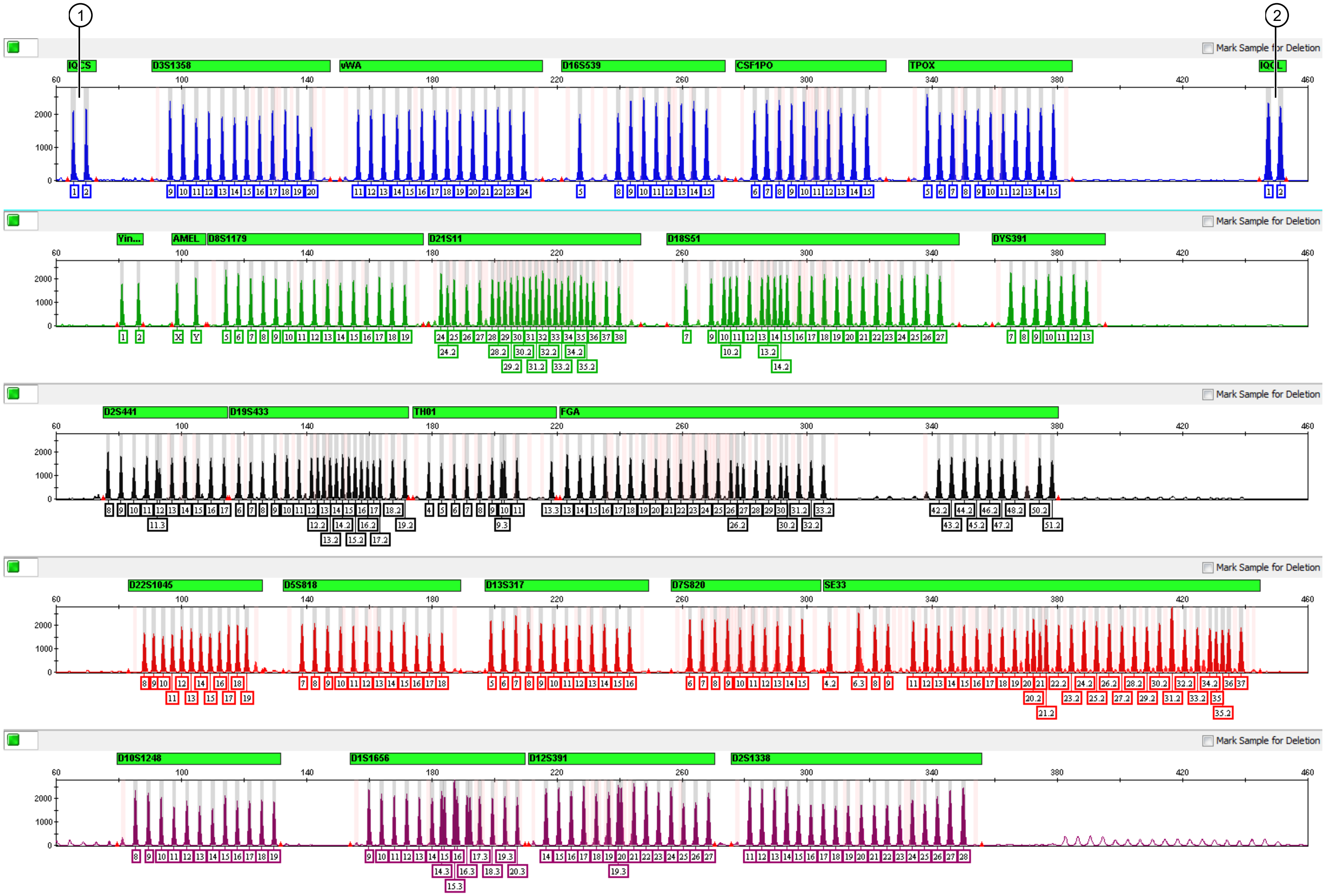

Figure 4 GeneMapper™ *ID-X* Software plot of the GlobalFiler™ IQC Allelic Ladder (Y-axis scale 0–2,500 RFU)

① IQCS (small) Allele 1 and 2

② IQCL (large) Allele 1 and 2

Contents and storage

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.

GlobalFiler™ kit contents and storage

The GlobalFiler™ kit contains sufficient quantities of the reagents for 200 (Cat. No. 4476135) or 1,000 (Cat. No. 4482815) amplification reactions at 25 µL/reaction.

Item	Description	Amount		Storage[1]
		200 reactions (Cat. No. 4476135)	1,000 reactions (Cat. No. 4482815)	
GlobalFiler™ Master Mix	Contains enzyme, salts, dNTPs, bovine serum albumin, and 0.05% sodium azide in buffer and salt.	1 × 1.5 mL	1 × 7.5 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 from cell line in 0.05% sodium azide and buffer.[2] See "DNA Control 007 profiles" on page 13, Figure 1.	1 × 0.3 mL	1 × 0.6 mL	
GlobalFiler™ Primer Set	Contains forward and reverse primers to amplify human DNA targets.	1 × 0.5 mL	1 × 2.5 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.
GlobalFiler™ Allelic Ladder	Contains amplified alleles. See "Allelic ladder profiles" on page 15, Figure 3.	1 × 0.065 mL	1 × 0.15 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.

GlobalFiler™ IQC kit contents and storage

The GlobalFiler™ IQC kit contains sufficient quantities of the reagents for 200 amplification reactions at 25 µL/reaction.

Item	Description	Amount	Storage[1]
		200 reactions (Cat. No. A43565)	
GlobalFiler™ Master Mix	Contains enzyme, salts, dNTPs, bovine serum albumin, and 0.05% sodium azide in buffer and salt.	1 × 1.5 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 from cell line in 0.05% sodium azide and buffer.[2] See "DNA Control 007 profiles" on page 13, Figure 2.	1 × 0.3 mL	
GlobalFiler™ IQC Primer Set	Contains forward and reverse primers to amplify human DNA targets and IQC markers.	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.
GlobalFiler™ IQC Allelic Ladder	Contains amplified alleles and IQC markers. See "Allelic ladder profiles" on page 15, Figure 4.	1 × 0.065 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 B, "Materials required but not supplied".

Instrument 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
GlobalFiler™ PCR Amplification Kit	
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
GlobalFiler™ IQC PCR Amplification Kit	
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 v1.5.2 or later

For more information

- For the instruments and software used during the GlobalFiler™ PCR Amplification Kit validation, see Chapter 6, “Experiments and results”.
- For the instruments and software used during the GlobalFiler™ IQC PCR Amplification Kit performance verification, see Chapter 9, “Performance verification with the GlobalFiler™ IQC kit”.
- For testing information on specific platforms, see the instrument or software user documentation.
- For ordering information, see Appendix B, “Materials required but not supplied”.

Workflow

GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits

Extract and quantify DNA

1. Extract DNA—Go to: www.thermofisher.com/hid-sampleprep
2. Quantify DNA—See "DNA quantification" on page 22

Perform PCR

1. "Prepare the amplification kit reactions" on page 24
2. "Perform PCR" on page 26

Perform capillary electrophoresis

1. "(Before first use of the kit) Set up the capillary electrophoresis instrument" on page 29
2. "Prepare samples for electrophoresis and start the run" on page 32)

Analyze data

1. "Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)" on page 35
2. "Create an analysis method" on page 41
3. "(If needed) Create a size standard definition file" on page 50
4. "Analyze and edit sample files with GeneMapper™ ID-X Software" on page 52
5. "Examine or edit a project" on page 52
6. Chapter 5, "Assess the PCR reaction with the Internal Quality Control System"

2

Perform PCR

Validated DNA input amounts and PCR cycles

The kits are validated for use with:

- 1.0 ng of DNA (15-µL input volume) for 29 cycles
- 500 pg of DNA (15-µL input volume) for 30 cycles, for increased sensitivity with low-concentration samples

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, clear (non-colored), polypropylene tube.

Component	Amount per reaction
Master mix	7.5 µL
Primer set[1]	2.5 µL

[1] The primer set in the GlobalFiler™ kit is different from the primer set in the GlobalFiler™ IQC kit. Do not interchange.

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 10 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 >1.0 ng for 29 cycles or >500 pg for 30 cycles, dilute with low-TE buffer to obtain a 15-µL input volume.
   - If the total sample input volume is <15 µL, bring to volume with low-TE buffer to obtain a 15-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	
	29-cycle protocol	30-cycle protocol
Negative control	15 µL of low-TE buffer	15 µL of low-TE buffer
Test sample	15 µL of DNA	15 µL of DNA
Positive control	Combine, then add to the reaction well or tube: • 10 µL of DNA Control 007 (0.1 ng/µL) • 5 µL of low-TE buffer	Combine, then add to the reaction well or tube: • 5 µL of DNA Control 007 (0.1 ng/µL) • 10 µL of low-TE buffer

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 26.

Perform PCR

IMPORTANT! The kit is optimized for use with the thermal cyclers that are listed in "Instrument and software compatibility" on page 19.

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1. Program the thermal cycler.
   a. Set the ramping mode to **9700 Simulation**.

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

Initial incubation step	Cycle (29 or 30 cycles)[1]		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C 1 minute	94°C 10 seconds	59°C 90 seconds	60°C 10 minutes	4°C ≤24 hours[2]

[1] See "Validated DNA input amounts and PCR cycles" on page 22.
[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.

(GlobalFiler™ kit only) Direct amplification

Note: The GlobalFiler™ IQC kit was not validated for the direct amplification workflow. At PCR cycle numbers <29, the IQC peaks may not be detected.

Note: The GlobalFiler™ kit is not fully validated for direct amplification of single-source samples. Perform validation studies before using this kit for direct amplification of single-source samples, or use the GlobalFiler™ Express PCR Amplification Kit.

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.

In addition, untreated papers such as 903 paper and swabs can be used with Prep-n-Go™ Buffer for direct amplification of biological samples that contain high-quality DNA, such as reference samples.

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.

For more information on direct amplification studies, go to **thermofisher.com**, then search for the *Technical Note: Direct Amplification of Reference Samples Using the GlobalFiler™ PCR Amplification Kit*, or contact your local Human Identification representative.

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.

Note: The primer set and the allelic ladder in the GlobalFiler™ kit are different from the primer set and the allelic ladder in the GlobalFiler™ IQC kit. Do not interchange.

(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 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 1 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_J6_36_POP4(xl)
		Size standard: GS600 LIZ (60–460)
		Dye set: J6 (DS-36)
		Run module: HID_J6_36_POP4 (xl)
		Injection conditions: 1.2 kV/15 seconds (xl: 24 seconds)
		Run conditions: 13 kV/1,550 seconds

Table 2 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:** GlobalFiler™ kit
		Injection conditions: 1.2 kV/10 seconds	**Dye set:** J6 (DS-36)
		Run conditions: 11 kV/1,120 seconds	**Size standard:** GS600 LIZ (60–460)

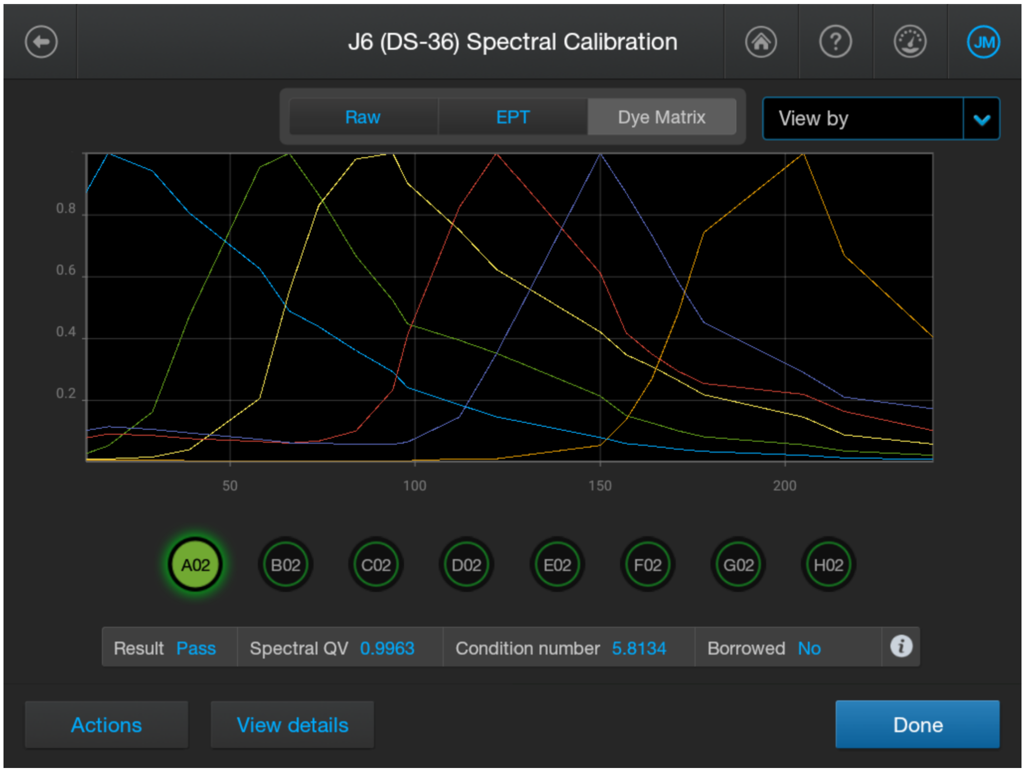
Table 3 Software setup: 3500 Series Genetic Analyzer for Human Identification

Operating system	3500 Data Collection Software	Run parameters
Windows™ 10	v4, v4.0.1	**Assay:** AB_J6_LS_POP4(xl)
		Instrument protocol: AB_HID36_POP4(xl)_J6_NT3200
		Run module: HID36_POP4(xl)
		Injection conditions: 1.2 kV/15 seconds (xl: 24 seconds)
		Run conditions: 13 kV/1,550 seconds
		Dye set: J6

Perform spectral calibration

Perform a spectral calibration using the DS-36 Matrix Standard Kit (Dye Set J6, 6-dye) (Cat. No. 4425042).

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



Result Pass | Spectral QV 0.9963 | Condition number 5.8134 | Borrowed No

Figure 5 Example spectral calibration: SeqStudio™ Flex Series Genetic Analyzer for Human Identification

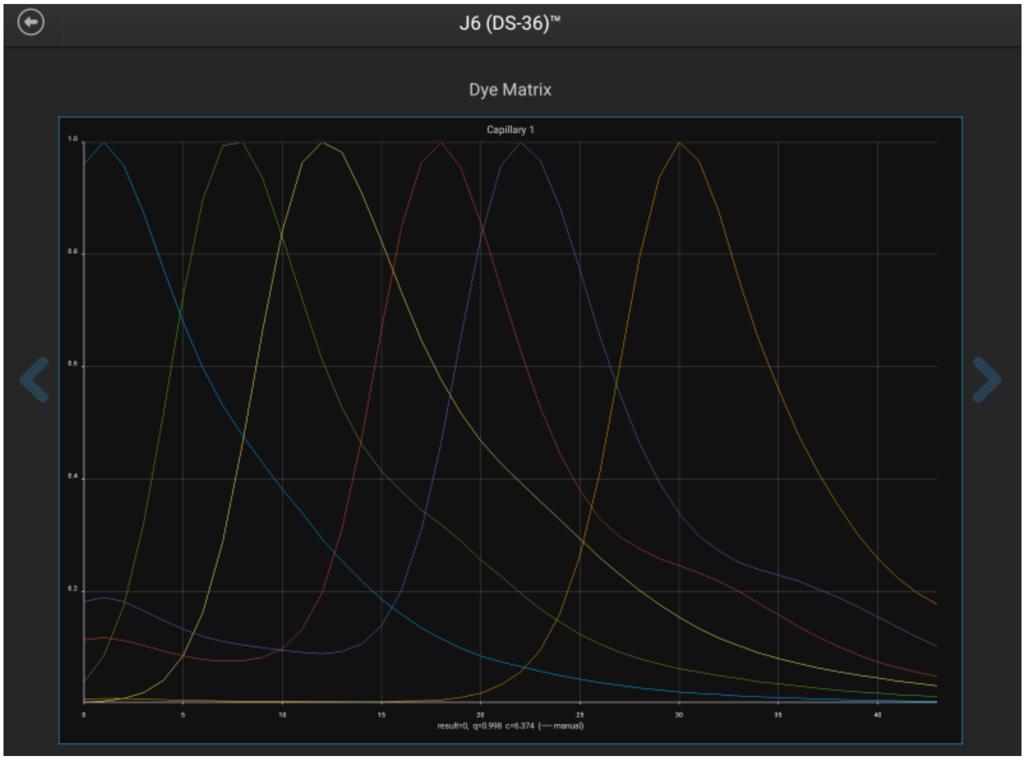


Figure 6 Factory-provided spectral calibration: SeqStudio™ Genetic Analyzer for HID

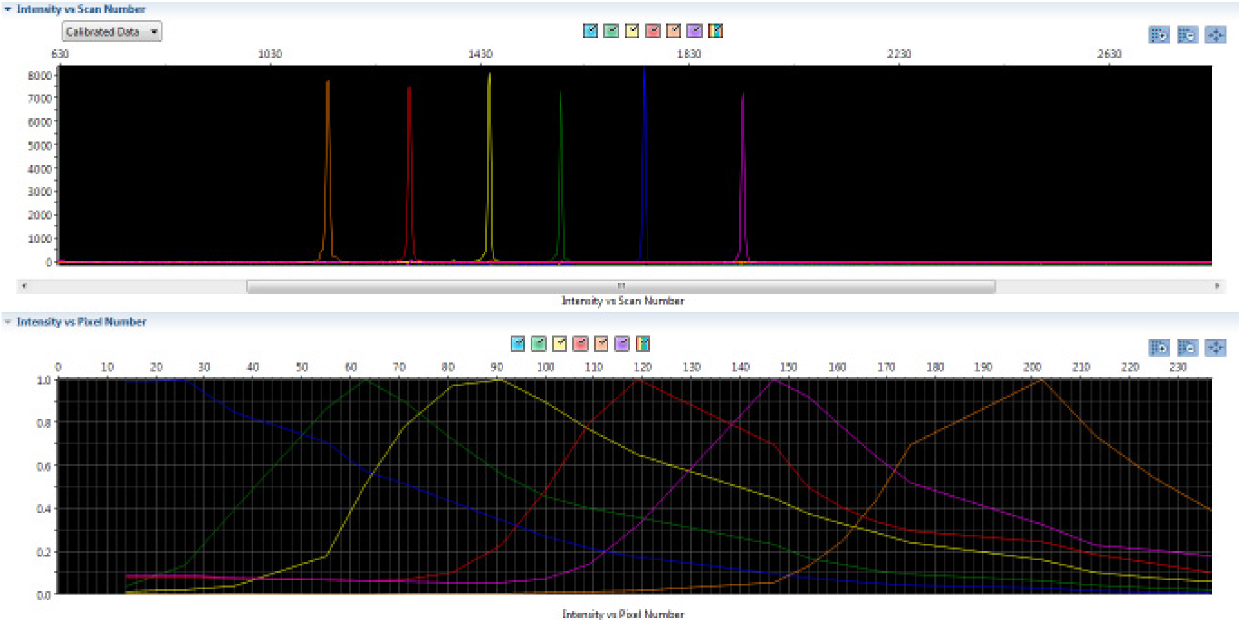


Figure 7 Example spectral calibration: 3500 Series Genetic Analyzer for Human Identification

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.

Component	Amount per reaction
GeneScan™ 600 LIZ™ Size Standard v2.0	0.4 µL
Hi-Di™ Formamide	9.6 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	10 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 “Instrument and software compatibility” on page 19.

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

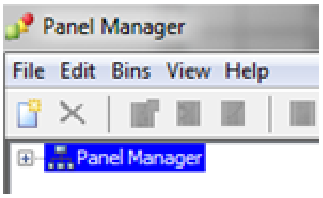
(If needed) Download newer versions of panel, bin, and stutter files

(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 36.
- If the latest files are already installed on your copy of the GeneMapper™ *ID-X* Software, skip to "Create an analysis method" on page 41.

(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.

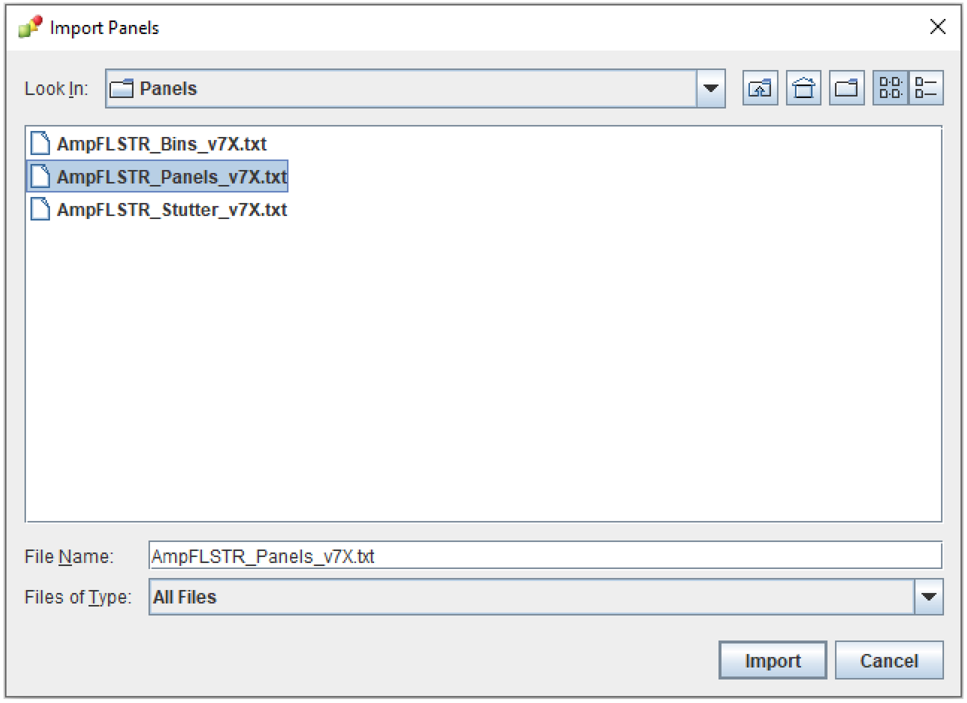
1. Start the GeneMapper™ *ID-X* Software, then sign in with the appropriate user name and password.

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 36.

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



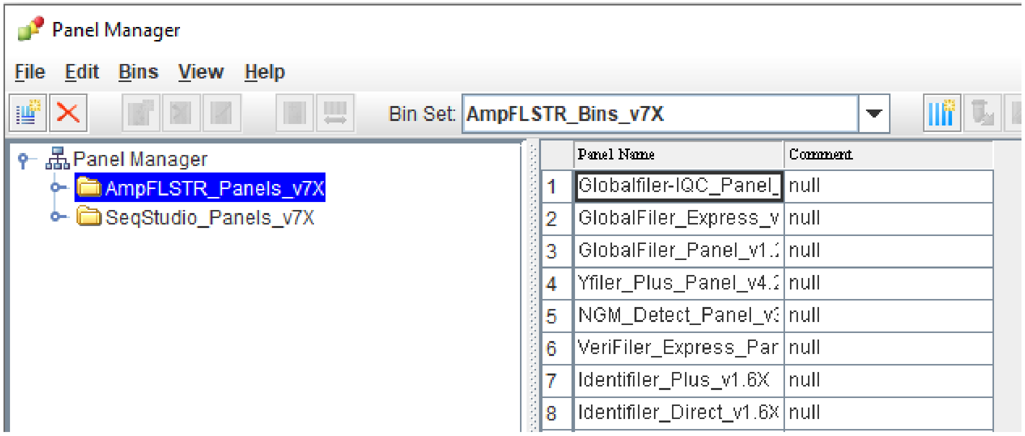
Look In: Panels

AmpFLSTR_Bins_v7X.txt
AmpFLSTR_Panels_v7X.txt
AmpFLSTR_Stutter_v7X.txt

File Name: AmpFLSTR_Panels_v7X.txt
Files of Type: All Files

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.



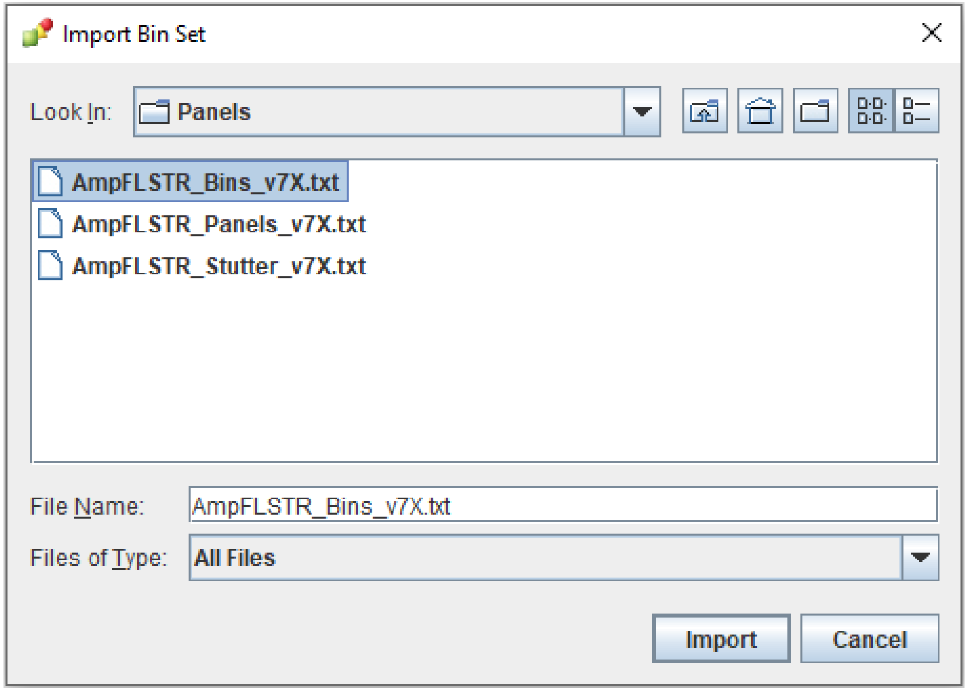
Bin Set: AmpFLSTR_Bins_v7X

Panel Manager
- AmpFLSTR_Panels_v7X
- SeqStudio_Panels_v7X

	Panel Name	Comment
1	Globalfiler-IQC_Panel_	null
2	GlobalFiler_Express_v	null
3	GlobalFiler_Panel_v1.:	null
4	Yfiler_Plus_Panel_v4.:	null
5	NGM_Detect_Panel_v:	null
6	VeriFiler_Express_Par	null
7	Identifiler_Plus_v1.6X	null
8	Identifiler_Direct_v1.6X	null

   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**.

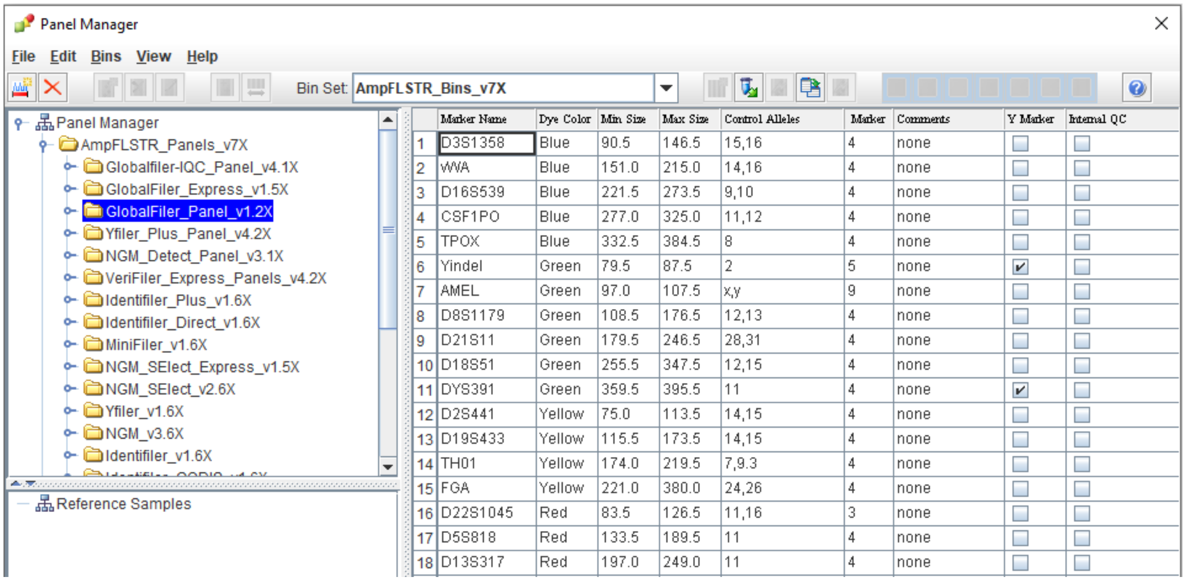


AmpFLSTR_Bins_v7X.txt
AmpFLSTR_Panels_v7X.txt
AmpFLSTR_Stutter_v7X.txt

File Name: AmpFLSTR_Bins_v7X.txt
Files of Type: All Files

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.



Bin Set: AmpFLSTR_Bins_v7X

	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker	Comments	Y Marker	Internal QC
1	D3S1358	Blue	90.5	146.5	15,16	4	none	☐	☐
2	vWA	Blue	151.0	215.0	14,16	4	none	☐	☐
3	D16S539	Blue	221.5	273.5	9,10	4	none	☐	☐
4	CSF1PO	Blue	277.0	325.0	11,12	4	none	☐	☐
5	TPOX	Blue	332.5	384.5	8	4	none	☐	☐
6	Yindel	Green	79.5	87.5	2	5	none	☑	☐
7	AMEL	Green	97.0	107.5	x,y	9	none	☐	☐
8	D8S1179	Green	108.5	176.5	12,13	4	none	☐	☐
9	D21S11	Green	179.5	246.5	28,31	4	none	☐	☐
10	D18S51	Green	255.5	347.5	12,15	4	none	☐	☐
11	DYS391	Green	359.5	395.5	11	4	none	☑	☐
12	D2S441	Yellow	75.0	113.5	14,15	4	none	☐	☐
13	D19S433	Yellow	115.5	173.5	14,15	4	none	☐	☐
14	TH01	Yellow	174.0	219.5	7,9.3	4	none	☐	☐
15	FGA	Yellow	221.0	380.0	24,26	4	none	☐	☐
16	D22S1045	Red	83.5	126.5	11,16	3	none	☐	☐
17	D5S818	Red	133.5	189.5	11	4	none	☐	☐
18	D13S317	Red	197.0	249.0	11	4	none	☐	☐

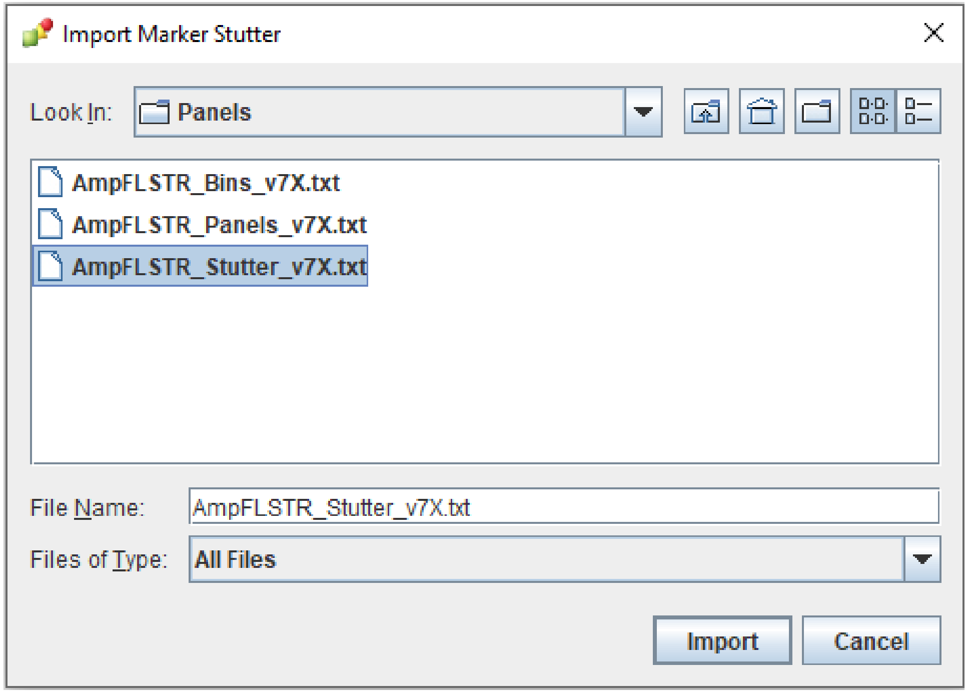
7. Import the stutter file.

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**.



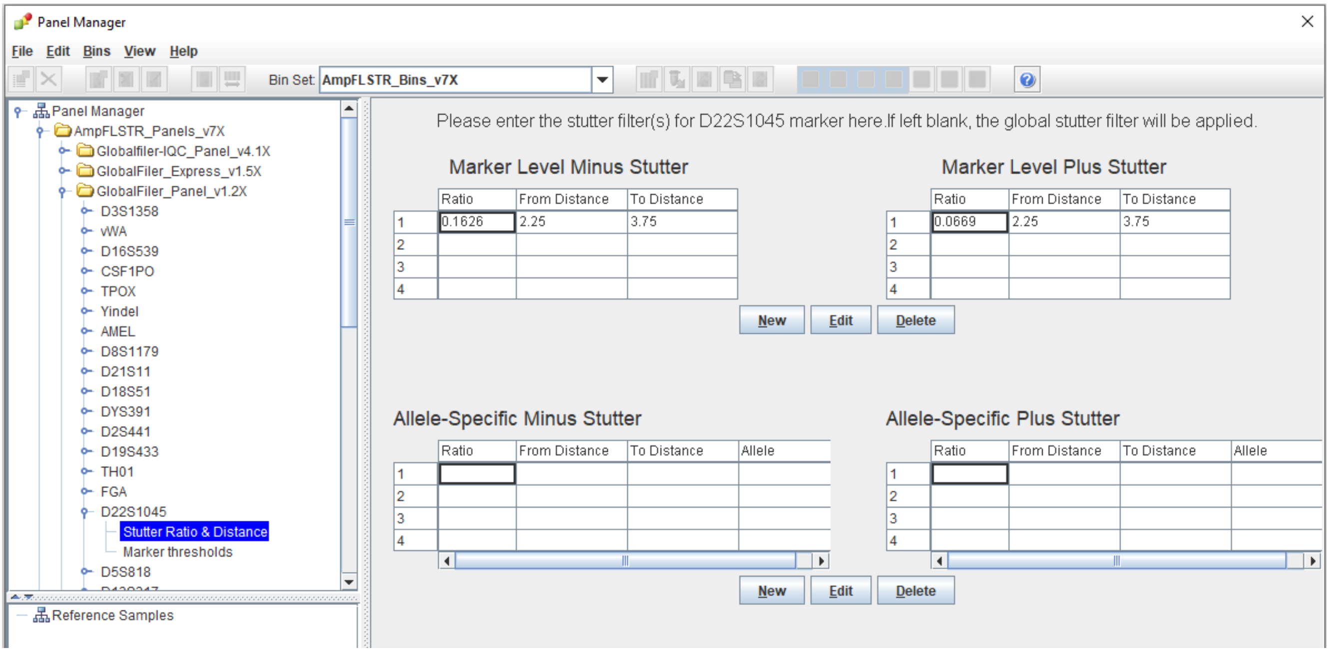
File Name: AmpFLSTR_Stutter_v7X.txt

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.



Bin Set: AmpFLSTR_Bins_v7X

Please enter the stutter filter(s) for D22S1045 marker here.If left blank, the global stutter filter will be applied.

Marker Level Minus Stutter

	Ratio	From Distance	To Distance
1	0.1626	2.25	3.75
2			
3			
4			

Marker Level Plus Stutter

	Ratio	From Distance	To Distance
1	0.0669	2.25	3.75
2			
3			
4			

Allele-Specific Minus Stutter

	Ratio	From Distance	To Distance	Allele
1				
2				
3				
4				

Allele-Specific Plus Stutter

	Ratio	From Distance	To Distance	Allele
1				
2				
3				
4				

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*.

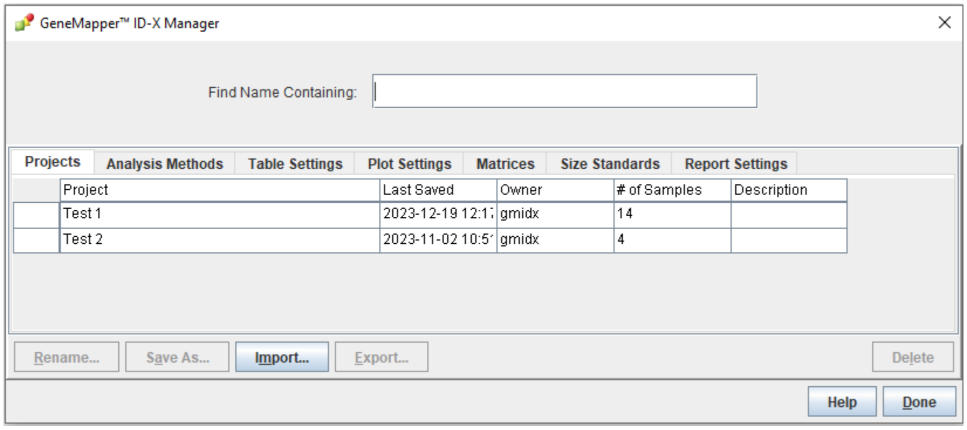
Create an analysis method

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**.



Project	Last Saved	Owner	# of Samples	Description
Test 1	2023-12-19 12:1	gmidx	14	
Test 2	2023-11-02 10:5	gmidx	4	

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.

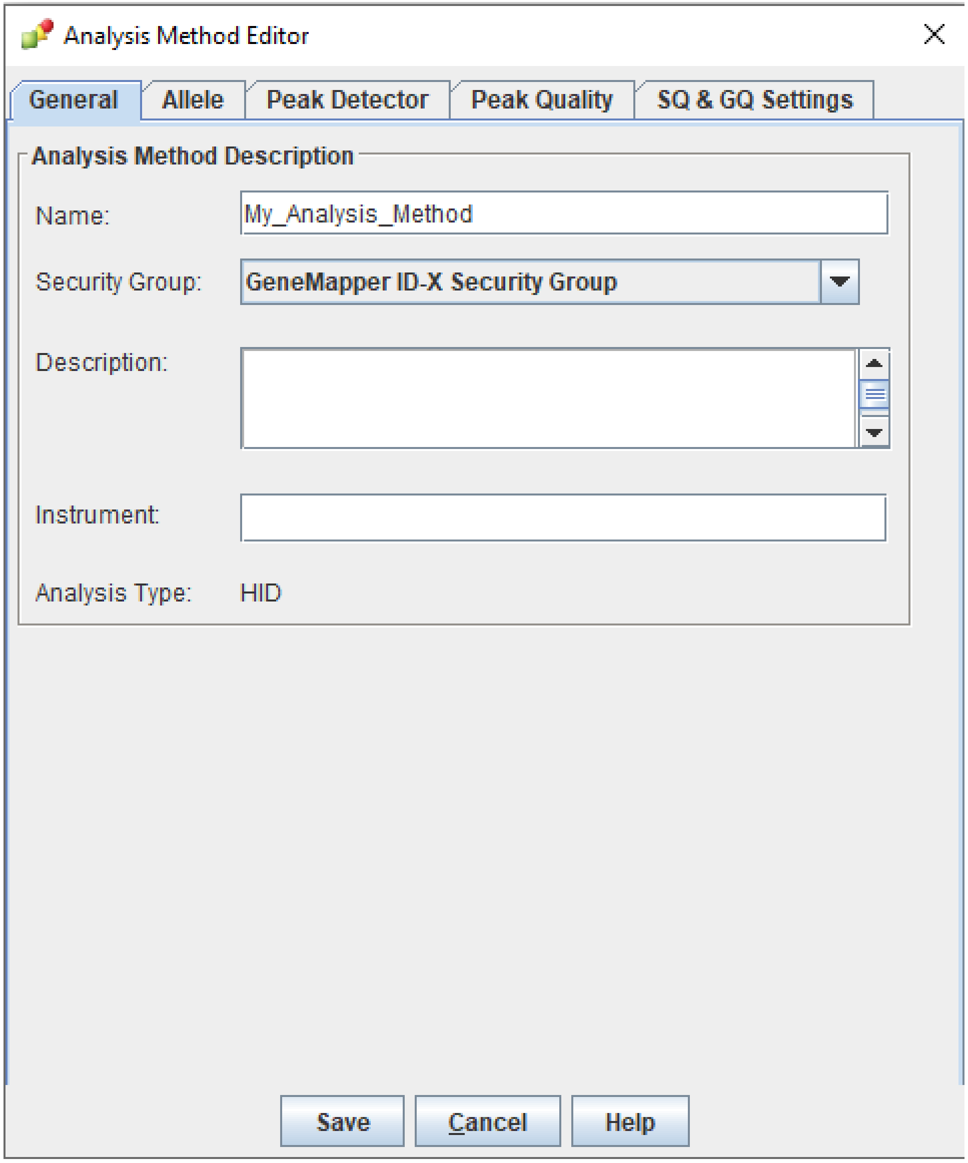
---

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.



Name: My_Analysis_Method

Security Group: GeneMapper ID-X Security Group

Description:

Instrument:

Analysis Type: HID

Figure 8 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.

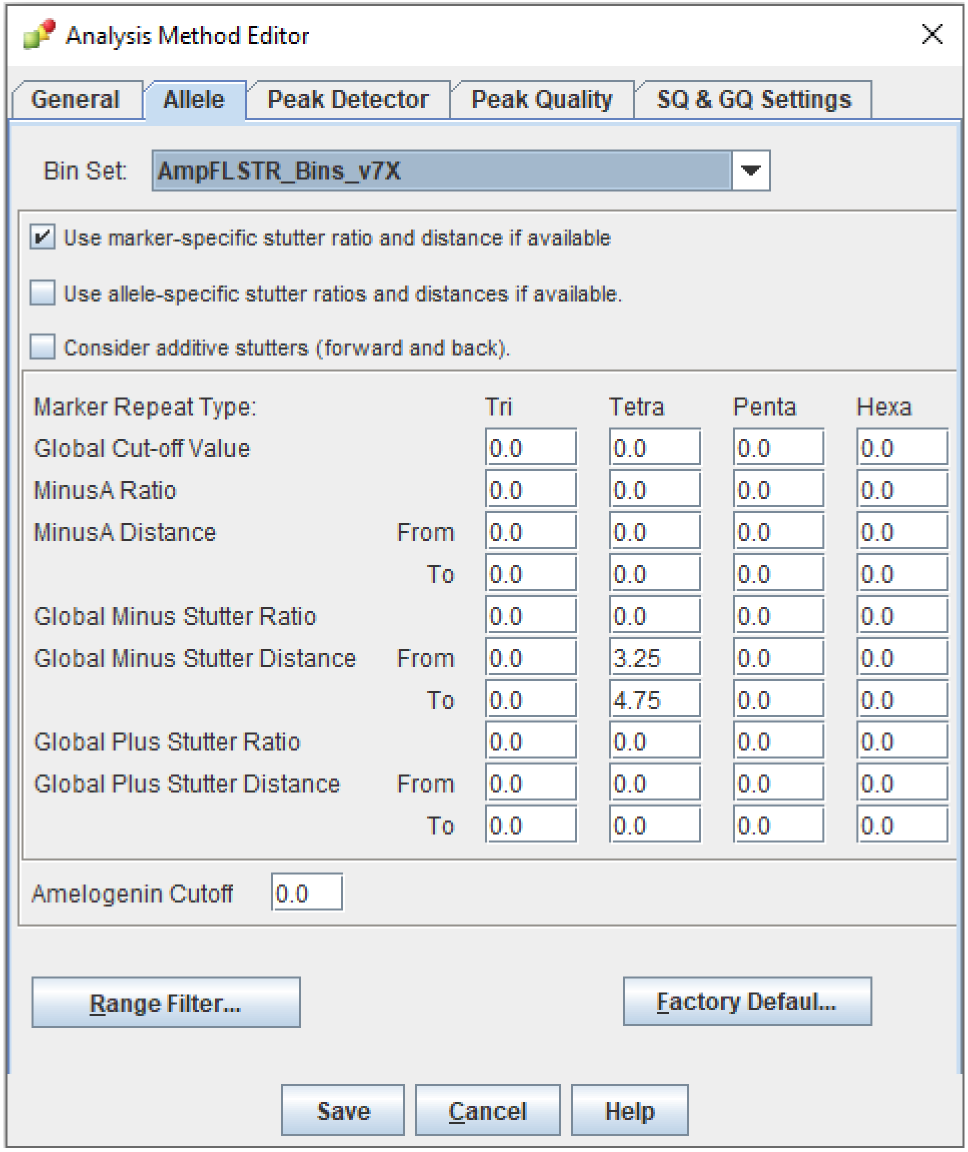
Option	Action	Additional information
Use marker-specific stutter ratio and distance if available	Select or deselect the checkbox, as needed.	To apply the stutter ratios that are contained in the Panel Manager, select the checkbox.
Use allele-specific stutter ratios and distances if available The checkbox 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.



Bin Set: AmpFLSTR_Bins_v7X

☑ Use marker-specific stutter ratio and distance if available

☐ Use allele-specific stutter ratios and distances if available.

☐ Consider additive stutters (forward and back).

Marker Repeat Type:		Tri	Tetra	Penta	Hexa
Global Cut-off Value		0.0	0.0	0.0	0.0
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio		0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From	0.0	3.25	0.0	0.0
	To	0.0	4.75	0.0	0.0
Global Plus Stutter Ratio		0.0	0.0	0.0	0.0
Global Plus Stutter Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0

Amelogenin Cutoff 0.0

Figure 9 Allele tab settings

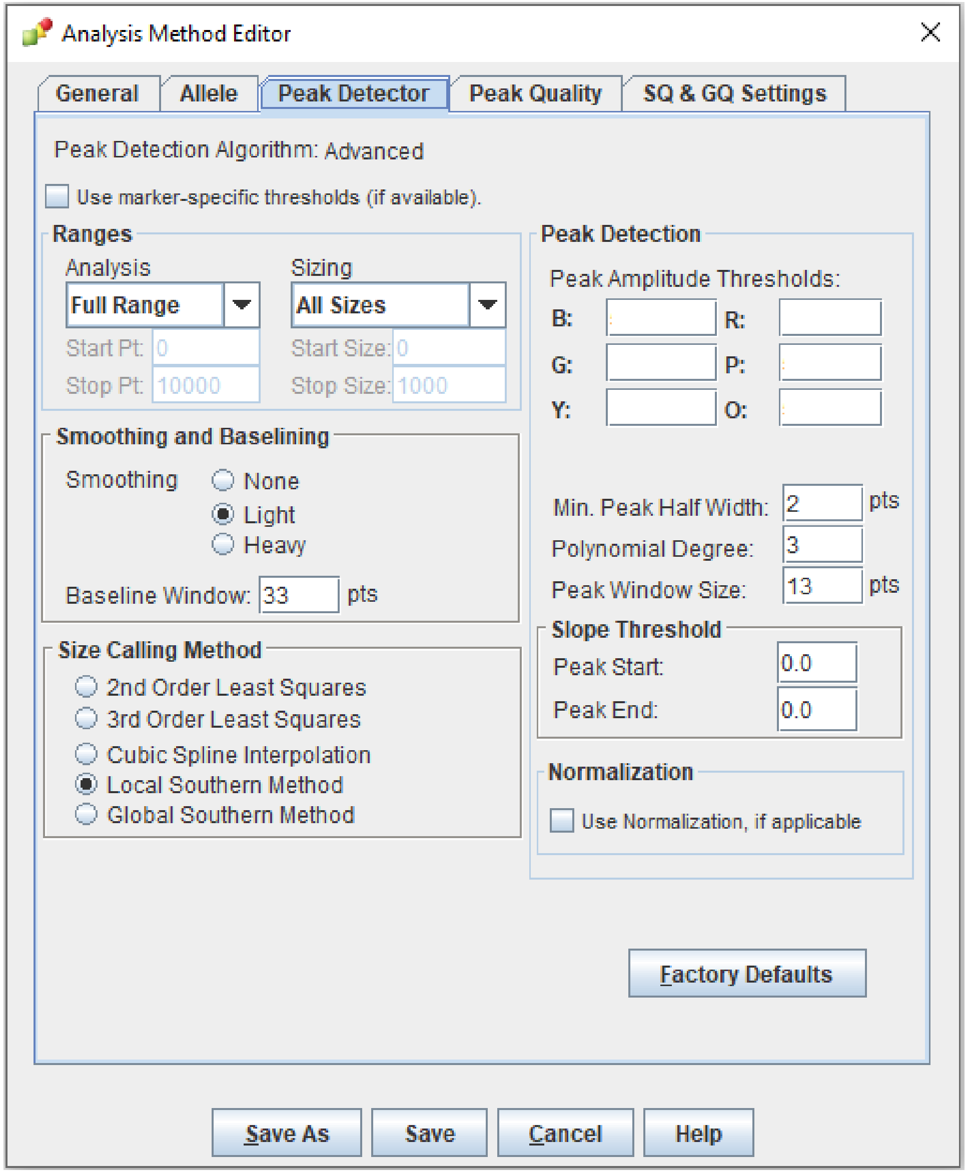
Enter Peak Detector tab settings

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 GlobalFiler™ kit was validated with the values listed. If your laboratory uses an unvalidated polymer, you may need to adjust these values.
	Baseline Window—Enter **33** pts.	
Size Calling Method	Select **Local Southern Method.**	The GlobalFiler™ kit was validated using the Local Southern 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 GlobalFiler™ 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 **13** 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.



Peak Detection Algorithm: Advanced

☐ Use marker-specific thresholds (if available).

Ranges

Analysis: Full Range
Start Pt: 0
Stop Pt: 10000

Sizing: All Sizes
Start Size: 0
Stop Size: 1000

Smoothing and Baselining

Smoothing ○ None ● Light ○ Heavy

Baseline Window: 33 pts

Size Calling Method

○ 2nd Order Least Squares
○ 3rd Order Least Squares
○ Cubic Spline Interpolation
● Local Southern Method
○ Global Southern Method

Peak Detection

Peak Amplitude Thresholds:
B:
R:
G:
P:
Y:
O:

Min. Peak Half Width: 2 pts
Polynomial Degree: 3
Peak Window Size: 13 pts

Slope Threshold

Peak Start: 0.0
Peak End: 0.0

Normalization

☐ Use Normalization, if applicable

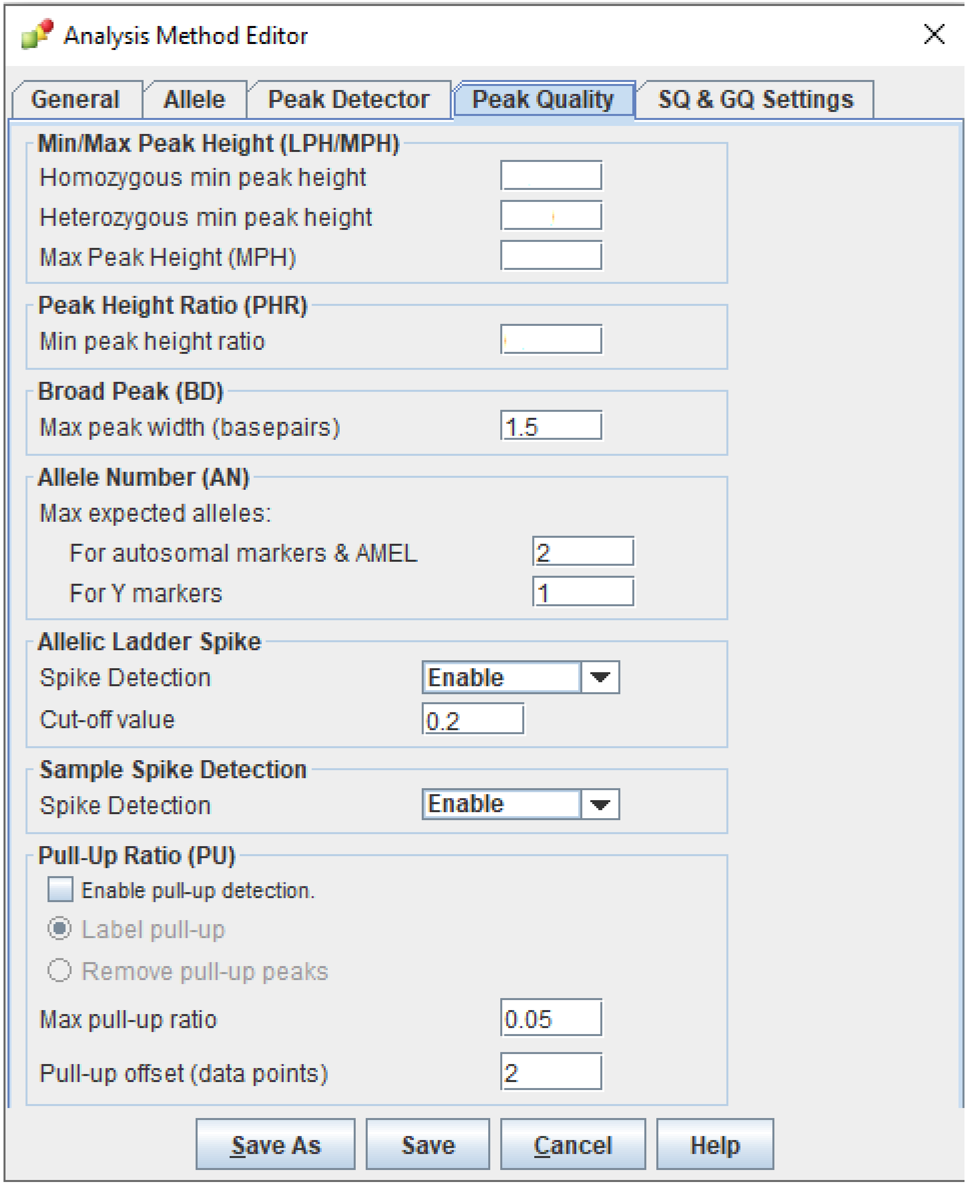
Figure 10 Peak Detector tab settings

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 11.

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).



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

Peak Height Ratio (PHR)
Min peak height ratio

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

Allele Number (AN)
Max expected alleles:
For autosomal markers & AMEL: 2
For Y markers: 1

Allelic Ladder Spike
Spike Detection: Enable
Cut-off value: 0.2

Sample Spike Detection
Spike Detection: Enable

Pull-Up Ratio (PU)
☐ Enable pull-up detection.
● Label pull-up
○ Remove pull-up peaks
Max pull-up ratio: 0.05
Pull-up offset (data points): 2

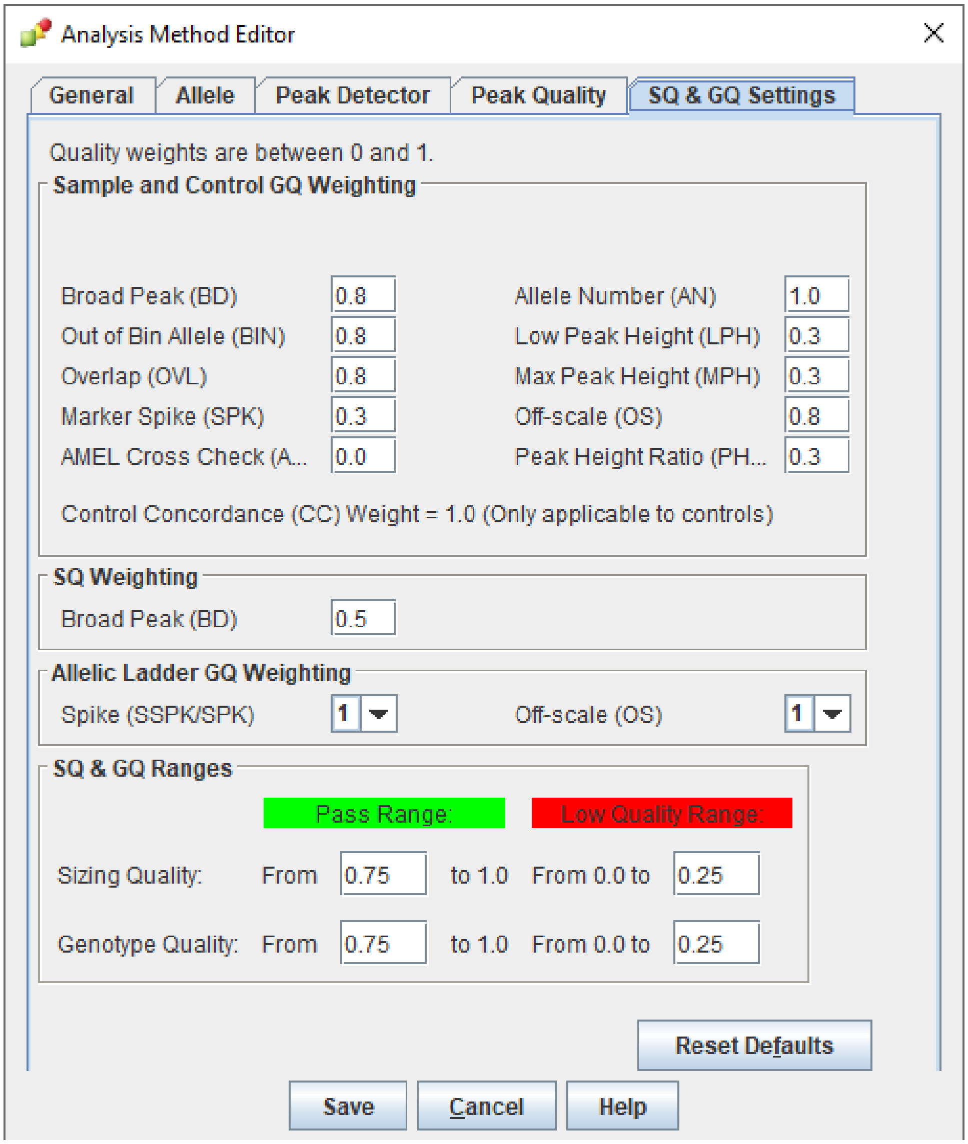
Figure 11 Peak Quality tab settings

Enter SQ & GQ tab settings

Enter the appropriate values.

IMPORTANT! The software default values are shown in Figure 12. 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.

Note: Set the **ACC GQ Weighting** according to the values you determine during internal validation studies of the **ACC PQV**. For example, set the **ACC GQ Weighting** to 0.3 or greater to flag samples in which the Amelogenin result is anything other than X, X or X, Y, or does not agree with the results for the DYS391 or the Y indel markers.



Quality weights are between 0 and 1.

Sample and Control GQ Weighting

Broad Peak (BD) 0.8
Out of Bin Allele (BIN) 0.8
Overlap (OVL) 0.8
Marker Spike (SPK) 0.3
AMEL Cross Check (A... 0.0
Allele Number (AN) 1.0
Low Peak Height (LPH) 0.3
Max Peak Height (MPH) 0.3
Off-scale (OS) 0.8
Peak Height Ratio (PH... 0.3

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

SQ Weighting

Broad Peak (BD) 0.5

Allelic Ladder GQ Weighting

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

SQ & GQ Ranges

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

Figure 12 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.

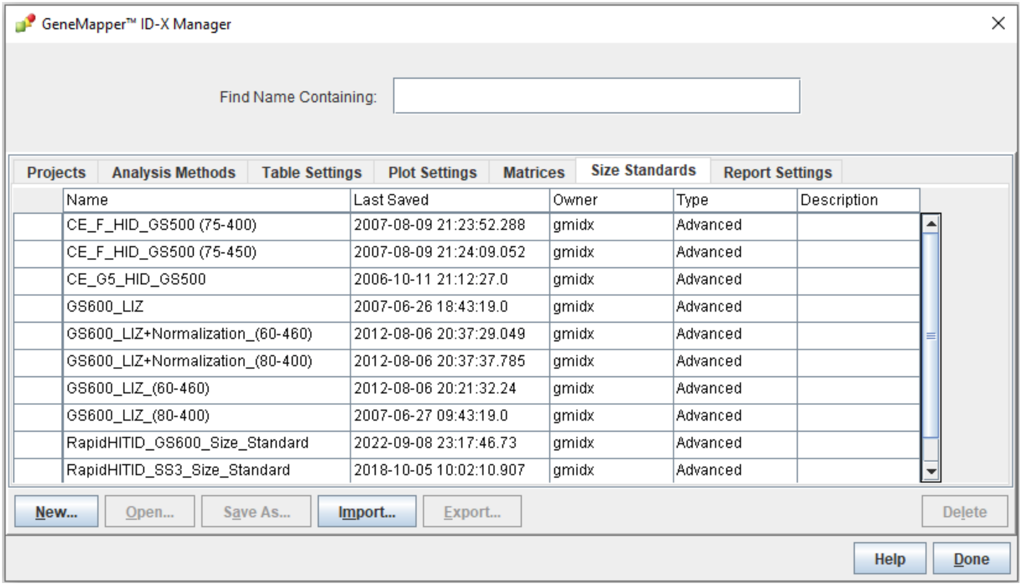
About the GS600_LIZ_(60–460) size standard definition file

The GS600_LIZ_(60–460) size standard definition file that is provided with GeneMapper™ *ID-X* Software and used with the Local Southern Method (size calling method) 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.

This size standard definition has been validated for use with this kit on the genetic analyzers listed in "Instrument and software compatibility" on page 19. If you need to create your own size standard definition, see "Create a size standard definition file" on page 50.

Create a size standard definition file

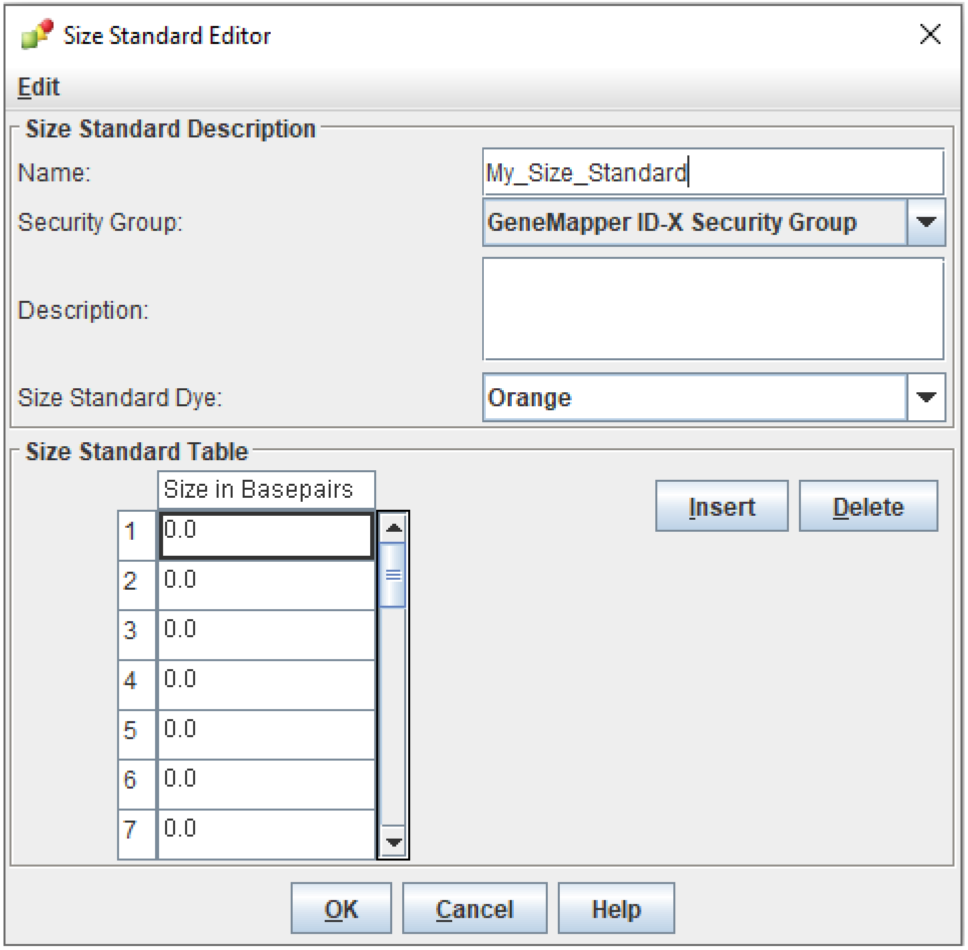
1. Select **Tools ▸ GeneMapper ID-X Manager** to open the **GeneMapper ID-X Manager**.
2. Click the **Size Standards** tab, then click **New**.



Name	Last Saved	Owner	Type	Description
CE_F_HID_GS500 (75-400)	2007-08-09 21:23:52.288	gmidx	Advanced	
CE_F_HID_GS500 (75-450)	2007-08-09 21:24:09.052	gmidx	Advanced	
CE_G5_HID_GS500	2006-10-11 21:12:27.0	gmidx	Advanced	
GS600_LIZ	2007-06-26 18:43:19.0	gmidx	Advanced	
GS600_LIZ+Normalization_(60-460)	2012-08-06 20:37:29.049	gmidx	Advanced	
GS600_LIZ+Normalization_(80-400)	2012-08-06 20:37:37.785	gmidx	Advanced	
GS600_LIZ_(60-460)	2012-08-06 20:21:32.24	gmidx	Advanced	
GS600_LIZ_(80-400)	2007-06-27 09:43:19.0	gmidx	Advanced	
RapidHITID_GS600_Size_Standard	2022-09-08 23:17:46.73	gmidx	Advanced	
RapidHITID_SS3_Size_Standard	2018-10-05 10:02:10.907	gmidx	Advanced	

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.



Size Standard Editor

Size Standard Description

Name: My_Size_Standard

Security Group: **GeneMapper ID-X Security Group**

Description:

Size Standard Dye: **Orange**

Size Standard Table

	Size in Basepairs
1	0.0
2	0.0
3	0.0
4	0.0
5	0.0
6	0.0
7	0.0

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 41.
Panel	Select the current kit panel. If needed, see "Check panel, bin, and stutter file versions on your computer" on page 35.
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 50.

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 191 for a list of available documents.

5

Assess the PCR reaction with the Internal Quality Control System

Overview of the Internal Quality Control system

The GlobalFiler™ IQC PCR Amplification Kit introduces synthetic DNA template and PCR primers that are specific for the Internal Quality Control (IQC) system. All other kit components, primer sequences, reaction setup, thermal cycling parameters, and electrophoresis conditions are the same for the GlobalFiler™ IQC and GlobalFiler™ kits.

The GlobalFiler™ IQC Allelic Ladder contains synthetic DNA template for two IQC markers:

- **IQCS (small)**—Approximately 70 bp
- **IQCL (large)**—Approximately 450 bp

The IQC markers bracket the read region at the small and large molecular weight ends of the electropherogram. The IQC markers confirm successful amplification and electrophoresis in non-compromised human DNA-positive or human DNA-negative samples. The relative peak height of the peaks in the markers can also help to distinguish between inhibited and degraded samples.

Note: In samples, one peak is present for the IQCS and IQCL markers with allele designation 2 (see Figure 2).
In the allelic ladder, two peaks are present for the IQCS and IQCL markers with allele designation 1 and 2 (see Figure 4). The GeneMapper™ *ID-X* Software requires two peaks per marker in the allelic ladder for analysis.

Evaluate the PCR reaction

To evaluate the PCR performance of the samples using the IQC markers, review the relative peak heights of the IQCS and IQCL markers and peak heights of the samples:

- Balanced IQC marker peak heights (peak heights are approximately equal) indicate successful PCR.
- Balanced IQC marker peak heights with unbalanced sample peak heights across the profile indicate possible DNA degradation.
- Unbalanced IQC marker peak heights indicate the presence of inhibitors.

Note: With high inputs of DNA (for example, >2 ng) some suppression of the IQC peaks may also be seen.

Table 4 IQC peak interpretation

Sample DNA profile	IQC peaks	Interpretation	Example
Balanced	Balanced	PCR performance is optimal, no indication of sample degradation or inhibition	See Figure 13
Ski slope	• IQCL peak height significantly decreased relative to the IQCS peak • IQCL peak not present	Indication of inhibition	See Figure 14 and Figure 15
Ski slope	Balanced	Indication of degradation	See “Ski slope profile with balanced IQC peaks (degradation)” on page 56
No peaks	Balanced	No DNA or very little sample DNA	See Figure 17
No peaks	No peaks	PCR failure	—
Low or no peaks	IQCL peak height significantly decreased or not present	PCR failure or extreme inhibition	See Figure 18

Balanced profile

A balanced profile indicates that PCR has successfully occurred. In a balanced profile:

- DNA profile peaks do not decrease at higher molecular weights
- Relative peak heights of the IQCS and IQCL markers are approximately equal, or the IQCL peak height is higher than the IQCS peak height

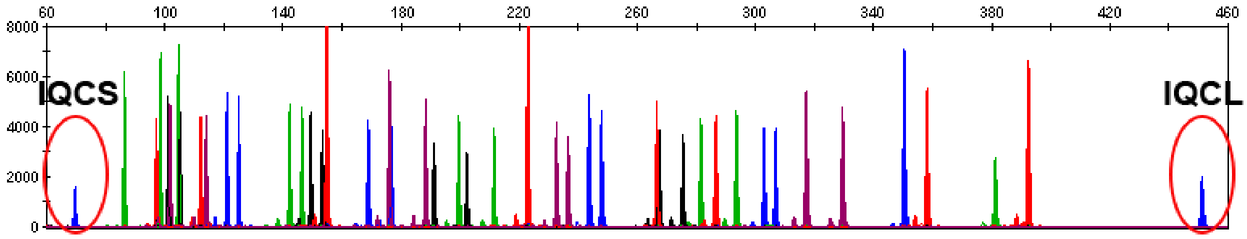


Figure 13 Balanced profile with 1.0 ng of DNA (Y-axis scale 0–8,000 RFU)

Ski slope profile with decreased or missing IQCL peak height (inhibition)

In the presence of hematin or other inhibitors:

- DNA profile peaks decrease at higher molecular weights (ski slope)
- IQCL peak height is significantly lower than the IQCS peak height or is not present

A ski slope pattern of sample peaks with a significantly lower IQCL peak height relative to the IQCS peak height is shown in Figure 14. This indicates that the PCR reaction has been compromised by inhibition. If high levels of PCR inhibition occur, lower IQCL peaks exhibiting some shouldering due to incomplete +A nucleotide addition may be observed.

The complete absence of an IQCL peak, indicating a high level of inhibition, is shown in Figure 15.

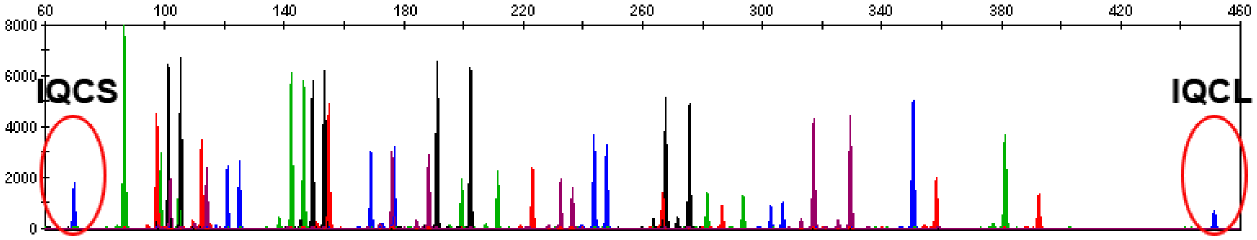


Figure 14 IQC peaks with 150 ng/µL of humic acid (Y-axis scale 0–8,000 RFU)

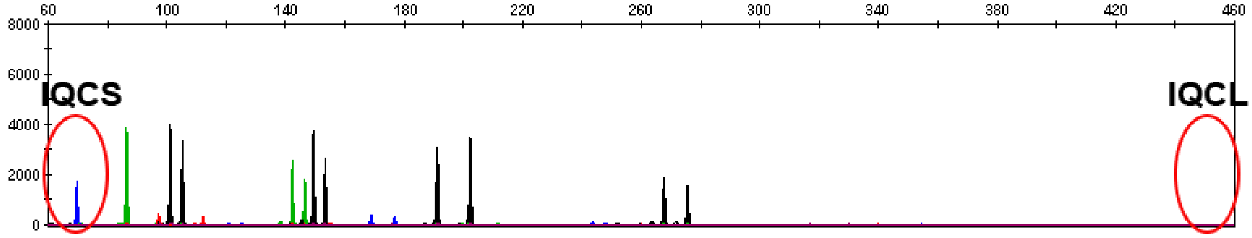


Figure 15 IQC peaks with 250 ng/µL of humic acid (Y-axis scale 0–8,000 RFU)

Ski slope profile with balanced IQC peaks (degradation)

In the presence of degraded DNA:

- DNA profile peaks decrease at higher molecular weights (ski slope)
- Relative peak heights of the IQCS and IQCL markers are approximately equal

The IQCL peak height does not decrease in the presence of degraded sample at low, medium, and high levels of degradation, as shown in Figure 16.

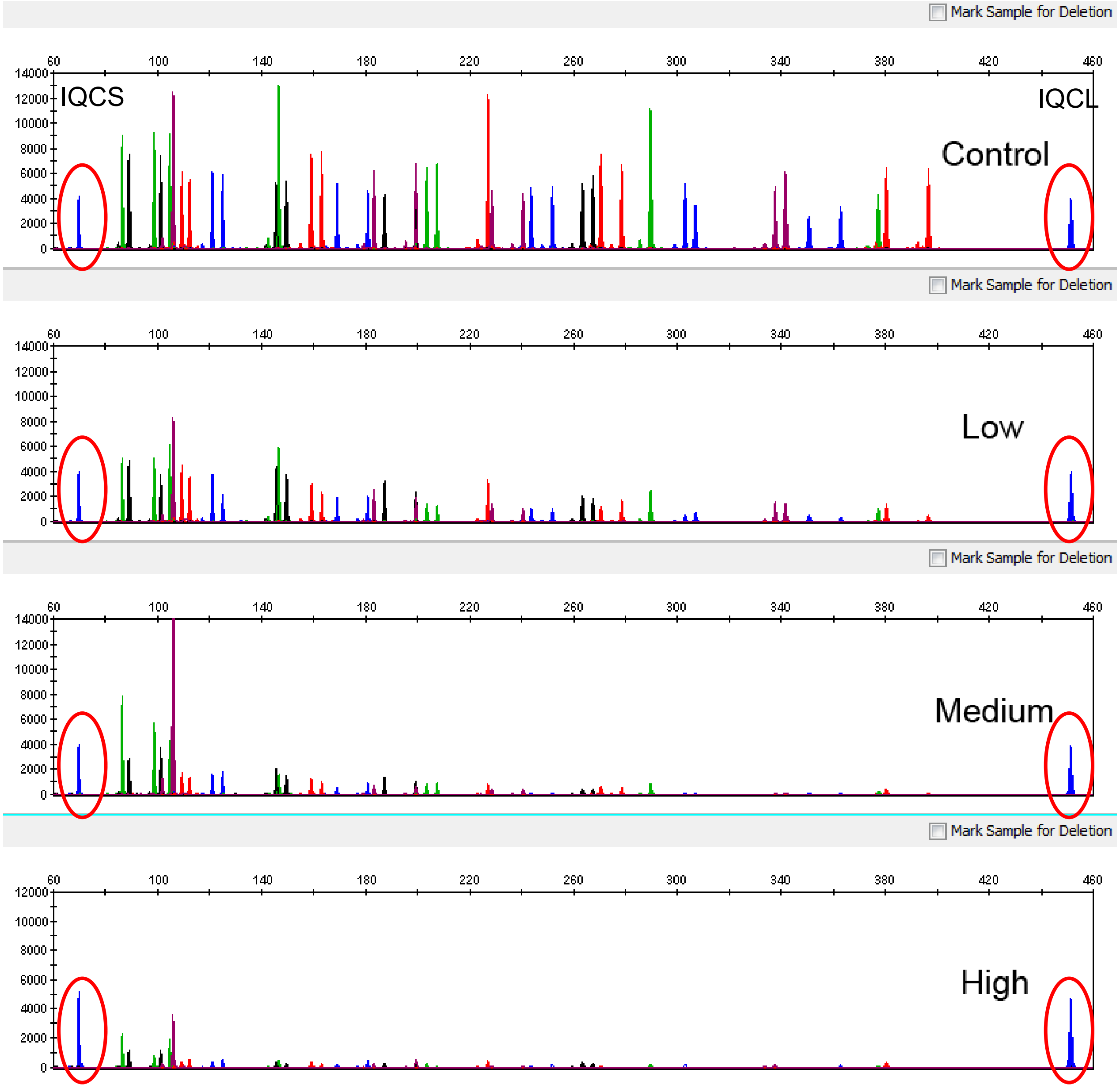


Figure 16 **IQC peaks with degraded DNA at 0.5 ng for 30 cycles (control Y-axis scale 0–14,000 RFU; low, medium, and high Y-axis scale 0–12,000 RFU).**

No sample peaks with balanced IQC peaks

Although there are no DNA profile peaks in the following figure, the presence of both the IQCS and IQCL peaks with balanced relative peak heights indicates that PCR has successfully occurred.

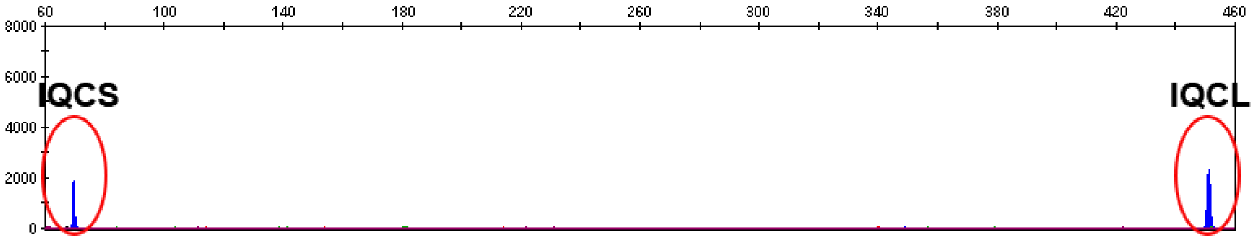


Figure 17 IQCS and IQCL peaks with 0 ng of DNA (Y-axis scale 0–8,000 RFU)

No sample peaks with extreme inhibition of IQC peaks

In the presence of high levels of inhibitor:

- DNA profile peaks may have very low signal or may be missing
- Peak heights of the IQCS and IQCL markers decreased or the peaks are missing

The decreased IQCS peak and the missing IQCL peak in the presence of high levels of inhibitor is shown in Figure 18.

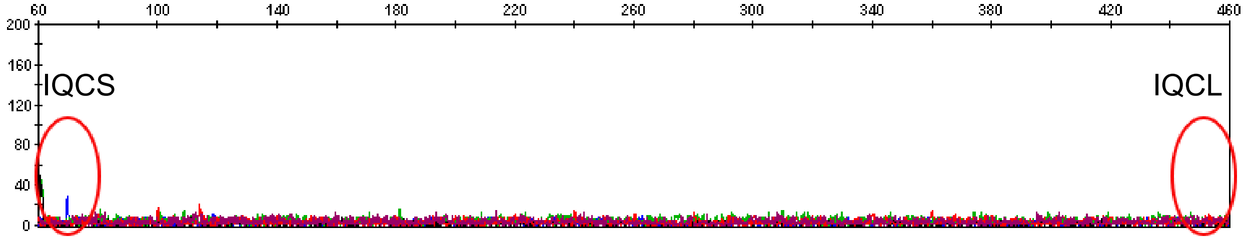


Figure 18 IQC peaks with very high hematin level (Y-axis scale 0–200 RFU)

6 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

We conducted developmental validation experiments according to the updated and revised guidelines from the Scientific Working Group on DNA Analysis Methods (SWGDAM, December 2012). Based on these guidelines, we conducted experiments that comply with guidelines 2.0 and 3.0 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).

We used conditions that produced optimum PCR product yield and that met reproducible performance standards. It is our opinion that while these experiments are not exhaustive, they are appropriate for a manufacturer of STR kits intended for forensic and/or parentage testing use.

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

Except where noted, all developmental validation studies were performed using the Veriti™ Thermal Cycler according to the protocol described in the Perform PCR chapter.

SWGDAM guideline 2.2.1

"Developmental validation is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic, database, known or casework reference samples." (SWGDAM, December 2012)

SWGDAM guideline 3.9.2

"The reaction conditions needed to provide the required degree of specificity and robustness should be determined. These include, but are not limited to, thermal cycling parameters, the concentration of primers, magnesium chloride, DNA polymerase, and other critical reagents." (SWGDAM, December 2012)

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, 1 ng of DNA Control 007 was amplified in the presence of varying concentrations of magnesium chloride, and the results were analyzed on a 3500xL Genetic Analyzer (Figure 19). The performance of the multiplex is most robust within ±20% of the optimal magnesium chloride concentration.

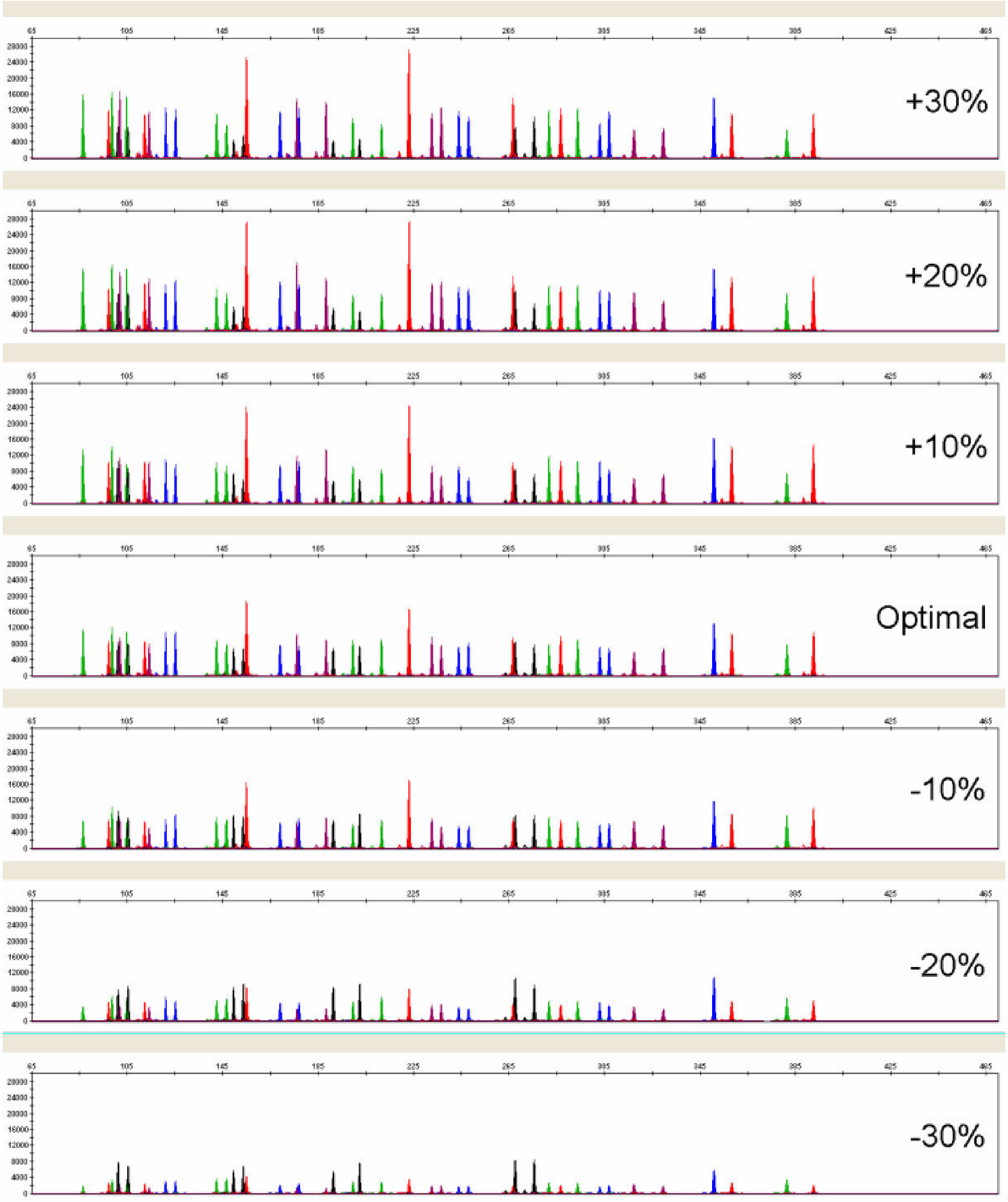


Figure 19 DNA Control 007 (1.0 ng) amplified with the GlobalFiler™ kit in the presence of varying concentrations of magnesium chloride and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0–30,000 RFU).

Thermal cycling temperatures

Thermal cycling parameters were optimized using a Design of Experiments (DOE) approach that attempts to identify the combination of temperatures and hold times that produce the best assay performance. Optimal assay performance was determined through evaluation of assay sensitivity, peak-height balance, and resistance to PCR inhibitors.

For example, annealing/extension temperatures of 55, 57, 59, 61, and 63°C were tested using a Veriti™ Thermal Cycler (Figure 20). The PCR products were analyzed using a 3500xL Genetic Analyzer.

Of the tested annealing temperatures, 57–61°C produced robust profiles. At 55°C and 63°C, the yield of the majority of loci was significantly reduced. The optimal combination of specificity, sensitivity, and resistance to PCR inhibition was observed at 59°C. Thermal cycler temperature is critical to assay performance; therefore, routine, regularly scheduled thermal cycler calibration is strongly recommended.

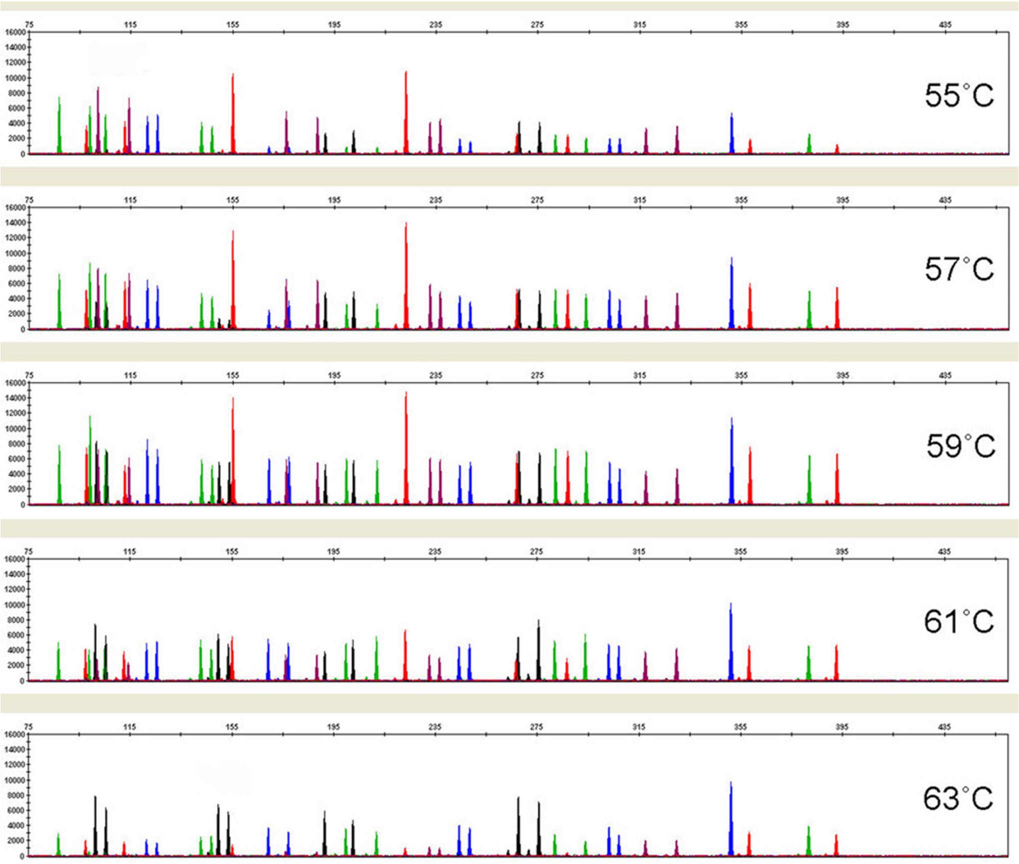


Figure 20 **Electropherograms obtained from amplification of 1.0 ng of DNA Control 007 at annealing temperatures of 55, 57, 59, 61, and 63°C, analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0–16,000 RFU).**

PCR cycle number

Reactions were amplified for 27, 28, 29, 30, and 31 cycles on the Veriti™ Thermal Cycler using 1 ng of DNA Control 007. As expected, the amount of PCR product increased with the number of cycles. A full profile was generated for all numbers of thermal cycles (27–31) and off-scale data were collected for several allele peaks at 30 and 31 cycles (Figure 21).

We recommend using 29 cycles to optimize signal peak height and minimize artifactual or other undesirable peaks.

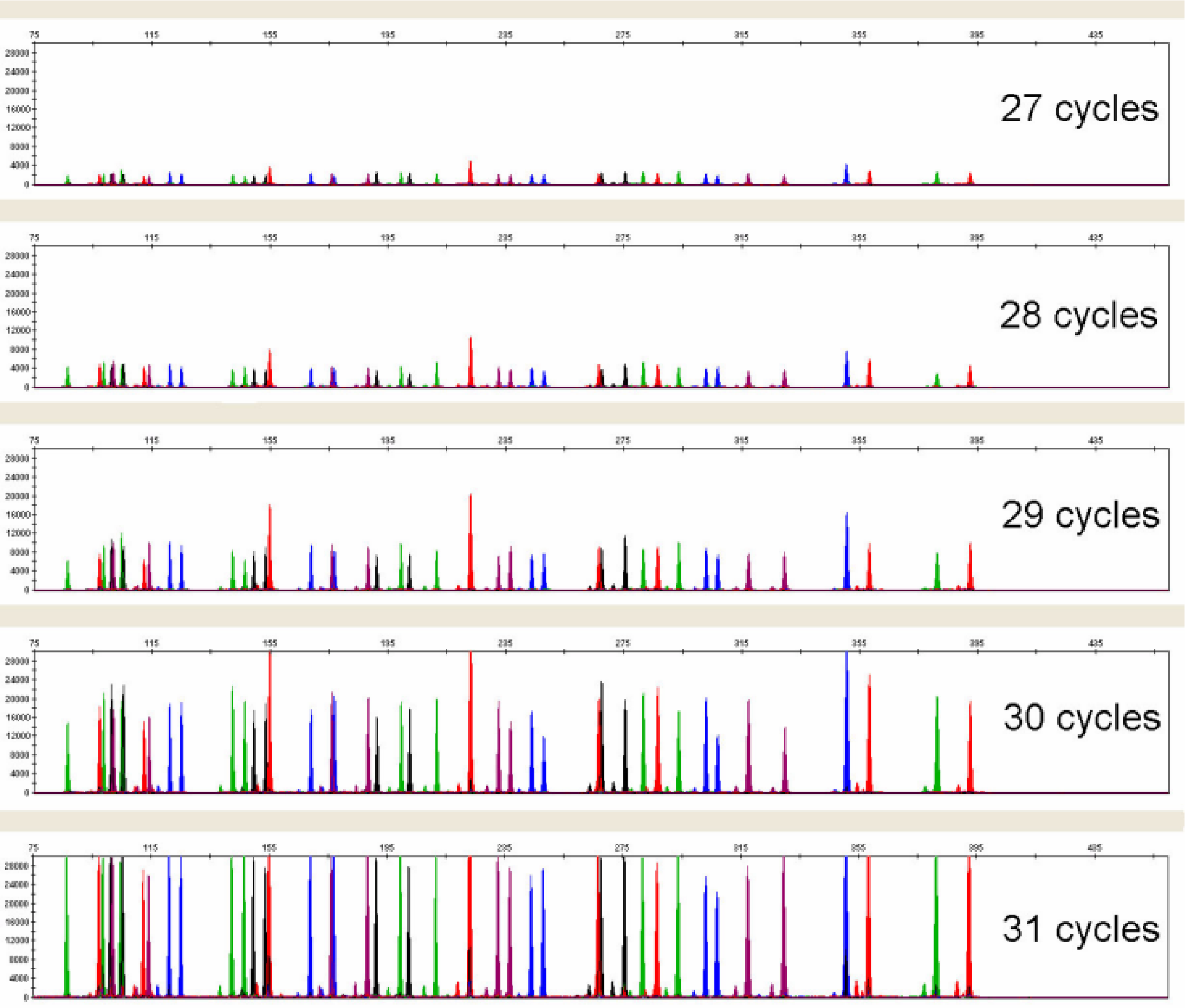


Figure 21 Representative GlobalFiler™ kit profiles obtained from amplification of 1.0 ng of DNA Control 007 using 27, 28, 29, 30, and 31 cycles, analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0–30,000 RFU).

Accuracy, precision, and reproducibility

SWGDAM guideline 3.5

> “Precision and accuracy of the assay should be demonstrated: Precision characterizes the degree of mutual agreement among a series of individual measurements, values and/or results. Precision depends only on the distribution of random errors and does not relate to the true value or specified value. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results. Accuracy is the degree of conformity of a measured quantity to its actual (true) value. Accuracy of a measuring instrument is the ability of a measuring instrument to give responses close to a true value.” (SWGDAM, December 2012)

Accuracy observation

The size differences that are typically observed between sample alleles and the GlobalFiler™ Allelic Ladder alleles on the 3130*xl*, 3500, and 3500xL instruments with POP-4™ Polymer are shown in Figure 22, Figure 23, and Figure 24. 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, regardless of the capillary electrophoresis platform.

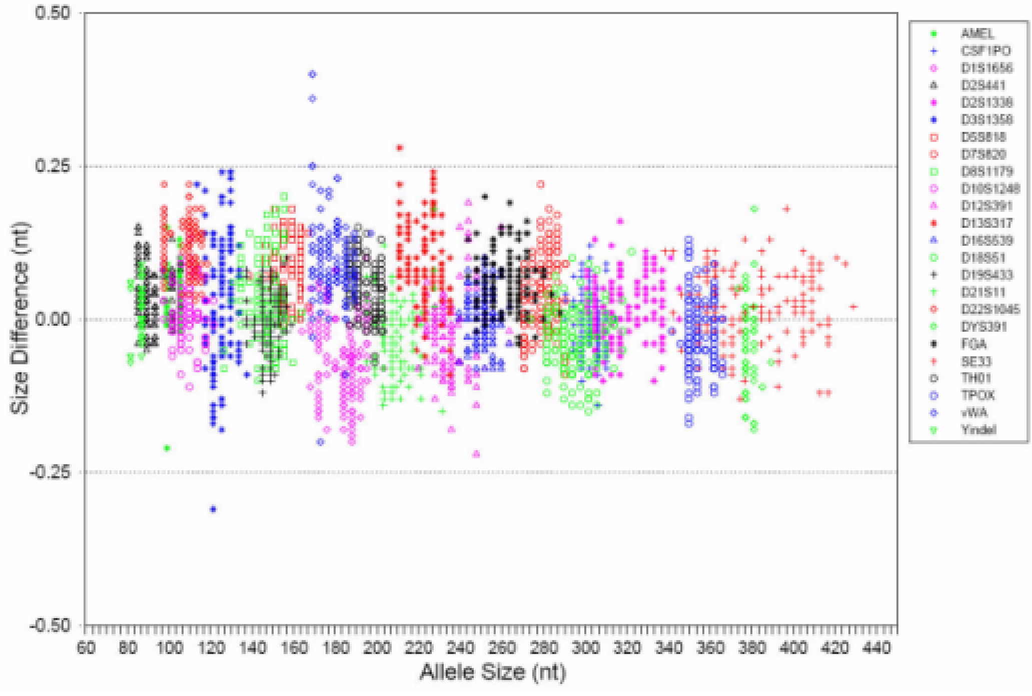


Figure 22 **Allele size vs. allelic ladder sizing for 84 samples analyzed on a 3130*xl* Genetic Analyzer. Size and ladder sizing for the GlobalFiler™ kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.**

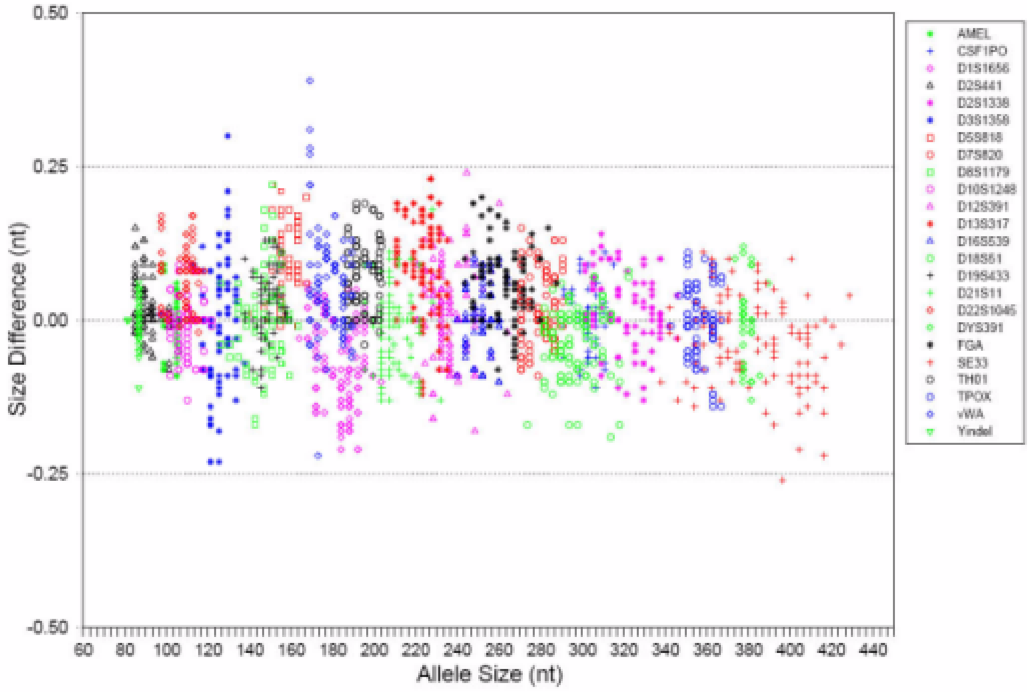


Figure 23 Allele size vs. allelic ladder sizing for 84 samples analyzed on a 3500 Genetic Analyzer. Size and ladder sizing for the GlobalFiler™ kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

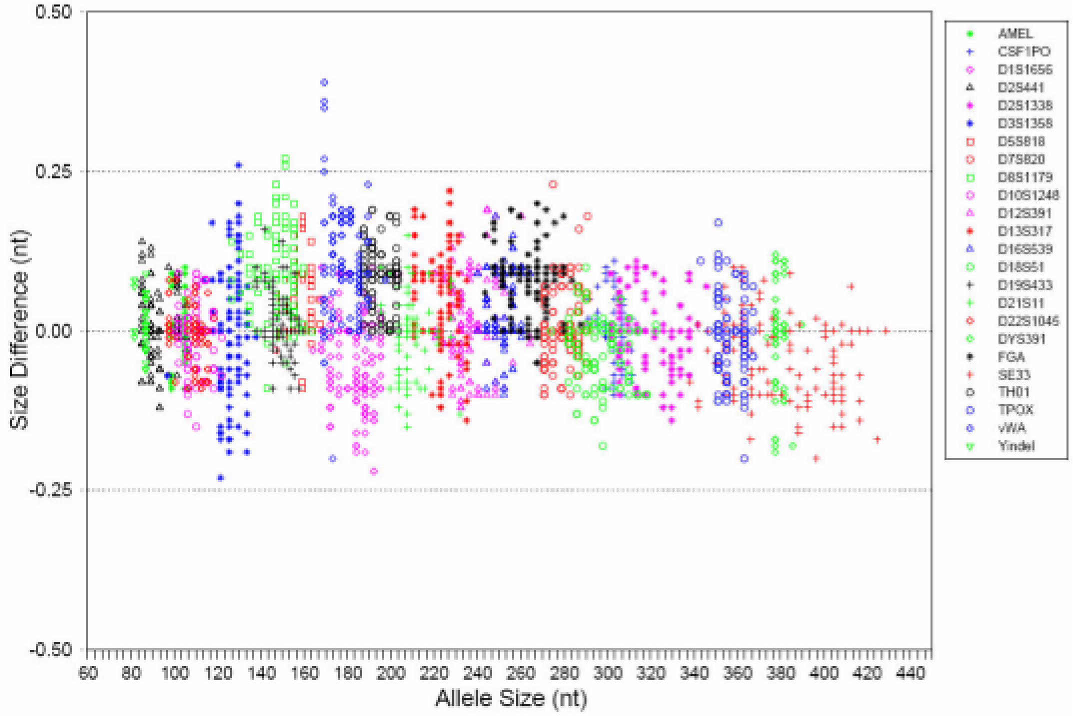


Figure 24 Allele size vs. allelic ladder sizing for 84 samples analyzed on a 3500xL Genetic Analyzer. Size and ladder sizing for the GlobalFiler™ kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

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 observation

Typical precision results obtained from multiple runs of the GlobalFiler™ Allelic Ladder using the GeneScan™ 600 LIZ™ Size Standard v2.0 are shown in Table 6. The results were obtained within a set of injections on a single capillary array. The number of repeated injections for each capillary electrophoresis platform is shown in Table 5.

Table 5 Repeated injections for each CE platform

CE platform	Capillaries	No. of injections	Sizing method
3130*xl*	16/injection	5	Local Southern, 60–460 bp
3500	8/injection	12	Local Southern, 60–460 bp
3500xL	24/injection	4	Local Southern, 60–460 bp

The mean sizes and the standard deviation for the allele sizing were calculated for all the alleles in each run (Table 6). The mean range and the standard deviation range show the lowest and highest values obtained across multiple runs.

Sample alleles can occasionally size outside of 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 with the smallest standard deviations in sizing. The tight clustering of allele sizes obtained, where the standard deviation in sizing is typically <0.15 bp, is illustrated in Figure 22 through Figure 24. 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 to 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 labeling 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 several 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.

IMPORTANT! To minimize the variation in sizing between runs and to help ensure accurate genotyping, follow the guidelines in “Allelic ladder requirements for data analysis” on page 34 and use allelic ladders obtained from the same run as samples to analyze the samples.

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

Table 6 Precision results of multiple runs of the GlobalFiler™ Allelic Ladder

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
AMEL						
X	98.86–98.89	0.022–0.027	98.63–98.68	0.031–0.051	98.58–98.62	0.042–0.047
Y	104.90–104.93	0.025–0.037	104.71–104.77	0.019–0.050	104.67–104.69	0.030–0.043
CSF1PO						
6	282.58–282.63	0.022–0.042	283.17–283.26	0.017–0.062	283.17–283.26	0.046–0.059
7	286.54–286.59	0.026–0.046	287.12–287.21	0.026–0.049	287.13–287.25	0.035–0.054
8	290.49–290.54	0.029–0.045	291.09–291.18	0.031–0.066	291.10–291.22	0.044–0.056
9	294.44–294.49	0.026–0.039	295.04–295.14	0.030–0.061	295.07–295.18	0.032–0.055
10	298.38–298.42	0.038–0.052	298.99–299.09	0.006–0.059	299.01–299.12	0.032–0.063
11	302.28–302.33	0.025–0.043	302.91–302.99	0.016–0.062	302.91–303.03	0.035–0.054
12	306.19–306.25	0.038–0.044	306.84–306.91	0.025–0.063	306.84–306.97	0.040–0.063
13	310.15–310.20	0.029–0.043	310.81–310.91	0.042–0.064	310.83–310.96	0.045–0.058
14	314.18–314.23	0.038–0.042	314.87–314.96	0.005–0.078	314.88–315.01	0.037–0.047
15	318.40–318.45	0.034–0.039	319.10–319.18	0.004–0.063	319.10–319.25	0.045–0.061
D10S1248						
8	85.31–85.33	0.025–0.036	85.39–85.46	0.024–0.051	85.37–85.40	0.031–0.046
9	89.40–89.43	0.025–0.035	89.53–89.58	0.022–0.055	89.49–89.52	0.033–0.045
10	93.47–93.49	0.030–0.037	93.62–93.68	0.035–0.057	93.58–93.63	0.040–0.053
11	97.54–97.57	0.026–0.040	97.73–97.79	0.006–0.054	97.70–97.73	0.045–0.050
12	101.62–101.65	0.029–0.036	101.84–101.90	0.017–0.053	101.80–101.86	0.041–0.045

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
13	105.70–105.73	0.029–0.037	105.96–106.03	0.034–0.052	105.90–105.96	0.037–0.043
14	109.75–109.79	0.030–0.038	110.02–110.09	0.014–0.065	109.98–110.05	0.038–0.048
15	113.74–113.79	0.027–0.039	114.05–114.13	0.001–0.058	114.01–114.08	0.019–0.048
16	117.65–117.69	0.026–0.035	117.95–118.02	0.001–0.046	117.90–117.97	0.038–0.050
17	121.52–121.56	0.029–0.043	121.85–121.91	0.004–0.054	121.81–121.86	0.032–0.048
18	125.40–125.45	0.028–0.041	125.77–125.85	0.022–0.062	125.73–125.79	0.036–0.048
19	129.32–129.37	0.027–0.045	129.73–129.78	0.028–0.049	129.68–129.75	0.040–0.053
D12S391						
14	216.01–216.08	0.040–0.040	216.47–216.57	0.019–0.061	216.54–216.61	0.040–0.060
15	220.06–220.12	0.040–0.045	220.52–220.59	0.039–0.067	220.61–220.67	0.040–0.055
16	224.08–224.14	0.040–0.047	224.56–224.64	0.018–0.081	224.66–224.72	0.050–0.059
17	228.02–228.08	0.029–0.047	228.53–228.62	0.030–0.095	228.61–228.69	0.052–0.065
18	231.99–232.07	0.033–0.043	232.53–232.60	0.017–0.070	232.58–232.68	0.044–0.057
19	235.95–236.01	0.030–0.044	236.50–236.55	0.026–0.076	236.55–236.64	0.050–0.058
19.3	239.02–239.08	0.029–0.046	239.52–239.62	0.001–0.071	239.60–239.70	0.048–0.065
20	239.95–240.03	0.028–0.054	240.51–240.61	0.001–0.068	240.58–240.68	0.036–0.061
21	244.03–244.09	0.033–0.045	244.55–244.65	0.025–0.061	244.62–244.72	0.047–0.055
22	247.99–248.04	0.033–0.042	248.51–248.60	0.039–0.063	248.57–248.69	0.046–0.055
23	251.99–252.05	0.024–0.039	252.50–252.58	0.028–0.056	252.57–252.66	0.051–0.056
24	255.97–256.02	0.031–0.047	256.50–256.58	0.014–0.055	256.54–256.64	0.038–0.053
25	259.86–259.90	0.033–0.042	260.41–260.52	0.036–0.076	260.46–260.56	0.037–0.059
26	263.80–263.84	0.032–0.039	264.37–264.46	0.033–0.058	264.41–264.52	0.045–0.061
27	267.90–267.95	0.033–0.049	268.46–268.55	0.028–0.064	268.54–268.63	0.042–0.052
D13S317						
5	198.77–198.81	0.023–0.035	198.95–199.01	0.004–0.055	198.96–198.99	0.045–0.055
6	202.77–202.82	0.024–0.043	202.92–202.97	0.035–0.065	202.94–202.97	0.039–0.048
7	206.75–206.79	0.022–0.034	206.87–206.93	206.87–206.93	206.89–206.94	0.036–0.046

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
8	210.82–210.87	0.027–0.037	210.93–210.99	0.009–0.065	210.96–210.98	0.044–0.050
9	214.85–214.89	0.028–0.037	214.95–214.99	0.008–0.054	214.97–215.00	0.032–0.049
10	218.95–218.97	0.022–0.033	219.04–219.09	0.032–0.054	219.08–219.09	0.028–0.053
11	223.00–223.02	0.022–0.041	223.08–223.15	0.010–0.064	223.13–223.16	0.045–0.054
12	227.11–227.13	0.024–0.040	227.17–227.25	0.031–0.061	227.24–227.25	0.052–0.067
13	231.05–231.09	0.029–0.040	231.15–231.20	0.020–0.058	231.19–231.21	0.043–0.050
14	235.01–235.05	0.029–0.036	235.11–235.17	0.020–0.063	235.15–235.18	0.042–0.057
15	239.05–239.08	0.031–0.039	239.15–239.21	0.005–0.062	239.19–239.22	0.023–0.055
16	243.18–243.21	0.025–0.036	243.26–243.31	0.005–0.046	243.29–243.32	0.035–0.049
D16S539						
5	227.17–227.21	0.031–0.038	227.46–227.54	0.029–0.063	227.32–227.40	0.044–0.057
8	239.33–239.37	0.028–0.044	239.66–239.73	0.001–0.057	239.49–239.58	0.042–0.051
9	243.46–243.51	0.029–0.038	243.77–243.87	0.005–0.049	243.63–243.72	0.027–0.046
10	247.59–247.63	0.025–0.045	247.90–247.98	0.001–0.049	247.76–247.84	0.039–0.047
11	251.65–251.68	0.027–0.039	251.95–252.03	0.008–0.057	251.79–251.89	0.045–0.055
12	255.61–255.65	0.025–0.037	255.93–256.00	0.006–0.050	255.78–255.86	0.039–0.045
13	259.55–259.59	0.022–0.036	259.89–259.98	0.001–0.059	259.72–259.84	0.051–0.057
14	263.55–263.60	0.025–0.038	263.91–264.00	0.015–0.059	263.75–263.86	0.050–0.054
15	267.59–267.63	0.027–0.041	267.93–268.04	0.025–0.056	267.79–267.89	0.043–0.055
D18S51						
7	261.06–261.11	0.024–0.038	261.26–261.37	0.005–0.060	261.21–261.29	0.033–0.048
9	269.20–269.24	0.025–0.041	269.40–269.49	0.028–0.056	269.33–269.39	0.038–0.054
10	273.27–273.32	0.026–0.043	273.45–273.53	0.024–0.049	273.41–273.45	0.048–0.054
10.2	275.27–275.32	0.028–0.040	275.43–275.52	0.014–0.050	275.42–275.45	0.034–0.045
11	277.35–277.37	0.022–0.035	277.50–277.59	0.009–0.058	277.48–277.52	0.043–0.059
12	281.39–281.44	0.026–0.037	281.55–281.64	0.008–0.054	281.51–281.54	0.050–0.053
13	285.42–285.46	0.027–0.038	285.58–285.66	0.018–0.056	285.52–285.55	0.042–0.047

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
13.2	287.38–287.42	0.028–0.037	287.54–287.62	0.025–0.052	287.49–287.53	0.049–0.053
14	289.43–289.46	0.033–0.037	289.60–289.67	0.025–0.058	289.52–289.57	0.042–0.051
14.2	291.41–291.43	0.027–0.035	291.56–291.64	0.023–0.060	291.50–291.54	0.041–0.052
15	293.43–293.47	0.022–0.032	293.57–293.66	0.035–0.063	293.53–293.57	0.041–0.045
16	297.44–297.47	0.024–0.039	297.58–297.69	0.008–0.055	297.54–297.57	0.034–0.048
17	301.40–301.43	0.025–0.035	301.55–301.64	0.009–0.058	301.49–301.55	0.052–0.056
18	305.36–305.39	0.028–0.046	305.51–305.60	0.028–0.065	305.47–305.52	0.043–0.045
19	309.38–309.41	0.021–0.039	309.50–309.60	0.019–0.065	309.47–309.54	0.037–0.063
20	313.44–313.48	0.030–0.040	313.55–313.66	0.000–0.065	313.53–313.59	0.033–0.063
21	317.69–317.71	0.027–0.040	317.78–317.91	0.021–0.065	317.78–317.83	0.038–0.057
22	322.00–322.01	0.028–0.044	322.07–322.20	0.012–0.067	322.06–322.10	0.026–0.056
23	326.04–326.05	0.020–0.036	326.12–326.23	0.030–0.060	326.11–326.15	0.052–0.063
24	330.13–330.15	0.024–0.035	330.21–330.30	0.030–0.055	330.19–330.25	0.036–0.058
25	334.18–334.22	0.030–0.037	334.27–334.36	0.027–0.072	334.28–334.30	0.046–0.057
26	338.22–338.26	0.025–0.049	338.29–338.41	0.008–0.052	338.30–338.35	0.036–0.053
27	342.29–342.34	0.026–0.038	342.37–342.47	0.011–0.058	342.38–342.42	0.045–0.063
D19S433						
6	118.28–118.35	0.024–0.047	118.50–118.61	0.026–0.068	118.52–118.55	0.042–0.048
7	122.03–122.10	0.024–0.045	122.28–122.39	0.042–0.085	122.33–122.34	0.040–0.052
8	125.82–125.89	0.022–0.050	126.11–126.21	0.025–0.063	126.15–126.16	0.046–0.061
9	129.64–129.68	0.031–0.049	129.94–130.05	0.040–0.067	129.99–130.01	0.051–0.054
10	133.46–133.52	0.033–0.047	133.81–133.93	0.034–0.073	133.86–133.89	0.039–0.058
11	137.33–137.38	0.036–0.048	137.69–137.82	0.044–0.072	137.74–137.78	0.047–0.058
12	141.22–141.27	0.021–0.044	141.63–141.76	0.044–0.068	141.67–141.73	0.042–0.058
12.2	143.21–143.27	0.030–0.051	143.63–143.75	0.042–0.084	143.69–143.74	0.038–0.055
13	145.17–145.22	0.028–0.047	145.60–145.73	0.032–0.068	145.65–145.71	0.044–0.058
13.2	147.16–147.23	0.031–0.04	147.61–147.73	0.037–0.065	147.67–147.71	0.034–0.060

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
14	149.11–149.17	0.036–0.045	149.56–149.69	0.041–0.076	149.64–149.69	0.047–0.062
14.2	151.11–151.18	0.029–0.051	151.58–151.71	0.035–0.074	151.63–151.71	0.046–0.068
15	153.07–153.13	0.029–0.053	153.56–153.67	0.032–0.072	153.61–153.67	0.047–0.056
15.2	155.06–155.15	0.030–0.040	155.57–155.68	0.036–0.075	155.63–155.69	0.050–0.057
16	157.02–157.11	0.032–0.052	157.53–157.66	0.031–0.077	157.60–157.66	0.046–0.063
16.2	159.03–159.12	0.038–0.050	159.57–159.67	0.036–0.071	159.61–159.68	0.030–0.060
17	160.98–161.07	0.038–0.048	161.49–161.61	0.007–0.072	161.56–161.62	0.040–0.070
17.2	162.97–163.04	0.036–0.047	163.50–163.62	0.015–0.073	163.57–163.63	0.051–0.061
18.2	166.99–167.07	0.030–0.045	167.55–167.66	0.024–0.067	167.61–167.68	0.052–0.060
19.2	170.89–170.97	0.028–0.044	171.45–171.56	0.034–0.074	171.52–171.61	0.038–0.059
D1S1656						
9	159.91–159.94	0.018–0.037	159.94–160.03	0.000–0.054	159.98–160.00	0.030–0.052
10	163.93–163.97	0.023–0.036	163.97–164.04	0.016–0.054	163.99–164.02	0.036–0.043
11	167.94–167.97	0.026–0.036	167.97–168.05	0.028–0.059	168.03–168.03	0.031–0.046
12	171.94–171.98	0.025–0.033	172.02–172.07	0.018–0.057	172.03–172.05	0.034–0.048
13	175.95–175.99	0.023–0.033	176.01–176.08	0.012–0.056	176.05–176.07	0.040–0.044
14	180.02–180.06	0.031–0.038	180.11–180.16	0.004–0.059	180.14–180.16	0.046–0.055
14.3	183.09–183.12	0.022–0.041	183.17–183.21	0.032–0.056	183.19–183.22	0.027–0.051
15	184.00–184.04	0.029–0.035	184.10–184.16	0.032–0.052	184.12–184.15	0.035–0.054
15.3	187.15–187.18	0.020–0.034	187.23–187.30	0.024–0.059	187.28–187.30	0.042–0.049
16	188.05–188.08	0.021–0.030	188.16–188.22	0.031–0.068	188.19–188.22	0.027–0.045
16.3	191.19–191.24	0.017–0.035	191.30–191.36	0.023–0.063	191.35–191.37	0.032–0.054
17	192.10–192.14	0.030–0.039	192.22–192.30	0.021–0.063	192.26–192.28	0.038–0.050
17.3	195.23–195.26	0.021–0.038	195.37–195.42	0.011–0.060	195.40–195.42	0.035–0.055
18.3	199.28–199.31	0.020–0.035	199.40–199.47	0.036–0.056	199.44–199.47	0.023–0.049
19.3	203.24–203.27	0.025–0.038	203.37–203.46	0.013–0.057	203.40–203.41	0.035–0.047
20.3	207.19–207.22	0.029–0.034	207.32–207.39	0.022–0.057	207.34–207.38	0.041–0.049

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
D21S11						
24	182.84–182.89	0.020–0.038	183.09–183.14	0.018–0.056	182.98–183.05	0.024–0.049
24.2	184.92–184.98	0.029–0.033	185.17–185.23	0.026–0.055	185.06–185.14	0.039–0.055
25	186.94–187.00	0.027–0.038	187.21–187.26	0.021–0.057	187.11–187.17	0.041–0.054
26	191.04–191.10	0.019–0.033	191.33–191.37	0.024–0.055	191.21–191.29	0.037–0.059
27	195.16–195.20	0.025–0.036	195.45–195.52	0.014–0.058	195.35–195.41	0.033–0.051
28	199.20–199.24	0.029–0.039	199.49–199.57	0.007–0.059	199.39–199.46	0.021–0.056
28.2	201.21–201.26	0.027–0.039	201.48–201.53	0.012–0.058	201.38–201.45	0.042–0.054
29	203.16–203.22	0.022–0.037	203.46–203.55	0.004–0.059	203.34–203.41	0.029–0.044
29.2	205.22–205.27	0.022–0.034	205.49–205.56	0.008–0.067	205.38–205.45	0.039–0.054
30	207.19–207.25	0.022–0.038	207.48–207.55	0.008–0.060	207.36–207.44	0.039–0.047
30.2	209.17–209.23	0.025–0.041	209.45–209.51	0.020–0.060	209.34–209.41	0.038–0.051
31	211.20–211.24	0.021–0.039	211.48–211.54	0.012–0.061	211.37–211.45	0.048–0.051
31.2	213.17–213.23	0.021–0.041	213.45–213.53	0.004–0.054	213.33–213.41	0.051–0.057
32	215.22–215.27	0.020–0.036	215.52–215.57	0.035–0.055	215.40–215.48	0.031–0.044
32.2	217.26–217.31	0.020–0.034	217.54–217.59	0.031–0.052	217.44–217.51	0.026–0.053
33	219.33–219.38	0.025–0.041	219.61–219.71	0.006–0.056	219.54–219.60	0.044–0.060
33.2	221.30–221.34	0.024–0.037	221.60–221.68	0.039–0.061	221.52–221.58	0.028–0.046
34	223.44–223.48	0.027–0.038	223.75–223.81	0.038–0.058	223.65–223.72	0.050–0.059
34.2	225.37–225.41	0.033–0.040	225.68–225.76	0.038–0.072	225.59–225.66	0.048–0.063
35	227.46–227.50	0.032–0.043	227.79–227.86	0.028–0.067	227.68–227.75	0.045–0.058
35.2	229.41–229.44	0.025–0.042	229.74–229.80	0.024–0.058	229.64–229.72	0.043–0.068
36	231.40–231.45	0.037–0.045	231.76–231.83	0.026–0.065	231.66–231.75	0.041–0.054
37	235.50–235.54	0.025–0.044	235.84–235.93	0.019–0.065	235.75–235.84	0.042–0.054
38	239.48–239.53	0.026–0.042	239.83–239.89	0.001–0.059	239.71–239.82	0.047–0.059

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
D22S1045						
8	88.49–88.51	0.024–0.027	88.33–88.40	0.006–0.045	88.29–88.33	0.029–0.038
9	91.47–91.48	0.023–0.032	91.33–91.39	0.006–0.047	91.29–91.32	0.026–0.043
10	94.46–94.47	0.027–0.037	94.31–94.38	0.004–0.051	94.28–94.31	0.034–0.042
11	97.44–97.46	0.027–0.033	97.30–97.37	0.006–0.050	97.28–97.29	0.030–0.040
12	100.44–100.46	0.018–0.031	100.31–100.36	0.032–0.053	100.28–100.29	0.027–0.043
13	103.47–103.49	0.018–0.035	103.36–103.39	0.011–0.070	103.31–103.33	0.028–0.050
14	106.49–106.50	0.022–0.034	106.36–106.43	0.001–0.054	106.32–106.34	0.034–0.041
15	109.48–109.49	0.028–0.039	109.36–109.41	0.014–0.053	109.33–109.34	0.040–0.049
16	112.46–112.48	0.032–0.041	112.34–112.39	0.006–0.065	112.30–112.33	0.044–0.051
17	115.39–115.41	0.023–0.034	115.27–115.34	0.001–0.051	115.24–115.26	0.024–0.037
18	118.28–118.30	0.025–0.028	118.15–118.23	0.001–0.052	118.10–118.13	0.014–0.033
19	121.15–121.17	0.029–0.037	121.06–121.10	0.014–0.051	120.98–121.00	0.029–0.048
D2S1338						
11	280.99–281.04	0.027–0.035	281.61–281.74	0.009–0.060	281.68–281.78	0.049–0.055
12	284.94–284.99	0.031–0.042	285.60–285.67	0.031–0.059	285.60–285.71	0.048–0.052
13	288.88–288.95	0.028–0.036	289.53–289.60	0.026–0.062	289.53–289.66	0.037–0.050
14	292.82–292.88	0.027–0.045	293.46–293.53	0.019–0.060	293.46–293.58	0.046–0.051
15	296.70–296.76	0.033–0.041	297.27–297.34	0.037–0.057	297.29–297.41	0.034–0.060
16	300.63–300.69	0.026–0.042	301.22–301.30	0.009–0.061	301.26–301.37	0.045–0.052
17	304.53–304.58	0.029–0.041	305.12–305.20	0.027–0.064	305.14–305.27	0.038–0.054
18	308.46–308.52	0.024–0.036	309.05–309.14	0.015–0.064	309.11–309.22	0.040–0.061
19	312.46–312.51	0.029–0.042	313.06–313.16	0.008–0.061	313.12–313.25	0.043–0.059
20	316.62–316.67	0.020–0.037	317.21–317.30	0.026–0.065	317.27–317.39	0.047–0.057
21	320.81–320.86	0.025–0.043	321.40–321.49	0.042–0.064	321.45–321.58	0.038–0.063
22	324.89–324.95	0.029–0.045	325.44–325.53	0.016–0.058	325.50–325.63	0.049–0.075
23	328.91–328.99	0.029–0.038	329.48–329.58	0.031–0.066	329.57–329.67	0.050–0.062

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
24	332.95–333.00	0.025–0.034	333.53–333.61	0.018–0.056	333.58–333.69	0.049–0.060
25	336.94–337.00	0.024–0.035	337.51–337.59	0.016–0.065	337.57–337.67	0.050–0.058
26	340.96–341.00	0.026–0.037	341.50–341.57	0.009–0.060	341.57–341.68	0.033–0.061
27	345.05–345.09	0.023–0.036	345.58–345.67	0.023–0.057	345.64–345.74	0.047–0.054
28	349.37–349.42	0.026–0.038	349.79–349.89	0.032–0.062	349.90–349.95	0.043–0.050
D2S441						
8	76.79–76.81	0.019–0.034	76.57–76.62	0.001–0.046	76.55–76.60	0.026–0.047
9	80.93–80.95	0.026–0.034	80.74–80.77	0.000–0.047	80.70–80.75	0.035–0.048
10	85.04–85.08	0.018–0.033	84.87–84.91	0.006–0.052	84.84–84.88	0.029–0.036
11	89.16–89.18	0.023–0.031	89.01–89.05	0.015–0.048	88.97–89.02	0.036–0.043
11.3	92.32–92.35	0.025–0.033	92.19–92.24	0.018–0.049	92.16–92.18	0.032–0.040
12	93.25–93.27	0.020–0.029	93.12–93.17	0.015–0.050	93.09–93.12	0.033–0.041
13	97.18–97.20	0.022–0.031	97.07–97.11	0.005–0.048	97.03–97.06	0.032–0.046
14	101.30–101.32	0.025–0.033	101.18–101.23	0.011–0.053	101.17–101.18	0.011–0.045
15	105.43–105.45	0.028–0.033	105.33–105.38	0.001–0.055	105.32–105.34	0.038–0.047
16	109.55–109.56	0.027–0.037	109.46–109.51	0.030–0.055	109.45–109.46	0.031–0.046
17	113.74–113.76	0.031–0.038	113.67–113.72	0.005–0.052	113.65–113.68	0.048–0.051
D3S1358						
9	96.60–96.64	0.022–0.033	96.49–96.55	0.008–0.047	96.47–96.49	0.039–0.049
10	100.76–100.79	0.026–0.037	100.66–100.71	0.004–0.052	100.63–100.65	0.044–0.051
11	104.95–104.97	0.026–0.037	104.81–104.90	0.016–0.051	104.80–104.83	0.040–0.048
12	108.98–109.00	0.024–0.039	108.83–108.92	0.023–0.046	108.83–108.85	0.040–0.044
13	113.23–113.25	0.032–0.037	113.08–113.17	0.000–0.061	113.07–113.09	0.047–0.052
14	117.21–117.24	0.024–0.041	117.09–117.14	0.015–0.047	117.06–117.07	0.015–0.035
15	121.09–121.12	0.023–0.034	120.96–121.04	0.004–0.053	120.92–120.94	0.031–0.050
16	125.20–125.24	0.030–0.036	125.09–125.15	0.024–0.061	125.05–125.06	0.033–0.047
17	129.33–129.37	0.023–0.038	129.22–129.29	0.013–0.063	129.18–129.20	0.037–0.047

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
18	133.36–133.40	0.020–0.035	133.26–133.32	0.018–0.055	133.22–133.23	0.044–0.052
19	137.35–137.39	0.026–0.036	137.24–137.31	0.014–0.058	137.21–137.21	0.041–0.048
20	141.66–141.71	0.027–0.036	141.56–141.62	0.005–0.054	141.52–141.54	0.038–0.046
D5S818						
7	138.53–138.55	0.025–0.039	138.60–138.68	0.004–0.050	138.58–138.60	0.038–0.056
8	142.59–142.60	0.027–0.034	142.66–142.71	0.034–0.053	142.63–142.66	0.040–0.051
9	146.68–146.71	0.013–0.034	146.75–146.82	0.017–0.048	146.74–146.75	0.036–0.048
10	150.82–150.83	0.022–0.032	150.90–150.97	0.020–0.054	150.89–150.90	0.030–0.042
11	154.91–154.93	0.029–0.039	155.00–155.06	0.015–0.057	154.98–155.02	0.041–0.052
12	159.00–159.03	0.025–0.038	159.10–159.17	0.038–0.056	159.09–159.12	0.037–0.053
13	163.04–163.09	0.019–0.032	163.16–163.21	0.015–0.051	163.13–163.17	0.037–0.053
14	167.07–167.10	0.026–0.034	167.16–167.24	0.030–0.055	167.17–167.20	0.040–0.044
15	171.08–171.12	0.021–0.032	171.20–171.26	0.025–0.051	171.20–171.22	0.034–0.056
16	175.09–175.13	0.029–0.032	175.22–175.27	0.038–0.062	175.22–175.25	0.038–0.055
17	179.10–179.14	0.021–0.039	179.25–179.31	0.005–0.052	179.25–179.27	0.005–0.050
18	183.16–183.20	0.021–0.035	183.32–183.39	0.026–0.062	183.30–183.37	0.027–0.049
D7S820						
6	262.40–262.42	0.025–0.040	262.54–262.60	0.034–0.057	262.55–262.60	0.035–0.051
7	266.41–266.43	0.027–0.044	266.56–266.62	0.020–0.058	266.55–266.61	0.043–0.052
8	270.41–270.43	0.025–0.033	270.57–270.63	0.027–0.068	270.58–270.62	0.037–0.053
9	274.43–274.45	0.029–0.034	274.59–274.64	0.027–0.051	274.62–274.64	0.035–0.050
10	278.44–278.46	0.026–0.032	278.61–278.69	0.007–0.060	278.63–278.66	0.038–0.059
11	282.43–282.45	0.023–0.032	282.62–282.67	0.011–0.066	282.60–282.64	0.035–0.048
12	286.40–286.41	0.028–0.036	286.58–286.64	0.033–0.057	286.57–286.60	0.042–0.054
13	290.35–290.38	0.026–0.032	290.54–290.61	0.025–0.057	290.54–290.58	0.036–0.045
14	294.30–294.33	0.027–0.037	294.49–294.57	0.015–0.062	294.51–294.54	0.033–0.054
15	298.25–298.26	0.026–0.051	298.46–298.54	0.006–0.060	298.46–298.49	0.042–0.052

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
D8S1179						
5	114.45–114.46	0.029–0.036	114.21–114.27	0.032–0.052	114.15–114.18	0.031–0.046
6	118.42–118.43	0.024–0.035	118.20–118.25	0.000–0.054	118.12–118.16	0.029–0.044
7	122.38–122.40	0.022–0.036	122.17–122.21	0.034–0.057	122.09–122.12	0.035–0.050
8	126.39–126.41	0.024–0.031	126.18–126.23	0.008–0.052	126.08–126.12	0.035–0.049
9	130.41–130.43	0.030–0.038	130.21–130.25	0.018–0.062	130.11–130.13	0.039–0.047
10	134.45–134.49	0.024–0.035	134.25–134.30	0.015–0.049	134.17–134.19	0.037–0.054
11	138.52–138.57	0.028–0.032	138.32–138.36	0.004–0.054	138.24–138.27	0.037–0.050
12	142.66–142.70	0.027–0.040	142.45–142.50	0.012–0.053	142.37–142.39	0.032–0.040
13	146.89–146.93	0.017–0.039	146.71–146.74	0.023–0.054	146.61–146.64	0.034–0.042
14	151.05–151.08	0.025–0.035	150.85–150.89	0.025–0.053	150.76–150.78	0.034–0.040
15	155.21–155.24	0.025–0.038	155.00–155.04	0.015–0.057	154.90–154.93	0.036–0.046
16	159.37–159.40	0.028–0.035	159.16–159.19	0.004–0.057	159.07–159.08	0.032–0.046
17	163.46–163.49	0.020–0.035	163.25–163.28	0.031–0.051	163.16–163.19	0.030–0.048
18	167.54–167.57	0.022–0.037	167.32–167.36	0.031–0.048	167.23–167.26	0.037–0.050
19	171.62–171.64	0.025–0.034	171.39–171.42	0.024–0.063	171.30–171.33	0.036–0.051
DYS391						
7	364.73–364.78	0.028–0.044	365.17–365.27	0.028–0.062	365.11–365.19	0.045–0.066
8	368.80–368.85	0.027–0.045	369.25–369.35	0.025–0.060	369.18–369.28	0.043–0.061
9	372.81–372.85	0.027–0.036	373.25–373.34	0.022–0.064	373.18–373.28	0.046–0.057
10	376.80–376.86	0.029–0.039	377.24–377.34	0.011–0.067	377.17–377.27	0.044–0.063
11	380.85–380.89	0.030–0.042	381.26–381.34	0.005–0.057	381.21–381.30	0.044–0.054
12	384.90–384.95	0.030–0.038	385.24–385.35	0.024–0.064	385.21–385.29	0.049–0.059
13	389.00–389.04	0.023–0.046	389.33–389.43	0.027–0.065	389.30–389.37	0.046–0.056

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
FGA						
13	223.48–223.53	0.028–0.038	223.35–223.42	0.038–0.080	223.43–223.50	0.036–0.060
14	227.48–227.55	0.037–0.043	227.38–227.46	0.029–0.079	227.47–227.53	0.045–0.061
15	231.52–231.59	0.024–0.041	231.40–231.48	0.034–0.073	231.50–231.56	0.050–0.065
16	235.55–235.62	0.031–0.039	235.43–235.49	0.024–0.077	235.53–235.59	0.047–0.062
17	239.58–239.64	0.017–0.036	239.45–239.52	0.004–0.071	239.55–239.60	0.025–0.052
18	243.70–243.75	0.027–0.035	243.55–243.64	0.039–0.087	243.65–243.72	0.042–0.051
19	247.82–247.87	0.022–0.036	247.63–247.71	0.011–0.065	247.76–247.81	0.053–0.056
20	251.86–251.89	0.023–0.034	251.68–251.75	0.004–0.070	251.78–251.83	0.024–0.046
21	255.81–255.84	0.027–0.039	255.61–255.70	0.006–0.070	255.74–255.79	0.032–0.047
22	259.73–259.77	0.031–0.039	259.55–259.61	0.000–0.072	259.66–259.71	0.024–0.056
23	263.74–263.78	0.028–0.041	263.55–263.62	0.031–0.078	263.68–263.72	0.050–0.056
24	267.74–267.79	0.022–0.029	267.55–267.62	0.025–0.067	267.65–267.73	0.038–0.055
25	271.79–271.84	0.031–0.038	271.58–271.66	0.024–0.067	271.72–271.77	0.041–0.053
26	275.80–275.87	0.024–0.032	275.61–275.67	0.027–0.063	275.73–275.80	0.044–0.054
26.2	277.84–277.88	0.026–0.035	277.62–277.70	0.009–0.064	277.76–277.84	0.044–0.059
27	279.82–279.85	0.028–0.038	279.59–279.66	0.040–0.059	279.70–279.78	0.025–0.060
28	283.81–283.84	0.029–0.038	283.59–283.65	0.008–0.064	283.68–283.74	0.042–0.053
29	287.79–287.84	0.030–0.048	287.57–287.63	0.027–0.048	287.67–287.75	0.040–0.055
30	291.80–291.85	0.034–0.046	291.57–291.63	0.029–0.065	291.69–291.75	0.044–0.051
30.2	293.58–293.63	0.027–0.038	293.35–293.42	0.019–0.057	293.46–293.53	0.043–0.050
31.2	297.55–297.61	0.034–0.037	297.31–297.37	0.008–0.055	297.42–297.50	0.044–0.053
32.2	301.49–301.54	0.032–0.044	301.24–301.33	0.006–0.066	301.38–301.44	0.031–0.057
33.2	305.42–305.46	0.028–0.040	305.17–305.25	0.028–0.064	305.31–305.37	0.036–0.056
42.2	342.26–342.29	0.028–0.049	341.96–342.00	0.008–0.060	342.11–342.18	0.036–0.052
43.2	346.34–346.37	0.033–0.042	346.01–346.09	0.022–0.071	346.17–346.27	0.036–0.060
44.2	350.42–350.45	0.025–0.047	350.10–350.16	0.027–0.077	350.24–350.34	0.042–0.047

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
45.2	354.49–354.55	0.022–0.041	354.18–354.24	0.028–0.077	354.34–354.42	0.050–0.062
46.2	358.43–358.46	0.027–0.053	358.12–358.18	0.008–0.064	358.27–358.37	0.028–0.060
47.2	362.45–362.49	0.030–0.043	362.13–362.19	0.046–0.066	362.29–362.37	0.051–0.058
48.2	366.52–366.54	0.027–0.041	366.21–366.24	0.017–0.065	366.37–366.45	0.044–0.060
50.2	374.50–374.53	0.026–0.038	374.16–374.21	0.029–0.074	374.32–374.41	0.037–0.062
51.2	378.51–378.57	0.027–0.040	378.13–378.21	0.042–0.065	378.32–378.41	0.047–0.066
SE33						
4.2	307.08–307.10	0.024–0.037	307.12–307.19	0.031–0.080	307.19–307.22	0.038–0.053
6.3	316.31–316.34	0.028–0.036	316.31–316.39	0.018–0.057	316.40–316.43	0.036–0.051
8	321.56–321.58	0.024–0.037	321.54–321.62	0.006–0.065	321.65–321.67	0.041–0.067
9	325.67–325.70	0.024–0.038	325.63–325.72	0.017–0.063	325.76–325.77	0.047–0.062
11	333.77–333.80	0.029–0.039	333.74–333.83	0.032–0.053	333.86–333.88	0.041–0.049
12	337.85–337.87	0.030–0.040	337.83–337.88	0.016–0.060	337.93–337.94	0.048–0.054
13	341.90–341.93	0.029–0.038	341.85–341.93	0.011–0.062	341.98–342.00	0.025–0.052
14	346.01–346.03	0.032–0.048	345.93–346.02	0.026–0.055	346.07–346.09	0.044–0.053
15	350.07–350.09	0.031–0.041	349.99–350.10	0.022–0.055	350.11–350.14	0.037–0.051
16	354.17–354.20	0.030–0.043	354.11–354.20	0.026–0.060	354.22–354.25	0.043–0.060
17	358.26–358.29	0.031–0.038	358.20–358.29	0.008–0.060	358.34–358.36	0.055–0.065
18	362.32–362.33	0.026–0.042	362.26–362.35	0.010–0.065	362.41–362.43	0.024–0.059
19	366.28–366.29	0.026–0.041	366.20–366.31	0.030–0.064	366.34–366.36	0.042–0.052
20	370.32–370.34	0.026–0.036	370.24–370.33	0.031–0.065	370.38–370.40	0.036–0.050
20.2	372.32–372.33	0.028–0.041	372.23–372.31	0.026–0.054	372.37–372.39	0.048–0.059
21	374.33–374.34	0.027–0.036	374.25–374.33	0.026–0.065	374.39–374.40	0.041–0.049
21.2	376.34–376.35	0.025–0.035	376.23–376.32	0.033–0.064	376.39–376.41	0.047–0.051
22.2	370.32–370.34	0.026–0.036	380.22–380.30	0.040–0.065	370.38–370.40	0.036–0.050
23.2	372.32–372.33	0.028–0.041	384.30–384.41	0.034–0.074	372.37–372.39	0.048–0.059
24.2	374.33–374.34	0.027–0.036	388.36–388.45	0.023–0.059	374.39–374.40	0.041–0.049

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
25.2	376.34–376.35	0.025–0.035	392.34–392.42	0.033–0.062	376.39–376.41	0.047–0.051
26.2	380.33–380.36	0.028–0.044	396.47–396.54	0.010–0.072	380.38–380.40	0.003–0.055
27.2	384.45–384.47	0.024–0.039	400.48–400.57	0.041–0.065	384.48–384.52	0.050–0.056
28.2	388.50–388.51	0.032–0.043	404.41–404.50	0.021–0.067	388.52–388.57	0.042–0.059
29.2	392.49–392.52	0.030–0.046	408.37–408.49	0.037–0.076	392.51–392.54	0.046–0.056
30.2	396.61–396.65	0.035–0.043	412.39–412.49	0.010–0.071	396.62–396.66	0.030–0.055
31.2	400.64–400.67	0.036–0.050	416.40–416.49	0.026–0.058	400.65–400.70	0.047–0.054
32.2	404.58–404.60	0.037–0.046	420.56–420.65	0.006–0.063	404.58–404.61	0.050–0.061
33.2	408.56–408.60	0.033–0.050	424.57–424.68	0.027–0.077	408.55–408.60	0.049–0.056
34.2	412.58–412.60	0.024–0.049	428.62–428.72	0.033–0.063	412.57–412.58	0.043–0.057
35	416.59–416.60	0.019–0.042	430.63–430.72	0.033–0.074	416.57–416.60	0.048–0.057
35.2	420.74–420.77	0.037–0.051	432.62–432.72	0.038–0.068	420.71–420.74	0.042–0.066
36	424.76–424.81	0.030–0.055	434.62–434.74	0.031–0.072	424.73–424.78	0.048–0.066
37	428.79–428.82	0.030–0.050	438.57–438.71	0.039–0.070	428.75–428.79	0.054–0.059
TH01						
4	178.72–178.81	0.022–0.042	179.17–179.24	0.001–0.080	179.17–179.24	0.046–0.059
5	182.76–182.83	0.030–0.047	183.21–183.29	0.034–0.070	183.21–183.28	0.045–0.054
6	186.80–186.87	0.031–0.042	187.28–187.36	0.034–0.068	187.29–187.36	0.043–0.054
7	190.85–190.92	0.023–0.042	191.34–191.43	0.025–0.064	191.35–191.42	0.045–0.052
8	194.88–194.96	0.026–0.040	195.39–195.49	0.031–0.070	195.39–195.46	0.042–0.047
9	198.91–198.99	0.029–0.043	199.44–199.53	0.000–0.080	199.43–199.50	0.050–0.057
9.3	201.95–202.04	0.025–0.041	202.46–202.55	0.010–0.067	202.47–202.53	0.047–0.053
10	202.86–202.94	0.027–0.044	203.38–203.48	0.013–0.065	203.36–203.44	0.039–0.053
11	206.83–206.90	0.031–0.041	207.31–207.45	0.026–0.071	207.32–207.40	0.047–0.057
13.3	217.84–217.91	0.029–0.042	218.35–218.43	0.017–0.088	218.37–218.44	0.041–0.052

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
TPOX						
5	337.43–337.50	0.024–0.046	338.35–338.51	0.008–0.061	338.30–338.51	0.049–0.060
6	341.43–341.54	0.030–0.052	342.41–342.59	0.012–0.065	342.35–342.56	0.055–0.065
7	345.60–345.67	0.030–0.050	346.57–346.71	0.021–0.053	346.49–346.69	0.050–0.059
8	349.60–349.66	0.035–0.042	350.55–350.76	0.021–0.061	350.51–350.72	0.040–0.055
9	353.66–353.71	0.026–0.048	354.66–354.79	0.028–0.078	354.58–354.79	0.046–0.064
10	357.69–357.76	0.035–0.051	358.71–358.85	0.006–0.061	358.64–358.84	0.051–0.068
11	361.72–361.77	0.031–0.045	362.73–362.88	0.011–0.066	362.66–362.89	0.047–0.075
12	365.71–365.78	0.035–0.056	366.73–366.89	0.028–0.075	366.68–366.89	0.055–0.075
13	369.72–369.79	0.035–0.046	370.76–370.92	0.030–0.062	370.70–370.91	0.054–0.066
14	373.71–373.79	0.035–0.052	374.77–374.93	0.022–0.071	374.70–374.91	0.055–0.077
15	377.72–377.79	0.037–0.050	378.78–378.94	0.005–0.065	378.70–378.92	0.059–0.067
Y indel						
1	81.26–81.31	0.022–0.035	81.08–81.17	0.015–0.049	81.05–81.10	0.033–0.045
2	86.53–86.58	0.028–0.034	86.38–86.43	0.011–0.060	86.34–86.35	0.028–0.046
vWA						
11	156.50–156.52	0.023–0.033	156.60–156.65	0.015–0.048	156.55–156.59	0.040–0.048
12	160.56–160.59	0.019–0.036	160.67–160.71	0.005–0.053	160.64–160.66	0.038–0.055
13	164.61–164.65	0.021–0.035	164.73–164.77	0.011–0.047	164.68–164.72	0.037–0.052
14	168.81–168.86	0.023–0.032	168.95–169.00	0.029–0.052	168.93–168.97	0.040–0.047
15	172.75–172.77	0.026–0.033	172.86–172.91	0.017–0.055	172.84–172.87	0.037–0.040
16	176.76–176.80	0.021–0.034	176.91–176.95	0.008–0.049	176.87–176.90	0.037–0.049
17	180.81–180.83	0.019–0.033	180.95–180.99	0.004–0.056	180.90–180.96	0.045–0.053
18	184.84–184.89	0.025–0.037	185.02–185.06	0.010–0.050	184.97–185.01	0.038–0.053
19	188.93–188.95	0.024–0.034	189.10–189.16	0.027–0.054	189.08–189.12	0.043–0.052
20	193.00–193.02	0.028–0.039	193.19–193.24	0.023–0.059	193.16–193.20	0.036–0.057
21	197.02–197.05	0.029–0.037	197.23–197.28	0.008–0.060	197.19–197.24	0.046–0.052

Allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
22	201.03–201.06	0.026–0.035	201.25–201.31	0.035–0.056	201.21–201.26	0.044–0.054
23	204.91–204.95	0.024–0.036	205.12–205.19	0.015–0.047	205.08–205.13	0.041–0.048
24	209.23–209.26	0.024–0.040	209.43–209.50	0.028–0.063	209.40–209.44	0.041–0.053

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 3500xL 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.

The stutter observed in the population study is shown in Figure 25 through Figure 31.

Additional marker-specific plus stutter observed in the population study with the GlobalFiler™ kit is listed in Table 7. Examples of non-standard stutter peaks at two loci are shown in "Example of non-standard stutter peaks observed at the D22S1045, D1S1656, and SE33 loci" on page 88.

Note: To detect the plus stutter, we lowered the threshold slightly below the typical 175-RFU threshold. Markers that showed negligible plus stutter are omitted.

The stutter filter settings derived from these data are listed in "Stutter filter settings provided with the GeneMapper™ ID-X Software" on page 90.

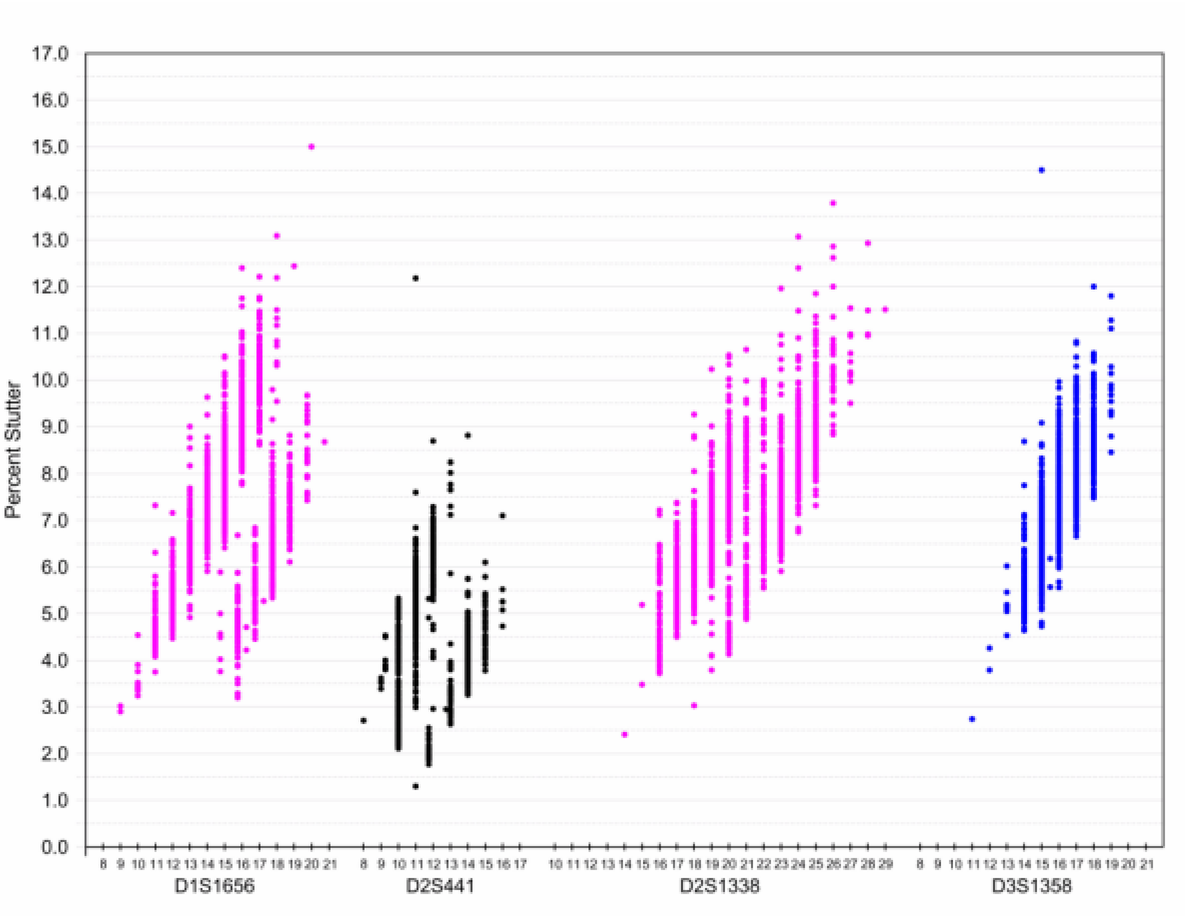


Figure 25 Stutter percentages for D1S1656, D2S441, D2S1338, and D3S1358 loci (Blue=FAM™ dye, black=NED™ dye, purple=SID™ dye)

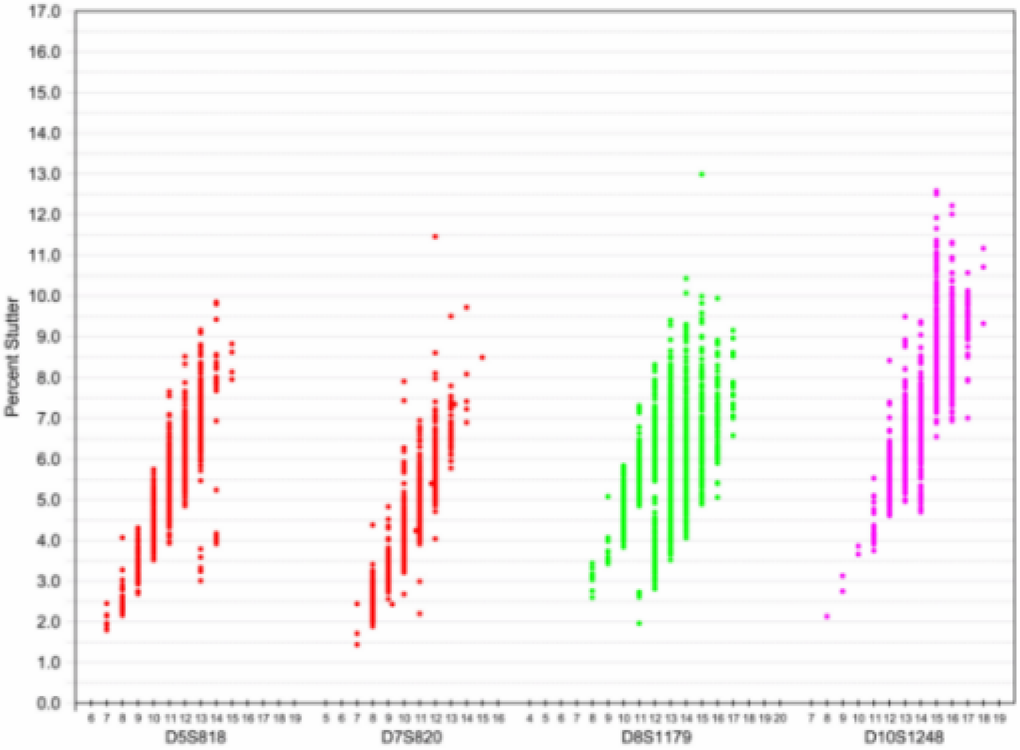


Figure 26 Stutter percentages for D5S818, D7S820, D8S1179, and D10S1248 (Green=VIC™ dye, red=TAZ™ dye, purple=SID™ dye)

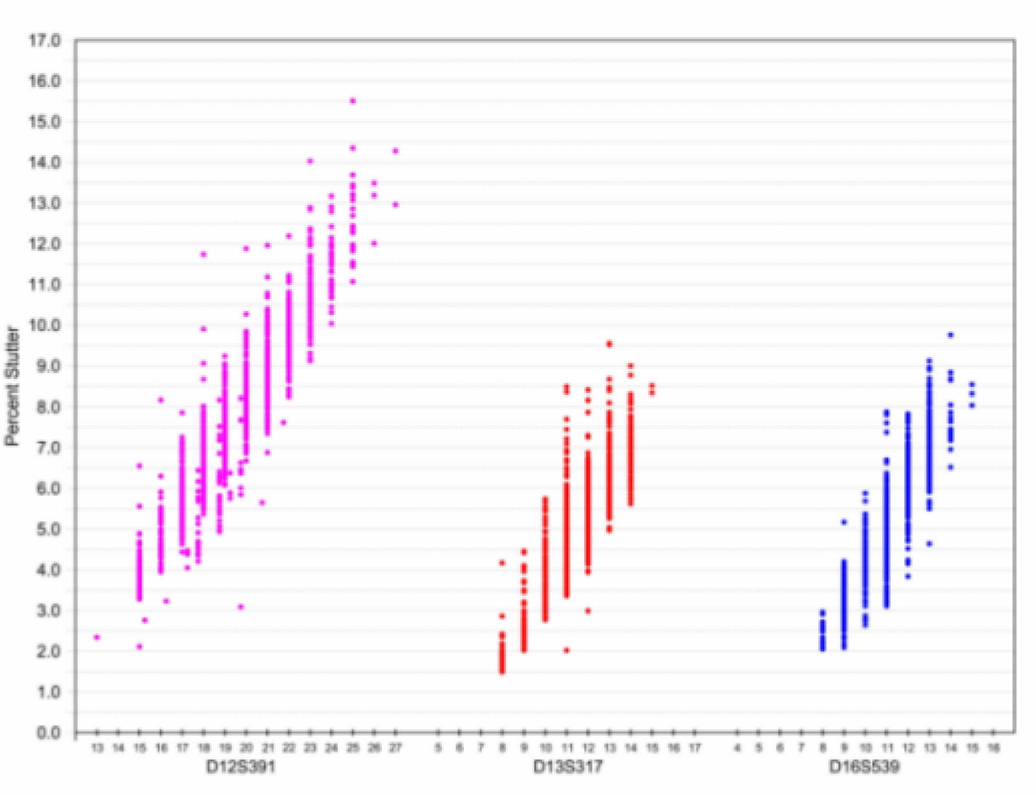


Figure 27 Stutter percentages for D12S391, D13S317, and D16S539 loci (Blue=FAM™ dye, red=TAZ™ dye, purple=SID™ dye)

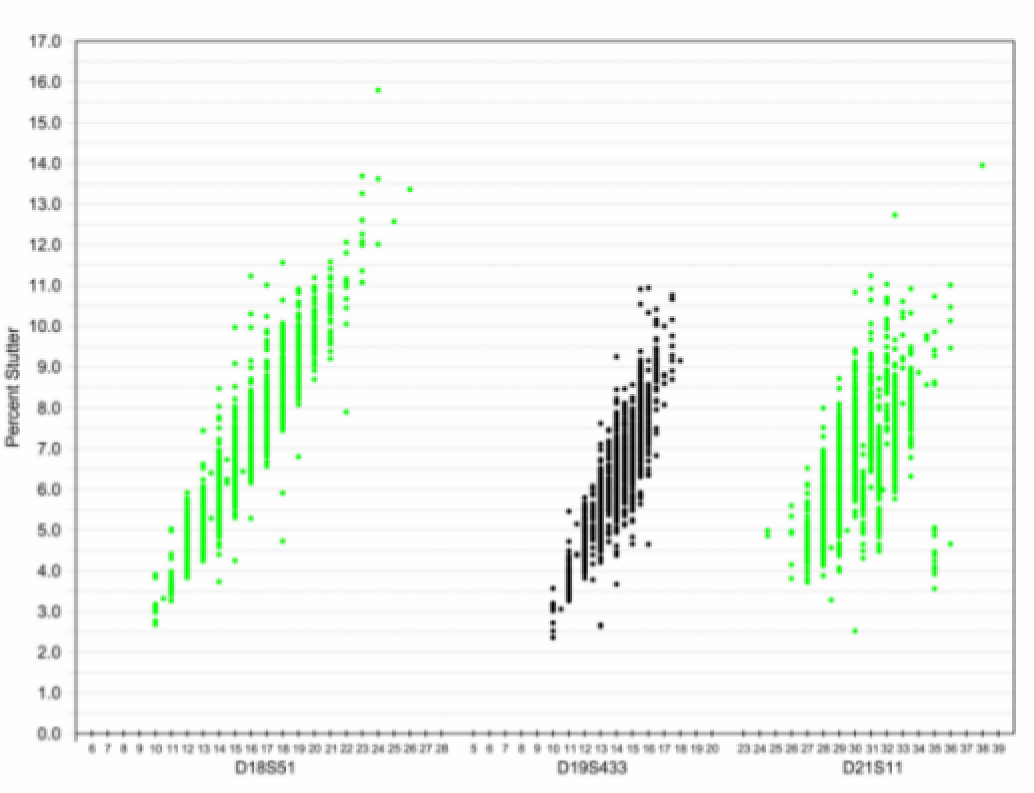


Figure 28 **Stutter percentages for D18S51, D19S433, and D21S11 loci (Green=VIC™ dye, black=NED™ dye)**

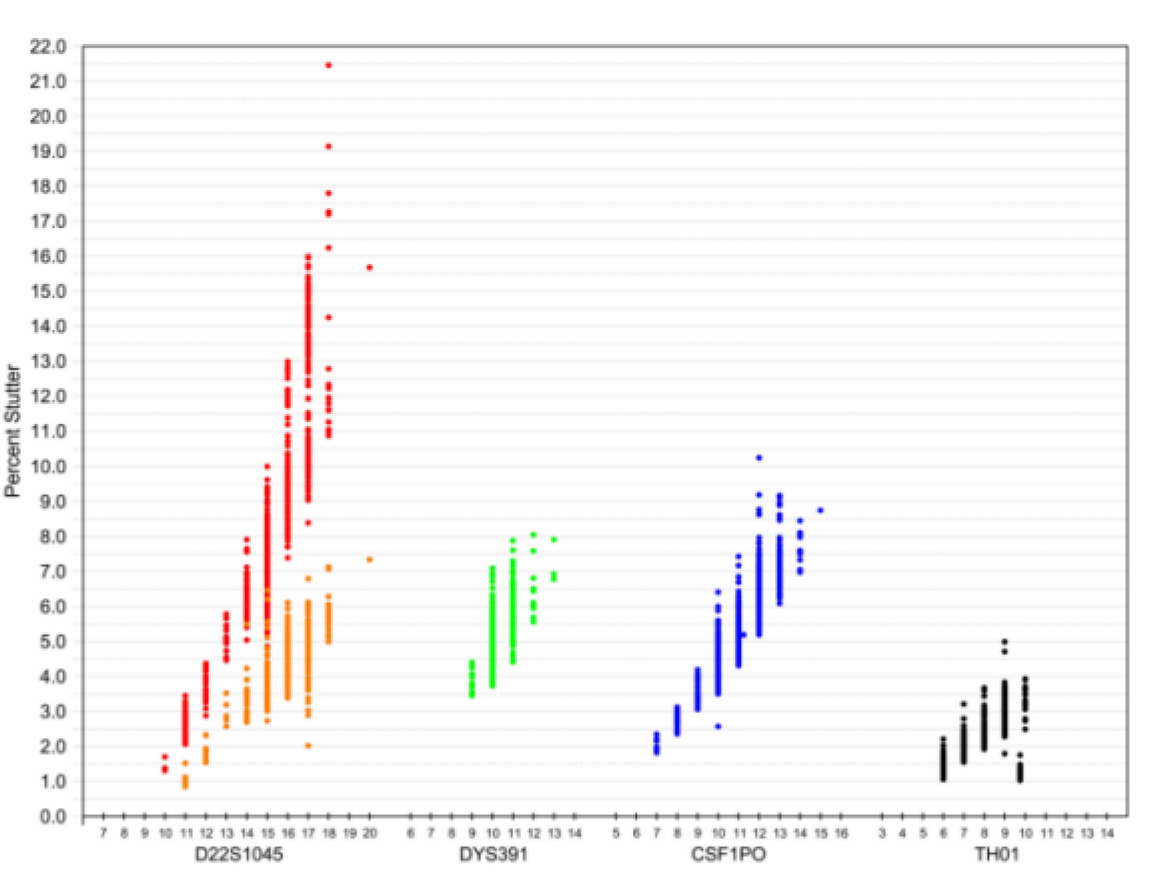


Figure 29 Stutter percentages for D22S1045, DYS391, CSF1PO, and TH01 loci (Blue=FAM™ dye, green=VIC™ dye, black=NED™ dye, red=TAZ™ dye). Red and orange data points associated with D22S1045 locus indicate minus and plus stutter, respectively.

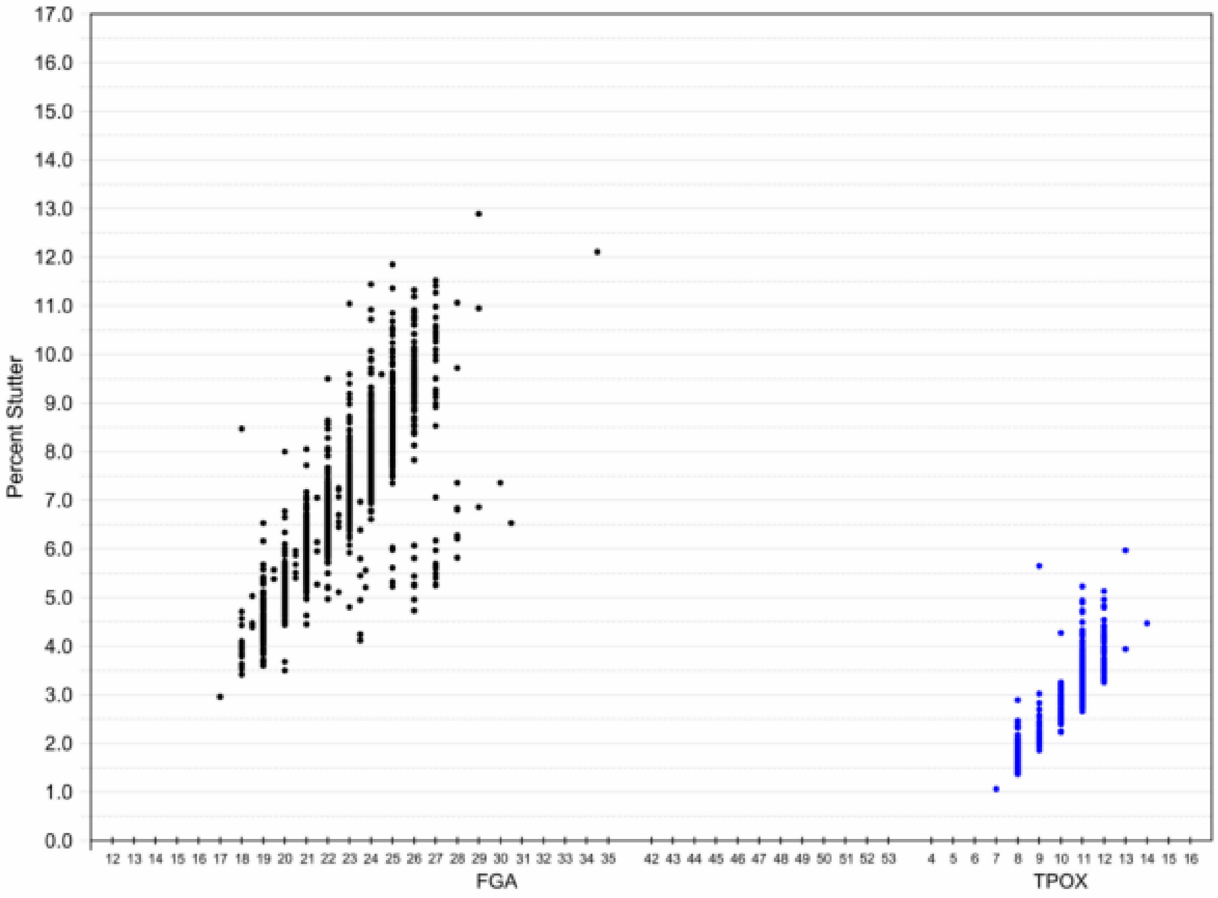


Figure 30 Stutter percentages for FGA and TPOX loci (Blue=FAM™ dye, black=NED™ dye)

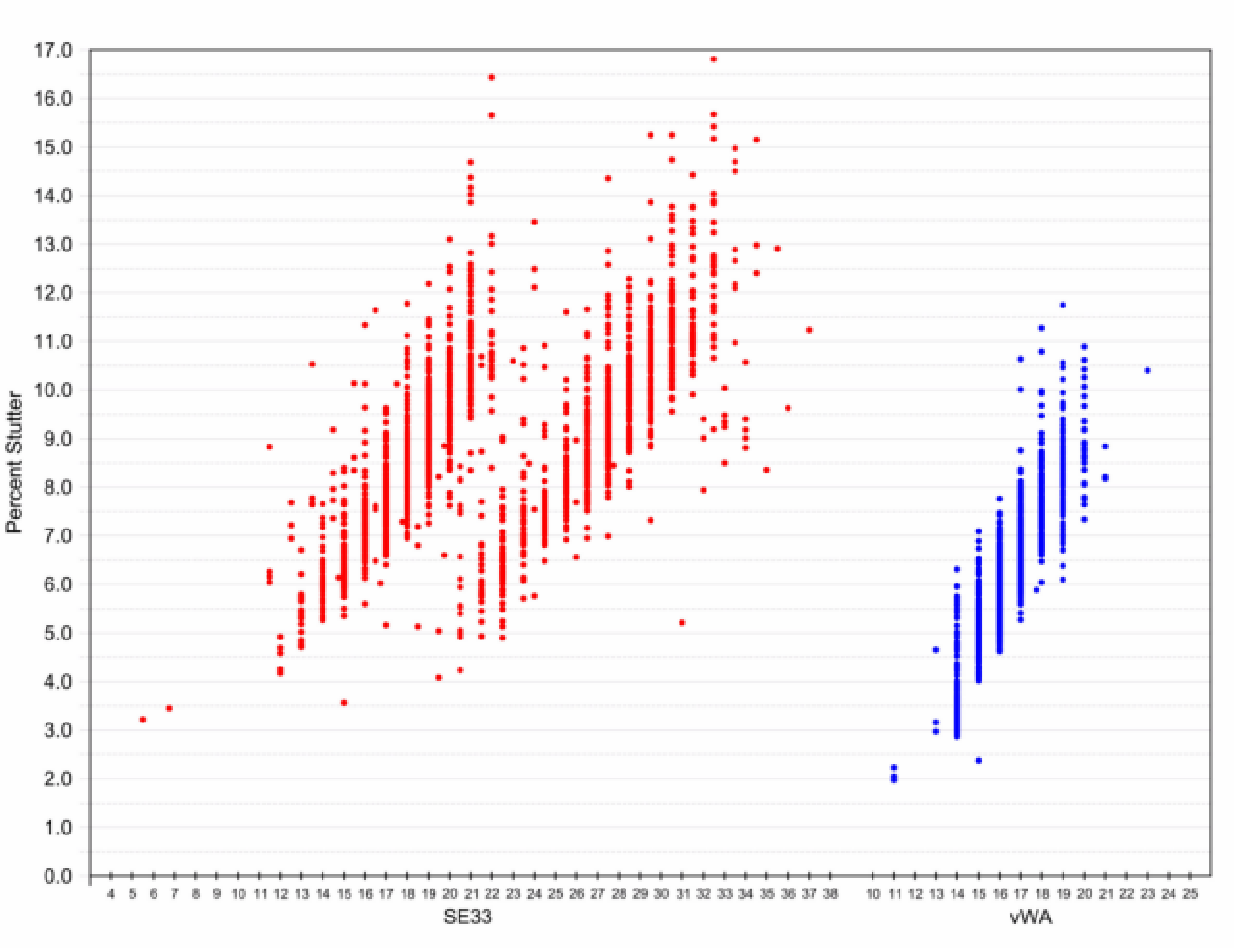


Figure 31 Stutter percentages for SE33 and vWA loci (Blue=FAM™ dye, red=TAZ™ dye)

Table 7 Marker-specific plus stutter observed in the population study with the GlobalFiler™ kit. Data produced on a 3500xL Genetic Analyzer using a threshold cutoff of 150 RFU. Markers that showed negligible plus stutter are omitted.

Locus	Observation	Mean	Standard deviation	Minimum	Maximum[1]
CSF1PO	19	1.533	0.496	0.85	2.64
D10S1248	40	2.076	1.104	0.56	6.77
D12S391	15	2.582	1.162	0.73	5.21
D13S317	28	1.803	1.232	0.57	7.06
D16S539	12	1.77	1.143	0.64	3.75
D18S51	27	3.106	2.25	0.62	9.8
D19S433	8	2.574	1.181	1.11	4.85
D1S1656	60	1.583	1.072	0.62	8.07
D21S11	28	1.655	1.064	0.64	4.38

Table 7 Marker-specific plus stutter observed in the population study with the GlobalFiler kit. Data produced on a 3500xL Genetic Analyzer using a threshold cutoff of 150 RFU. Markers that showed negligible plus stutter are omitted. *(continued)*

Locus	Observation	Mean	Standard deviation	Minimum	Maximum[1]
D22S1045 (N + 3)	1002	4.258	0.8103	0.85	7.34
D2S1338	14	3.516	2.06	1.13	8.34
D2S441	24	2.313	3.126	0.54	15.31
D3S1358	8	1.798	1.137	0.56	4.07
D5S818	48	1.493	0.817	0.73	5.88
D8S1179	46	1.6	0.778	0.6	4.36
DYS391	10	2.556	1.69	0.95	6.32
FGA	24	2.569	2.265	0.81	11.37
SE33 (N + 4)	76	2.48	1.164	0.85	6.13
vWA	8	1.495	1.426	0.65	4.97

[1] The maximum value represents outliers. 95% of data was obtained was within the mean ±2 standard deviations.

Example of non-standard stutter peaks observed at the D22S1045, D1S1656, and SE33 loci

STR loci such as D22S1045 (Figure 32), D1S1656, and SE33 (Figure 33) include more complex nucleotide sequences including regions of tri- and dinucleotide repeats that can yield additional stutter peaks. If these stutter peaks exceed the peak amplitude threshold (typically 175 RFU), they may be detected as additional alleles in the profile. The stutter file that is provided with the GeneMapper™ *ID-X* Software for analysis of GlobalFiler™ kit data contains stutter filters for these loci to prevent these peaks from being called in normal profiles (see Table 8).

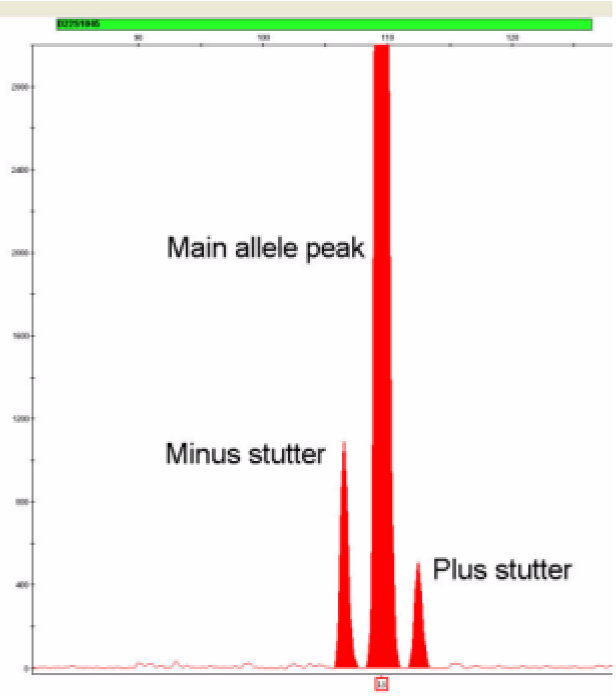


Figure 32 **GlobalFiler™ kit electropherogram showing plus stutter associated with the D22S1045 STR locus. Data produced on a 3500xL Genetic Analyzer.**

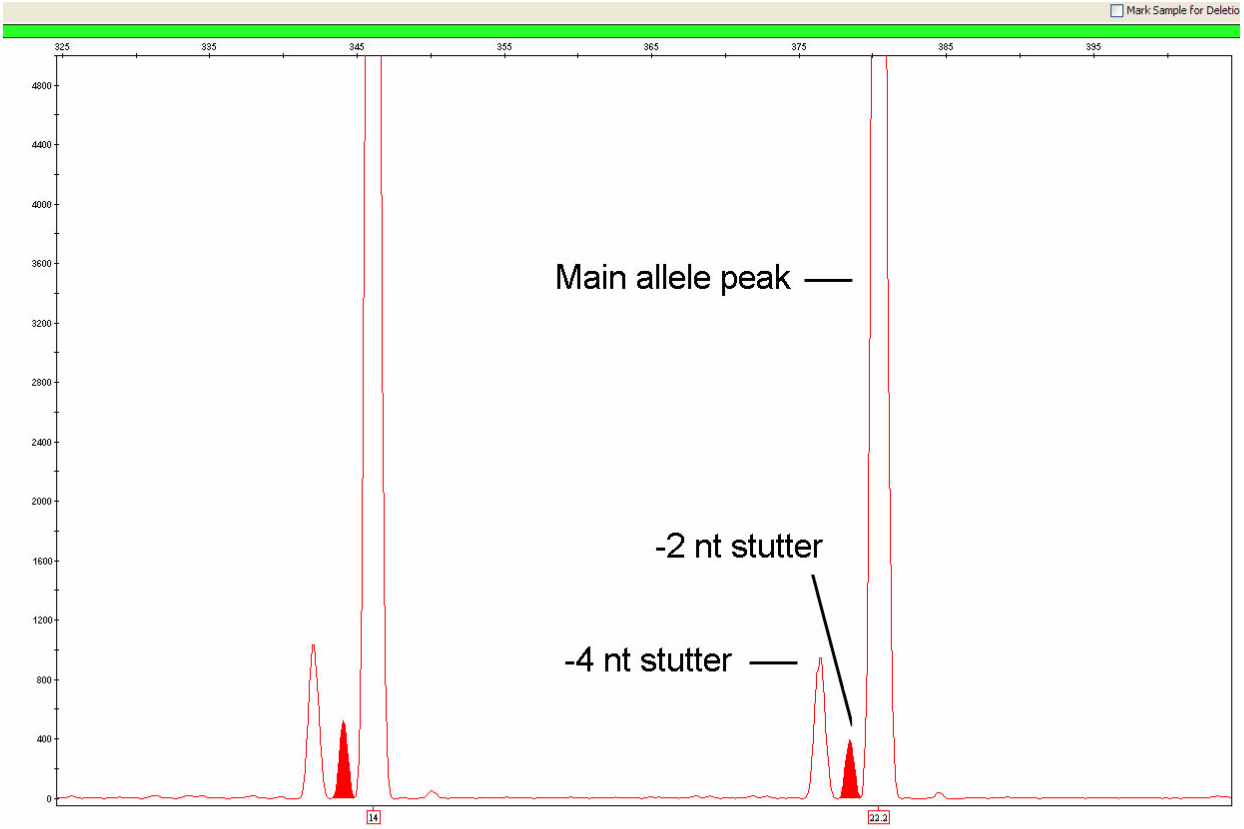


Figure 33 **Example of a –2 bp reproducible artifact at the SE33 locus. Data produced on a 3500xL Genetic Analyzer.**

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

The data that are shown in “Stutter observation” on page 80 were used to derive the settings in Table 8. 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
CSF1PO	8.77
D10S1248	11.46
D12S391	13.66
D13S317	9.19
D16S539	9.48
D18S51	12.42
D19S433	9.97
D1S1656	12.21
D1S1656 (–2 bp)	2.45
D21S11	10.45
D22S1045	16.26
D22S1045 (+3 bp)	6.69
D2S1338	11.73
D2S441	8.10
D3S1358	10.98
D5S818	9.16
D7S820	8.32
D8S1179	9.60
DYS391	7.43
FGA	11.55
SE33	14.49

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

Locus	% Stutter
SE33 (–2 bp)	3.97
TH01	4.45
TPOX	5.55
vWA	10.73

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 10 minutes at 60°C.

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products. Examples of incomplete and normal +A addition are shown in Figure 34. Final extension incubation for longer than the recommended time can result in double +A addition, in which two nontemplate adenosine residues are added to the PCR product. Double +A addition can cause "shoulders" on the right side of main allele peaks, and is therefore to be avoided.

Due to improved PCR buffer chemistry, the lack of +A addition is generally less of an issue with the GlobalFiler™ kit than with earlier generation kits. However, "shouldering" of allele peaks can still be observed if the amount of input DNA is greater than the recommended concentration. Amplification of excess input DNA can also result in off-scale data.

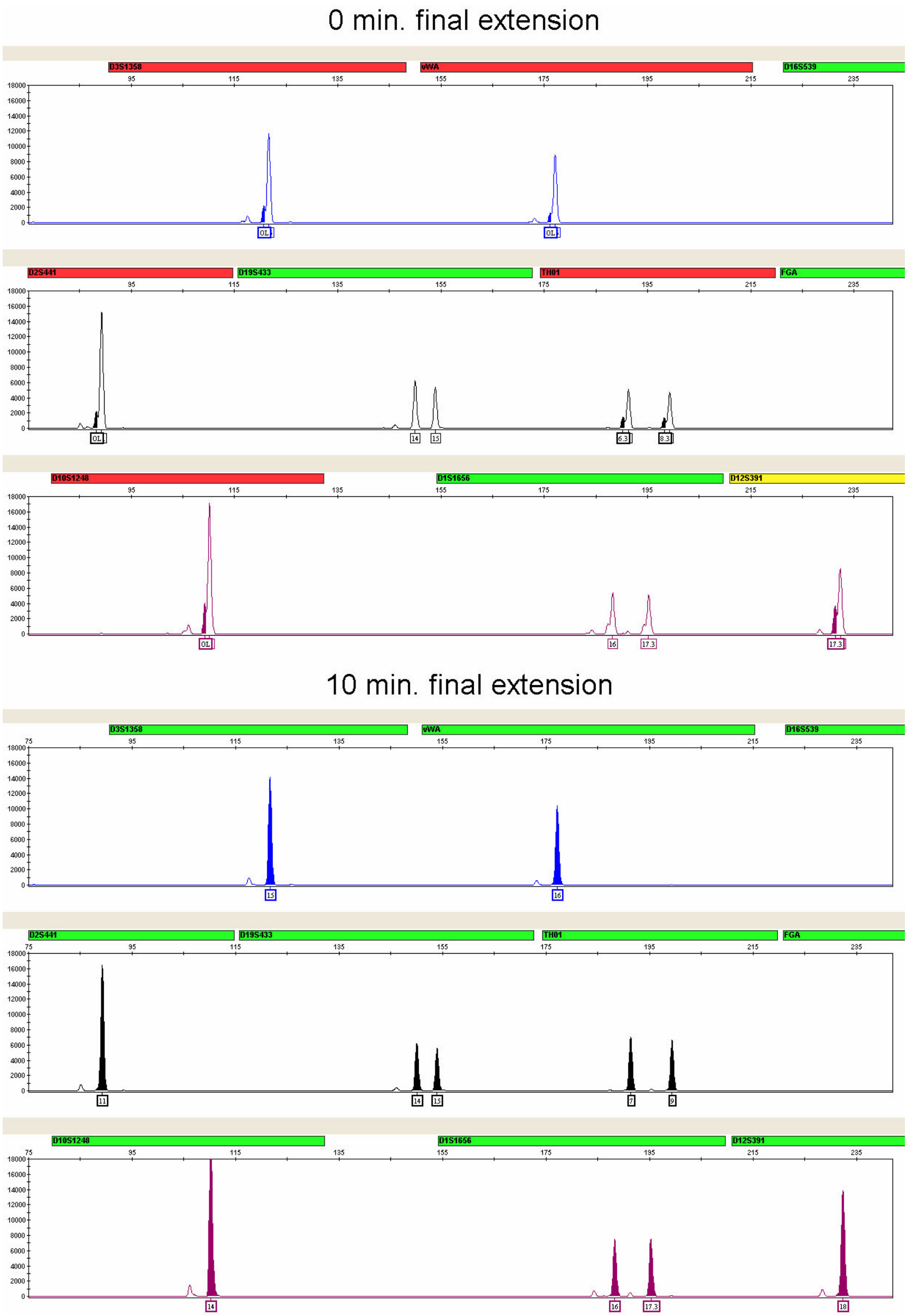


Figure 34 Omitting the final extension step results in shoulders on main allele peaks due to incomplete A nucleotide addition. Examples shown are the smaller amplicons of FAM™, NED™, and SID™ dye channel data from a 3500xL Genetic Analyzer using the GlobalFiler™ kit.

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).

Dye artifact observation

Due to improvements in PCR primer manufacturing processes, the incidence of artifacts has been greatly reduced in the GlobalFiler™ kit. Kit electropherograms are essentially free of reproducible dye artifacts within the kit read region of 74–444 bp for commonly used analytical thresholds. The low baseline-level fluorescence that is observed in a typical negative control PCR is shown in Figure 35.

However, it is important to consider noise and other amplification-related artifacts when interpreting data.

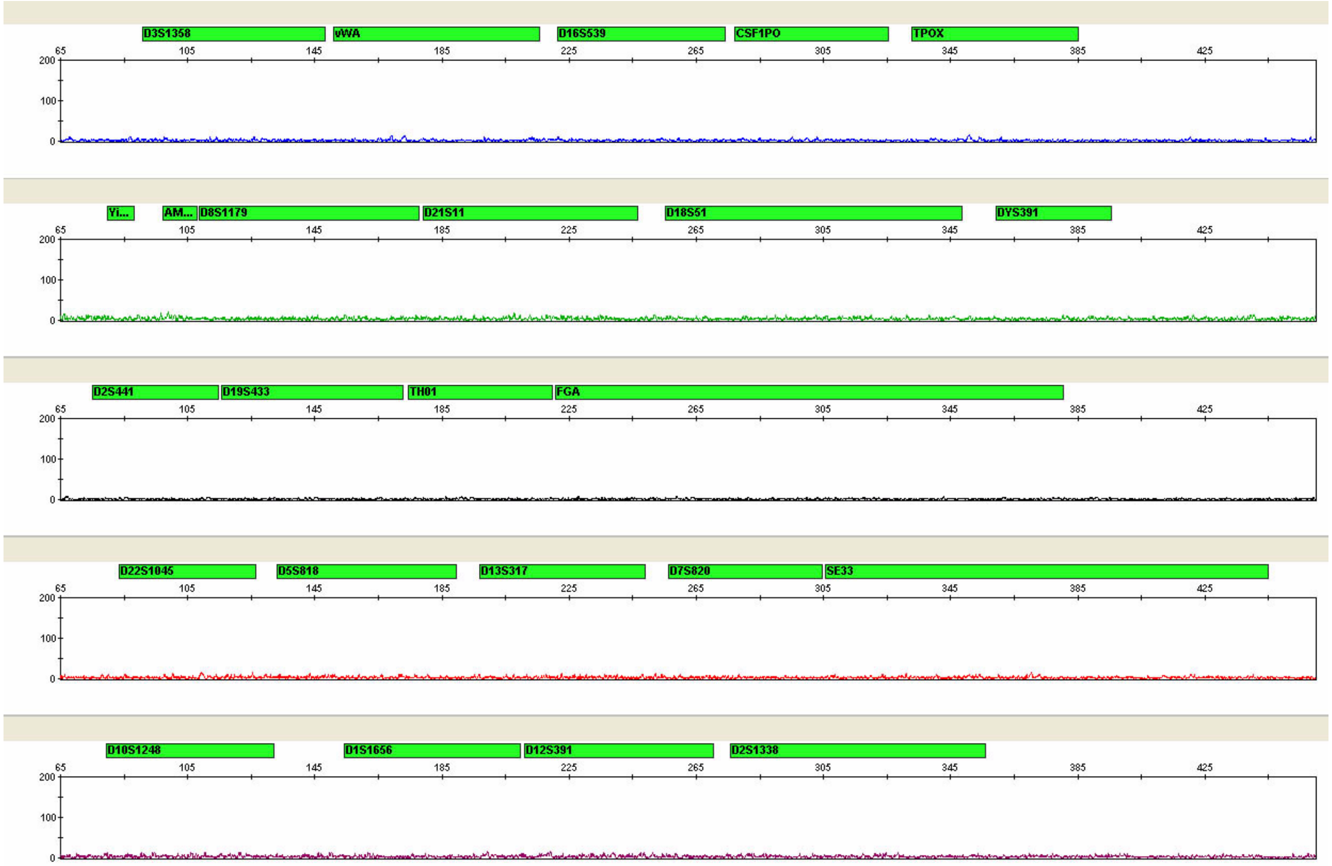


Figure 35 Examples of fluorescence background in data produced on a 3500xL Genetic Analyzer (Y-axis scale 0–200 RFU).

Amplification-related artifacts

This section shows artifacts that may be generated during amplification.

Note: The sample electropherograms shown in this section are magnified to show artifacts.

TH01 locus artifact

A reproducible artifact that is intrinsic to the GlobalFiler™ kit assay has been identified at the TH01 locus (Figure 36):

- Typically observed N–12 nucleotides from the true TH01 peak(s), at 0.4% to 0.9% of the parent peak.
- Probable cause—Secondary structure
- Typical signal intensity:
  - Low, may not be called as a peak if it is below the peak detection threshold used for analysis
  - Affected by DNA input amount

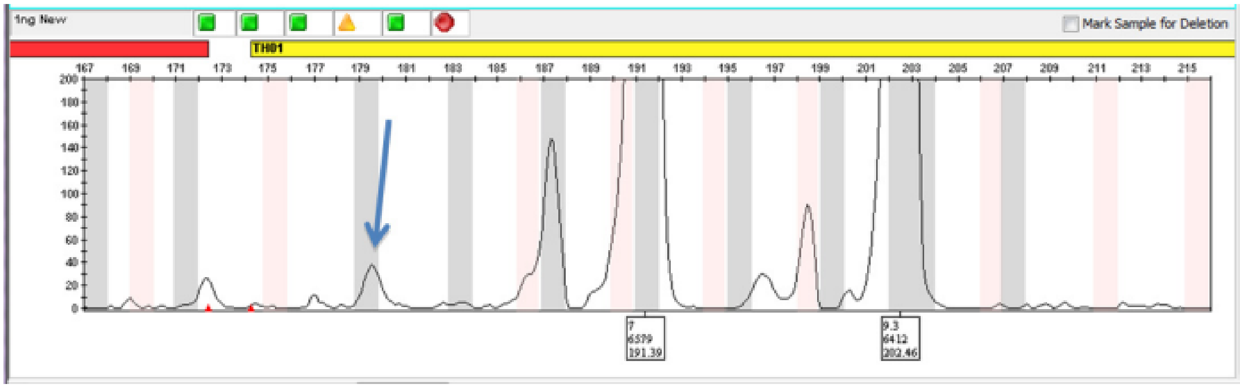


Figure 36 N-12 artifact (see arrow) associated with TH01 allele 7

TPOX locus artifact

A reproducible artifact intrinsic to the GlobalFiler™ kit assay has been identified at the TPOX locus (Figure 37):

- Typically observed N–24 nucleotides from the true TPOX peak(s).

  These artifacts have also been observed in the Identifiler™ Plus kit at (N–14 to N–15) nucleotides from the true TPOX peak(s).
- Probable cause—Secondary structure
- Typical signal intensity:
  - Low, may not be called as a peak if it is below the peak detection threshold used for analysis
  - Affected by DNA input amount

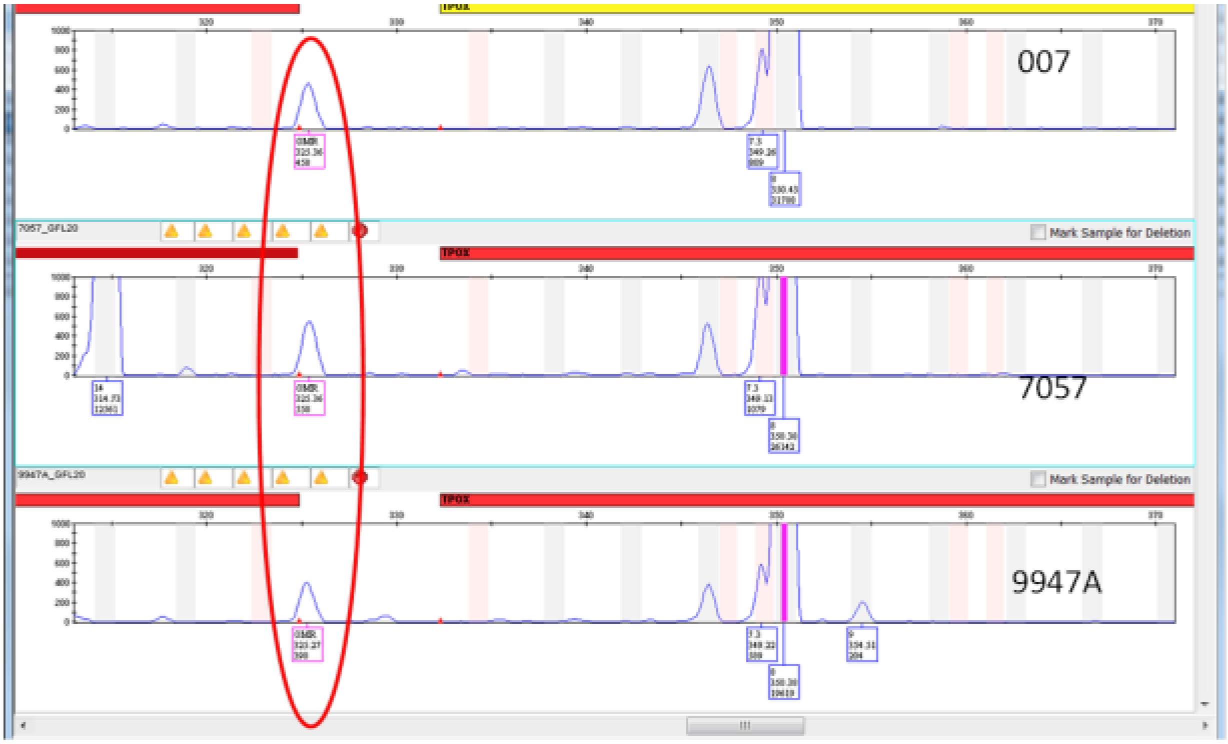


Figure 37 FAM™ dye artifacts in GlobalFiler™ kit profiles (size = 325 bp), three DNA samples at N-24 nucleotides from the TPOX peak depicted, 1 ng of DNA/30 cycles.

Amelogenin and Yindel loci artifact

A reproducible artifact that is intrinsic to the GlobalFiler™ kit assay has been identified at between the Amelogenin and Yindel loci (Figure 38):

- Typically observed at ~94.5 bp, at 0.3% to 0.7% of the parent peak, and does not fall within an allelic bin. Peak height is directly proportional to the peak height of the X allele peak.
- Probable cause—Secondary structure
- Typical signal intensity:
  - Low, may not be called as a peak if it is below the peak detection threshold used for analysis
  - Affected by DNA input amount

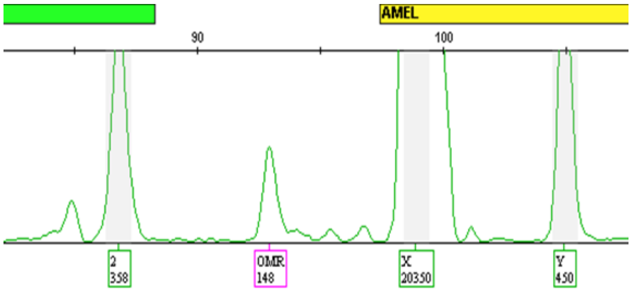


Figure 38 Artifact between the Amelogenin and Yindel loci (OMR=Outside Marker Range flag)

D21 locus artifact

A reproducible artifact that is intrinsic to the GlobalFiler™ kit assay has been identified at the D21 locus (Figure 39):

- Typically observed at ~207 bp
- Dependant upon sample type:
  - Has been observed in samples with low or undetermined quantitation results
  - Has not been observed in PCR positive or negative controls
- Probable cause—Presence and amplification of non-human DNA

  Sequencing studies have shown that the amplicon produced is homologous to yeast and fungal species commonly found in the environment, such as from plants, soil and decaying organic matter with no sequence homology to the human genome.

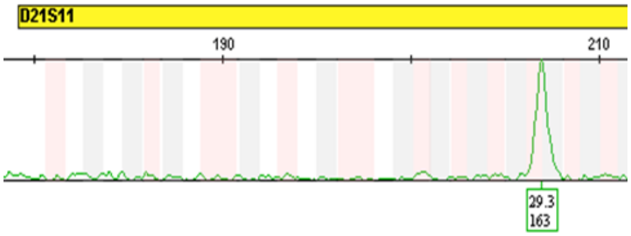


Figure 39 Artifact at the D21 locus

vWA locus artifact

A reproducible artifact that is intrinsic to the GlobalFiler™ kit assay has been identified at the vWA locus (Figure 40):

- Typically observed at ~204 bp
- Dependant upon sample type:
  - Has been observed in samples with low or undetermined quantitation results
  - Has not been observed in PCR positive or negative controls
- Probable cause—Presence and amplification of non-human DNA

  Sequencing studies have shown that the amplicon produced is homologous to yeast and fungal species commonly found in the environment with no sequence homology to the human genome.

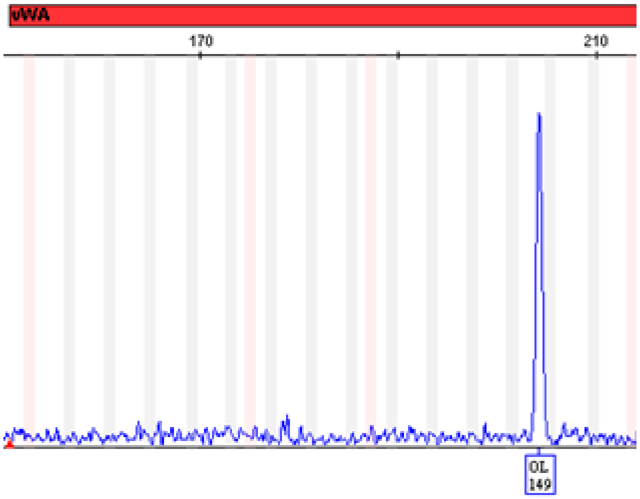


Figure 40 Artifact at the vWA locus

D5 locus artifact

A reproducible artifact that is intrinsic to the GlobalFiler™ kit assay has been identified at the D5 locus (Figure 41):

- Typically observed at ~180 bp
- Has been observed in samples
- Has not been observed in PCR negative controls
- Probable cause—non-STR amplification by-product
- Typical signal intensity:
  - Low, may not be called as a peak if it is below the peak detection threshold used for analysis
  - Affected by DNA input amount

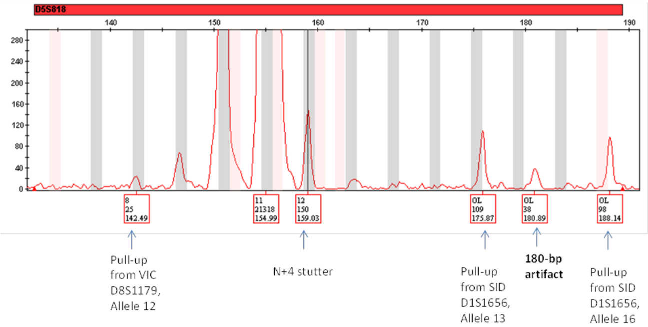


Figure 41 Artifact at D5 locus

D3 locus artifact

A reproducible artifact that is intrinsic to the GlobalFiler™ kit assay has been identified at the D3 locus (Figure 42):

- Typically observed at ~132 bp
- Has been observed in samples
- Has not been observed in PCR negative controls
- Probable cause—Non-STR amplification by-product
- Typical signal intensity:
  - Low, may not be called as a peak if it is below the peak detection threshold used for analysis
  - Affected by DNA input amount

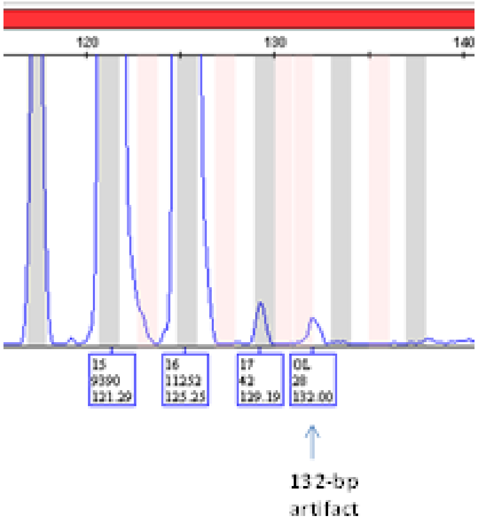


Figure 42 Artifact at D3 locus

Characterization of loci

SWGDAM guideline 3.1

“The basic characteristics of a genetic marker should be determined and documented.” (SWGDAM, December 2012)

Loci in this kit

This section describes basic characteristics of the 21 autosomal STR loci, Y STR locus, Y indel locus, and sex-determining marker (Amelogenin) that are amplified with the GlobalFiler™ 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 in intron 1 of the X homolog. Amplification generates 99-bp and 105-bp products from the X and Y chromosomes, respectively. (Sizes are the actual nucleotide size according to sequencing results, including 3´ A nucleotide addition, and size may not correspond exactly to allele mobility observed on capillary electrophoresis platforms.) Except for D22S1045, a trinucleotide STR, the remaining loci are tetranucleotide short tandem repeat (STR) loci. The length differences among alleles of a particular locus are caused by differences in the number of repeat units.

We have sequenced all the alleles in the GlobalFiler™ kit Allelic Ladder, including microvariants. In addition, other groups in the scientific community have sequenced alleles at some of these loci (Nakahori *et al*., 1991; Puers *et al*., 1993; Möller *et al*., 1994; Barber *et al*., 1995; Möller and Brinkmann, 1995; Barber *et al.*, 1996; Barber and Parkin, 1996; Brinkmann *et al*., 1998; Momhinweg *et al*., 1998; Watson *et al*., 1998). Among the various sources of sequence data on the 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 GlobalFiler™ 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).

Genetic linkage

Three sets of STR loci in the GlobalFiler™ kit are located on the same chromosome arms, as shown in Table 9. Of these, the most closely spaced are vWA and D12S391, which are located 6.3 million bp apart on the p arm of chromosome 12. Linkage disequilibrium analysis was conducted on vWA and D12S391 genotype results from 1,034 individuals of three ethnic groups (350 African-American, 349 Caucasian, and 335 Hispanic) using the Linkage Disequilibrium module of GenePop software version 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008).

The results of this analysis (not shown) indicated that there is no statistically significant linkage disequilibrium found between vWA and D12S391.

However, an inheritance analysis of the CEPH pedigree families demonstrated a degree of linkage between vWA and D12S391 that does not support the assumption of independence for kinship analysis (Budowle, *et al.*, 2010).

For examples of additional research on this topic, see: Burri, *et al*., 2015; Liu, *et al*., 2013.

Table 9 STR loci in the GlobalFiler™ kit located on the same chromosome arms

Locus	Chromosome location[1]		Distance apart (Mb)
	Map	Mb units	
D5S818	5q23.2	123.139	26.297
CSF	5q33.1	149.436	
vWA	12p13.31	5.963	6.378
D12S391	12p13.2	12.341	
TPOX	2p25.3	1.472	66.741
D2S441	2p14	68.213	

[1] STR locus mapping data was obtained from the NCBI Map Viewer http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9606 or the UCSC Genome Browser (http://genome.ucsc.edu/). GenePop LD analysis probability results (p values) >0.05 were considered to indicate that linkage disequilibrium between the loci within the population tested was not statistically significant.

Species specificity

SWGDAM Guideline 3.2

"The ability to detect genetic information from non-targeted species (e.g., detection of microbial DNA in a human assay) should be determined. The detection of genetic information from non-targeted species does not necessarily invalidate the use of the assay, but may help define the limits of the assay." (SWGDAM, December 2012)

Nonhuman study observation

The GlobalFiler™ 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, and macaque (1.0 ng each)
- **Non-primates**—Mouse, dog, sheep, pig, rabbit, cat, horse, hamster, rat, chicken, and cow (10.0 ng each)
- **Microorganisms**—*Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Bacillus subtilis*, and *Lactobacillus rhamnosus* (equivalent to 105 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 in the 70–400 bp region (gorilla data not shown). Macaque DNA produced an Amelogenin X peak, a 6-FAM™ dye peak at 359 bp, a NED™ dye peak at 278 bp, and two small SID™ dye peaks at 304 bp and 328 bp.

Among the non-primate species, most produced no peaks over a threshold of 175 RFU. Horse yielded reproducible VIC™ dye peaks at 94 bp (<1,800 RFU) due to Amelogenin cross-reactivity. Pig yielded reproducible TAZ™ dye peaks at 424 bp (<1,200 RFU). Chicken yielded reproducible TAZ™dye peak at 328 bp (<200 RFU). Individual replicate PCRs of dog, mouse, and chicken yielded single, small (<50 RFU), non-reproducible peaks. These non-reproducible cross-reactivities were not detectable when the dog or mouse DNA were amplified in the presence of 1 ng of human genomic DNA (data not shown). All breeds, subspecies, or microorganisms could not be tested. Minor variations can exist with different test samples, such as slightly different peaks or peak sizes. However, this data shows that the likelihood of obtaining an allelic profile consistent with that from a human sample from non-primates or microorganisms is extremely low.

Example electropherogram results from the species specificity tests are shown in Figure 43.

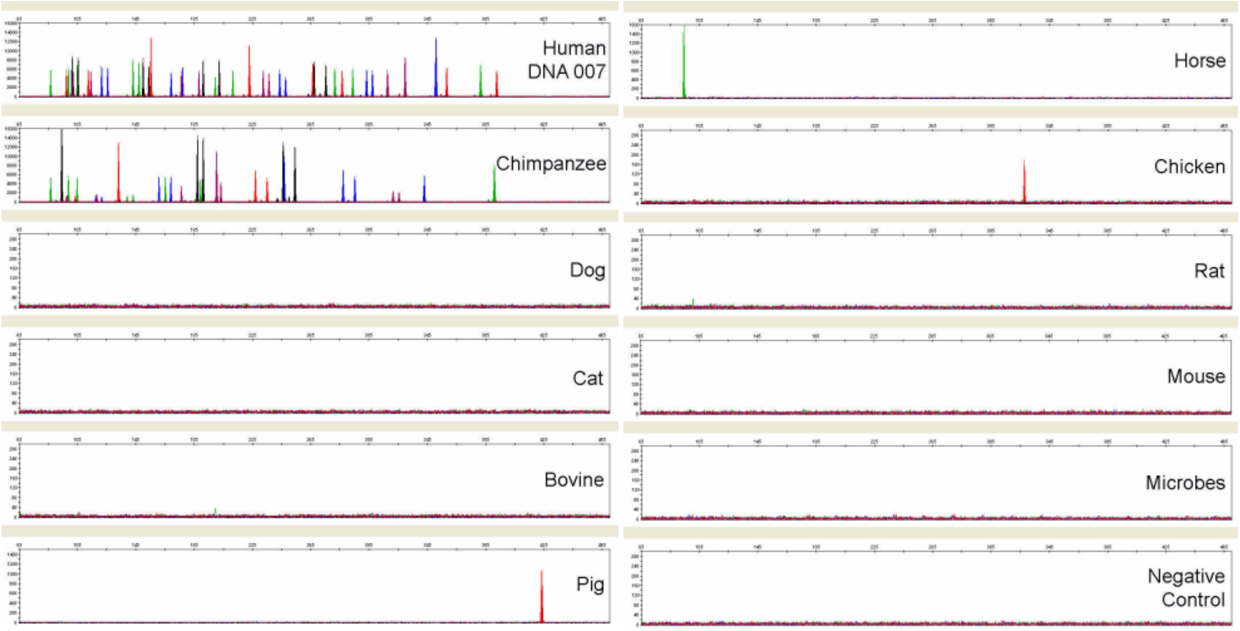


Figure 43 Representative electropherograms for some species tested in a species specificity study. Data produced on a 3500xL Genetic Analyzer.

Sensitivity

SWGDAM guideline 3.3

"The ability to obtain reliable results from a range of DNA quantities, to include the upper and lower limits of the assay, should be evaluated." (SWGDAM, December 2012)

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 recommended amount of input DNA for the GlobalFiler™ kit is 1.0 ng for 29-cycle PCR, based on quantification with kits such as the Quantifiler™ Human DNA Quantification Kit or the Quantifiler™ Duo DNA Quantification Kit.

To determine the optimum input DNA amount, perform studies according to the quantification kit that you use. If the sample contains degraded or inhibited DNA, amplification of a higher amount of DNA may be beneficial.

The amplification results of serially diluted DNA Control 007 (1.0–0.031 ng) are shown in Figure 44. Full profiles (43 PCR products) were consistently obtained at 0.125 ng, but occasional partial profiles resulted at lower concentrations.

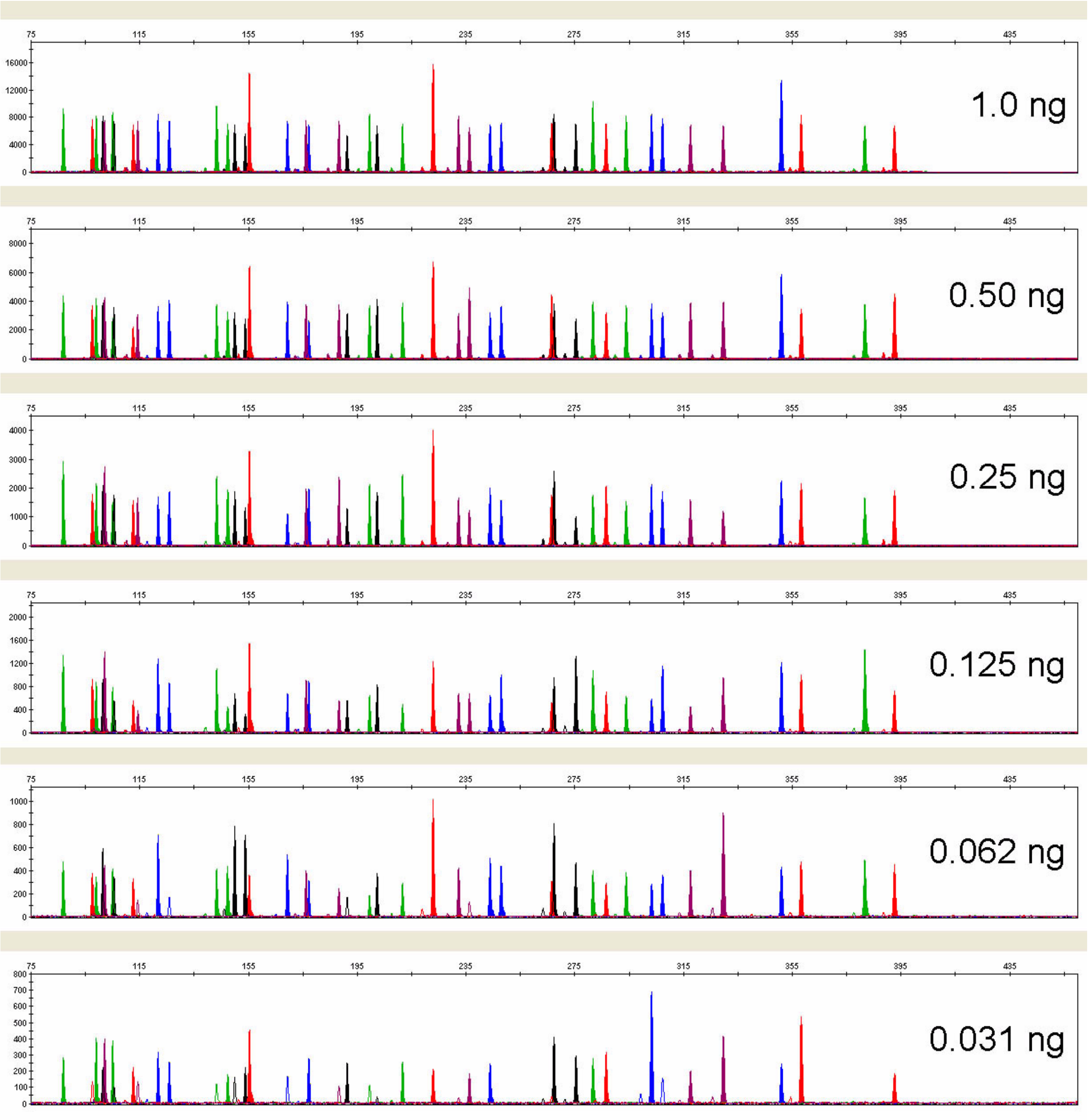


Figure 44 Electropherograms for amplifications using 1, 0.50, 0.25, 0.125, 0.062, and 0.031 ng of DNA Control 007. Electrophoresis was performed on a 3500xL Genetic Analyzer. Note that as the DNA input is serially diluted by 2-fold, the Y-axis scale is also magnified by 2-fold for the smaller input amounts of DNA.

Stability

SWGDAM guideline 3.4

"The ability to obtain results from DNA recovered from biological samples deposited on various substrates and subjected to various environmental and chemical insults should be evaluated. 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 to determine the effects of such factors." (SWGDAM, December 2012)

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 A3121 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 agarose gel analysis to determine the average size of the DNA fragments at each time point.

1.0 ng of degraded DNA was amplified using the GlobalFiler™ kit. As the DNA became progressively degraded, the loci failed to amplify robustly in order of decreasing size. Preferential amplification was not observed.

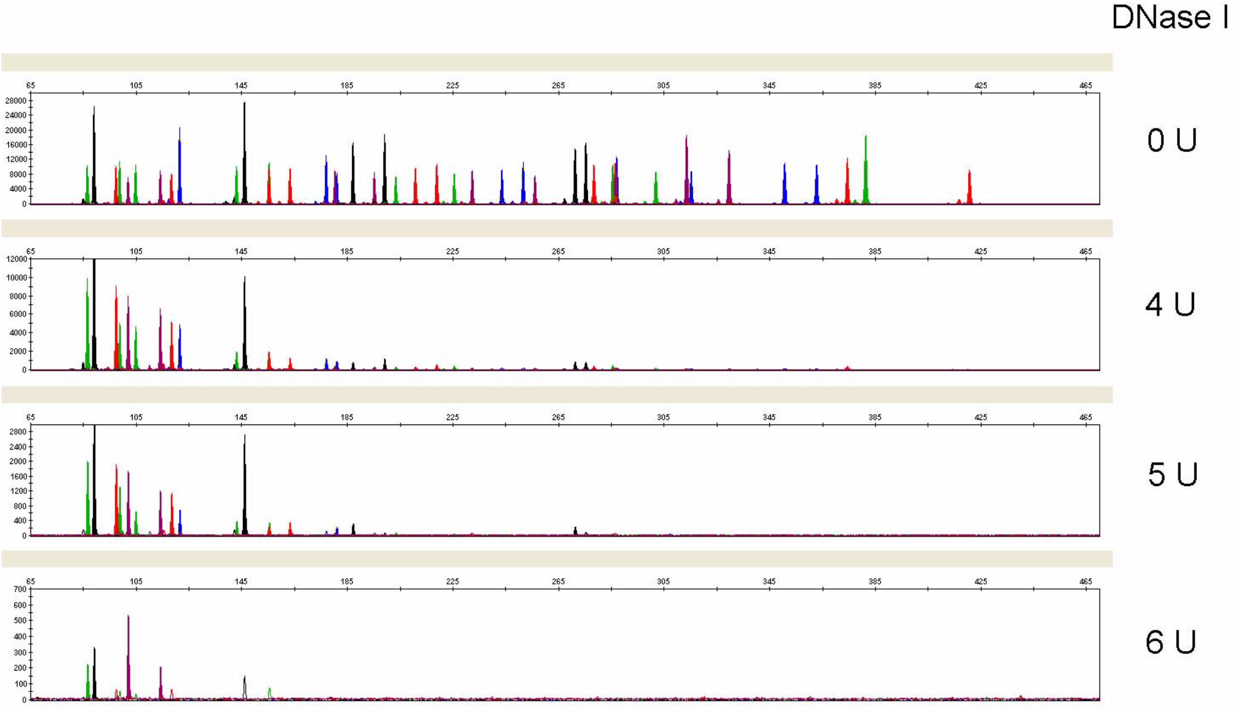


Figure 45 **Amplification of A3121 DNA samples sonicated and incubated with increasing doses of DNase I. Panels 1, 2, 3, and 4 correspond to 0, 4, 5, and 6 Units of DNase I. Note that the Y-axis scale is magnified for more degraded samples, which generate lower peak heights (Y-axis scale 0–700, 0–2,800, 0–12,000, or 0–28,000).**

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 GlobalFiler™ kit, 1.0 ng of DNA Control 007 was amplified in the presence of increasing concentrations of hematin for 29 cycles of amplification (Figure 46). The concentrations of hematin used were 0, 200, 300, and 400 µM (Table 10).

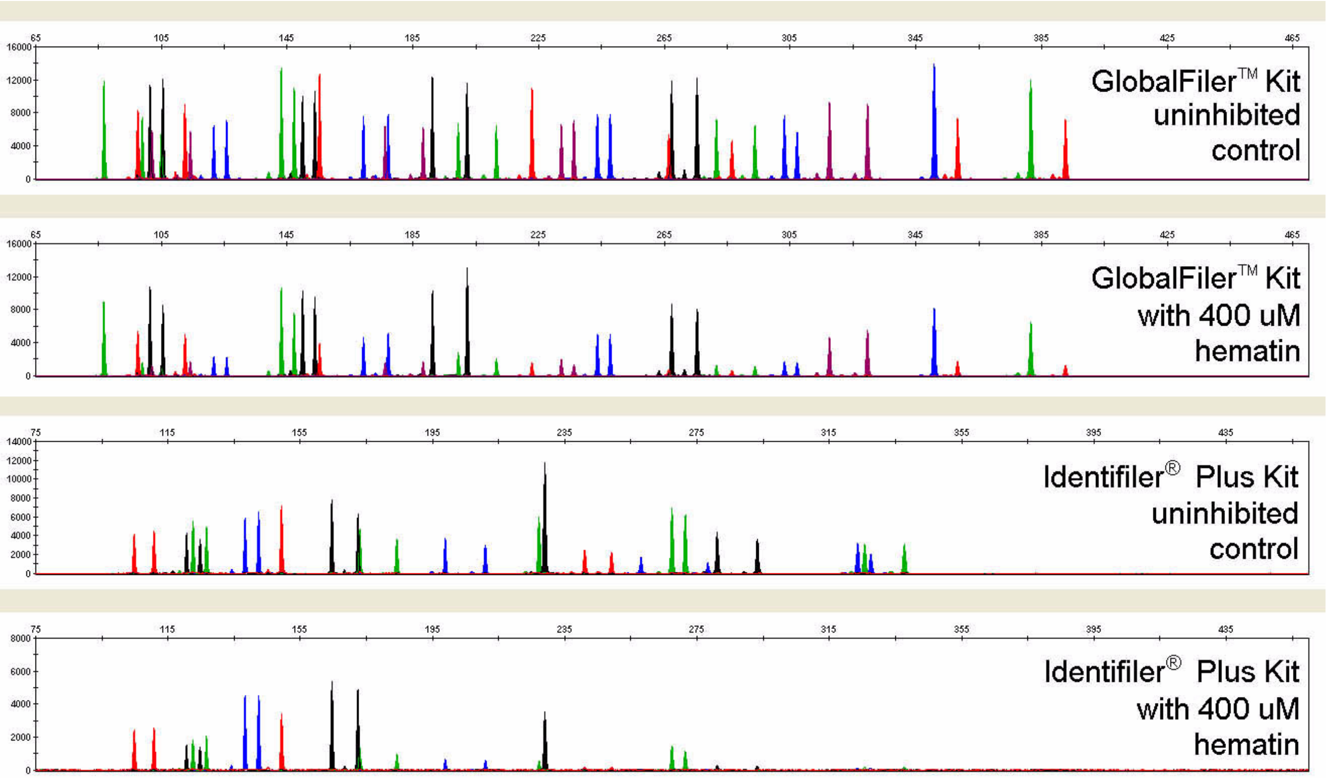


Figure 46 Electropherograms for the GlobalFiler™ kit and the Identifiler™ Plus kit show the improved performance of the GlobalFiler™ kit in the presence of hematin compared with previous AmpFℓSTR™ kits. The same set of inhibited samples was analyzed with the GlobalFiler™ kit and the Identifiler™ Plus kit for 29 cycles of amplification.

Table 10 GlobalFiler™ kit performance in simulated hematin inhibition (n=3).

Hematin concentration	Number of alleles detected[1]
0 µM	43, 43, 43
200 µM	43, 43, 43
300 µM	43, 43, 43
400 µM	43, 43, 43

[1] Only those peaks ≥175 RFU were counted. A complete profile with DNA Control 007 yields 43 peaks using the GlobalFiler™ kit.

Effect of inhibitors—humic acid

Traces of humic acid can inhibit the PCR amplification of DNA evidence collected from soil. Amplification of 1.0 ng of DNA Control 007 in the presence of increasing amounts of humic acid was performed using the GlobalFiler™ kit for 29 cycles of amplification (Figure 47). The concentrations of humic acid tested were 0, 70, 140, and 210 ng/µL (Table 11).

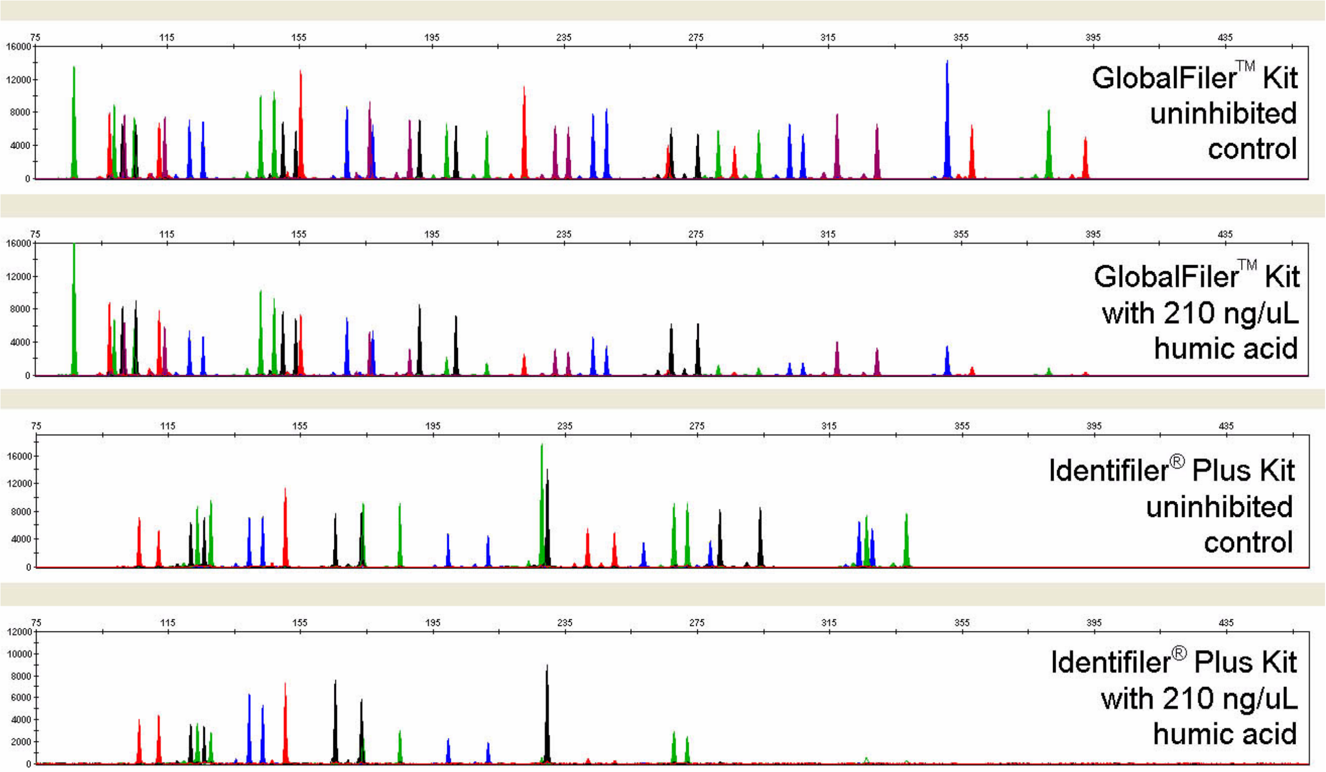


Figure 47 **Electropherograms for the GlobalFiler™ kit and the Identifiler™ Plus kit show the improved performance of the GlobalFiler™ kit in the presence of humic acid compared with previous AmpFℓSTR™ kits. The same set of inhibited samples was analyzed with the GlobalFiler™ kit and the Identifiler™ Plus kit for 29 cycles of amplification.**

Table 11 **GlobalFiler™ kit performance in simulated humic inhibition (n=3).**

Humic acid concentration	Number of alleles detected[1]
0 ng/µL	43, 43, 43
70 ng/µL	43, 43, 43
140 ng/µL	43, 43, 43
210 ng/µL	43, 43, 39

[1] Only those peaks ≥175 RFU were counted. A complete profile with DNA Control 007 yields 43 peaks using the GlobalFiler™ kit.

Mixture studies

SWGDAM guideline 3.8

"The ability to obtain reliable results from mixed source samples should be determined." (SWGDAM, December 2012)

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 GlobalFiler™ kit loci in unmixed human population database samples are shown in Figure 48. The population samples used are listed in "Population samples used in these studies" on page 113.

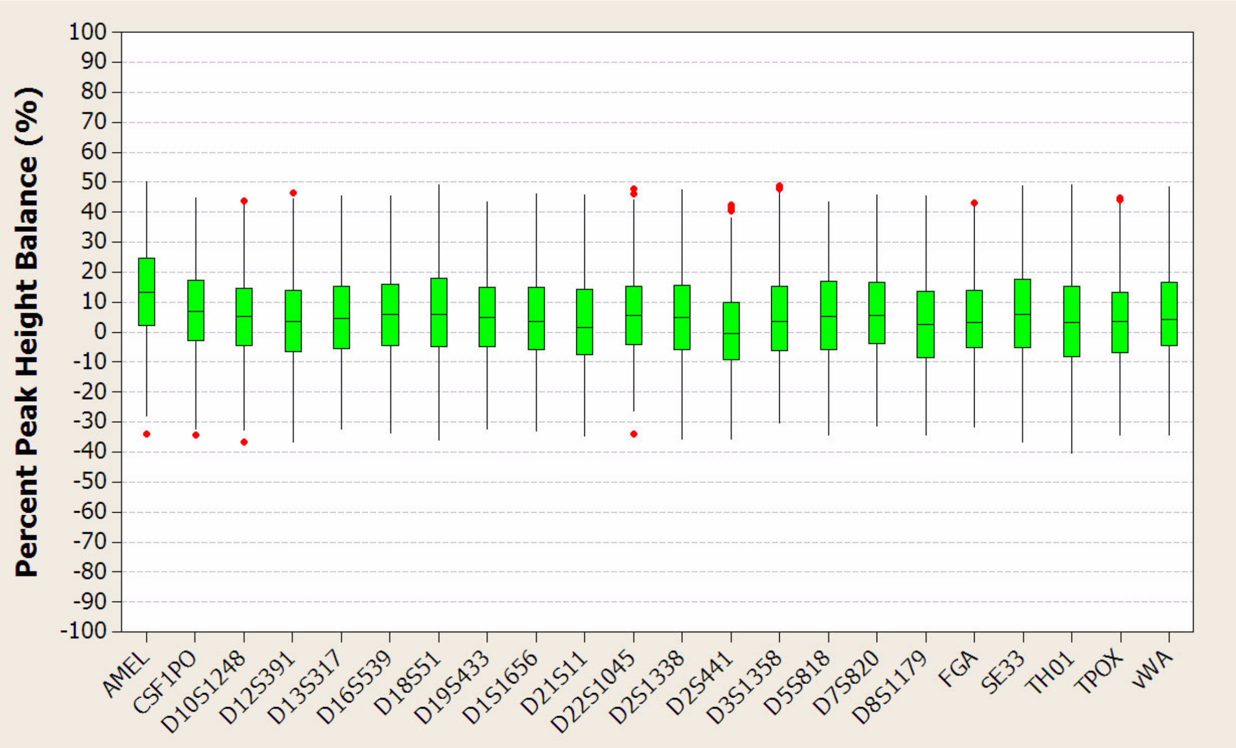


Figure 48 Heterozygote ratios for 1 ng of input DNA. The distribution of intra-locus peak height ratios are expressed as plus and minus percent, by locus. Green boxes show the middle 50% or interquartile range (IQR). Box halves below and above median show the second and third quartile, respectively. "Whiskers" indicate 1.5 IQR from the upper and lower margins of the IQR. Red diamonds are outlier data points more than 1.5 IQR from the median.

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, 5:1, 8:1, 1:0). The total amount of genomic input DNA mixed at each ratio was 1 ng. The samples were amplified in a Veriti™ 96-Well Thermal Cycler, then electrophoresed and detected using a 3500xL Genetic Analyzer.

The results of the mixed DNA samples are shown in Figure 49, 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 5:1 (0.833:0.167 ng) and 8:1 (0.889:0.111 ng). Generally, 15:1 ratios resulted in partial profiles for the minor component (data not shown). The genotypes of these samples are described in Table 12.

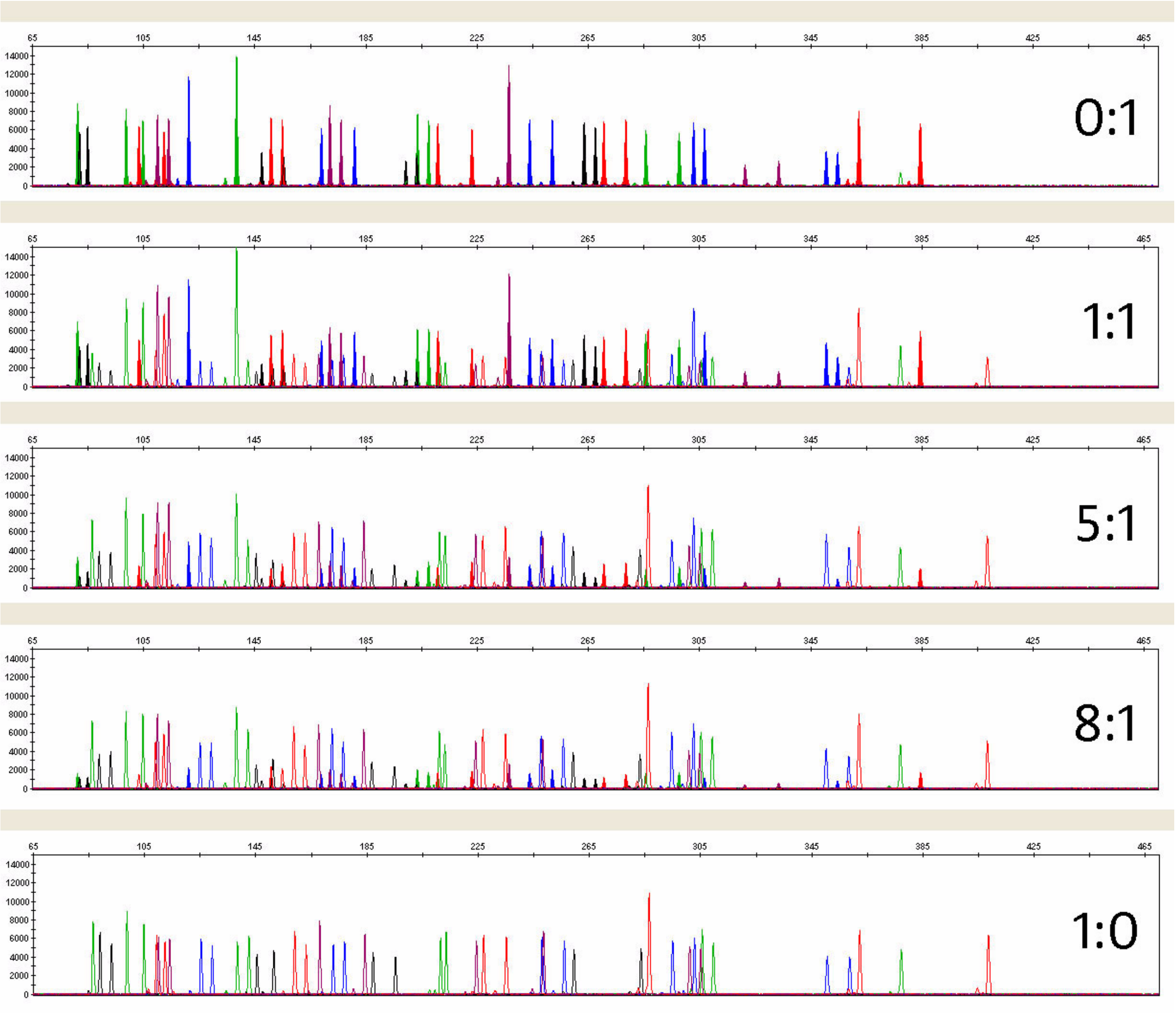


Figure 49 Amplification of DNA mixtures at various ratios. Panels show electropherograms for (top to bottom) 0:1 (minor contributor only), 1:1 mixture, 5:1 mixture, 8:1 mixture, and 1:0 (major contributor only).

Table 12 Genotypes of mixed DNA samples

Locus	Sample A genotype	Sample B genotype
D3S1358	15	16, 17
vWA	14, 17	15, 16
D16S539	9, 11	10, 12
CSF1PO	11, 12	9, 11
TPOX	8, 9	8, 10
Y indel	1	2

Table 12 Genotypes of mixed DNA samples *(continued)*

Locus	Sample A genotype	Sample B genotype
Amelogenin	X, Y	X, Y
D8S1179	11	11, 12
D21S11	29, 30	31, 31.2
D18S51	13, 16	18, 19
DYS391	10	10
D2S441	9.1, 10	11, 12
D19S433	13.2, 15.2	13, 14.2
TH01	9, 10	6, 8
FGA	23, 24	22, 28
D22S1045	13, 16	15, 16
D5S818	10, 11	12, 13
D13S317	8, 11	12, 14
D7S820	8, 10	12
SE33	18, 23.2	18, 29.2
D10S1248	14, 15	14, 15
D1S1656	12, 13	11, 15
D12S391	19	16, 22
D2S1338	21, 24	16, 17

Population data

SWGDAM guideline 3.7

“The distribution of genetic markers in populations should be determined in relevant population groups.” (SWGDAM, December 2012)

Loci in the kit

The GlobalFiler™ kit contains loci for which extensive population data are available. For additional information on the loci shared between many of the AmpFℓSTR™ kits, see the population data and additional studies section of the *AmpFℓSTR™ NGM SElect™ PCR Amplification Kit User Guide* (Pub. No. MAN0029842) and the *AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit User Guide* (Pub. No. MAN0029850).

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.

Population samples used in these studies

The GlobalFiler™ kit was used to generate the population data provided in this section. Whole blood samples were provided by the Interstate Blood Bank (Memphis, Tennessee) and Boca Biolistics (Coconut Creek, Florida). Samples were collected in the United States (with no geographical preference) from randomly selected individuals of known ethnicities. Ethnicities of sample donors were:

- African-American—330 samples
- Asian—153 samples
- Caucasian—343 samples
- Hispanic—368 samples

DNA was extracted with a 6100 Nucleic Acid Prep Station.

In addition to the alleles that we observed and recorded in our databases, other alleles have been published or reported to us by other laboratories (see the STRBase at **https://strbase.nist.gov/**).

Concordance studies

The primer sequences used in the GlobalFiler™ kit and GlobalFiler™ Express kit are identical. We compared allele calls between the two kits. Genotype data from 200 blood samples on FTA™Classic Cards showed 100% concordance between the two kits. The GlobalFiler™ kit genotypes of the above population data were also compared against the genotypes generated using the Identifiler™ Plus kit and the NGM SElect™ kit. The few discordant genotypes observed were exclusively found in loci where degenerate primers were added in the GlobalFiler™ kit to rescue known SNPs found in the primer binding sites.

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 autosomal STR allele frequencies at the GlobalFiler™ kit loci by population group are shown in Table 13.

The Y-specific allele frequencies by population group for the GlobalFiler™ kit DYS391 and Y indel loci are shown in Table 14. The Y-specific allele frequencies were not included in the probability of identity calculation.

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

Table 13 Autosomal allele frequencies by population group for GlobalFiler™ kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.)

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
CSF1PO				
5	*	*	*	*
6	0.15*	*	*	*
7	3.79	*	*	0.95
8	9.39	0.33*	0.29*	0.54*
9	3.79	2.61	2.77	2.58
10	26.21	26.14	27.99	25.14
11	23.33	21.9	31.78	27.45
11.1	*	*	*	0.14*
12	27.88	38.56	30.76	37.91
13	4.39	9.8	5.98	4.62
14	1.06	0.65*	0.44*	0.54*
15	*	*	*	0.14*
16	*	*	*	*
D10S1248				
7	0.15*	*	*	*
8	*	*	*	0.14*
9	0.15*	*	*	0.14*
10	*	0.33*	*	0.14*
11	3.64	*	0.58*	0.27*
12	14.09	10.78	3.5	4.48

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
13	22.88	36.93	29.45	25.95
14	27.88	22.55	29.74	36.14
15	18.48	22.55	19.39	22.69
16	10.15	5.23	13.41	7.74
17	2.27	1.63	3.64	2.31
18	0.30*	*	0.29*	*
19	*	*	*	*
D12S391				
13	*	*	*	0.14*
14	*	*	*	0.14*
15	7.58	2.61	4.37	4.08
15.1	0.15*	*	*	*
16	5.15	0.98*	3.35	5.03
16.1	0.15*	*	*	*
17	16.52	8.17	10.35	7.34
17.1	0.45*	*	*	0.27*
17.3	0.61*	*	1.9	1.22
18	24.55	28.43	16.18	19.7
18.3	1.21	*	2.19	2.17
19	13.94	23.86	12.54	18.75
19.1	0.61*	*	*	*
19.3	0.30*	*	0.58*	1.22
20	11.52	16.99	9.77	17.12
20.3	*	*	0.15*	*
21	7.27	10.78	13.56	8.7
21.3	0.15*	*	0.15*	*
22	5	3.59	10.79	6.79
23	3.64	3.27	8.16	3.67

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
24	0.61*	0.98*	3.64	1.9
25	0.61*	0.33*	1.9	1.36
26	*	*	0.29*	0.27*
27	*	*	0.15*	0.14*
D13S317				
5	*	*	*	*
6	*	*	*	*
7	*	*	0.15*	*
8	2.27	29.74	10.93	8.97
9	2.27	12.09	7.14	16.3
10	3.03	13.73	6.85	9.65
11	29.24	25.49	29.01	22.83
12	43.79	14.38	30.76	27.45
13	14.55	3.92	10.64	10.05
14	4.55	0.33*	4.52	4.76
15	0.30*	0.33*	*	*
16	*	*	*	*
17	*	*	*	*
D16S539				
4	*	*	*	*
5	*	*	*	*
6	*	*	*	0.14*
8	3.33	*	1.46	2.04
9	21.67	31.05	12.68	10.19
10	11.52	14.05	4.08	15.76
11	30	20.59	32.22	31.79
12	19.09	21.57	30.9	24.18
13	13.03	11.44	16.76	14.4

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
14	1.36	1.31*	1.75	1.22
15	*	*	0.15*	0.27*
16	*	*	*	*
D18S51				
6	*	*	*	*
7	*	*	*	*
9	*	*	*	0.14*
10	0.15*	*	1.17	0.68
10.2	0.15*	*	*	*
11	0.45*	1.31*	0.87	1.22
12	6.21	5.56	15.01	10.46
13	3.94	17.32	11.95	11.41
13.2	0.30*	*	*	*
14	5.91	22.88	17.64	16.3
14.2	0.45*	*	*	0.14*
15	16.52	16.99	15.31	12.23
15.2	*	*	*	0.14*
16	18.18	12.42	11.95	12.91
17	16.36	6.54	10.79	17.39
18	14.09	4.9	8.31	7.74
19	9.7	5.23	4.08	3.53
20	4.7	1.96	1.31	1.9
20.2	0.15*	*	*	*
21	1.82	1.96	1.02	2.17
22	0.61*	0.98*	0.29*	0.68
23	0.30*	0.98*	0.29*	0.54*
24	*	0.65*	*	0.27*
25	*	*	*	0.14*

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
26	*	0.33*	*	*
27	*	*	*	*
28	*	*	*	*
D19S433				
5.2	*	*	*	*
6	*	*	*	*
7	*	*	*	*
8	*	*	*	*
9	0.30*	0.33*	*	*
10	1.21	*	0.15*	0.41*
10.2	0.15*	*	*	*
11	9.85	*	*	1.63
11.2	0.30*	*	*	0.27*
12	10.45	4.58	7.29	8.42
12.1	*	*	0.15*	*
12.2	3.94	0.33*	0.15*	1.49
13	27.88	28.1	27.26	18.48
13.2	5.3	2.61	1.6	6.93
14	18.94	23.2	35.13	30.71
14.2	5.3	9.48	2.04	4.62
15	6.67	7.52	16.18	13.04
15.2	4.39	20.26	3.5	6.79
16	1.52	0.33*	5.69	4.08
16.2	3.18	2.61	0.29*	2.17
17	*	*	0.29*	0.54*
17.2	0.61*	0.65*	0.15*	0.41*
18	*	*	0.15*	*
18.2	*	*	*	*

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
19.2	*	*	*	*
D1S1656				
8	*	*	*	*
9	0.15*	*	*	0.14*
10	1.36	*	0.29*	0.41*
11	5.3	3.59	6.27	3.94
12	8.48	4.25	15.74	9.38
13	11.06	13.73	7	7.07
14	25	6.21	6.27	11.28
14.3	0.91	*	0.29*	0.27*
15	16.97	20.26	15.31	15.49
15.3	1.82	*	8.75	2.99
16	10	31.05	9.33	15.08
16.1	*	*	*	0.27*
16.3	7.27	0.65*	4.96	5.16
17	2.73	14.05	4.96	6.79
17.1	*	*	0.29*	*
17.3	5.76	3.59	12.68	15.76
18	0.45*	0.33*	0.29*	0.82
18.3	1.82	1.63	5.98	4.48
19	0.15*	*	*	*
19.3	0.61*	0.33*	1.6	0.68
20	*	0.33*	*	*
20.3	0.15*	*	*	*
21	*	*	*	*
D21S11				
23.2	*	*	*	*
24	*	*	*	*

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
24.2	*	*	*	0.27*
25	*	*	*	*
26	0.30*	*	0.58*	0.41*
27	5.91	*	2.62	1.49
28	25.15	4.9	16.76	11.41
28.2	*	0.65*	*	0.14*
29	15.61	26.8	23.76	21.06
29.2	*	*	0.15*	*
29.3	0.15*	*	0.15*	*
30	20.76	30.72	23.18	27.17
30.2	1.67	0.65*	2.77	1.77
31	8.79	9.48	6.85	5.16
31.2	4.55	3.92	8.89	11.14
31.3	*	0.33*	*	*
32	1.36	2.61	2.33	1.36
32.2	7.12	14.38	9.62	12.5
33	0.91	0.98*	*	0.14*
33.2	3.18	4.58	1.9	5.3
34	0.15*	*	*	*
34.2	*	*	0.44*	0.14*
35	3.64	*	*	0.27*
35.2	*	*	*	*
36	0.76	*	*	0.14*
37	*	*	*	*
38	*	*	*	0.14*
39	*	*	*	*
D22S1045				
7	*	*	*	*

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
8	0.61*	*	*	*
9	*	*	*	*
10	4.09	*	0.44*	0.68
11	14.7	15.36	13.85	7.61
12	6.21	0.33*	0.58*	0.95
13	0.30*	0.33*	1.02	1.09
14	7.88	0.33*	3.35	2.04
15	23.33	33.66	36.3	43.48
16	20.3	23.86	36.3	34.65
17	20.45	24.18	7.58	8.42
18	2.12	1.96	0.58*	0.95
19	*	*	*	*
20	*	*	*	0.14*
D2S1338				
10	*	*	*	*
11	*	*	*	*
12	*	*	*	*
13	0.15*	*	0.15*	*
14	*	*	0.15*	*
15	0.30*	*	0.15*	*
16	5.3	1.63	4.08	3.8
17	10	14.05	18.37	17.8
18	4.85	13.07	8.31	6.52
19	16.21	16.67	14.14	17.53
20	10.45	8.82	15.74	13.86
21	11.97	2.94	2.92	3.67
22	12.42	5.88	1.75	6.52
23	9.24	18.3	10.06	14.27

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
24	8.79	11.11	10.2	8.83
25	6.97	5.88	12.1	5.43
26	2.58	*	1.6	1.49
27	0.76	0.33*	0.29*	0.14*
28	*	0.98*	*	0.14*
29	*	0.33*	*	*
D2S441				
8	0.15*	*	*	*
9	*	*	0.58*	0.14*
9.1	*	2.94	*	*
10	9.09	20.59	19.83	30.3
11	35.61	36.27	33.09	31.93
11.3	2.88	2.61	5.1	4.62
12	20.45	20.92	4.08	3.8
12.3	0.15*	*	0.29*	0.41*
13	3.48	6.21	3.35	1.9
14	26.21	9.8	28.86	23.1
15	1.97	0.65*	4.37	3.4
16	*	*	0.44*	0.41*
17	*	*	*	*
D3S1358				
8	*	*	*	*
9	0.30*	*	*	0.14*
10	*	*	*	*
11	*	*	0.29*	*
12	0.15*	0.33*	*	0.14*
13	0.61*	*	0.15*	0.41*
14	9.09	2.61	15.16	9.1

Table 13 **Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.)** *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
15	28.18	49.02	27.26	34.65
15.2	0.30*	*	*	*
16	32.42	21.9	24.34	26.9
17	22.27	19.61	19.68	17.93
18	6.06	6.54	11.66	9.92
19	0.61*	*	1.46	0.82
20	*	*	*	*
21	*	*	*	*
D5S818				
6	*	*	*	*
7	0.30*	1.63	*	5.3
8	6.21	0.33*	0.73	1.49
9	1.97	9.15	5.39	5.03
10	7.27	22.22	5.54	4.35
11	25	28.76	33.82	38.18
12	35.45	24.51	37.61	30.16
13	21.82	12.75	14.87	14.54
14	1.67	0.65*	1.75	0.95
15	0.30*	*	0.29*	*
16	*	*	*	*
17	*	*	*	*
18	*	*	*	*
19	*	*	*	*
D7S820				
5	*	*	*	*
6	0.30*	*	*	*
7	0.45*	0.33*	1.31	1.09
8	21.67	16.99	16.47	12.5

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
9	11.67	7.52	16.62	8.29
9.1	*	0.33*	*	*
10	30.45	20.26	27.26	25.14
10.3	*	*	*	0.14*
11	19.85	31.37	20.99	29.35
11.3	*	*	*	0.14*
12	13.03	20.26	14.58	19.02
13	2.27	2.61	2.33	3.94
13.1	0.15*	*	*	*
14	0.15*	0.33*	0.29*	0.41*
15	*	*	0.15*	*
16	*	*	*	*
D8S1179				
4	*	*	*	*
5	*	*	*	*
6	*	*	*	*
7	*	*	*	*
8	0.30*	*	2.04	0.68
9	0.30*	*	1.31	0.27*
10	3.33	9.8	10.5	9.51
11	5.61	9.48	6.71	5.03
12	11.36	13.4	15.16	12.5
13	18.18	24.18	33.24	33.15
14	35.91	15.69	18.8	23.23
15	18.03	22.22	9.04	11.41
16	5.91	4.25	2.77	3.53
17	1.06	0.98*	0.44*	0.68
18	*	*	*	*

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
19	*	*	*	*
20	*	*	*	*
FGA				
12.2	*	*	*	*
13	*	*	*	*
14	*	*	*	*
15	*	*	*	*
16	*	0.33*	0.15*	*
16.1	0.30*	*	*	*
17	*	0.33*	0.15*	*
18	0.91	3.27	1.02	0.68
18.2	0.61*	*	*	*
19	6.97	4.25	5.69	7.61
19.2	0.45*	*	*	*
20	6.82	3.92	14.87	8.7
20.2	0.30*	*	0.44*	0.27*
21	11.67	13.07	18.22	13.45
21.2	0.15*	0.33*	0.29*	*
22	17.27	14.38	19.24	14.4
22.2	0.15*	*	0.87	0.54*
23	17.27	27.12	14.87	12.91
23.2	*	0.65*	0.44*	0.41*
23.3	0.30*	*	*	*
24	18.94	18.3	14.43	15.62
24.2	*	0.33*	*	*
25	9.55	9.8	6.71	13.72
26	4.09	3.27	1.9	7.07
26.2	*	*	*	*

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
27	2.58	0.65*	0.58*	3.12
28	1.21	*	0.15*	0.95
29	*	*	*	0.41*
30	0.15*	*	*	0.14*
30.2	0.15*	*	*	*
31.2	*	*	*	*
32.2	*	*	*	*
33.2	*	*	*	*
34.2	0.15*	*	*	*
42.2	*	*	*	*
43.2	*	*	*	*
44.2	*	*	*	*
45.2	*	*	*	*
46.2	*	*	*	*
47.2	*	*	*	*
48.2	*	*	*	*
50.2	*	*	*	*
51.2	*	*	*	*
SE33				
4.2	*	*	*	*
5.2	0.15*	*	*	*
6.3	*	*	0.15*	*
8	*	*	*	*
9	*	*	*	*
11	*	*	*	*
11.2	0.76	*	*	0.14*
12	0.15*	0.33*	0.44*	0.14*
12.1	*	*	0.15*	*

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
12.2	0.30*	*	0.15*	0.14*
13	1.36	*	0.87	1.22
13.2	0.45*	*	*	0.14*
14	3.33	*	3.64	1.77
14.2	0.15*	*	*	0.82
14.3	*	*	0.15*	*
15	4.24	1.31*	3.64	4.89
15.2	0.15*	*	0.15*	0.14*
16	6.97	3.59	5.39	5.57
16.2	0.30*	*	*	0.27*
16.3	*	*	*	0.14*
17	7.73	5.23	6.56	8.7
17.2	0.15*	*	*	*
17.3	*	*	0.15*	*
18	10.76	4.9	7.87	10.05
18.2	0.15*	*	*	0.27*
19	15	9.48	8.31	8.15
19.2	0.30*	*	0.29*	*
19.3	*	*	0.29*	*
20	9.55	6.86	5.25	4.48
20.2	0.91	0.33*	0.87	0.82
21	5.76	6.21	2.04	3.12
21.2	0.91	1.63	1.17	1.09
22	1.97	2.61	0.58*	1.09
22.2	1.36	2.29	3.35	2.31
23	0.30*	*	*	*
23.2	0.61*	2.61	2.62	2.85
23.3	*	*	*	0.14*

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
24	0.30*	0.33*	0.15*	0.14*
24.2	1.67	6.54	4.52	2.31
25.2	2.42	7.19	3.79	3.12
26	0.15*	*	*	0.27*
26.2	5.61	7.52	4.52	6.39
27.2	5.91	3.59	6.85	7.07
27.3	*	*	*	0.14*
28.2	3.94	7.84	7.73	6.25
29.2	2.58	8.5	7.87	5.84
30.2	1.21	7.52	4.66	3.8
31	*	*	*	0.14*
31.2	1.06	1.63	2.77	2.31
32	*	*	0.44*	*
32.2	0.76	1.31*	1.6	2.04
33	*	*	0.29*	0.41*
33.2	0.45*	0.33*	0.15*	0.54*
34	*	*	0.29*	0.41*
34.2	0.15*	*	*	0.27*
35	*	*	0.15*	*
35.2	*	0.33*	*	*
36	*	*	0.15*	*
37	*	*	*	0.14*
38	*	*	*	*
TH01				
3	*	*	*	*
4	*	*	*	*
5	0.45*	*	0.15*	*
6	15.45	13.07	21.72	27.17

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
6.1	0.15*	*	*	*
7	37.42	26.14	17.64	32.74
8	20.61	3.59	11.37	8.7
9	16.06	51.63	17.06	12.77
9.3	8.33	4.25	31.2	17.12
10	1.52	1.31*	0.87	1.49
11	*	*	*	*
13.3	*	*	*	*
TPOX				
4	*	*	*	*
5	*	*	*	*
6	8.03	*	0.15*	0.54*
7	2.27	0.98*	*	0.14*
8	35.91	49.35	50.15	47.83
9	19.09	13.07	12.97	8.02
10	9.55	3.59	4.66	6.11
11	21.67	29.74	28.28	26.36
12	3.33	3.27	3.79	10.73
13	0.15*	*	*	0.14*
14	*	*	*	0.14*
15	*	*	*	*
16	*	*	*	*
vWA				
10	*	*	*	*
11	0.45*	*	*	0.14*
12	*	*	*	0.27*
13	0.91	*	0.15*	0.14*
14	7.27	23.53	8.75	6.52

Table 13 Autosomal allele frequencies by population group for GlobalFiler kit STR loci. (Asterisks indicate alleles not detected or not detected in significant quantities.) *(continued)*

Allele	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
15	20.91	1.63	12.24	9.78
16	27.58	15.36	22.3	30.57
17	19.85	29.74	27.41	27.17
17.3	*	*	*	0.14*
18	13.79	19.61	17.78	18.07
19	6.52	9.15	10.06	6.39
20	1.97	0.98*	1.31	0.82
21	0.61*	*	*	*
22	*	*	*	*
23	0.15*	*	*	*
24	*	*	*	*
25	*	*	*	*

Table 14 Y-specific frequencies by population group for GlobalFiler™ kit DYS391 and Y indel loci. (Asterisks indicate alleles not detected or not detected in significant quantities.)

Allele	African-American (n=246)	Asian (n=65)	U.S. Caucasian (n=233)	U.S. Hispanic (n=182)
DYS391				
6	*	*	*	*
7	*	*	*	*
8	*	*	*	*
9	1.22	3.08*	1.72	6.59
10	71.54	83.08	44.64	52.75
11	26.42	13.85	51.93	36.26
12	0.41*	*	1.72	3.3
13	0.41*	*	*	1.10*
14	*	*	*	*
Y indel				
1	1.22	67.69	*	0.55*
2	98.78	32.31	100	99.45

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

Locus	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
CSF1PO	0.0850	0.1317	0.1333	0.1353
D10S1248	0.0693	0.1045	0.0943	0.1131
D12S391	0.0377	0.0664	0.0231	0.0318
D13S317	0.1451	0.0817	0.0761	0.0564
D16S539	0.0727	0.0915	0.1043	0.0809
D18S51	0.0322	0.0402	0.0311	0.0281
D19S433	0.0388	0.0663	0.0862	0.0484
D1S1656	0.0340	0.0564	0.0223	0.0247
D21S11	0.0453	0.0671	0.0520	0.0487
D22S1045	0.0559	0.1073	0.1309	0.1604
D2S1338	0.0225	0.0337	0.0316	0.0316
D2S441	0.1030	0.0961	0.0976	0.1079
D3S1358	0.0984	0.1689	0.0749	0.0949
D5S818	0.0968	0.0883	0.1341	0.1122
D7S820	0.0784	0.0875	0.0680	0.0790
D8S1179	0.0762	0.0527	0.0631	0.0661
FGA	0.0322	0.0555	0.0384	0.0282
SE33	0.0118	0.0139	0.0085	0.0081
TH01	0.0949	0.1750	0.0801	0.0902
TPOX	0.0833	0.1788	0.1757	0.1456
vWA	0.0622	0.0840	0.0650	0.0926
Combined	6.18×10^{-27}	3.34×10^{-24}	3.71×10^{-26}	3.09×10^{-26}

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

Allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), match probability (MP), and p-value of each locus was calculated using a software program developed by Ge (Li *et al*., 2013) and shown in the following table.

Departures from Hardy-Weinberg Equilibrium (HWE) expectations of each locus were derived using Arlequin (Excoffier *et al*., 2010). After Bonferroni correction (Weir, 1990), (p-value = 0.05/21 = 0.0024), no departures from HWE were observed at any locus.

The average observed heterozygosity across the 21 autosomal STR loci was 0.815 in the African-American population, 0.779 in the Asian population, 0.804 in the U.S. Caucasian population, and 0.789 in the U.S. Hispanic population. The most heterozygous locus was SE33 (mean observed heterozygosity across all populations of 0.948), and the least heterozygous STR locus was TPOX (mean observed heterozygosity across all populations of 0.652). The cumulative match probability (including the Y chromosome loci) was as follows:

- African-American—2.17×10^{-27}
- Asian—2.26×10^{-25}
- U.S. Caucasian—5.27×10^{-27}
- U.S. Hispanic—5.0×10^{-27}

Table 16 Allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), match probability (MP), and p-value of STR loci

Locus	African-American				Asian				U.S. Caucasian				U.S. Hispanic			
	Ho	He	MP	p-value	Ho	He	MP	p-value	Ho	He	MP	p-value	Ho	He	MP	p-value
Y indel	—	—	0.976	—	—	-	0.556	—	—	—	1.000	—	—	—	1.000	—
DYS391	—	—	0.540	—	—	—	0.709	—	—	—	0.472	—	—	—	0.409	—
D3S1358	0.772	0.762	0.094	0.902	0.686	0.682	0.151	0.012	0.753	0.786	0.079	0.698	0.698	0.758	0.098	0.291
vWA	0.752	0.798	0.068	0.116	0.829	0.784	0.080	0.309	0.841	0.807	0.064	0.621	0.842	0.782	0.080	0.463
D16S539	0.772	0.797	0.071	0.026	0.800	0.764	0.092	0.457	0.801	0.749	0.103	0.047	0.770	0.774	0.084	0.835
CSF1PO	0.772	0.773	0.086	0.020	0.743	0.721	0.125	0.128	0.721	0.720	0.131	0.745	0.727	0.710	0.137	0.619
TPOX	0.693	0.755	0.093	0.579	0.671	0.665	0.168	0.770	0.633	0.644	0.181	0.598	0.669	0.667	0.153	0.102
D8S1179	0.782	0.793	0.068	0.662	0.814	0.828	0.052	0.795	0.793	0.797	0.065	0.122	0.755	0.802	0.064	0.518
D21S11	0.861	0.849	0.039	0.553	0.800	0.791	0.069	0.667	0.873	0.837	0.046	0.385	0.827	0.839	0.044	0.315
D18S51	0.931	0.868	0.031	0.324	0.829	0.853	0.038	0.572	0.873	0.872	0.030	0.962	0.849	0.870	0.031	0.945
D2S441	0.772	0.756	0.099	0.421	0.714	0.746	0.101	0.238	0.757	0.766	0.090	0.077	0.791	0.763	0.094	0.611
D19S433	0.812	0.825	0.051	0.663	0.714	0.802	0.064	0.130	0.785	0.774	0.083	0.859	0.820	0.834	0.046	0.446
TH01	0.762	0.747	0.102	0.418	0.614	0.656	0.171	0.381	0.753	0.783	0.081	0.326	0.770	0.767	0.091	0.845
FGA	0.782	0.866	0.033	0.082	0.900	0.841	0.044	0.240	0.829	0.857	0.037	0.337	0.842	0.882	0.025	0.127
D22S1045	0.842	0.822	0.055	0.062	0.743	0.742	0.112	0.966	0.705	0.714	0.131	0.026	0.698	0.672	0.162	0.064
D5S818	0.752	0.761	0.094	0.799	0.800	0.786	0.079	0.430	0.761	0.716	0.127	0.197	0.727	0.727	0.115	0.219
D13S317	0.762	0.695	0.138	0.217	0.786	0.799	0.069	0.327	0.769	0.777	0.081	0.718	0.799	0.815	0.059	0.855
D7S820	0.842	0.786	0.077	0.404	0.814	0.778	0.081	0.062	0.789	0.805	0.067	0.278	0.748	0.789	0.076	0.830
SE33	0.960	0.929	0.009	0.776	0.943	0.936	0.008	0.526	0.968	0.947	0.005	0.532	0.921	0.941	0.007	0.597
D10S1248	0.792	0.789	0.075	0.823	0.757	0.764	0.091	0.928	0.785	0.769	0.090	0.630	0.691	0.724	0.124	0.336

Table 16 Allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), match probability (MP), and p-value of STR loci *(continued)*

Locus	African-American				Asian				U.S. Caucasian				U.S. Hispanic			
	Ho	He	MP	p-value	Ho	He	MP	p-value	Ho	He	MP	p-value	Ho	He	MP	p-value
D1S1656	0.921	0.863	0.033	0.351	0.757	0.818	0.056	0.043	0.912	0.899	0.019	0.550	0.871	0.896	0.020	0.048
D12S391	0.861	0.864	0.032	0.190	0.771	0.808	0.063	0.650	0.904	0.896	0.020	0.450	0.842	0.874	0.028	0.071
D2S1338	0.911	0.894	0.020	0.763	0.871	0.872	0.030	0.356	0.880	0.878	0.027	0.230	0.906	0.877	0.027	0.929

The PE values of the GlobalFiler™ kit STR loci (individually and combined) are shown in Table 17.

Table 17 Probability of paternity exclusion values for the GlobalFiler™ kit STR loci

Locus	African-American (n=330)	Asian (n=153)	U.S. Caucasian (n=343)	U.S. Hispanic (n=368)
CSF1PO	0.5878	0.4904	0.4507	0.4644
D10S1248	0.6623	0.5353	0.5649	0.4644
D12S391	0.7401	0.6310	0.8032	0.6588
D13S317	0.4521	0.6063	0.5544	0.5770
D16S539	0.5548	0.6063	0.5915	0.5623
D18S51	0.7892	0.6560	0.7557	0.7121
D19S433	0.6332	0.5238	0.5135	0.6431
D1S1656	0.7462	0.5703	0.8032	0.7338
D21S11	0.7280	0.6063	0.7264	0.7013
D22S1045	0.7038	0.4795	0.4507	0.3970
D2S1338	0.8140	0.7463	0.7498	0.7392
D2S441	0.5228	0.5353	0.4986	0.5051
D3S1358	0.4918	0.3976	0.5338	0.4689
D5S818	0.4717	0.5942	0.4839	0.4959
D7S820	0.5767	0.5942	0.5808	0.5970
D8S1179	0.5990	0.6063	0.6187	0.5381
FGA	0.7280	0.8397	0.6632	0.7175
SE33	0.8639	0.8800	0.9231	0.8781
TH01	0.5124	0.3424	0.5036	0.5381
TPOX	0.4817	0.3602	0.3435	0.3620
vWA	0.6103	0.6186	0.6576	0.6276
PEi	2.0564×10^{-10}	2.7761×10^{-09}	4.1986×10^{-10}	2.1709×10^{-09}
Combined	0.9999999998	0.9999999972	0.9999999996	0.9999999978

7

Performance verification after BSA component replacement

BSA component overview

Starting with the following lot numbers, GlobalFiler™ PCR Amplification Kit configuration was introduced starting with the following lot numbers:

- 200 reaction: Lot 1503018 and later
- 1,000 reaction: Lot 1503014 and later

The new configuration addresses these issues:

- Long-term stability and robustness of the GlobalFiler™ kit master mix
- Higher than expected peaks in the NED™ dye channel
- A "reverse ski slope" effect in the SID™dye channel

Our internal investigation suggested that these effects were caused by a lack of accessibility within the PCR reaction to $MgCl_2$, a critical component for robust amplification. The BSA component of the GlobalFiler™ kit master mix contains a phosphate buffer verified to be involved in the formation of a $Mg_3(PO_4)_2$ complex, resulting in a decreased concentration of accessible $^{2+}$ ions.

In the new kit configuration:

- Manufacturing of the master mix transitioned from a raw-material BSA formulation prepared with phosphate buffer to a raw-material BSA formulation prepared with Tris-HCl buffer.
- The Additive tube has been removed from the kit.

All other kit components are unchanged in the new configuration (BSA powder raw material, BSA concentration, all other components and component concentrations, primer sequences, and primer concentrations).

Experiment overview

We performed validation studies to compare the performance of the GlobalFiler™ kit master mix containing the BSA formulated in Tris-HCl buffer (updated kit) with the performance of the GlobalFiler™ kit master mix containing the BSA formulated in phosphate buffer. We focused on studies most relevant to forensic DNA testing (see SWGDAM Guidelines effective December, 2012). These studies, although not exhaustive, are in our opinion appropriate for a manufacturer.

The studies we performed are:

- Sensitivity
- Sensitivity
- Performance with degraded DNA samples
- Genotype concordance (population study)
- Performance with 2-contributor mixtures

Materials and methods

Materials

- Two lots of GlobalFiler™ kit master mix were formulated with Tris-buffered BSA according to the standard manufacturing protocol (V1 and V2 master mix).
- One lot of GlobalFiler™ kit master mix was formulated using the original potassium phosphate-buffered BSA as a control according to the standard manufacturing protocol (Kpi master mix).

  With the exception of the buffer used to formulate the BSA, master mix lot formulations V1, V2 and Kpi used the same raw materials.
- GlobalFiler™ PCR Amplification Kit lot 1411014 was used as an on-market control. The primer set, control DNA and allelic ladder from this lot were used for all experiments to minimize other sources of variation between the master mix lots.

Component	V1	V2	Kpi	Lot 14
Master mix	V1 (fresh)	V2 (fresh)	Kpi (fresh)	Lot 1410010[1]
Primer set	Lot 1410006[1]	Lot 1410006[1]	Lot 1410006[1]	Lot 1410006[1]
Control DNA	Lot 1411008[1]	Lot 1411008[1]	Lot 1411008[1]	Lot 1411008[1]

[1] Components from GlobalFiler™ kit lot 1411014

Methods

We performed all amplifications with a Veriti™ Thermal Cycler, using the amplification conditions and cycle numbers recommended for the GlobalFiler™ kit . We ran all data on a 3500xL Genetic Analyzer with 3500 Series Data Collection Software v2. We analyzed the data with GeneMapper™ *ID-X* Software v1.4, and performed subsequent data analysis with Minitab™ or JMP™ statistical software.

Samples

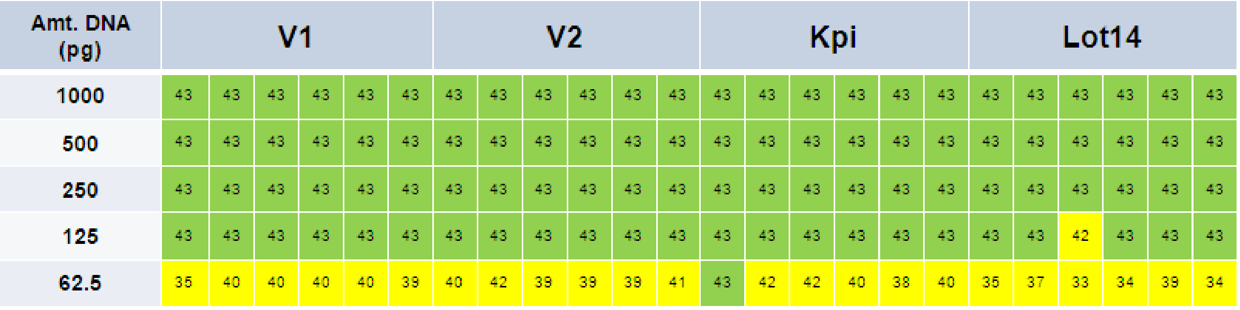
Test case	Description
Sensitivity study	DNA Control 007 was amplified at five DNA input amounts – 62.5 pg,125 pg, 250 pg, 500 pg and 1,000 pg. Six replicates were tested for each concentration with each master mix formats.
Stability study	1 ng of DNA Control 007 was treated with 0, 150 µM, 300 µM, and 450 µM of hematin and 0, 90 ng/µL, 150 ng/µL, and 210 ng/µL of humic acid. Each inhibitor concentration was amplified in 6 replicates using each master mix formulation. Control Raji DNA was sonicated and treated with varying amounts of DNAse I (4 U, 5 U, and, 6 U) for 20 minutes. 1 ng of each degraded sample was amplified in 4 replicates using each of the master mixes.
Population study	72 genomic DNA samples were amplified with each master mix formats and evaluated for genotype concordance, intralocus balance and intracolor balance.
Mixture study	Mixtures of Raji and DNA Control 007 genomic DNA samples were prepared in ratios of 0:1, 8:1, 5:1, 3:1, 1:1 and 1:0. 1 ng total input DNA was added to 3 replicate amplifications for each master mix formulation.

Sensitivity study results

Sensitivity study results: Allele recovery, average peak height, stutter comparison, and NTCs

Allele recovery

All master mix formulations yielded full profiles at 125 pg of input DNA, except for a single allele dropout for one replicate for the Lot 14 on-market kit master mix (Figure 50). These results can be explained by stochastic variation and sampling from dilute DNA solutions. Allelic dropout results can therefore be considered equivalent between the Test and Control mixes.



Amt. DNA (pg)	V1						V2						Kpi						Lot14					
1000	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
500	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
250	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
125	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	42	43	43	43
62.5	35	40	40	40	40	39	40	42	39	39	39	41	43	42	42	40	38	40	35	37	33	34	39	34

Figure 50 Allele recoveries for each replicate generated from input amounts ranging from 62.5 pg to 1 ng. The maximum number of recoverable alleles is 43.

Mean sample peak heights

Mean sample peak height observations were consistent between all Test and Control mixes (Figure 51). For the 500-pg and 1-ng input amounts, the average peak heights for the V1 and V2 master mixes were approximately 20% higher than peak heights obtained using the fresh Kpi master mix.

No significant differences in average allele peak heights were observed between the freshly formulated master mixes at the 125-pg and 250-pg DNA input amounts. All three freshly prepared master mixes (V1, V2, and Kpi) yielded up to 39% higher average peak heights than the on-market kit Lot 14, consistent with some degree of Mg depletion. Lot-to-lot peak heights have been shown to be highly variable.

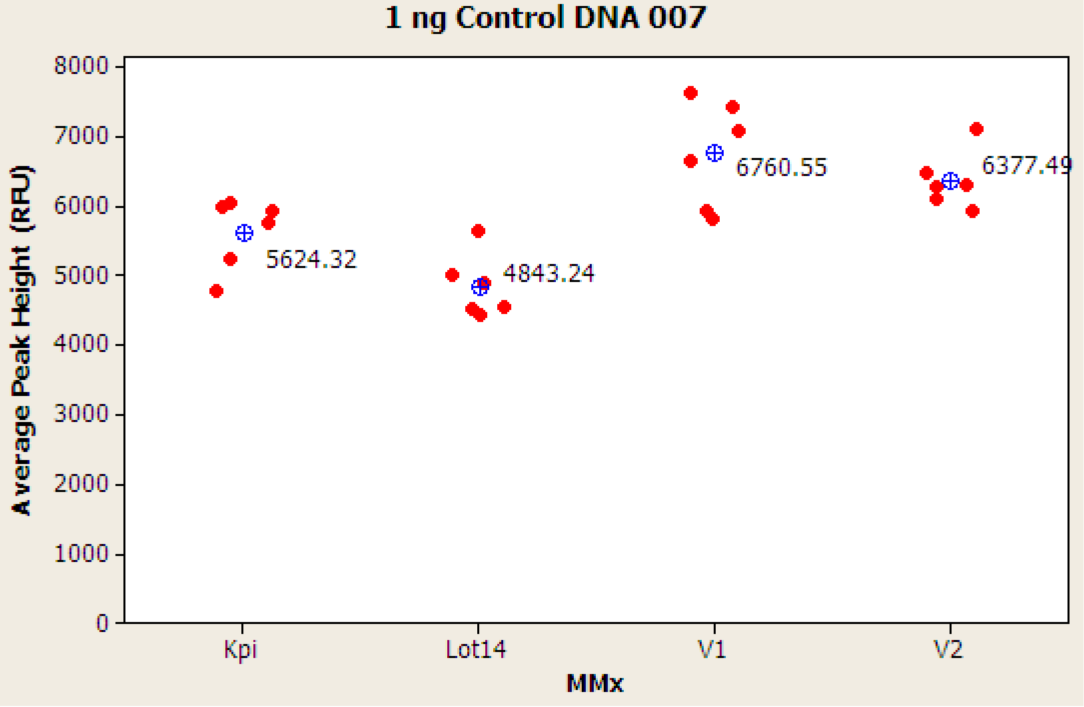


Figure 51 Average peak heights in RFU for the 1-ng replicates from the sensitivity study generated using the V1, V2, Kpi, and Lot 14 master mixes.

Stutter value comparison

Average stutter values at each locus were calculated for the 1 ng and 500-pg replicates. No stutter value differences of >0.5% were observed between the freshly prepared master mixes V1, V2, and Kpi. Only 6 of the 22 loci in the fresh master mixes exhibited differences in stutter values >0.5% in the Lot 14 master mix. For these 6 loci, the stutter values for the Lot 14 master mix were slightly lower than stutter values the fresh master mixes. This finding is consistent with a direct relationship between Mg^{2+} concentration and stutter percentage (Mulero, *et al*.). In all cases, stutter peaks were successfully filtered out using the stutter settings listed in “Stutter filter settings provided with the GeneMapper™ ID-X Software” on page 90.

Non-template controls (NTCs)

No reproducible artifacts above 175 RFU were observed in the read region for the non-template controls generated using all master mixes (Figure 52).

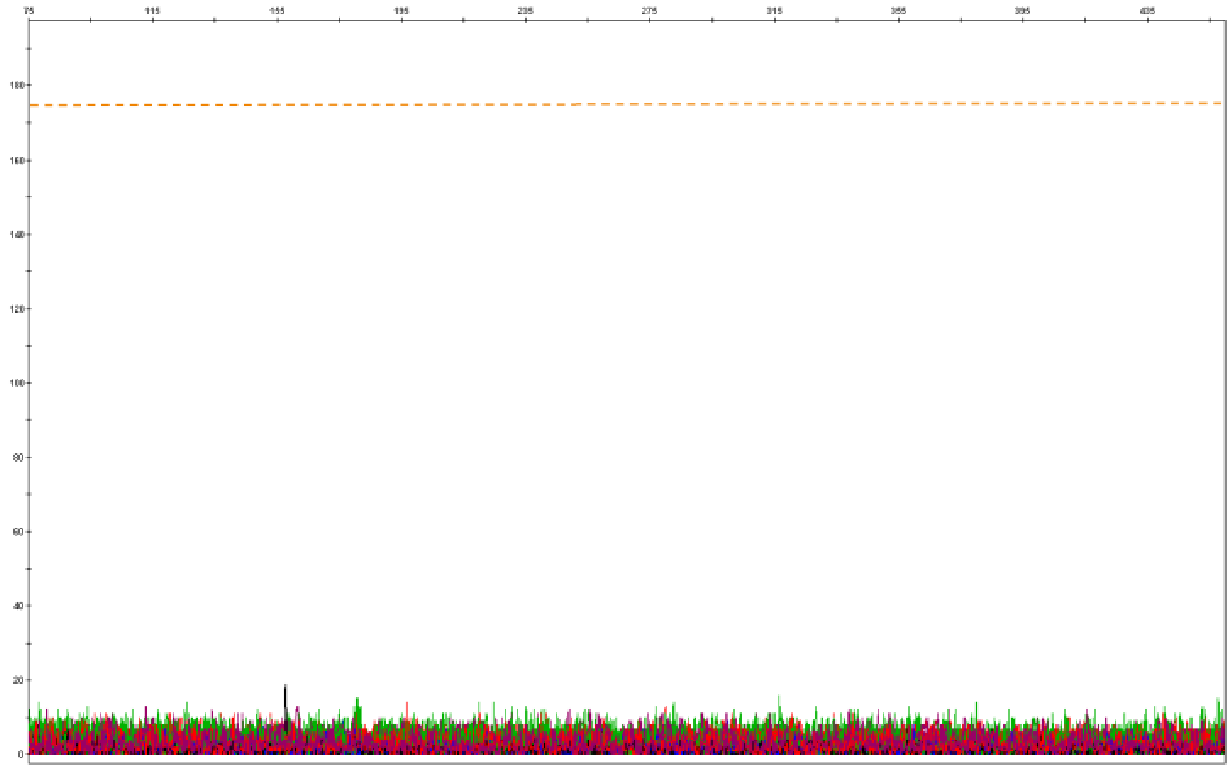


Figure 52 **NTC overlay of 6 replicates from the V1 master mix. The Y-scale was set at 200 RFU; the orange line represents the analytical threshold of 175 RFU. The baseline noise was only ~20 RFU. Comparable results were obtained for the V2 and Kpi master mixes.**

Sensitivity study results: Intralocus, intracolor, and intercolor balance

Intralocus balance (Peak Height Ratio)

Intra-locus balance percentage was calculated for heterozygous loci by dividing the lower of the two heterozygous peak by the higher of the two heterozygous peak then multiplied by 100. All master mixes yielded intralocus balances >65% for heterozygous loci at the 1 ng input amount as shown in the figure below with no significant differences in performance (Figure 53).

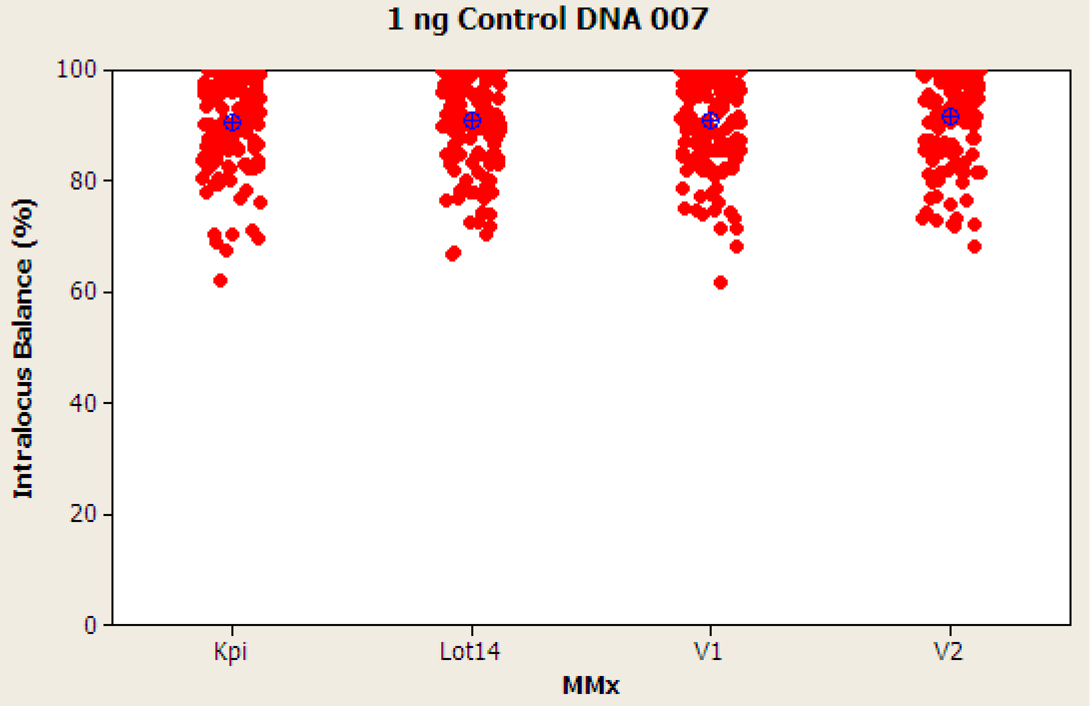


Figure 53 Average intralocus balance percentage for heterozygous loci for the 1-ng replicates from the sensitivity study generated using the Kpi, Lot 14, V1 and V2 master mixes.

Intracolor balance (ICB)

With the exception of Yindel and DYS391, homozygous peak heights were divided by 2 and heterozygous peak heights were averaged to obtain normalized peak heights. Intracolor balance was calculated by dividing the lowest normalized peak in each dye channel by the highest normalized peak in each dye channel then multiplied by 100. All master mixes yielded intracolor balances >40% for the 1 ng input amount (Figure 54). No significant differences in performance were observed for the freshly prepared master mixes (V1, V2 and Kpi). Lot 14 master mix exhibited slightly higher ICB for the NED™ dye channel and slightly lower ICB for the SID™ dye channel compared to the freshly prepared master mixes.

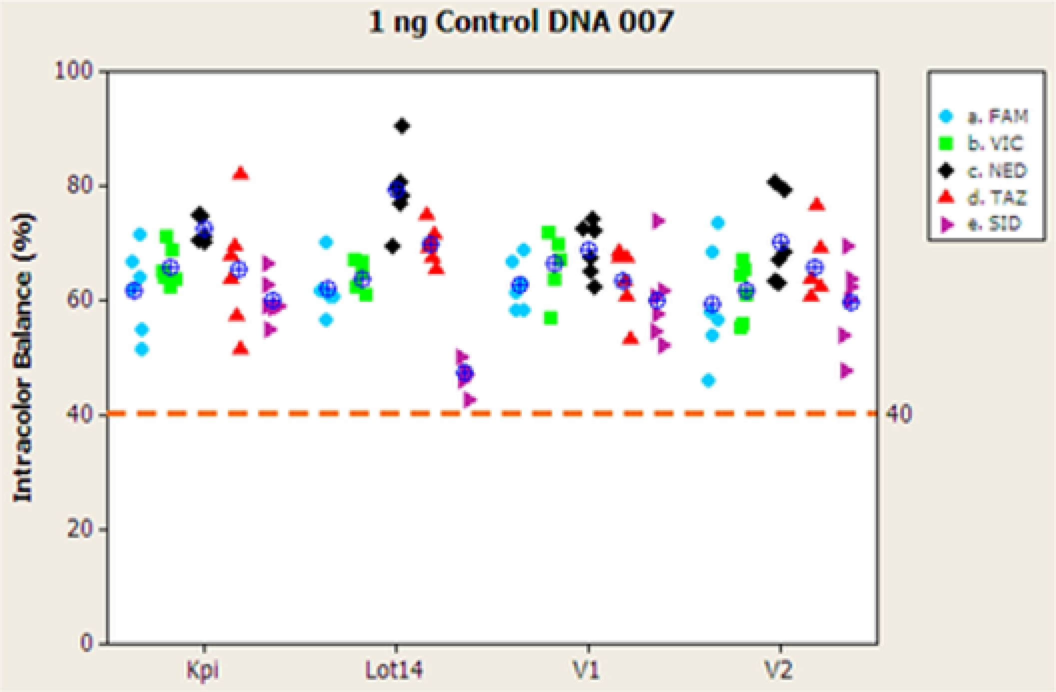


Figure 54 Average intracolor balance percentage plotted by dye channel for the 1 ng replicates from the sensitivity study generated using the, Kpi, Lot 14, V1 and V2 master mixes.

Intercolor balance

Intercolor balance was calculated by dividing the total normalized average signal in each dye channel by the total normalized signal from all dye channels. A completely balanced profile would be indicated by all dye colors representing 20% of the total signal. No significant differences were observed between the freshly prepared master mixes V1, V2 and Kpi with regard to intercolor balance for the 1 ng input DNA samples (Figure 55). The NED™ dye channel peaks for profiles generated using the Lot 14 master mix were higher than the other dye channels and yielded a higher intercolor balance percentage than NED™ dye channel peaks generated using the fresh master mixes.

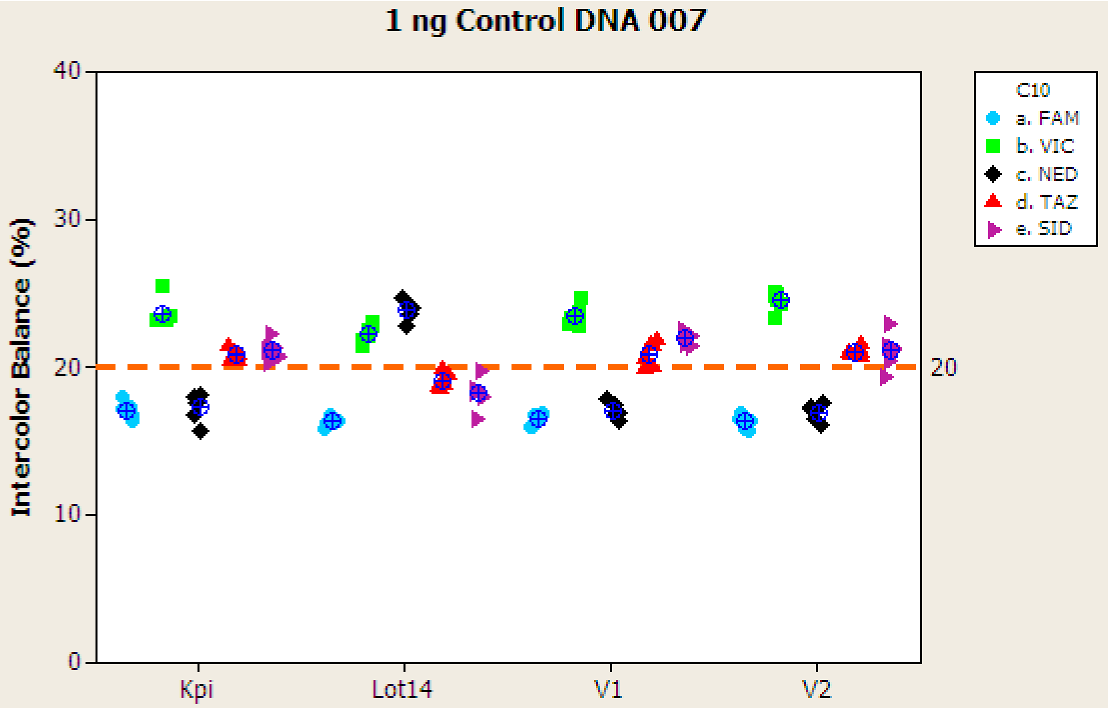
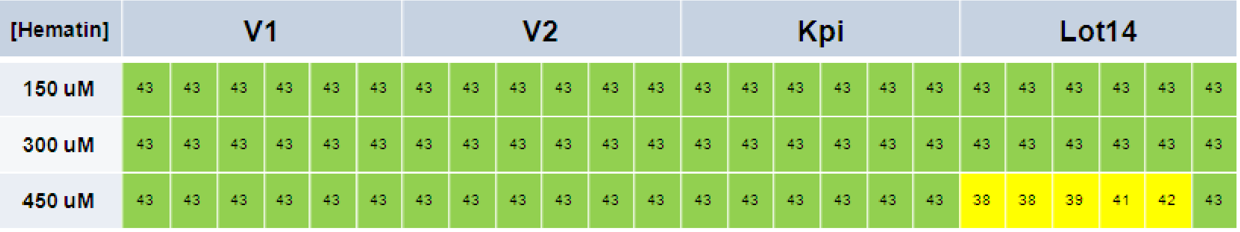


Figure 55 Average intercolor balance percentage plotted by dye channel for the 1 ng replicates from the sensitivity study generated using the Kpi, Lot 14, V1 and V2 master mixes.

Stability study results

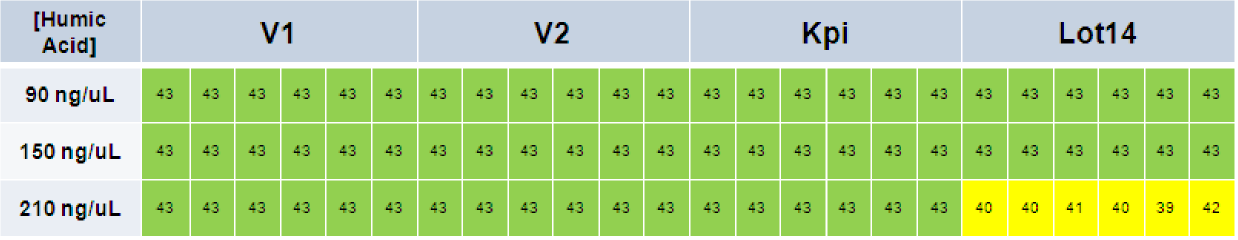
Inhibitor study

Comparable allele recoveries were obtained for the freshly prepared V1, V2, and Kpi master mixes (Figure 56 and Figure 57). Some allele dropout was observed with the Lot 14 master mix at the highest inhibitor concentrations. Compared to the freshly prepared master mixes, lower average peak heights were also observed with the Lot 14 master mix.



[Hematin]	V1						V2						Kpi						Lot14					
150 uM	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
300 uM	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
450 uM	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	38	38	39	41	42	43

Figure 56 Allele recoveries for each replicate generated from 1-ng input DNA amounts containing hematin concentrations ranging from 150 µM to 450 µM using each of the master mix formulations. The maximum number of alleles recoverable is 43.

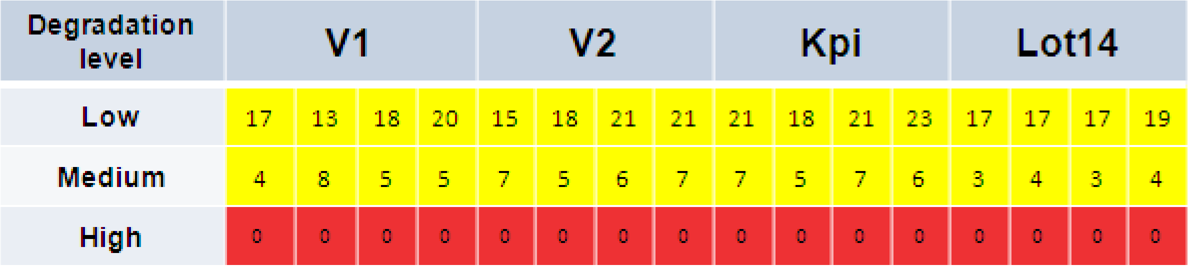


[Humic Acid]	V1						V2						Kpi						Lot14					
90 ng/uL	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
150 ng/uL	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
210 ng/uL	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	40	40	41	40	39	42

Figure 57 Allele recoveries for each replicate generated from 1-ng input DNA amounts containing humic acid concentrations ranging from 90 ng/µL to 210 ng/µL using each of the master mix formulations. The maximum number of alleles recoverable is 43.

Degraded DNA

Control Raji DNA was sonicated and treated with varying amounts of DNase I (4-6 U) for 20 minutes. 1 ng of each degraded sample was amplified in 4 replicates using each of the master mixes. Comparable allele recoveries were obtained for the fresh master mixes V1, V2, and Kpi (Figure 58). A slightly higher degree of allele dropout was observed with Lot 14 master mix under medium degradation conditions.



Degradation level	V1				V2				Kpi				Lot14			
Low	17	13	18	20	15	18	21	21	21	18	21	23	17	17	17	19
Medium	4	8	5	5	7	5	6	7	7	5	7	6	3	4	3	4
High	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 58 Allele recoveries for each replicate generated from 1-ng input DNA sonicated and treated with low (4U), medium (5U), and high (6U) amounts of DNase I. The maximum number of recoverable alleles is 43.

Population study results

Genotypes-intralocus balance

All 72 genomic DNA samples yielded full, concordant genotypes for all master mixes.

All 22 heterozygous loci yielded intralocus balances >65% for all master mixes.

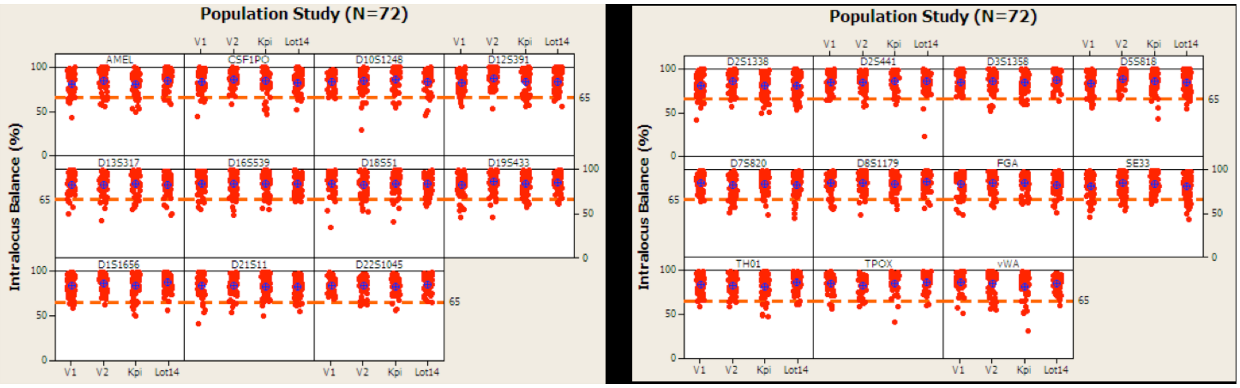


Figure 59 Average intralocus balance percentage plotted by locus for the 22 heterozygous loci from the population study (N=72) generated using the V1, V2, Kpi and Lot 14 master mixes.

All dye channels for data generated from all master mixes yielded average intracolor balances of >40% (Figure 59). The average SID™ intracolor balance was slightly lower for Lot 14 master mix than for data generated from the fresh master mixes V1, V2 and Kpi.

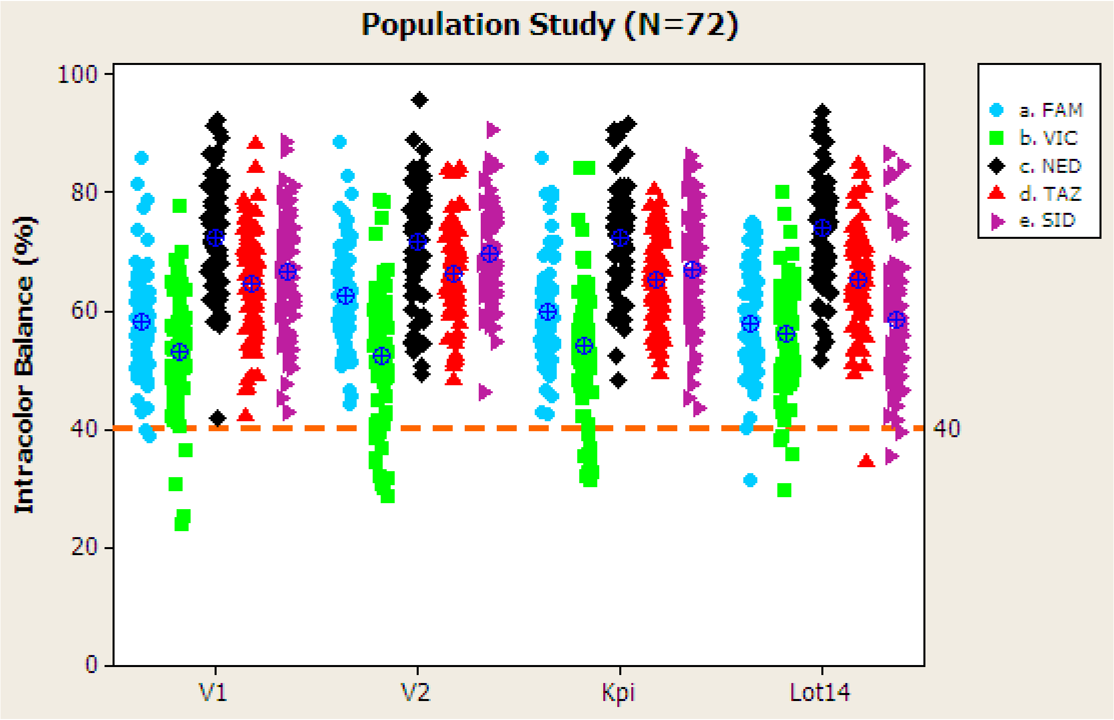
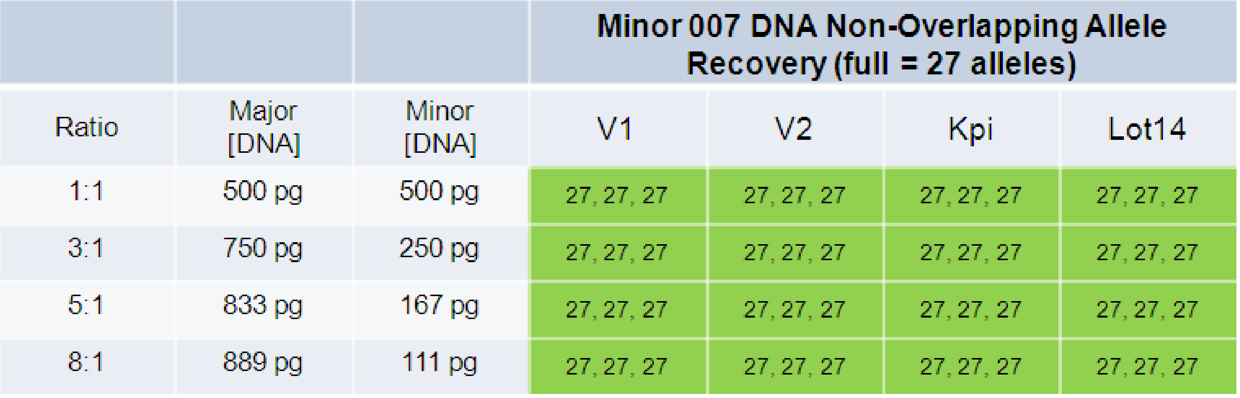


Figure 60 Average intracolor balance (%) plotted by dye channel for the 72 genomic DNA samples generated using the V1, V2, Kpi, and Lot 14 master mixes.

Mixture study results

All 27/27 non-overlapping alleles from the DNA Control 007 minor contributor were recovered in all mixture samples amplified using all 4 master mix formulations (Figure 61, Figure 62, and Figure 63). The lowest minor contributor represented approximately 111 pg of the minor contributor template DNA added.



			Minor 007 DNA Non-Overlapping Allele Recovery (full = 27 alleles)			
Ratio	Major [DNA]	Minor [DNA]	V1	V2	Kpi	Lot14
1:1	500 pg	500 pg	27, 27, 27	27, 27, 27	27, 27, 27	27, 27, 27
3:1	750 pg	250 pg	27, 27, 27	27, 27, 27	27, 27, 27	27, 27, 27
5:1	833 pg	167 pg	27, 27, 27	27, 27, 27	27, 27, 27	27, 27, 27
8:1	889 pg	111 pg	27, 27, 27	27, 27, 27	27, 27, 27	27, 27, 27

Figure 61 Recovery of the minor contributor non-overlapping alleles in Raji:007 DNA mixtures. The maximum number of recoverable non-overlapping alleles is 27.

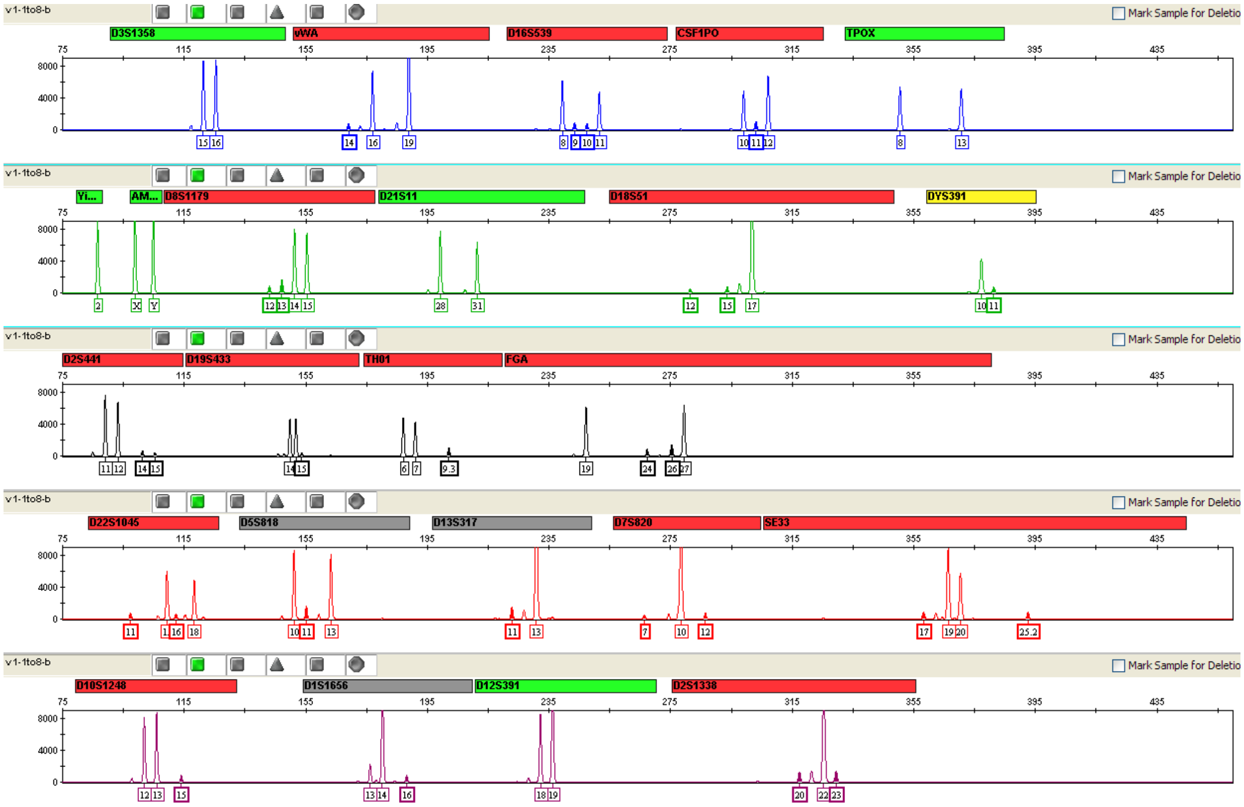


Figure 62 Electropherogram depicting the recovery of all non-overlapping minor alleles in the 8:1 Raji:007 DNA mixture with the V1 master mix formulation. The minor alleles are highlighted.

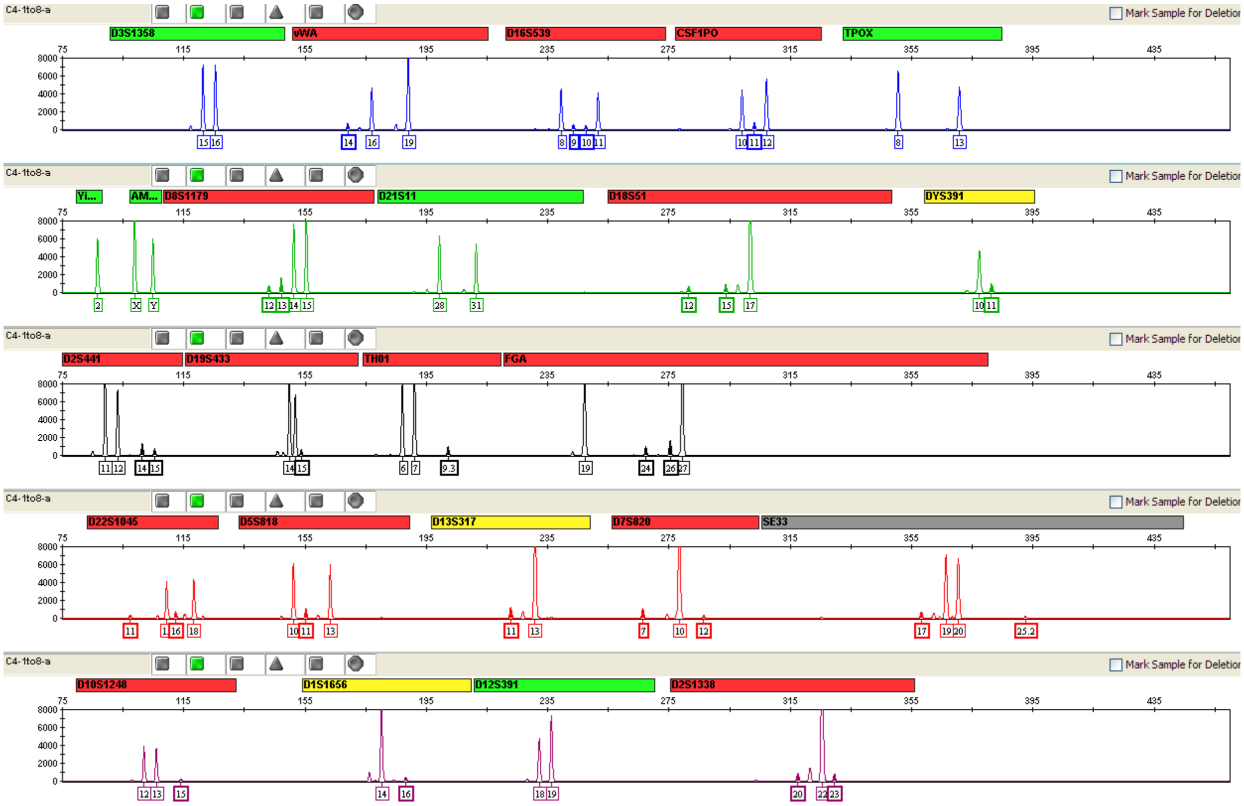


Figure 63 Electropherogram depicting the recovery of all non-overlapping minor alleles in the 8:1 Raji:007 DNA mixture with the Lot 14 master mix formulation. The minor alleles are highlighted.

Conclusion

The freshly prepared master mixes using BSA formulated in Tris buffer (V1 and V2) performed comparably to the freshly prepared master mix using the original phosphate buffer formulation (Kpi) in studies of degraded and inhibited samples and detection of minor contributor alleles in 2-person mixtures.

The sensitivity data set yielded comparable results for intralocus balance, intracolor balance, intercolor balance, and stutter percentage. The V1 and V2 master mixes yielded higher average peak heights than the Kpi master mix, resulting in a statistically significant difference. However, this difference was only significant for the 1-ng and 500-pg input amounts, and peak height has been demonstrated to be highly variable.

100% concordant genotyping results were obtained from a population data set of 72 genomic DNA samples. The on-market Lot 14 master mix performed comparably to the V1, V2 and Kpi formulations for allele recovery at low input DNA amounts, intralocus balance, and minor nonoverlapping allele recovery from mixture samples. Samples amplified with Lot 14 master mix yielded lower average peak heights, lower SID™ intracolor balance, and higher NED™ intercolor balance than the freshly prepared master mixes, which is consistent with Mg^{2+} depletion. However, all formulations met the specifications for allele recovery (full profile at 125 pg), intralocus balance (>65%), and intracolor balance (>40%), and yielded no reproducible artifacts in the non-template controls.

Based on our internal validation study results, equivalent quality profiles can be obtained across a wide range of forensic samples when using the GlobalFiler™ kit containing the BSA formulated in Tris-HCl buffer compared to the original kit containing the BSA formulated in phosphate buffer.

8

Performance verification with 30 PCR cycles

Experiment overview

Analysis of challenging samples or samples with a low quantity of DNA may not yield full profiles. Extending the PCR for an additional cycle can increase the sensitivity of the assay and improve the genotyping result for these samples.

We conducted the following studies to compare the performance of the GlobalFiler™ kit at 29 and 30 PCR cycles.

- Minimum threshold and contamination study
- Sensitivity study
- Inhibited sample study
- Degraded sample study

Materials, methods, and samples

Samples were prepared and amplified side-by-side:

- One lot of the GlobalFiler™ kit was used
- One male and one female genomic DNA samples were used for the sensitivity study and were quantified with the Quantifiler™ Trio DNA Quantification Kit on a 7500 Real-Time PCR System. Serial dilutions of 16 pg–2 ng were prepared using TE buffer (10 mM Tris-HCl pH 8.0 and 0.1 mM EDTA). Dilution series of both DNA samples were amplified in triplicate.
- Samples were amplified with 29 and 30 PCR cycles on a 96-well GeneAmp™ PCR System 9700 using the thermal cycling conditions listed in the PCR chapter.
- Sample files were acquired on 3500xL Genetic Analyzer with 3500 Data Collection Software v3.1 using injection conditions listed in the electrophoresis chapter.
- Sample files were analyzed with GeneMapper™ *ID-X* Software v1.5 using analysis settings listed in the analysis chapter.

Study	Samples
Minimum threshold and contamination	15 negative control samples (samples that do not contain DNA) with 1 RFU as the peak amplitude threshold. Data from 60–460 bp were collected.
Sensitivity	• Male genomic DNA sample obtained from the NIGMS Human Genetic Call Repository at the Coriell Institute for Medical Research: NA07057. • Female genomic DNA sample: IMR90. Dilution series of 16 pg–2 ng of both DNA samples were amplified in triplicate.
Degraded sample	Artificially degraded male DNA samples. Two levels of degraded DNA samples (low-degraded and medium-degraded) at two input amounts (1 ng and 0.5 ng) were amplified.
Inhibited sample	3 concentrations of hematin and humic acid, 3 replicates of each inhibitor.

Minimum threshold and contamination study results

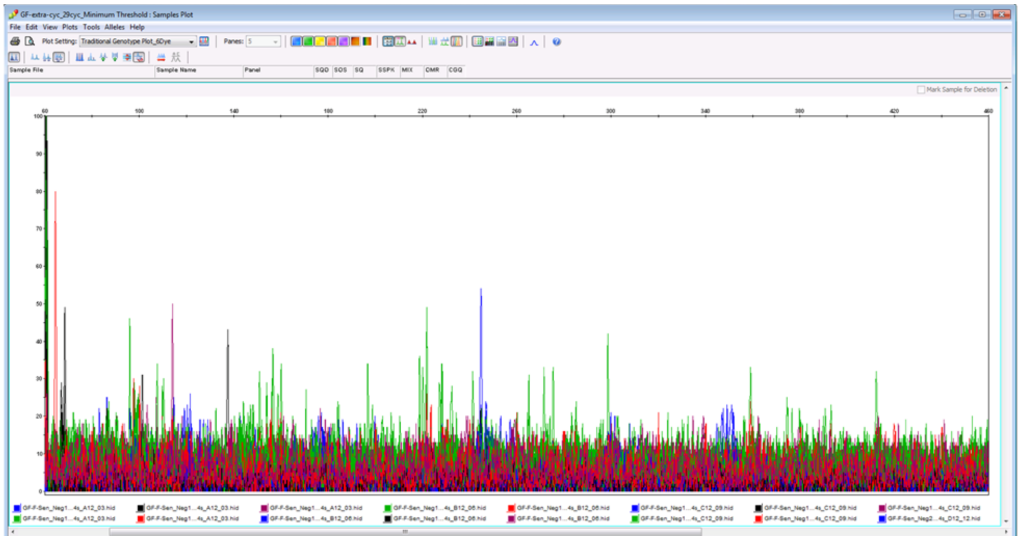
The following statistics were calculated for the peak heights (RFU) observed in each dye channel for 29 and 30 cycles:

- Maximum
- Average
- Standard deviation
- Limit of Detection (LOD): average plus 3 standard deviations; the RFU value below which 99.7% of the background “noise” peaks should be observed.
- Limit of Quantification (LOQ): average plus 10 standard deviations, provides an upper limit value below which all or nearly all background “noise” is expected to fall.

Figure 64 and Figure 65 show the results of the study. The background noise level is low for samples without human DNA. The minimum thresholds were established by rounding the LOQ to the nearest multiple of 5. At the standard 29 cycles, the average peak heights of background were 4–10 RFU, and the minimum thresholds were 35–60 RFU on blue, green, yellow, red and purple channels. The average peak heights of background increased to 5–11 RFU, and the minimum thresholds were increased to 45–80 RFU by one extra cycle (30 cycles).

Table 18 Calculated Minimum Threshold of 29 and 30 cycles

Average Peak Height (RFU) Dye	Maximum Peak Height (RFU)		Average Peak Height (RFU)		Standard Deviation		Average + 3 Standard Deviations (LOD)		Average + 10 Standard Deviations (LOQ)		Minimum Threshold (RFU)	
	Cycles		Cycles		Cycles		Cycles		Cycles		Cycles	
	29	30	29	30	29	30	29	30	29	30	29	30
Blue (6-FAM™)	54	54	5	6	2.9	4.7	14	20	34	53	35	55
Green (VIC™)	96	99	10	11	5.2	6.7	26	31	62	78	60	80
Yellow (NED™)	96	100	4	5	3.7	4.7	16	19	41	52	40	50
Red (TAZ™)	80	78	8	8	3.2	3.7	17	19	40	45	40	45
Purple (SID™)	50	38	9	9	3.1	3.5	18	20	40	44	40	45



Plot Setting: Traditional Genotype Plot_6Dye

Panes: 5

Figure 64 All negative samples at 29 cycles overlaid in all dyes except for LIZ™dye (Y-axis scale 0 to 100 RFU)

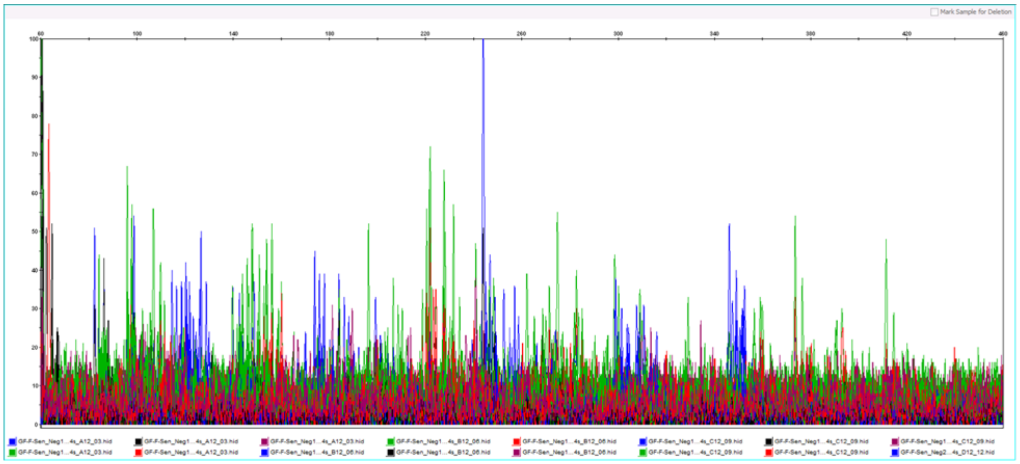


Figure 65 All negative samples at 30 cycles overlaid in all dyes except for LIZ™dye (Y-axis scale 0 to 100 RFU)

Sensitivity study results

The results in Table 19 show that one extra cycle (30 cycles) improved the genotyping results in single-source low-input DNA samples at 0.031 ng and lower with no or minimal artifacts when compared to results generated by 29 cycles.

Table 19 Average allele call percentages at low DNA input levels—29 vs 30 cycles

DNA input	Male DNA 07057		Female DNA IMR90	
	29 cycles	30 cycles	29 cycles	30 cycles
0.5 ng	100%	100%	100%	100%
0.25 ng	100%	100%	100%	100%
0.125 ng	100%	100%	100%	100%
0.063 ng	98%	99%	100%	100%
0.031 ng	71%	90%	56%	89%
0.16 ng	11%	53%	7%	51%

Inhibited sample study results

Allele dropout was observed in samples with high concentration (210 ng/uL) of humic acid (Figure 66). No allele dropout was observed in samples with low (90 ng/uL) or medium (150 ng/uL) concentration of humic acid, or in samples with any concentration of hematin.

One extra cycle (30 cycles) did not recover the allele dropout presented at 29 cycles in humic acid inhibited samples, thus had no effect in improving the genotyping results in the presence of this inhibitor.

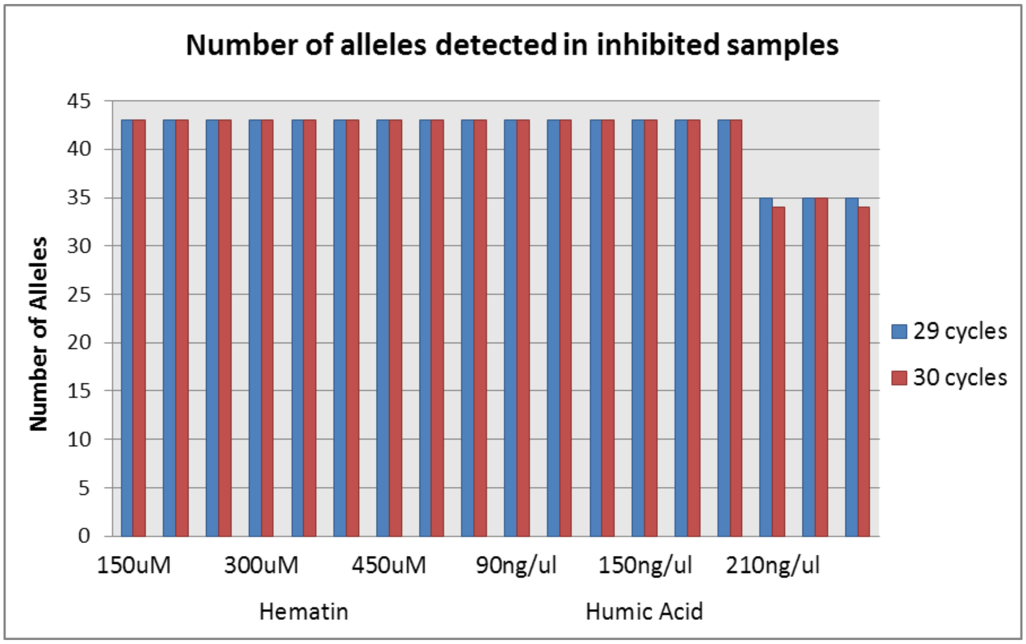


Figure 66 Number of allele calls for inhibited samples—29 vs 30 cycles

Degraded sample study

One extra cycle (30 cycles) decreased the allele dropout rate in both low-degraded and medium-degraded samples at both 1 ng and 0.5 ng DNA input, compared to standard 29 cycles (Figure 67).

In the 1 ng medium-degraded sample, off-scale peaks, pull-up peaks, elevated and additional stutter peaks, and other artifacts were observed.

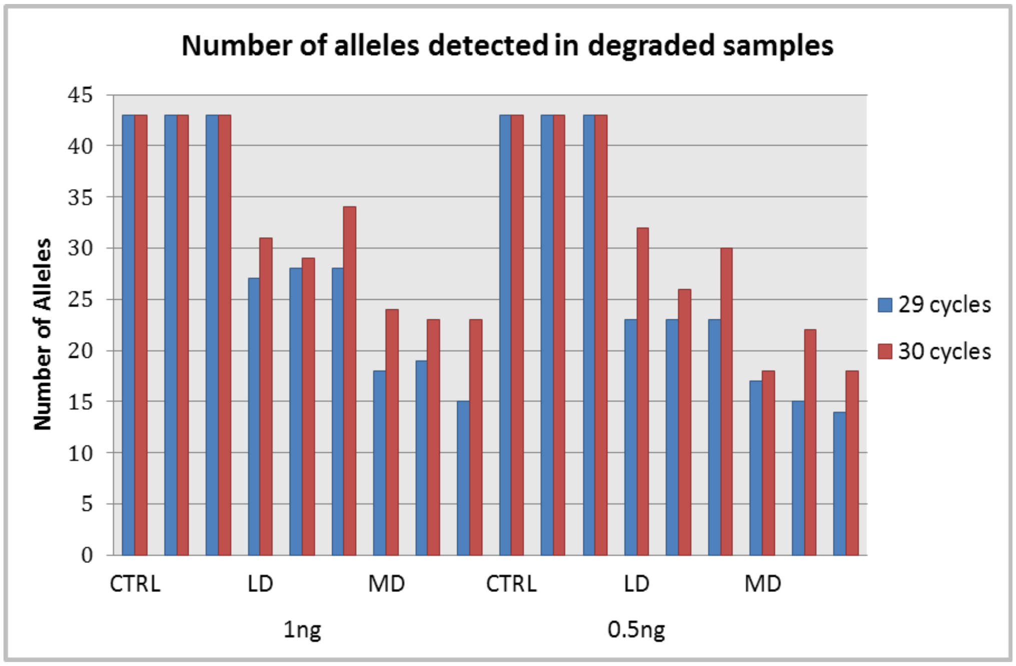


Figure 67 **Number of allele calls for degraded samples—29 vs 30 cycles. CTRL=DNA Control 007, LD=low degradation, MD=medium degradation,**

Conclusion

One additional PCR cycle improved allele recovery with minimal side effects. The greatest improvement was seen with samples containing low concentrations of DNA, with improvements in allele recovery also seen in degraded samples to a lesser extent. An additional PCR cycle is a viable option to improve performance of challenging samples.

9

Performance verification with the GlobalFiler™ IQC kit

Experiment overview

We performed validation studies to compare the performance of the GlobalFiler™ IQC kit and the GlobalFiler™ kit.

We focused on studies most relevant to forensic DNA testing (see SWGDAM Guidelines effective December 2016). These studies, although not exhaustive, are in our opinion appropriate for a manufacturer:

- Sensitivity
- Performance with inhibited and degraded samples
- PCR component concentration and PCR cycle analysis
- IQC Peak Sizing precision
- Species specificity
- Genotype concordance (population study)
- Mixture (2-contributor)
- Extra peaks in the electropherogram

Other performance characteristics, such as STR allele frequencies in human populations, random match probabilities, and sizing accuracy and precision of STR alleles have already been extensively characterized for the GlobalFiler™ kit and also apply to the GlobalFiler™ IQC kit. For more information, see:

- Chapter 6, "Experiments and results"
- Chapter 7, "Performance verification after BSA component replacement"

Materials, methods, and samples

Materials

The following kits were used in the studies:

- GlobalFiler™ IQC PCR Amplification Kit—One lot
- GlobalFiler™ PCR Amplification Kit—Lot 1809087

Methods

Unless otherwise noted, experiments were performed with the following instruments and software using the conditions recommended in Chapters 2, 3, and 4:

- ProFlex™ PCR System
- 3500xL Genetic Analyzer with 3500 Series Data Collection Software v3.1 or v4.0.1
- 3130*xl* Genetic Analyzer with Data Collection Software v4
- GeneMapper™ *ID-X* Software v1.5.2 or 1.6
- JMP™ statistical software for subsequent data analysis

Samples and test cases

All test cases were run on a 3500xL Genetic Analyzer. Test cases in which a 3130*xl* Genetic Analyzer was also used are noted below.

Test case	Description
Sensitivity	DNA Control 007 was amplified at 8 input amounts for 29 PCR cycles: 2 ng, 1 ng, 500 pg, 250 pg, 125 pg, 62 pg, 31 pg, and 16 pg. Four replicates were tested for each concentration with the GlobalFiler™ IQC and GlobalFiler™ kits.
Inhibited DNA	DNA Control 007 was amplified at 1 ng input for 29 PCR cycles in the presence of hematin (500 µM, 600 µM and 700 µM) and humic acid (150 ng/µL, 225 ng/µL, and 300 ng/µL). Four replicates were tested for each concentration with the GlobalFiler™ IQC and GlobalFiler™ kits.

(continued)

Test case	Description
Degraded DNA	A human male genomic DNA was ultrasonically sheared, then incubated with DNase I enzyme for varying times to create fractions with different degrees of degradation. Four fractions (undegraded control, low, medium, and high degraded fractions) were amplified at 0.5 ng input for 30 PCR cycles with the GlobalFiler™ IQC and GlobalFiler™ kits.
PCR component and PCR cycle analysis	• Four replicates of DNA Control 007 were amplified at 1 ng for 29 PCR cycles with varying GlobalFiler™ IQC and GlobalFiler™ primer set and master mix concentrations. • Four replicates of DNA Control 007 were amplified at 1 ng for 28, 29, 30, and 31 cycles with the GlobalFiler™ IQC kit.
IQC peak sizing precision	24 replicate samples of the GlobalFiler™ IQC Allelic Ladder were injected 4 times (serially) on 3500xL instrument. The experiment was repeated on the 3130*xl* instrument with 16 replicates.
Species specificity	The following samples were tested for cross-reactivity with the GlobalFiler™ IQC and GlobalFiler™ kits: • DNA for dog, cat, horse, hamster, rat, chicken, pig, rabbit and cow (10 ng input) • DNA for primates: gorilla, chimpanzee, and macaque (1.0 ng input) • Pool of microorganism DNA (approximately 100,000 genomic equivalents) • Total microbial population DNA extracted from soil (30 ng input)
Genotype concordance (population study)	• NIST SRM 2391c genotyping standard reference material was amplified at 1ng input with the GlobalFiler™ IQC kit for 29 cycles. • 82 human population samples were amplified at 1ng input with the GlobalFiler™ IQC kit for 29 cycles. Samples were run on two 3500xL instruments and one 3130*xl* instrument.
Mixture study	Two-contributor DNA mixtures were prepared with male DNA Control 007 and human female Control DNA 9947A in different mixing ratios to test with the GlobalFiler™ IQC kit. Samples were prepared so they always contained 1.0 ng of total DNA between the two contributors in mixture ratios of 1:0, 0:1, 1:1, 1:7 and 7:1 (male:female).
Extra peaks in the electropherograms (stutter and artifact)	DNA Control 007 was amplified with the GlobalFiler™ IQC and GlobalFiler™ kits at the same time using the same lot of master mix. Twelve replicate reactions with each kit, each reaction containing 1 ng of input DNA, were amplified for 29 cycles. Resulting stutter levels for different STR loci were compared between kits.

Sensitivity study results

A study was performed to compare the detection sensitivity of the GlobalFiler™ IQC kit and GlobalFiler™ kit with a dilution series of DNA Control 007 (human male genomic DNA). Dilutions were made in 2-fold increments to give a range of 2 ng down to 0.016 ng of DNA per 25 µL PCR reaction. PCR reactions were amplified for 29 cycles.

Example GlobalFiler™ IQC kit electropherograms for some of the DNA Control 007 dilutions are shown in Figure 68. (GlobalFiler™ kit profiles are not shown but gave very similar results.) Both kits gave full profiles of DNA Control 007 alleles (43 alleles total) in all four replicate PCRs with 125 pg of input DNA. While partial allele profiles were obtained for lower-concentration dilutions (some full profiles with 62.5 pg and all partial profiles for 31.2 pg and 16 pg) two-tailed t-tests (α=0.05) showed that the allele counts for replicate PCR reactions between the two kits were statistically equivalent for all dilutions.
A peak height comparison between the GlobalFiler™ IQC kit and GlobalFiler™ kit for 1-ng samples, by locus, is shown in Figure 69.

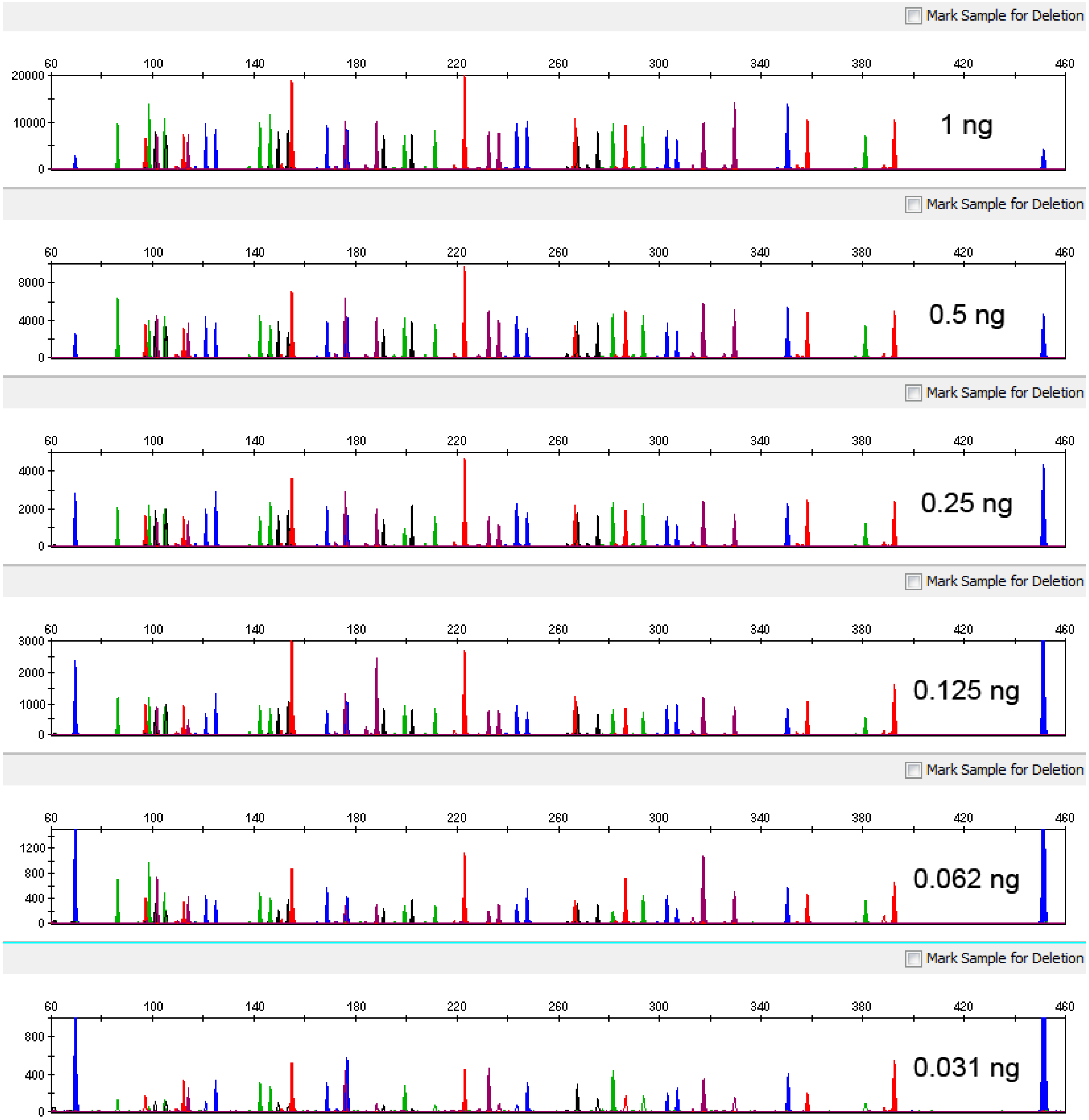


Figure 68 Example electropherograms are shown for GlobalFiler™ IQC kit reactions of a 2-fold dilution series of DNA Control 007 (human male genomic DNA). The Y-axis was adjusted to provide better visibility of lower-concentration dilutions.

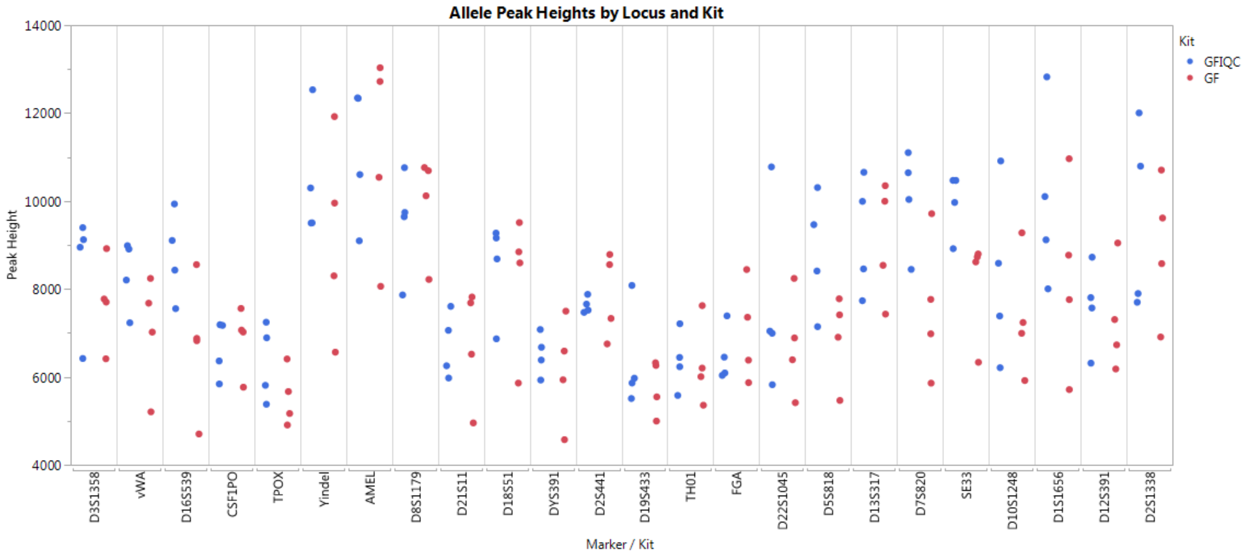


Figure 69 **Mean heterozygote peak heights are shown for replicate PCRs of the 1‑ng DNA Control 007 dilution, plotted by locus (blue=GlobalFiler™ IQC kit data, red=GlobalFiler™ kit).**

Two other important performance metrics were extracted from results for the 1‑ng DNA Control 007 dilution samples:

- **Intralocus balance** (ILB, the peak height ratio)—The mean ILB percentage for the GlobalFiler™ IQC kit and the GlobalFiler™ kit for 1‑ng reactions was 88.9% and 87.8%, respectively. These values are statistically equivalent (two-tailed t-test, α=0.05).
- **Intracolor balance** (ICB, the ratio of the highest and lowest average peak heights per locus within each dye channel)—ICB, assessed separately for each dye channel, showed a range from a low of ~56% for the green (VIC™ dye) channel in both kits, to a high of 73–80% for the yellow (NED™ dye) channel. The ICB was statistically equivalent for all dye channels in the GlobalFiler™ IQC kit and GlobalFiler™ kit (two-tailed t-tests, α=0.05).

Note: The FAM™-dye -labeled IQC peaks were omitted from ILB and ICB calculations.

Inhibited sample study results

Studies with models of PCR inhibition were performed to mimic the challenging sample types that are sometimes encountered in forensic casework analysis. To examine and compare the effects of two such model systems with the GlobalFiler™ IQC kit and GlobalFiler™ kit, samples were prepared with 1.0 ng of human male DNA Control 007 and varying concentrations of humic acid and hematin, including a control sample that did not contain PCR inhibitor. Humic acid concentrations were 150 ng/µL, 250 ng/µL, and 350 ng/µL. Hematin concentrations were 500 µM, 600 µM, and 700 µM (final concentrations in PCR reactions). PCR reactions were amplified for 29 cycles.

Example electropherograms with humic acid and hematin are shown in Figure 70 and Figure 71. With both inhibitors, peak height decreased (and was most pronounced for the IQCL marker) with increasing inhibitor concentrations. The IQCL peak was completely absent in the two highest inhibitor concentrations.

Inhibited and degraded DNA samples can have similar-looking profiles with poor amplification of larger STR alleles resulting in relatively low peak heights or drop-out peaks ("ski slope" profile shown below). In an inhibited sample, the IQCL peak height decreases in the presence of inhibitors. In a degraded sample, the IQCL peak does not decrease.

Note: Electropherograms and allele count information for degraded samples are shown in Figure 72 on page 163 and Figure 73 on page 164.

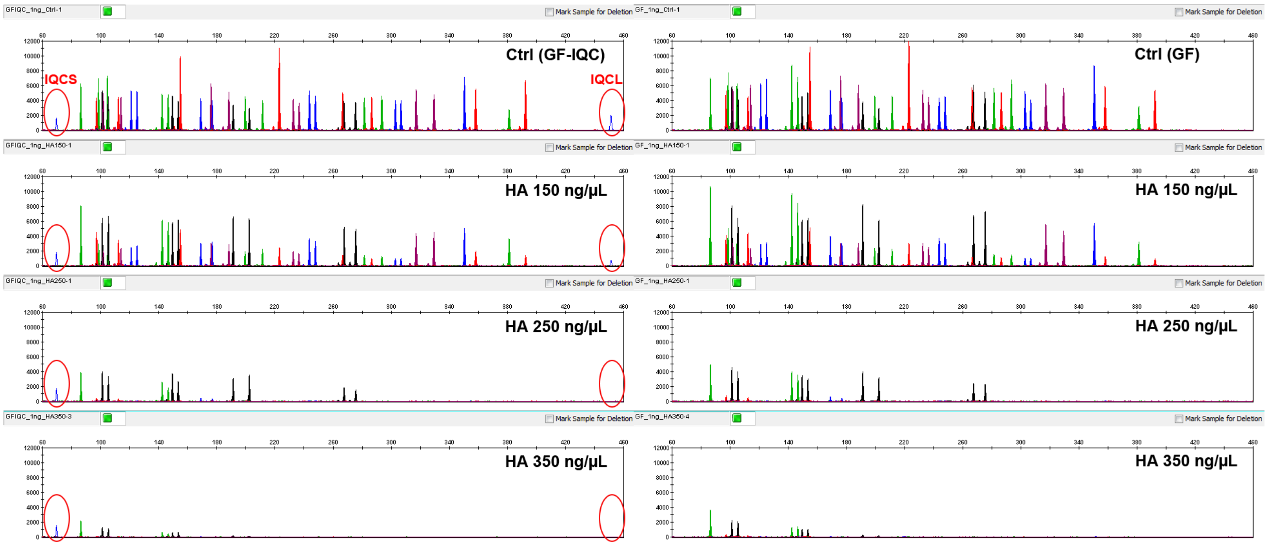


Figure 70 GlobalFiler™ IQC kit (left) and GlobalFiler™ kit (right) with humic acid. The red ovals in the GlobalFiler™ IQC kit electropherograms indicate the IQCS and IQCL peaks (Y-axis scale 12,000 RFU).

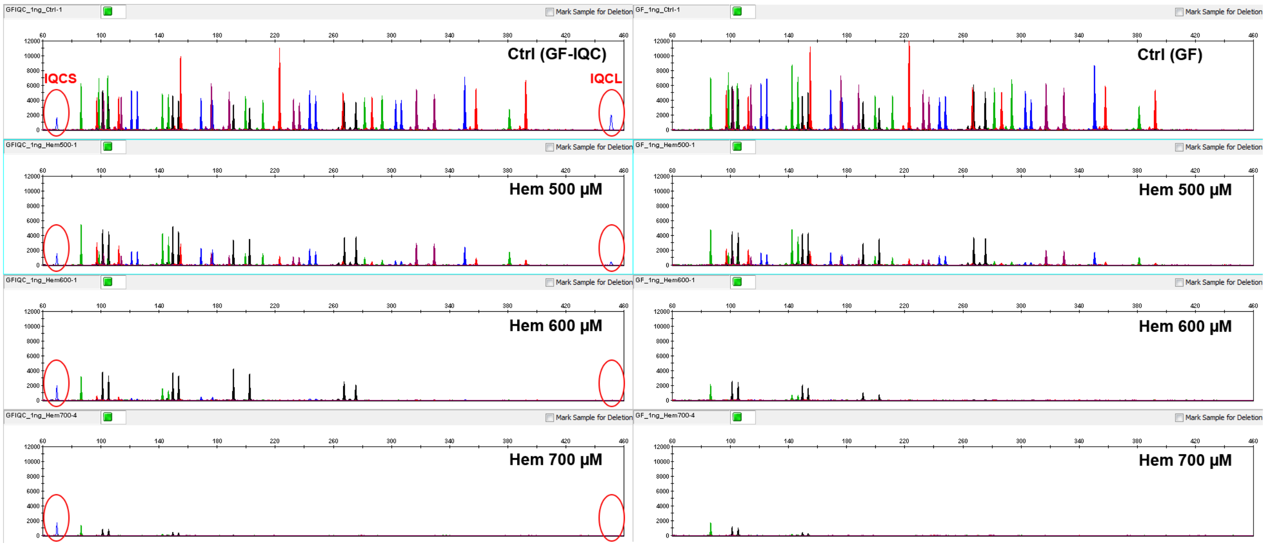


Figure 71 GlobalFiler™ IQC kit and GlobalFiler™ kit with hematin. The red ovals in the GlobalFiler™ IQC kit electropherograms indicate the IQCS and IQCL peaks (Y-axis scale 12,000 RFU).

Degraded sample study results

Degraded DNA was prepared by treating purified human male genomic DNA with a combination of ultrasonic shearing followed by digestion with DNase I exonuclease enzyme. This produced a series of fractions of the same genomic DNA with different levels of degradation. Four DNA fractions were analyzed (un-degraded control, and low, medium, and high degraded fractions).

The degraded fractions were added to GlobalFiler™ IQC and GlobalFiler™ kit reactions in amounts that were equalized to provide 0.5 ng of each DNA fraction per 25 µL PCR reaction. Thermal cycling was performed for 30 cycles.

Figure 72 shows example GlobalFiler™ IQC and GlobalFiler™ kit electropherograms from the degraded DNA study. Both kits produced similar numbers of alleles for the different fractions.

In contrast to inhibited samples, the IQCS and IQCL peaks are unaffected by the degree of DNA degradation in the samples.

Inhibited and degraded DNA samples can have similar-looking profiles with poor amplification of larger STR alleles resulting in relatively low peak heights or drop-out peaks ("ski slope" profile). In an inhibited sample, the IQCL peak height decreases in the presence of inhibitors. In a degraded sample, the IQCL peak does not decrease (as shown below).

Note: Figure 70 on page 161 and Figure 71 on page 161 show electropherograms for inhibited samples.

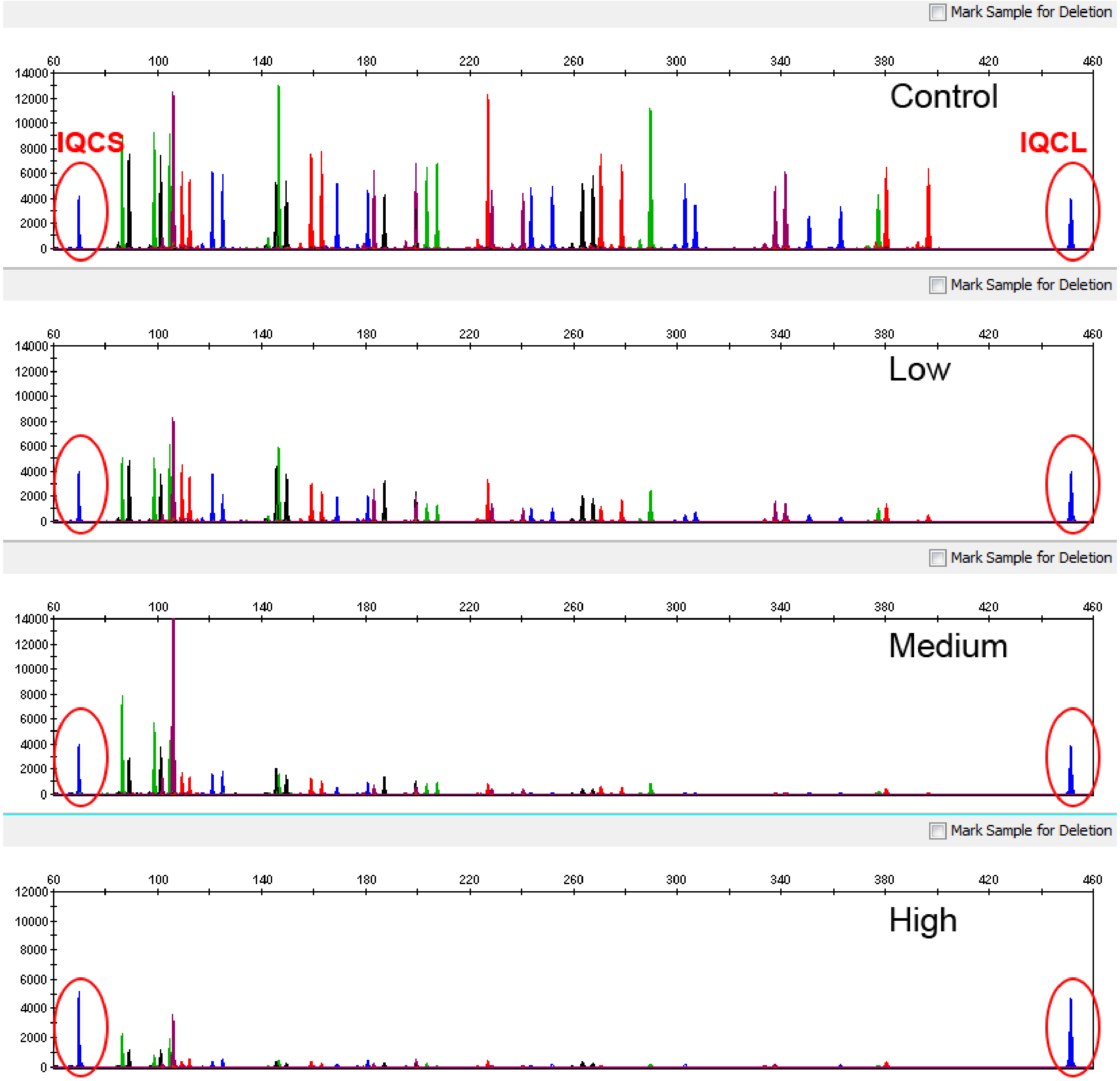


Figure 72 GlobalFiler™ IQC kit example electropherograms for degraded DNA

Figure 73 shows allele counts per replicate for degraded DNA. Low, medium and high inhibitor levels corresponded to 150 ng/µL, 250 ng/µL, and 350 ng/µL humic acid, or 500 µM, 600 µM, and 700 µM hematin; for degraded DNA, the levels corresponded to the extent of exonuclease digestion. Control samples were untreated and gave full allele profiles. Allele counts for each sample between the two kits were statistically equivalent (two-tailed t-tests, α = 0.05).

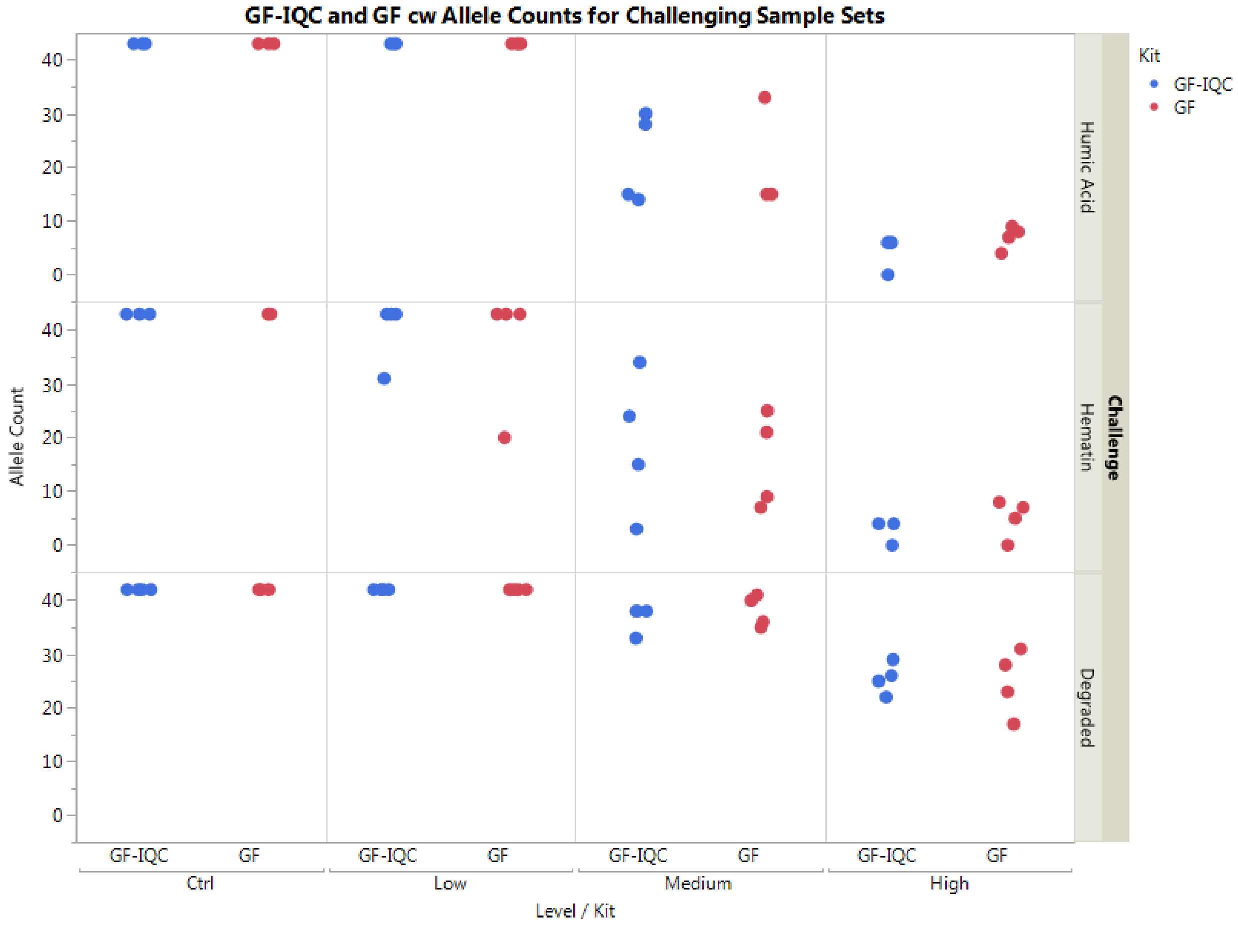


Figure 73 **GlobalFiler™ IQC kit (blue) and GlobalFiler™ kit (red) allele counts for inhibited and degraded DNA samples**

Figure 74 shows IQC Ratios (IQCL Peak Ht ÷ IQCS Peak Ht) for GlobalFiler™ IQC kit reactions with the three challenging sample types: humic acid (top), hematin (middle), and degraded DNA (bottom). Low, medium and high inhibitor levels corresponded to 150 ng/µL, 250 ng/µL, and 350 ng/µL humic acid, or 500 µM, 600 µM, and 700 µM hematin; for degraded DNA, the levels correspond to the extent of exonuclease digestion. Control samples were untreated.

Note that the IQC Ratio responds strongly to the level of PCR inhibitor and falls to 0 in the most highly inhibited samples, whereas it remains high for all degraded DNA samples.

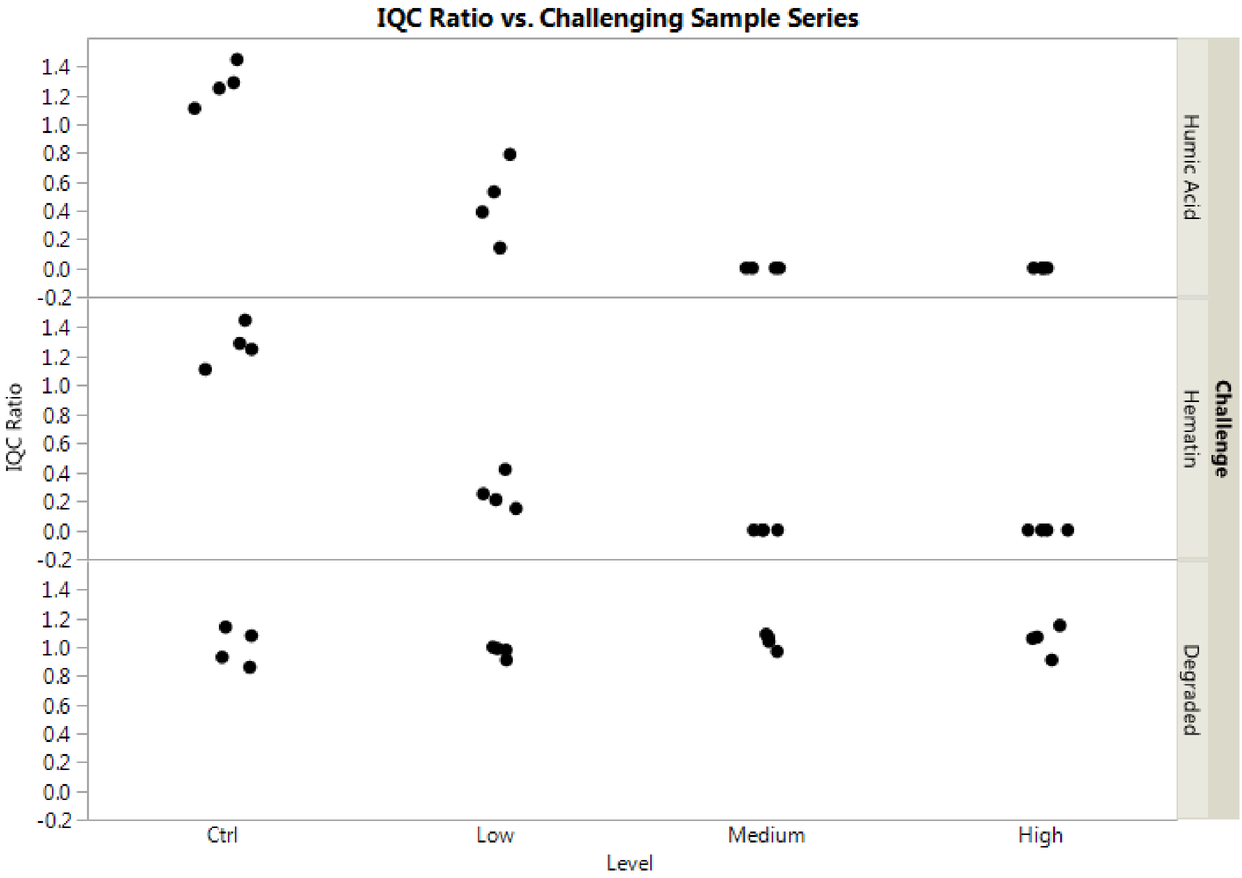


Figure 74 IQC Ratios (IQCL Peak Ht ÷ IQCS Peak Ht) for GlobalFiler™ IQC kit

PCR component concentration analysis results

Because the master mix formulation is identical between the GlobalFiler™ IQC kit and GlobalFiler™ kit, guard band studies that were originally performed on master mix components such as $MgCl_2$ for the GlobalFiler™ kit validation were not repeated (see Chapter 6, "Experiments and results"). However, a robustness performance check was performed by varying the relative amounts of primer set and master mix added to GlobalFiler™ IQC kit reactions.

In these experiments, the relative volumes of either master mix or primer set were varied (separately) between ±20% in 25 µL reaction volumes. Replicate amplification reactions with 1.0 ng of DNA Control 007 for each condition were amplified for 29 cycles.

Electropherograms were assessed for peak heights, intralocus balance (ILB), intracolor balance (ICB), and IQC ratio.

The ILB and ICB were largely insensitive to variations in primer set or master mix amount, as shown in Figure 75 and Figure 76. However, the average allele peak heights and the IQC ratio were higher or lower in response to higher or lower amounts of primer set or master mix. Importantly, both the GlobalFiler™ IQC kit and GlobalFiler™ kit showed similar responses to the variations in each component amount.

Note: The GlobalFiler™ kit does not contain the IQC markers. Therefore, the top-right area of the figures are blank.

Figure 75 Primer set amount variation for the GlobalFiler™ IQC kit (left) and GlobalFiler™ kit (right). Input is ±20% of the standard 2.5-µL input per 25-µL reaction (with the standard master mix amount) with 1.0 ng of DNA Control 007.

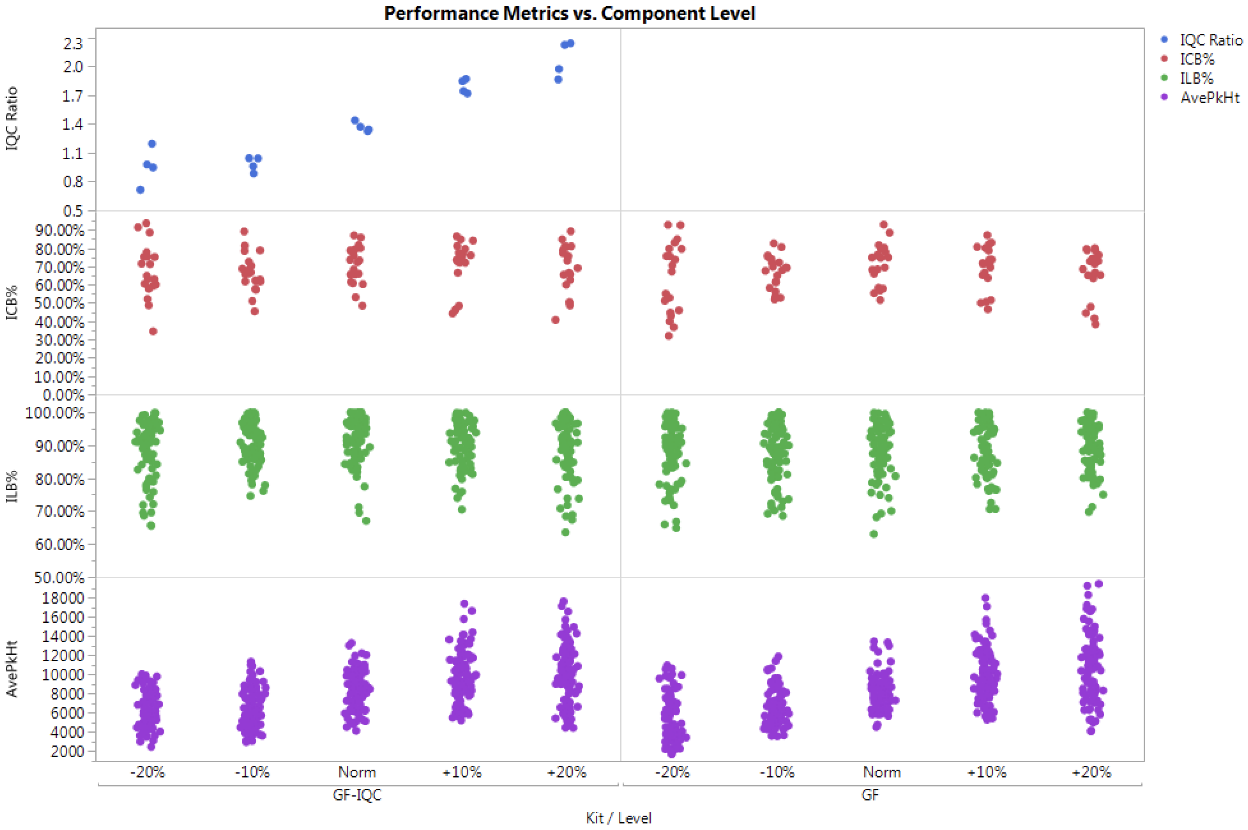


Figure 76 Master mix amount variation for the GlobalFiler™ IQC kit (left) and GlobalFiler™ kit (right). Input is ± 20% of the standard 7.5-µL input per 25-µL reaction (with the standard primer set amount) with 1.0 ng of DNA Control 007.

PCR cycle number analysis results

For this experiment, replicate PCR reactions were prepared with 1 ng of DNA Control 007 per reaction and distributed on four separate 96-well assay plates (4 replicate reactions per plate). The different plates were amplified for 28, 29, 30, and 31 cycles (Figure 77).

The GlobalFiler™ IQC kit and GlobalFiler™ kit are optimized for 29 or 30 cycles with 1.0 ng or 0.5 ng of human genomic DNA input, respectively. Full profiles were obtained for higher cycle numbers. However, off-scale peaks were observed for 30 and 31 cycles, and especially for 31 cycles. Minus-A shoulders were observed with several allele peaks, which indicate insufficient 3' adenylation of the labeled amplicon DNAs (a common occurrence in over-amplified PCR).

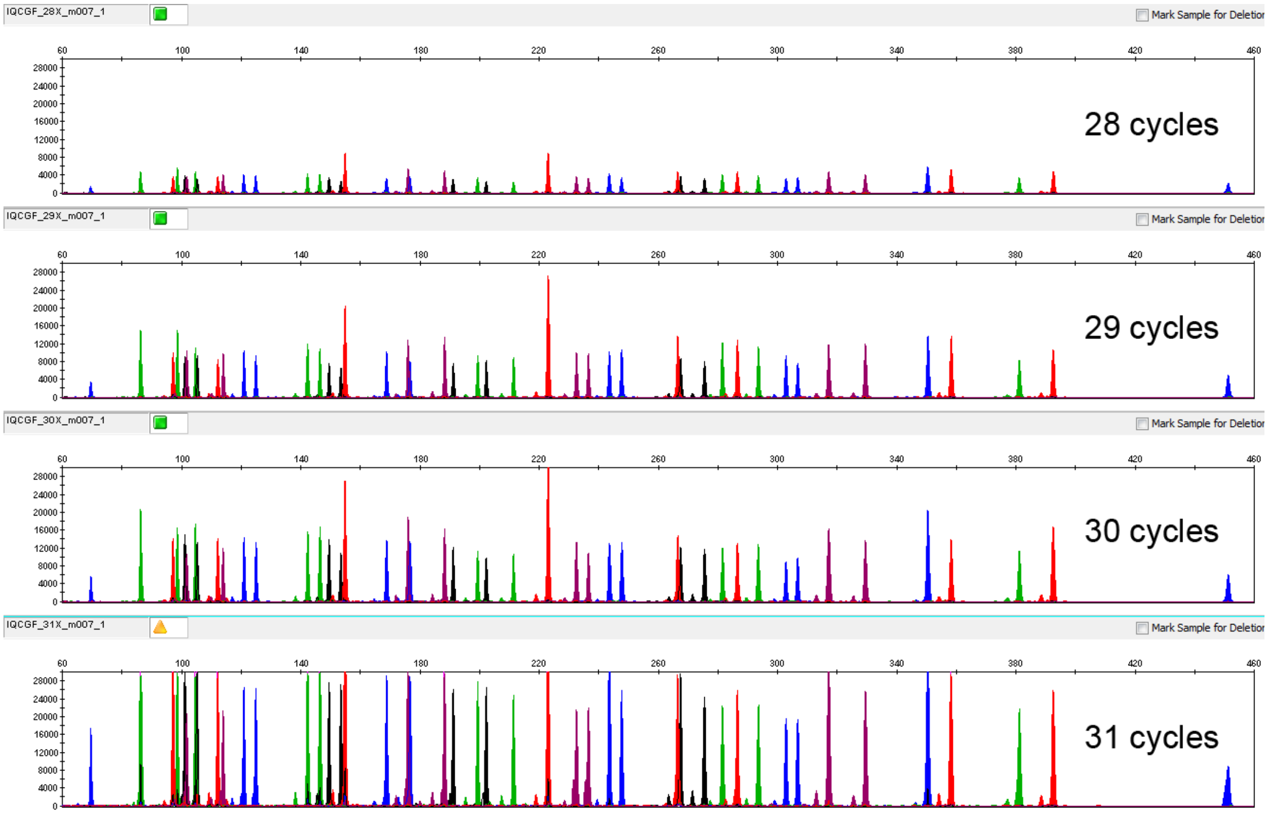


Figure 77 Representative GlobalFiler™ IQC kit electropherograms obtained from amplifying 1.0 ng of DNA Control 007 for 28, 29, 30, and 31 cycles (Y-axis scale 0–30,000 RFU).

IQC peak sizing precision

The sizing precision study of alleles for the 24 genetic loci in the GlobalFiler™ kit is presented in Chapter 6, “Experiments and results”. We performed a smaller study to assess the sizing precision of the IQCS and IQCL peaks.

Note: GeneMapper™ *ID-X* Software requires two alleles per marker for the IQC markers in the allelic ladder. In samples, you will observe only Allele 2 for the IQC peaks.

In this experiment, 24 replicate samples of the GlobalFiler™ IQC Allelic Ladder were prepared and dispensed into a 96-well MicroAmp™ 0.2-mL plate. After heat-denaturing and quenching on ice, the allelic ladder samples were injected 4 times (serially) on 3500xL and 3130*xl* instruments. Within each injection (24 wells on the 3500xL instrument and 16 wells on the 3130*xl* instrument), the minimum-maximum size ranges (in bp) and sizing standard deviations were calculated for the two "alleles" in the IQCS and IQCL markers in the allelic ladder.

The sizing standard deviation was less than ±0.15 bp for the IQC peaks.

Locus	3500xL		3130*xl*	
	Mean size per run	Std dev per run	Mean size per run	Std dev per run
IQCS-1	65.46–65.48	0.028–0.033	65.43–65.46	0.024–0.037
IQCS-2	69.6–69.61	0.028–0.032	69.55–69.58	0.023–0.042
IQCL-1	447.44–447.46	0.053–0.06	446.92–447.01	0.043–0.083
IQCL-2	451.32–451.32	0.041–0.058	450.78–450.86	0.061–0.078

Species specificity results

The GlobalFiler™ IQC kit and GlobalFiler™ kit provide the required specificity for detecting human alleles. Species specificity testing was performed to help ensure that there is minimal cross-reactivity with nonhuman DNA.

The following species were tested (in the specified amounts) using standard PCR and capillary electrophoresis conditions for both kits:

- **Primates**—Gorilla, chimpanzee, and macaque (1.0 ng each)
- **Non-primates**—Mouse, dog, pig, rabbit, cat, horse, hamster, rat, chicken, and bovine (10 ng each)
- **Human-associated microorganism pool**—*Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Bacillus subtilis*, and *Lactobacillus rhamnosus* (equivalent to 10^5 copies of each species per PCR)
- **Microbial total genomic DNA extracted from different soil environments**—Creek bed, forest and bay lands soils (30 ng per reaction).

Results were evaluated for the presence of any reproducible amplified peaks that would indicate cross-reactivity of the kits with any of the non-human species. Example electropherograms from species in which reproducible amplified peaks were observed are shown in Figure 78.

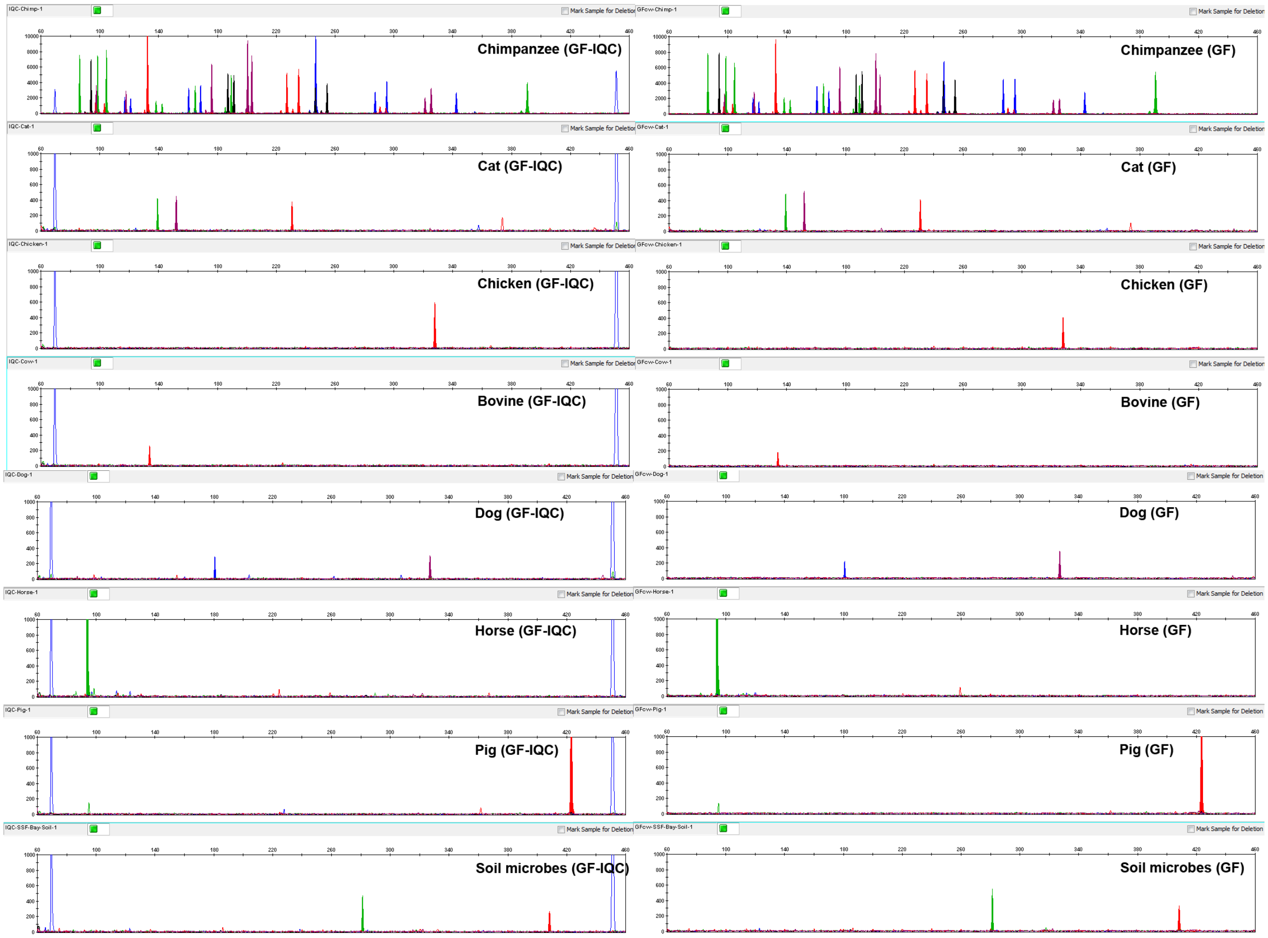


Figure 78 Example electropherograms for nonhuman species that produced reproducible cross-reactive peaks with the GlobalFiler™ IQC kit (left) and GlobalFiler™ kit (right). The Y-axis for chimpanzee DNA profiles (top) is 10,000 RFU; the Y-axis for all other profiles is 1,000 RFU. The blue (FAM™ dye-labeled) peaks at the low- and high-MW ends of the GlobalFiler™ IQC kit profiles are the IQCS and IQCL peaks, respectively.

The reproducible peaks that were observed in non-primate, nonhuman species are listed in Table 20.

Table 20 Reproducible peaks for nonhuman species with the GlobalFiler™ IQC kit and GlobalFiler™ kit

Species	Dye channel	Size	GlobalFiler™ IQC kit	GlobalFiler™ kit
Cat	G	139	476	447
	R	231	355	395
	R	374	245	151
	P	152	505	483
Chicken	R	328	560	475
Cow	R	134	257	182
Dog	B	181	314	255

Table 20 Reproducible peaks for nonhuman species with the GlobalFiler IQC kit and GlobalFiler kit *(continued)*

Species	Dye channel	Size	GlobalFiler™ IQC kit	GlobalFiler™ kit
Dog	P	327	298	262
Horse	G	94	4115	3999
Pig	G	95	178	138
	R	423	2223	2155
SSF Bay soil	G	281	419	505
	R	408	310	246

Genotype concordance with population samples and NIST SRM-2391c

The genotyping accuracy of the GlobalFiler™ kit was extensively tested in population studies (see Chapter 6, "Experiments and results"). We performed smaller-scale testing of the GlobalFiler™ IQC kit to verify that the addition of the IQC system does not impact genotyping accuracy. The genotype concordance was checked for a set of 82 in-house population samples and the NIST Standard Reference Material 2391c PCR-based Profiling Standard.

The 82 in-house population samples were from individuals of Caucasian ethnicity. The NIST SRM-2391c consisted of 4 human genomic DNA samples: 3 single-source samples (Components A, B and C) and one sample (Component D) that was a 3:1 mixture of Components A and C. Approximately 1 ng per reaction of all samples was amplified for 29 cycles with the GlobalFiler™ IQC kit and run on three CE instruments: a 3500xL instrument with Data Collection software v3.1 (DC3.1), a 3500xL instrument with DC4, and a 3130*xl* instrument with v4 software. The run files were analyzed with GeneMapper™ *ID-X* Software v1.5.2 (3500xL DC3.1 and 3130*xl* runs), and GeneMapper™ *ID-X* Software v1.6 (for the 3500xL DC4 run).

Note: On the 3130*xl* instrument, off-scale data was produced at the default injection time (10 seconds). This data was included in the analysis and was accurately genotyped. Optimize the injection time for your instrument.

For the NIST SRM-2391c Components A, B, C and D, the genotypes obtained with the GlobalFiler™ IQC kit were concordant with known genotypes provided with the samples.

For the 82 population samples run on the 3500xL instruments, all genotypes were concordant, with the following exception: on the 3500xL with DC3.1, three samples could not be sized or genotyped.

For the 82 population samples run on the 3130*xl* instrument:

- 1 sample genotype was incorrect for an individual with D12S391 alleles 17.9 and 18. The 17.9 allele was not resolved because of 1-bp spacing between the D12 alleles, and spectral pull-up from an off-scale peak in a different dye channel.
- 3 samples could not be sized or analyzed because of excessive off-scale data from samples that had higher DNA concentrations than expected.

The following table contains a summary of results for the 82 population samples.

Instrument	3500xL DC3.1	3500xL DC4	3130*xl*
GMID-X version	**v1.5.2**	**v1.5.2 & 1.6**	**v1.5.2**
# Correct genotypes after editing	79	82	79
Samples unable to genotype	3 samples with loss of resolution	0	4 samples impacted by off-scale data

Overall, this study confirmed that correct genotypes were obtained for most samples; the only failures were due to non-optimal CE run data.

Mixture study results

Two-contributor DNA mixtures were prepared with DNA Control 007 and human female Control DNA 9947A. Samples were prepared so they contained 1.0 ng of total DNA between the two contributors, in mixture ratios of 1:0, 0:1, 1:1, 1:7 and 7:1 (male:female).

An example electropherogram of the 1:7 sample (125 pg of DNA Control 007 and 875 pg of Control DNA 9947A) run with the GlobalFiler™ IQC kit is shown in Figure 79.

The DNA mixture composition of each sample and resulting allele counts of each contributor are shown in Table 21. Only alleles that were unique to each contributor (the allele was not shared with the other contributor and was not in a stutter position relative to another allele) were counted.

Full profiles of unique alleles for both major and minor contributors were obtained in the 1:7, 7:1, and 1:1 mixture samples.

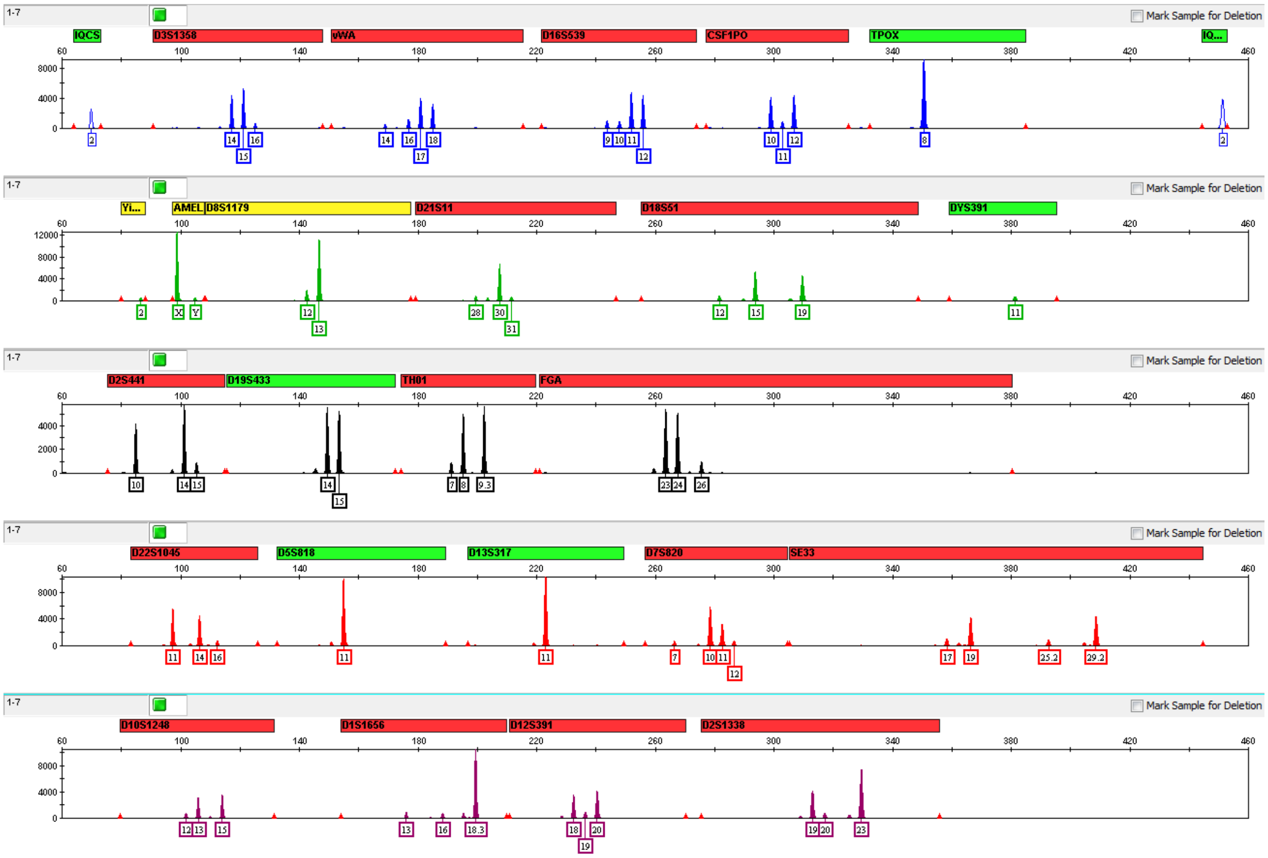


Figure 79 1:7 male:female mixture contained 125 pg of the DNA Control 007 minor contributor (male) and 875 pg of the Control DNA 9947A major contributor (female)

Table 21 DNA composition and mean allele counts per replicate GlobalFiler™ IQC kit reaction (full profiles of unique alleles for DNA Control 007 and Control DNA 9947A were 19 and 14, respectively)

Sample	Amount per reaction		Mean unique alleles per replicate	
	DNA Control 007	Control DNA 9947A	DNA Control 007	Control DNA 9947A
1:0	1.0	0	19	0
1:1	0.5	0.5	19	14
1:7	0.125	0.875	19	14
7:1	0.875	0.125	19	14
0:1	0	1.0	0	14

Extra peaks in the electropherogram

Chapter 5 contains extensive information about extra peaks that have been characterized in the GlobalFiler™ kit, such as Minus-A peaks, dye artifacts, and stutter. Those studies are also relevant for the GlobalFiler™ IQC kit and were not duplicated. We performed a comparison study to demonstrate that overall stutter levels were similar between the GlobalFiler™ and GlobalFiler™ IQC kits and to determine if artifacts were observed for the IQC marker peaks.

Extra peaks: Stutter

To compare stutter levels between the GlobalFiler™ IQC kit and GlobalFiler™ kit, DNA Control 007 was amplified with both kits at the same time, using the same lot of master mix. For each kit, 12 replicate reactions containing 1 ng of DNA Control 007 were amplified for 29 cycles. A stutter peak was evaluated only if it did not overlap with other allele or stutter peaks. The peak height ratio of each stutter peak was calculated as a fraction relative to its parent allele peak.

The results of the DNA Control 007 stutter experiment with stutter calculations at all STR loci except TH01 are shown in Figure 80. TH01 stutter product was often below the 175-RFU detection threshold and was not included in the study.

The average difference in stutter for all loci combined was +1.65% between the GlobalFiler™ kit and GlobalFiler™ IQC kit. This difference was not statistically significant.

Some loci showed distinct, separate groupings of data points (for example, D1S1656, D22S1045, and D7S820), which was a function of more widely spaced alleles in the same locus with very different stutter ratios.

One individual locus (DYS391) had an overall stutter difference as large as –7.48% (it was higher in the GlobalFiler™ kit than in the GlobalFiler™ IQC kit), which was likely due to stochastic effects and other sources of experimental error.

Overall, this study did not indicate any systematic differences in stutter between the two kits. Therefore, the current GlobalFiler™ kit stutter filter values can be used for the GlobalFiler™ IQC kit.

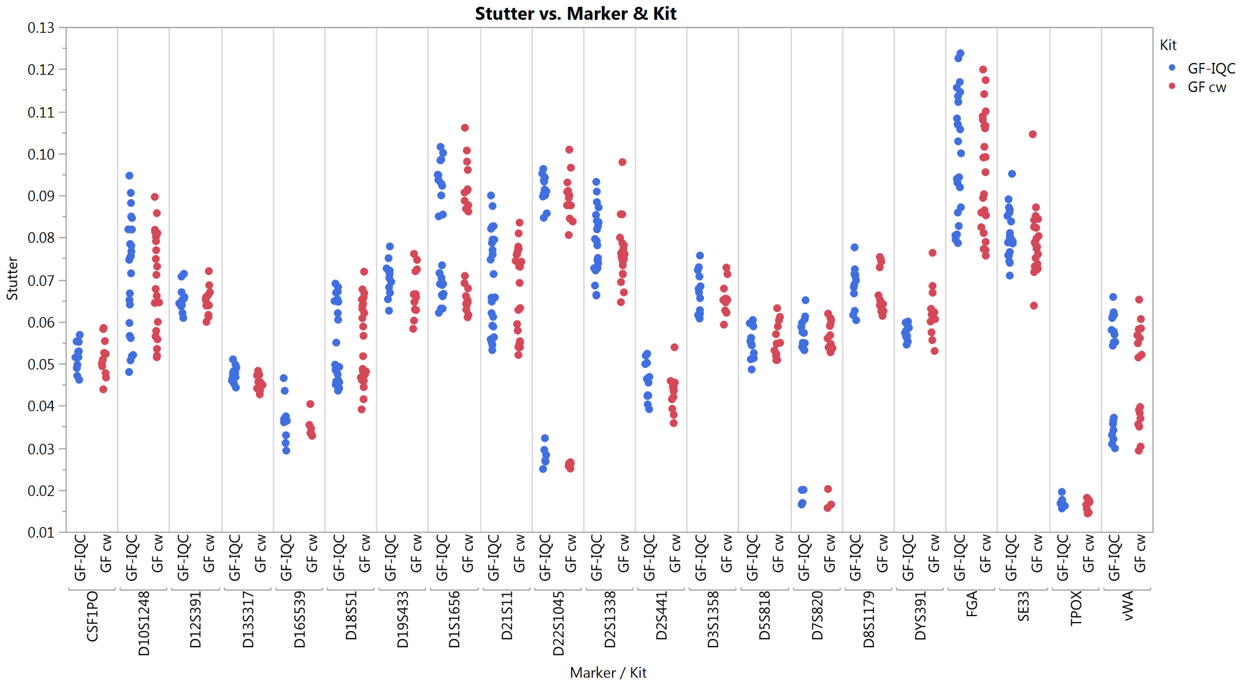


Figure 80 **Stutter measured for the GlobalFiler™ IQC kit (blue) and GlobalFiler™ kit (red) for all unobstructed main-allele/stutter-peak pairs of DNA Control 007 genomic DNA**

Extra peaks: IQCS artifact peaks

Two IQC artifact peaks were observed in GlobalFiler™ IQC kit samples:

- A minor artifact peak that amplifies above the peak amplitude threshold only with longer thermal cycles >29 was observed.

  This artifact may occasionally appear at 30 cycles. The peak appeared in data from both the 3500xL and 3130*xl* instruments, and the cause of the IQCS artifact is unknown.

  Because the IQCS peak is isolated from actual STR alleles, and the IQCS "allele call" itself is not included in the sample genotype results, this artifact peak does not affect results. Figure 81 shows the appearance of the artifact in a sample that was amplified for 31 cycles (higher than the recommended cycle number) to emphasize its appearance.

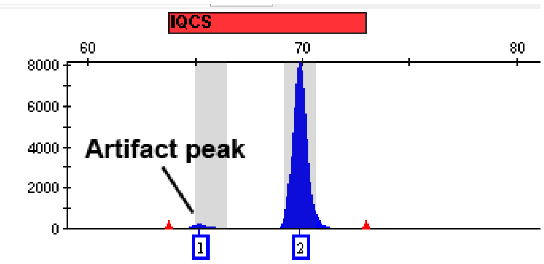


Figure 81 **IQCS artifact peak that appears when a sample was amplified for 31 cycles**

- An artifact that can appear in 3130*xl* sample data only if the oven is not sufficiently warmed up before a run. The artifact does not appear in the GlobalFiler™ IQC Allelic Ladder.

  This artifact may be caused by a transient association of the labeled-IQCS-amplicon DNA with the unlabeled-IQCS-PCR primer because of the lower-than-optimal temperature in the capillary array. The transient, partial double-stranded structure migrates slightly faster than normal labeled-IQCS-amplicon DNA and can form a shoulder peak. This artifact can be minimized by pre-heating the 3130*xl* oven at 60°C for ≥30 minutes before a run. Figure 82 shows an example of this artifact.

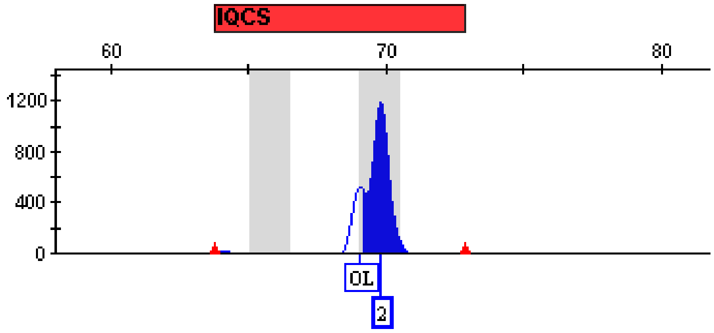


Figure 82   IQCS artifact peak that appears in 3130*xl* sample data only if the oven is not sufficiently warmed up before a run

Results summary

Test case	Expected result	Status
Sensitivity	Comparable performance between GlobalFiler™ IQC and GlobalFiler™ kits for intralocus balance, intracolor balance, average peak heights, and stutter percentage.	Pass
Inhibited DNA	Comparable performance between GlobalFiler™ IQC and GlobalFiler™ kits.	Pass
Degraded DNA	Comparable performance between GlobalFiler™ IQC and GlobalFiler™ kits.	Pass
PCR component and PCR cycle analysis	Comparable performance between GlobalFiler™ IQC and GlobalFiler™ kits when using the standard PCR protocol developed for the GlobalFiler™ kit.	Pass
IQC peak sizing precision	The IQC peaks in the GlobalFiler™ IQC Allelic Ladder are accurately analyzed in the GeneMapper™ *ID-X* Software v1.5.2 or v1.6.	Pass
Species specificity	Comparable cross-reactivity between the GlobalFiler™ IQC and GlobalFiler™ kits to the samples that were tested.	Pass
Genotype concordance (population study)	100% concordance between the GlobalFiler™ IQC and GlobalFiler™ kits when analyzing population samples and NIST Standard Reference Material 2391c PCR-based Profiling Standard.	Pass[1]

(continued)

Test case	Expected result	Status
Mixture study	Comparable performance with two-contributor DNA mixtures between the GlobalFiler™ IQC and GlobalFiler™ kit.	Pass
Extra peaks in the electropherograms (stutter and artifact)	Comparable performance when comparing stutter products and identifying artifacts between the GlobalFiler™ IQC and GlobalFiler™ kit. **Note:** Minor artifact peaks we observed only in the IQC peak region and do not affect kit performance.	Pass

[1] For information on samples that were not included in the genotype concordance testing, see "Genotype concordance with population samples and NIST SRM-2391c" on page 171.

Conclusion

The GlobalFiler™ IQC kit performed comparably to the GlobalFiler™ kit in all studies. The studies demonstrated the enhanced utility of the IQC system for forensic sample analysis.

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 1 minute 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.
	Evaporation.	Ensure that the plate is properly sealed with film.
	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.
	The tubes or plate were not seated tightly in the thermal cycler during amplification.	Push the tubes or plate firmly into the block after the first cycle.
	The wrong PCR reaction tubes or plate were used.	Use MicroAmp™ Reaction Tubes with Caps or a MicroAmp™ Optical 96-Well Reaction Plate.
	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 1.0 ng of DNA. For low concentration samples, add up to 15 µL of the DNA sample to the reaction mix, or consider using the 30-cycle protocol.

Observation	Possible cause	Recommended action
Positive signal from DNA Control 007 but partial or no signal from the test samples *(continued)*	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 or use the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit.
	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).
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.
	Stutter product (–1 repeat unit position) was amplified.	See Chapter 6, “Experiments and results”.
	The test sample contained mixed DNA.	See Chapter 6, “Experiments and results”.
	Incomplete 3′ A base addition (n–1 bp position) occurred.	In the PCR, include the final extension step: 10 minutes at 60°C.
		See Chapter 6, “Experiments and results”.
	The signal exceeds the dynamic range of the instrument and is causing signal "pull-up" into adjacent channels.	Ensure that you are using the recommended number of PCR cycles. Decrease the input DNA amount and repeat the PCR amplification, or interpret the off-scale data according to your laboratory procedure.
	Poor spectral separation occurred.	Perform a spectral calibration.
		Confirm that Filter Set J6 modules are installed and used for analysis.
	Too much DNA was present in the reaction.	Use the recommended amount of template DNA: 1.0 ng for 29 PCR cycles or 500 pg for 30 PCR cycles.
	The double-stranded DNA was not completely denatured.	Use the recommended amount of Hi-Di™ Formamide and heat the sample plate for 3 minutes at 95°C.

Observation	Possible cause	Recommended action
Poor peak height balance	Incorrect thermal cycler conditions were used.	Use the correct thermal cycler conditions.
Some, but not all, loci are visible on test sample electropherograms	The PCR reaction volume that you used is less than the volume required for the amplification.	Use the correct PCR reaction volume: 25 µL.
STR profiles contain many off-scale alleles	DNA quantification was not performed or was not accurate.	Ensure that DNA quantification is accurate.

B

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	Source
GeneScan™ 600 LIZ™ Size Standard v2.0 **IMPORTANT!** Do not use GeneScan™ 350 ROX™, GeneScan™ 500 ROX™, or GeneScan™ 500 LIZ™ Size Standards with this kit.	2 × 200 µL	4408399
TE Buffer [low-TE buffer; 10 mM Tris-HCl (pH 8.0) and 0.1 mM EDTA]	100 mL	12090015[1]
Hi-Di™ Formamide	25 mL	4311320

[1] Alternatively, you can prepare your own TE buffer. See "(Optional) Prepare low-TE buffer" on page 24.

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-36 Matrix Standard Kit (Dye Set J6)	4425042
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	Thermo Fisher Scientific[1]
SeqStudio™ Genetic Analyzer Cartridge v2	A41331
SeqStudio™ Genetic Analyzer Cathode Buffer Container	A33401
Reservoir Septa (for Cathode Buffer Container)	A35640

(continued)

Item	Source
SeqStudio™ Integrated Capillary Protector	A31923
Septa for SeqStudio™ Genetic Analyzer, 96 well	A35641
Septa for SeqStudio™ Genetic Analyzer, 8 strip	A35643
DS-36 Matrix Standard Kit (Dye Set J6)	4425042

[1] Available on **apps.thermofisher.com** or for download at **thermofisher.com**.

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-36 Matrix Standard Kit (Dye Set J6)	4425042
POP-4™ (960) Performance Optimized Polymer	4393710
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
(GlobalFiler™ IQC PCR Amplification Kit only: Required patch for GeneMapper™ ID-X Software v1.5) GeneMapper™ *ID-X* Software v1.5.2 patch[3]	Thermo Fisher Scientific[2]
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**.

[3] The patch enables full functionality of the data quality assessment tools when using the GlobalFiler™ IQC PCR Amplification Kit.

Note: For a list of GeneMapper™ *ID-X* Software versions that are compatible with your kit and capillary electrophoresis instrument, see "Instrument and software compatibility" on page 19.

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
MicroAmp™ 96-Well Tray/Retainer Set (Adapter for 8-Tube Strip)	403081
MicroAmp™ 96-Well Base	N8010531

(continued)

Item	Source
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

C

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 "Instrument and software compatibility" on page 19.

D

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, 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**

E

Documentation and support

Related documentation

Table 22 STR and quantification kits

Document title	Pub. No.
STR kits	
GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits—PCR Amplification and CE Quick Reference	MAN0029970
GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits— PCR Setup Quick Reference	MAN0029971
Technical Note: Artifacts Identified Post-Developmental Validation: GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits	Thermo Fisher Scientific[1]
Technical Note: Direct Amplification of Reference Samples Using the GlobalFiler™ PCR Amplification Kit	
Technical Note: Handling STR Kits and Ladder Decontamination	
Quantification kits	
Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide	4485355

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

Table 23 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 24 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 25 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 25 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**.

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