GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits

USER GUIDE

for use with:

GlobalFiler™ PCR Amplification Kit (Cat. No. 4476135 and 4482815)

GlobalFiler™ IQC PCR Amplification Kit (Cat. No. A43565)

Thermo Fisher
SCIENTIFIC

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

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Revision history: Pub. No. 4477604

Revision	Date	Description
F	03 October 2019	Add information 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. Update 3500 software information: pages 19, 28, 175, 184. Update GeneMapper™ *ID-X* Software: pages 176, 186.
E	07 July 2016	Add information on 30 cycles with 500-ng sample concentration to Chapter 1, Chapter 2, and Chapter 5. Add references to 3500 Series Data Collection 3 and GeneMapper ID-X v1.5. Add section on amplification artifacts to Chapter 5. Non-technical changes: Reorganize Chapter 1 and Chapter 5.
D	September 2015	Remove Master Mix Additive. Add the ProFlex™ PCR Systems. Add information about artifacts identified at the TH01 locus and TPOX locus. Add Section 5.2 "Performance verification after BSA component replacement". Update branding.
C	September 2014	Change storage conditions from 2 months to 6 months. Add Veriti™ Thermal Cycler verification information.
B	April 2014	Add data to Chapter 5 concerning the evaluation of Hardy-Weinberg equilibrium. Add information about the Quantifiler™ HP and Trio DNA Quantification Kits. Update the troubleshooting table.
A	August 2013	New document

Contents

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◼ **CHAPTER 8   Performance verification with 30 PCR cycles** **142**

1 Product information

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

Product description

Kit overview

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

The kits amplify:

* 21 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338)

* 1 Y-STR (DYS391)

* 1 insertion/deletion polymorphic marker on the Y chromosome (Y indel)

* Amelogenin (sex determining marker)

Both kits contain the 13 original CODIS loci with 7 from the expanded European Standard Set of Loci (ESSL) and the highly discriminating SE33 locus. The kits deliver 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.

In addition to the loci listed above, the GlobalFiler™ IQC PCR Amplification Kit amplifies 2 Internal Quality Control (IQC) markers.

For more information on the IQC markers, see Chapter 5, "Assess the PCR reaction with the Internal Quality Control System".

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

Validated DNA input amounts and PCR cycles	The kits are validated for use with:
	• 1.0 ng DNA (15 µL input volume) for 29 cycles
	• 500 pg DNA (15 µL input volume) for 30 cycles for increased sensitivity with low-concentration samples

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	

Dye	Color	Label
SID™	Purple	Samples, allelic ladders, and controls
LIZ™	Orange	GeneScan™ 600 LIZ™ Size Standard v2.0

Loci amplified by the kit

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

Table 1 GlobalFiler™ and GlobalFiler™ IQC kit loci and alleles

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	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
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3		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

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007
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	TAZ™	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 panel of standards needed for PCR amplification, PCR product sizing, and genotyping:

- **DNA Control 007**—A positive control for evaluating the efficiency of the amplification step and STR genotyping using the GlobalFiler™ Allelic Ladder. DNA Control 007 is included in the kit. See Figure 3 on page 15 and Figure 4 on page 16.

- **GeneScan™ 600 LIZ™ Size Standard v2.0**—Used for obtaining sizing results. This standard, which has been evaluated as an internal size standard, yields precise sizing results for PCR products. Order the GeneScan™ 600 LIZ™ Size Standard v2.0 (Cat. No. 4408399) separately.

- **GlobalFiler™ Allelic Ladder** or **GlobalFiler™ IQC Allelic Ladder**—Developed for accurate characterization of the alleles amplified by the kit. The allelic ladder is included in the kit and allows automatic genotyping of most of the reported alleles for the loci in the kit. See Figure 1 on page 13 and Figure 4 on page 16.

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

GlobalFiler™ Allelic Ladder profile
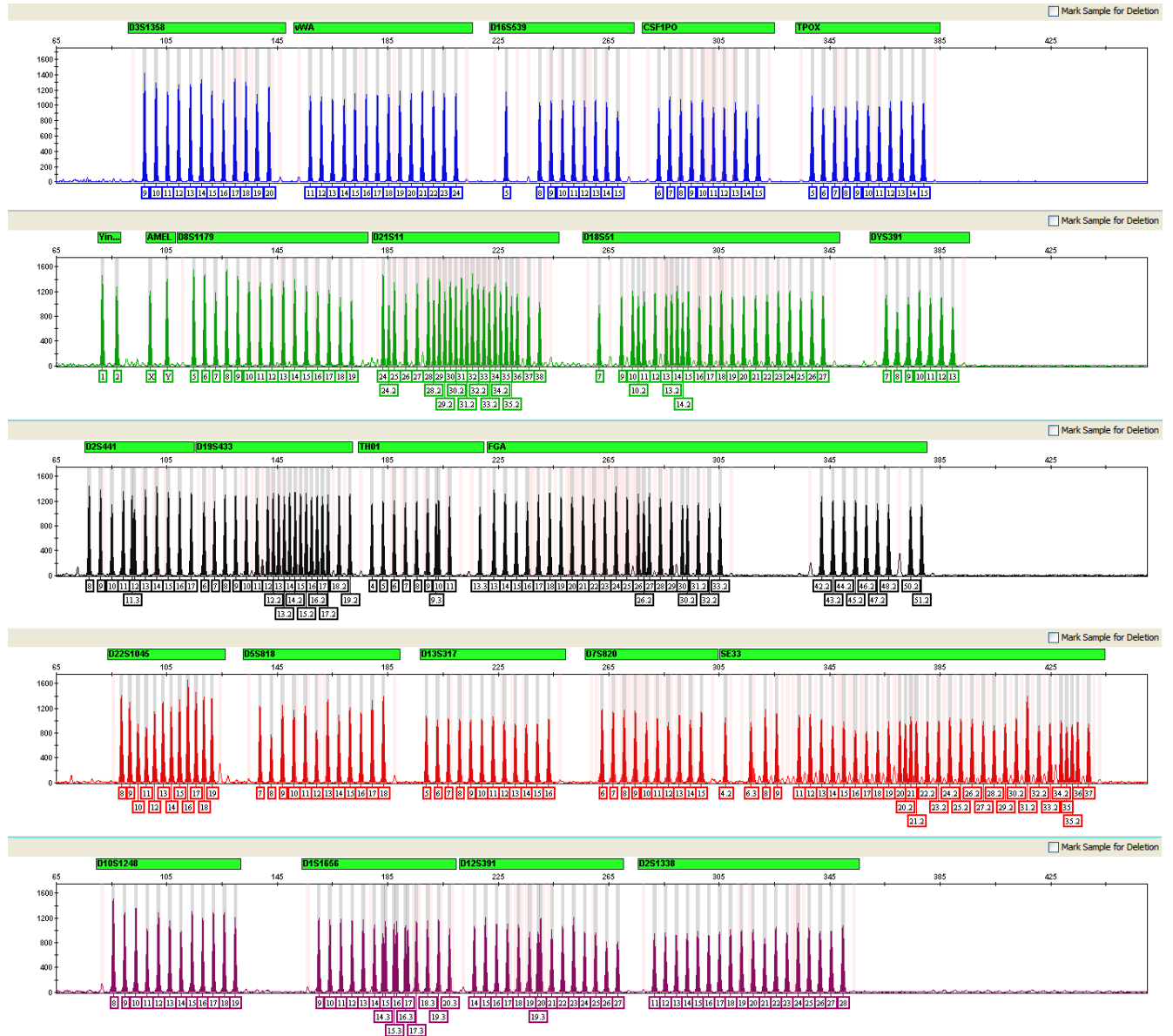
Figure 1 GeneMapper™ *ID-X* Software plot of the GlobalFiler™ Allelic Ladder

GlobalFiler™ IQC Allelic Ladder profile
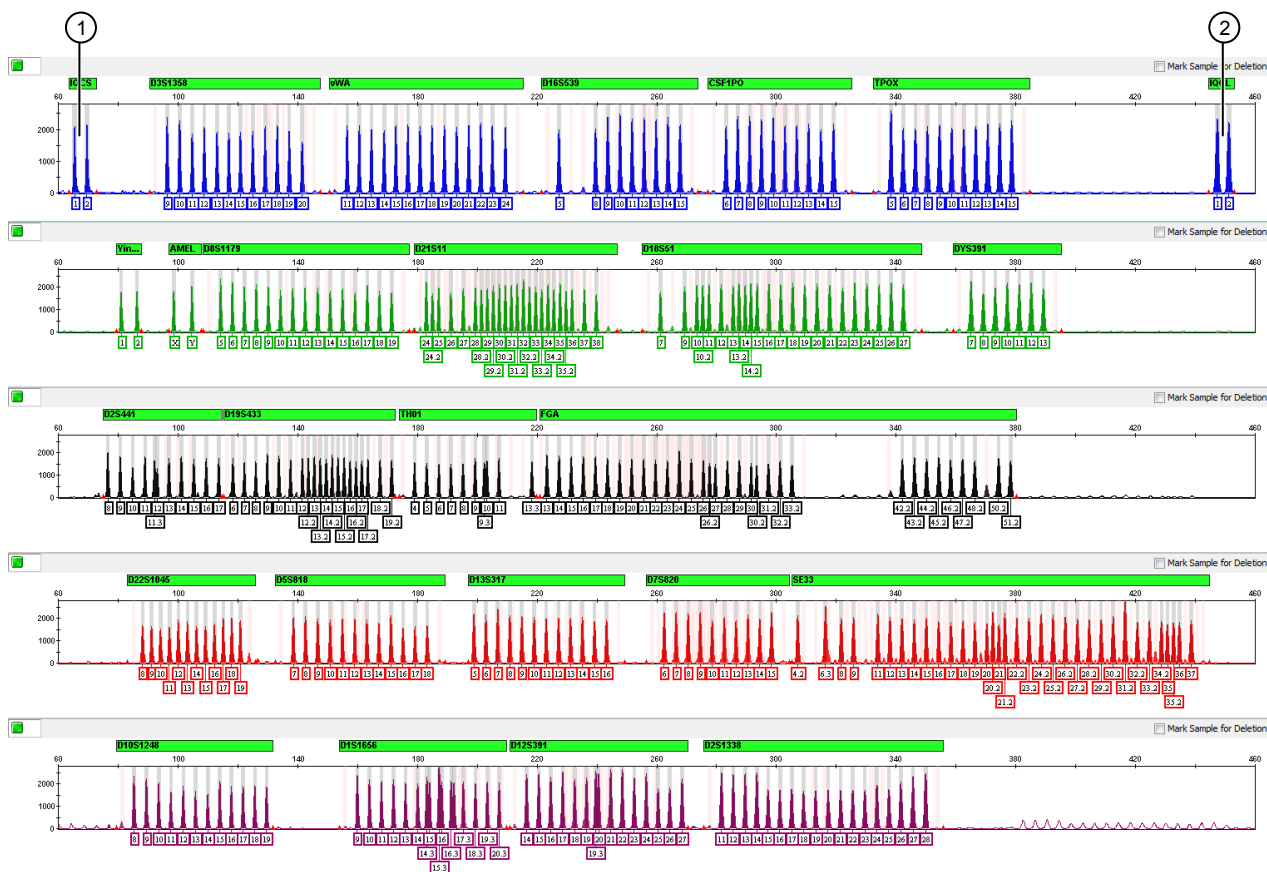
Figure 2 GeneMapper™ *ID-X* Software plot of the GlobalFiler™ IQC Allelic Ladder (Y-axis scale 0–2,700 RFU)

① IQCS (small) Allele 1 and 2

② IQCL (large) Allele 1 and 2

Note: In samples, one peak is present for the IQCS and IQCL markers with allele designation 2 (as shown in Figure 4 on page 16).
In the allelic ladder, two peaks are present for the IQCS and IQCL markers with allele designation 1 and 2 (see Figure 2 on page 14). The GeneMapper™ *ID-X* Software requires two peaks per marker in the allelic ladder for analysis.

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

GlobalFiler™ kit DNA Control 007 profile
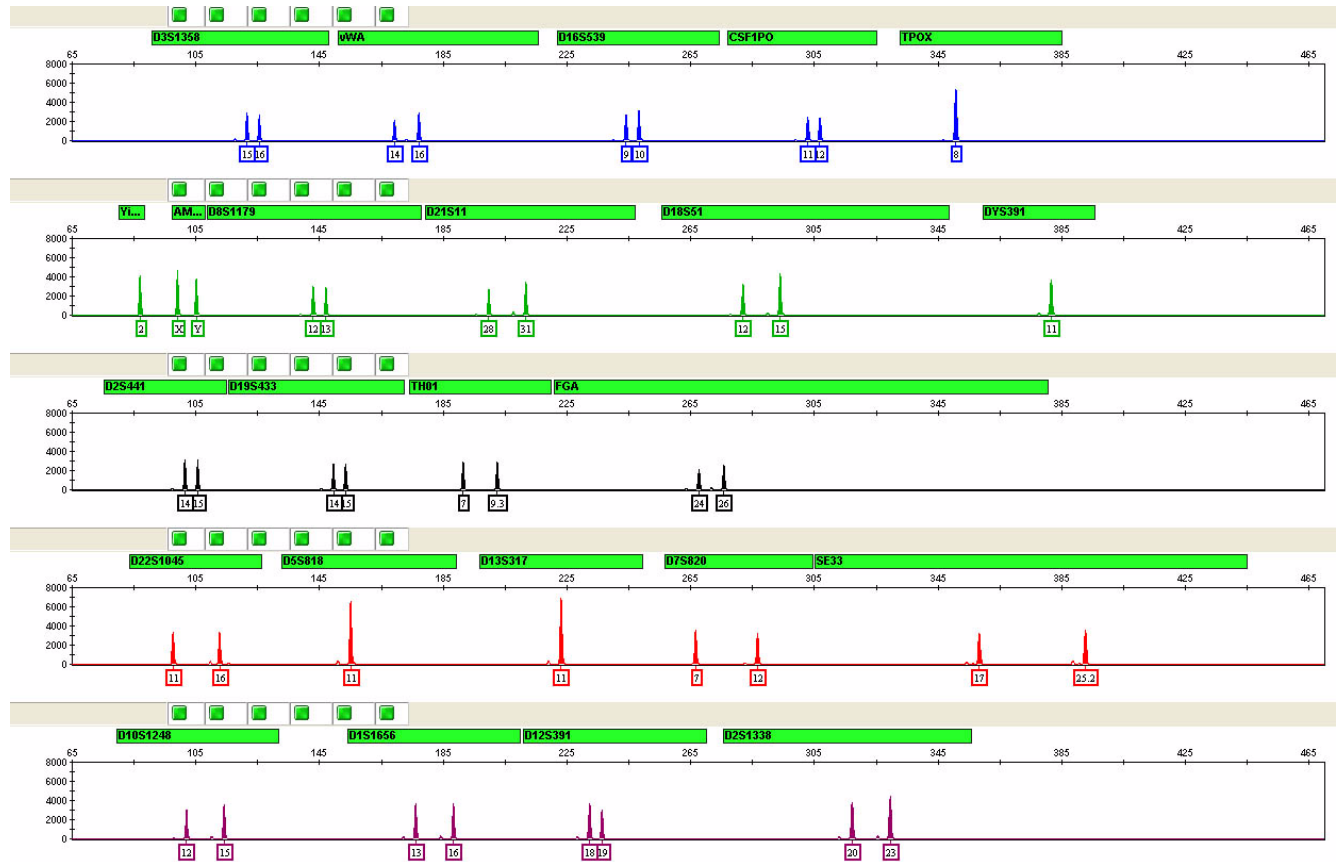
Figure 3 DNA Control 007 (1 ng) amplified with the GlobalFiler™ kit and analyzed on an Applied Biosystems™ 3500xL Genetic Analyzer (Y-axis scale 0 to 8,000 RFU).

GlobalFiler™ IQC kit DNA Control 007 profile
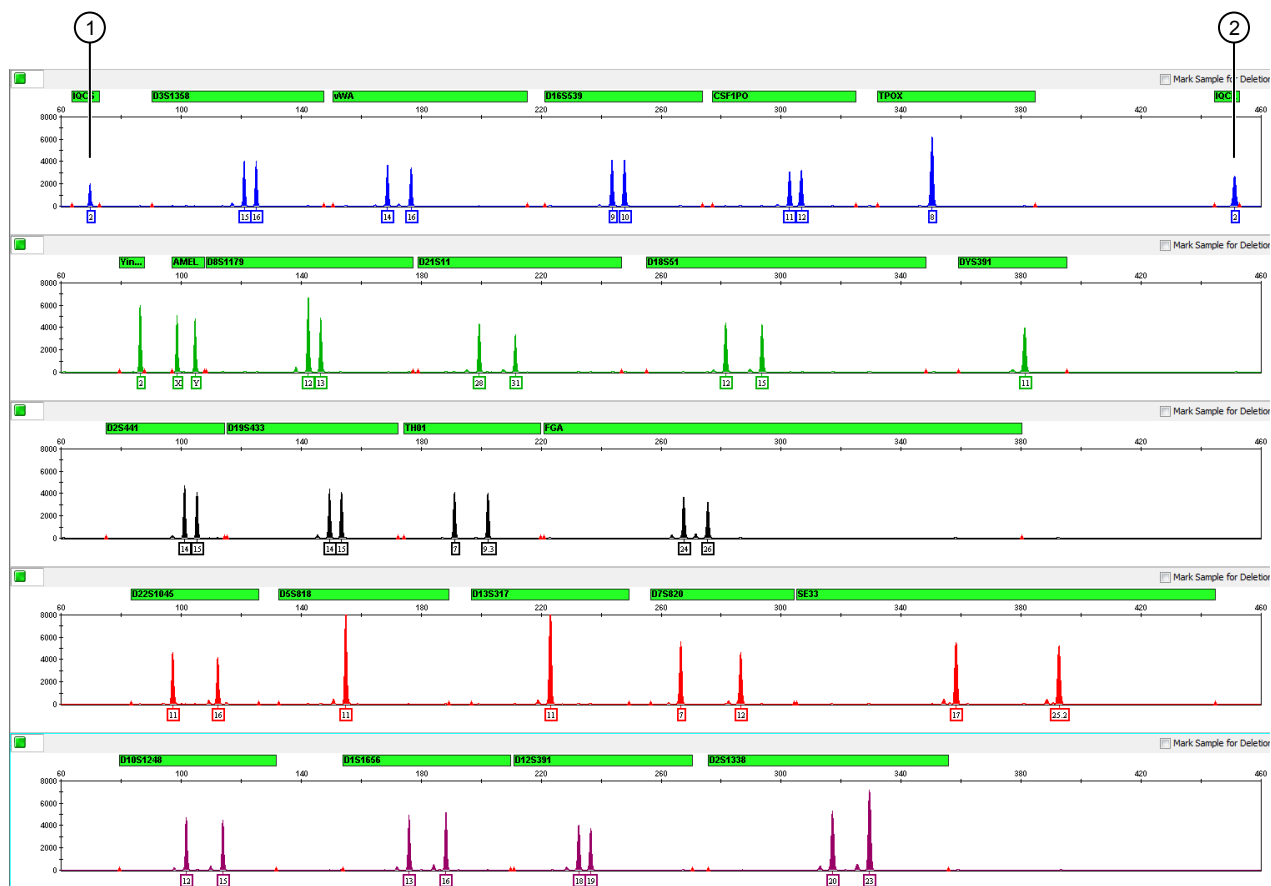
Figure 4 DNA Control 007 (1 ng) amplified with the GlobalFiler™ IQC kit and analyzed on an Applied Biosystems™ 3500xL Genetic Analyzer (Y-axis scale 0–8,000 RFU)

① IQCS peak
② IQCL peak

Contents and storage

GlobalFiler™ kit contents and storage

The GlobalFiler™ kit contains sufficient quantities of the following reagents to perform 200 (Cat. No. 4476135) or 1,000 (Cat. No. 4482815) amplifications at 25 µL/amplification:

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.

IMPORTANT! Do not refreeze kit components after thawing.

Contents	Description	200 reaction (Cat. No. 4476135)	1,000 reaction (Cat. No. 4482815)	Storage
GlobalFiler™ Master Mix	Contains enzyme, salts, dNTPs, bovine serum albumin, enzyme, 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°C to 8°C after first use for up to 6 months or up to the expiration date stated on the kit (whichever comes first).
GlobalFiler™ Primer Mix	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°C to 8°C after first use for up to 6 months or up to the expiration date stated on the kit (whichever comes first). Store protected from light.
GlobalFiler™ Allelic Ladder	Contains amplified alleles. See "GlobalFiler™ Allelic Ladder profile" on page 13 for information.	1 × 0.065 mL	1 × 0.15 mL	−25°C to −15°C on receipt. 2°C to 8°C after first use up to the expiration date stated on the kit. Store protected from light.
DNA Control 007	Contains 0.1 ng/µL human male genomic DNA from cell line in 0.05% sodium azide and buffer[1] See "GlobalFiler™ kit DNA Control 007 profile" on page 15 for information.	1 × 0.3 mL	1 × 0.6 mL	−25°C to −15°C on receipt. 2°C to 8°C after first use up to the expiration date stated on the kit.

[1] DNA Control 007 is included at a concentration that is appropriate for use as an amplification control (that is, to provide confirmation of the capability of the kit reagents to generate a profile of expected genotype). It is not designed for use as a DNA quantification control. If you quantify aliquots of 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 following reagents to perform 200 amplifications at 25 µL/amplification:

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer mix, amplified DNA, allelic ladder, and size standard from light when not in use.

IMPORTANT! Do not refreeze kit components after thawing.

Contents	Description	200 reaction (Cat. No. A43565)	Storage
GlobalFiler™ Master Mix	Contains enzyme, salts, dNTPs, bovine serum albumin, enzyme, and 0.05% sodium azide in buffer and salt.	1 × 1.5 mL	−25°C to −15°C on receipt. 2°C to 8°C after first use for up to 6 months or up to the expiration date stated on the kit (whichever comes first).
GlobalFiler™ IQC Primer Mix	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°C to 8°C after first use for up to 6 months or up to the expiration date stated on the kit (whichever comes first). Store protected from light.
GlobalFiler™ IQC Allelic Ladder	Contains amplified alleles and IQC markers. See "GlobalFiler™ IQC Allelic Ladder profile" on page 14 for information.	1 × 0.065 mL	−25°C to −15°C on receipt. 2°C to 8°C after first use up to the expiration date stated on the kit. Store protected from light.
DNA Control 007	Contains 0.1 ng/µL human male genomic DNA from cell line in 0.05% sodium azide and buffer[1] See "GlobalFiler™ IQC kit DNA Control 007 profile" on page 16 for information.	1 × 0.3 mL	−25°C to −15°C on receipt. 2°C to 8°C after first use up to the expiration date stated on the kit.

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

Instrument type	Validated models
Thermal cyclers	• ProFlex™ 96-well PCR System (Cat. No. 4484075) • ProFlex™ 2 × 96-well PCR System[1] (Cat. No. 4484076) • ProFlex™ 3 × 32-Well PCR System[1] (Cat. No. 4484073) • Veriti™ 96-Well Thermal Cycler (Cat. No. 4479071) • GeneAmp™ PCR System 9700, 96-Well Silver (Cat. No. N8050001) • GeneAmp™ PCR System 9700, 96-Well Gold-Plated (Cat. No. 4314878) **IMPORTANT!** The GlobalFiler™ and GlobalFiler™ IQC kits are NOT validated for use with: • ProFlex™ 2 × Flat PCR System (Cat. No. 4484078) • ProFlex™ 2 × 384-well PCR System (Cat. No. 4484077) • Veriti™ *Fast* 96-Well Thermal Cycler (Cat. No. 4375305) • GeneAmp™ PCR System 9700 with the aluminium 96-well block (Cat. No. 4314879)

Genetic analyzers[2]	• SeqStudio™ Genetic Analyzer
	• 3500/3500xL Genetic Analyzer with any of the following:
	  – 3500 Data Collection v4 Software (Windows™ 10 operating system)
	  – 3500 Data Collection v3.1 Software (Windows™ 7 operating system)
	  – 3500 Data Collection v2 Software[3] (Windows™ 7 operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670)
	  – 3500 Data Collection Software v1[3] (Windows™ Vista operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670)
	• 3130/3130xl Genetic Analyzer with:
	  – Data Collection Software v4 (Windows™ 7 operating system)
	  – 3130/3730 Data Collection v4 6-Dye Module v1

Analysis software	**GlobalFiler™ kit**: GeneMapper™ *ID-X* Software v1.4 or later **GlobalFiler™ IQC kit**: GeneMapper™ *ID-X* Software v1.5.2 or later

[1] The GlobalFiler™ IQC kit was not validated with thermal cycler.
[2] We conducted validation studies using the 3130xl, 3500, and 3500xL configurations.
[3] The GlobalFiler™ IQC kit was not validated with this version of software.

Workflow

Extract DNA, see:

www.thermofisher.com/hid-sampleprep

▼

Quantify DNA, see:

"DNA quantification" on page 21

▼

Perform PCR, see:

"Prepare the amplification kit reactions" on page 24

"Perform PCR" on page 25

▼

Perform electrophoresis, see:

"Set up the 3500/3500xL instruments for electrophoresis (before first use of the kit)" on page 28 or

"Set up the 3130/3130xl instruments for electrophoresis (before first use of the kit)" on page 30

▼

"Prepare samples for electrophoresis (3500 Series and 3130 Series instruments)" on page 32

▼

Analyze data, see:

"Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)" on page 36

"Create an analysis method" on page 41

"Create a size standard definition file if needed" on page 48

"Analyze and edit sample files with GeneMapper™ ID-X Software" on page 50

"Examine or edit a project" on page 51

2

Perform PCR

DNA quantification

Importance of quantification before STR analysis

DNA quantification can be used to determine:

- If the sample contains sufficient human DNA and/or human male DNA to proceed with short tandem repeat (STR) analysis.
- The amount of sample to use in STR analysis applications.
- For the Quantifiler™ Trio DNA Quantification Kit, the relative quantities of human male and female DNA in a sample that can help in the selection of the appropriate STR chemistry.
- The DNA quality, regarding inhibition level (all kits) or the inhibition level and the DNA degradation level (Quantifiler™ HP DNA Quantification Kit and Quantifiler™ Trio DNA Quantification Kit only). This metric is useful for determining the likelihood of recovery of STR loci with larger amplicon sizes.
- 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 provides discrimination of individuals similar to STR genotype match probabilities. Also, the Precision ID Ancestry Panel infers biogeographical ancestry for investigative leads.
- If PCR inhibitors are present in a sample that may require additional purification before proceeding to STR analysis.

Effect of DNA quantity on results

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

- Fluorescence intensity that exceeds the linear dynamic range for detection by the instrument ("off-scale" data).

Off-scale data is a problem because:

– Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause the corresponding stutter peak to appear higher in relative intensity, thus increasing the calculated percent stutter.

– Multicomponent analysis of off-scale data is not accurate. This inaccuracy results in poor spectral separation ("pull-up").

• Incomplete +A nucleotide addition.

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

If too little DNA is added to the PCR reaction, the total number of allele copies added to the PCR is extremely low and unbalanced amplification of the alleles may occur because of stochastic fluctuation.

Methods of quantifying DNA

For information on recent innovations in quantification chemistry, go to **thermofisher.com**.

Kit and user guide	Detects	How it works
Quantifiler™ Human DNA Quantification Kit (Cat. No. 4343895) For more information, see *Quantifiler™ Human DNA Quantification Kit and Y Human Male DNA Quantification Kit User Guide* (Pub. No. 4344790)	• Total human DNA	Uses a target-specific 5′ nuclease assay: • Two locus-specific PCR primers • One TaqMan™ MGB probe labeled with FAM™ dye for detecting the target sequence Uses an Internal PCR control (IPC) 5′ nuclease assay: • IPC template DNA (a synthetic sequence not found in nature) • Two primers for amplifying the IPC template • One TaqMan™ MGB probe labeled with VIC™ dye for detecting the IPC target
Quantifiler™ Duo DNA Quantification Kit (Cat. No. 4387746) For more information, see *Quantifiler™ Duo DNA Quantification Kit User Guide* (Pub. No. 4391294)	• Total human DNA • Human male DNA	Uses two target-specific 5′ nuclease assays: • Two locus-specific PCR primers per target (one for total human DNA and one for human male DNA) • Two TaqMan™ MGB probes labeled with VIC™ dye (for total human DNA) and FAM™ dye (for human male DNA) for detecting the target sequences Uses an Internal PCR control (IPC) 5′ nuclease assay: • IPC template DNA (a synthetic sequence not found in nature) • Two primers for amplifying the IPC template • One TaqMan™ MGB probe labeled with NED™ dye for detecting the IPC target

Kit and user guide	Detects	How it works
Quantifiler™ HP DNA Quantification Kit (Cat. No. 4482911) For more information, see *Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide* (Pub. No. 4485354)	• 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 to 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) For more information, see *Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide* (Pub. No. 4485354)	• 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.

Before you begin

Prepare low-TE buffer

For optimal results, we recommend using low-TE buffer for sample preparation. Prepare it as described in this procedure or buy it from Teknova (Cat. No. T0223).

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

     Note: Adjust the volumes accordingly for specific needs.

2. Aliquot, then autoclave the solutions.

3. Store the aliquots at room temperature.

Thaw reagents (before first use of the kit)

Thaw the Master Mix and Primer Set.

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.

IMPORTANT! Thawing is required only during first use of the kit. After first use, reagents are stored at 2°C to 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, amplified DNA, allelic ladder, and size standard from light when not in use.

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

2. Pipette the required volumes of components into an appropriately sized, clear (non-colored), polypropylene tube:

Reaction component	Volume per reaction
Master mix	7.5 µL
Primer set[1]	2.5 µL

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

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

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

4. Dispense 10 µL of reaction mixture into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate or each MicroAmp™ tube.

5. Adjust the sample input amount and volume if needed:
   - If total sample input amount is >1.0 ng for 29 cycles or >500 pg for 30 cycles, dilute with low-TE buffer to achieve a 15-µL input volume.
   - If total sample input volume is <15 µL, bring to volume with low-TE buffer to achieve a 15-µL input volume.

6. Prepare samples as shown in the following table, then add them to the appropriate well or tube (final reaction volume is 25 µL).

Sample	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 control DNA (0.1 ng/µL) • 5 µL of low-TE buffer	Combine, then add to the reaction well or tube: • 5 µL of control DNA (0.1 ng/µL) • 10 µL of low-TE buffer

7. Seal the MicroAmp™ Optical 96-Well Reaction Plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film, or cap the tubes.

8. Centrifuge the tubes or plate at 3,000 rpm for about 20 seconds in a tabletop centrifuge (with plate holders, if using 96-well plates).

9. Amplify the samples.

   IMPORTANT! See "Instrument and software compatibility" on page 19 for a list of validated thermal cyclers.

Perform PCR

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

1. Program the thermal cycling conditions.

   IMPORTANT! Set the thermal cycler you are using as indicated: ProFlex™ PCR System: 9700 Simulation ramping mode; Veriti™ Thermal Cycler: 100% ramping rate, *do not* use 9600 emulation mode; GeneAmp™ PCR System 9700: Max ramping mode.

Initial incubation step	Cycle (29 or 30 cycles)		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, Up to 24 hours[1]

[1] The infinity (∞) setting allows an unlimited hold time.

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

   IMPORTANT! If you are using adhesive clear film instead of caps to seal the plate wells, place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. The ProFlex™ PCR System and the Veriti™ Thermal Cycler do not require a compression pad.

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

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

   IMPORTANT! Protect the amplified DNA from light.

Direct amplification (GlobalFiler™ kit only)

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.

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

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 disc contains approximately 5 ng–20 ng of DNA. Because of the high quality of DNA, a lower cycle number is required to produce on-scale data.

Note: This 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.

For more information on direct amplification studies, go to **thermofisher.com**, then search for the technical note *Direct Amplification of Reference Samples—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(s)
3500	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
3500*xl*	1 per injection	24 samples	23 samples + 1 allelic ladder
3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3130*xl*	1 per injection	16 samples	15 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. Perform internal validation studies to verify the required allelic ladder injection frequency, to ensure accurate genotyping of all samples in your laboratory environment.

It is critical to genotype using an allelic ladder run under the same conditions as the samples. 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 than the primer set and the allelic ladder in the GlobalFiler™ IQC kit. Do not interchange.

Materials required for electrophoresis

Appendix B, "Materials required but not supplied" lists the required materials that are not supplied with this kit.

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

Set up the 3500/3500xL instruments for electrophoresis (before first use of the kit)

Electrophoresis software setup

The following table lists the data collection software and the run modules that you can use to analyze PCR products generated by this kit. For details on the procedures, see the documents listed in "Documentation and support" on page 184.

Note: We conducted validation studies for the kit using the 3130*xl*, 3500, or 3500xL configurations.

Genetic analyzer	Operating system	3500 Data Collection Software	Additional software	Plate templates, assays, run modules, and conditions
3500 3500xL	Windows™ 10	v3.3, v4	None	Plate templates: 6dye_36_POP4 (and _xl) Assay (DC4): AB_J6_LS_POP4 (and _xl), which contains instrument protocol AB_HID36_POP4_J6_NT3200.
3500 3500xL	Windows™ 7	v3, v3.1	None	Assays (DCv3.3 and earlier): GF+Norm_POP4 (and _xl) and GF_POP4 (and _xl), which contain instrument protocol HID36_POP4 (and_xl)_J6_NT3200.
3500 3500xL	Windows™ 7	v2	HID Updater 3500 DC v2 (Cat. No. 4480670)	All assays use the following conditions: • Run module: HID36_POP4
3500 3500xL	Windows™ Vista	v1	HID Updater 3500 DC v2 (Cat. No. 4480670)	• Injection conditions: 1.2 kV/15 sec (24 sec for xl) • Run conditions: 13 kV/1550 sec • Dye Set J6

Chapter 3 Perform electrophoresis
Set up the 3500/3500xL instruments for electrophoresis (before first use of the kit)

3

Perform spectral calibration

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

The following figure is an example of a passing 6-dye spectral calibration.
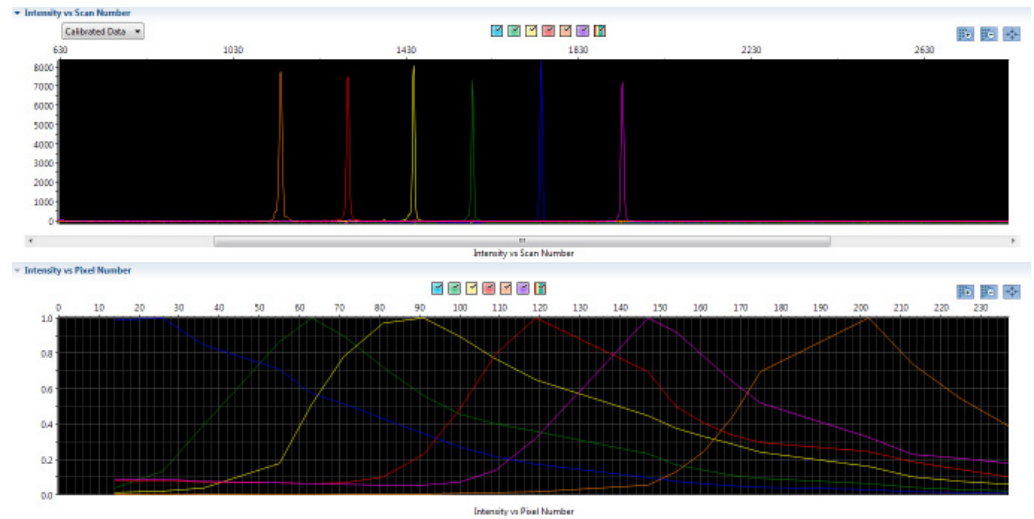
Set up the 3130/3130*xl* instruments for electrophoresis (before first use of the kit)

Electrophoresis software setup

The following table lists the data collection software and the run modules that you can use to analyze PCR products generated by this kit. For details on the procedures, see the documents listed in "Documentation and support" on page 184.

Depending on instrument sensitivity, DNA input amounts ≥1 ng amplified at 29 cycles can demonstrate off-scale peak heights for homozygous peaks. For more information, go to **thermofisher.com**, then search for the technical note *Evaluation of a decreased cycle number (28 cycles) and decreased injection time (5 seconds) when the GlobalFiler™ PCR Amplification Kit is run on a 3130 xl Genetic Analyzer*, or contact your local Human Identification representative.

Genetic analyzer	Operating system	Data Collection Software	Additional software	Run modules and conditions
3130	Windows™7	v4	3130/3730 DC v4 6-Dye Module v1	• HIDFragmentAnalysis36_POP4_1 • Injection conditions: 3 kV/5 sec • Run conditions: 15 kV/1500 sec • Dye Set J6
3130*xl*[1]				• HIDFragmentAnalysis36_POP4_1 • Injection conditions: 3 kV/5–10 sec[2] • Run conditions: 15 kV/1500 sec • Dye Set J6

[1] We conducted validation studies using the 3130*xl*, 3500, and 3500xL configurations.

[2] If you observe off-scale peaks, optimize the injection time for your instrument.

Obtain and activate 6-dye license

1. Confirm that you are running Data Collection Software v4 (**Help ▸ About**).

2. Obtain a 3130 DC v4 6-Dye Module v1 License key. Contact your local Human Identification representative for information.

3. Ensure that all network cards in the computer are enabled.

   IMPORTANT! You can run the 3130 Series Data Collection Software v4 using only the network cards that are enabled when you activate the software license. For example, if you activate the software when your wireless network card is disabled, you will not be able to run the software when the wireless network card is enabled.

Chapter 3 Perform electrophoresis
Set up the 3130/3130xl instruments for electrophoresis (before first use of the kit)

3

4. Select **Tools ▸ License Manager** to display the **Software Activation** dialog box.

5. Request the software license file by performing steps 1a, 1b, and 1c as listed on the activation screen. The license file will be emailed to you.

6. Obtain the software license file from your email.

7. Make a copy of the software license file and keep it in a safe location.

8. Copy the software license file to the desktop of the Data Collection Software v4 computer.

9. If the Software Activation dialog box has closed, select **Tools ▸ License Manager**.

10. Click **Browse**, then navigate to the software license file saved on your computer.

11. Click **Install and Validate License**.
    A message is displayed when the license is installed and validated.

12. Click **Close**.

Perform spectral calibration

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

The following figure is an example of a passing 6-dye spectral calibration.
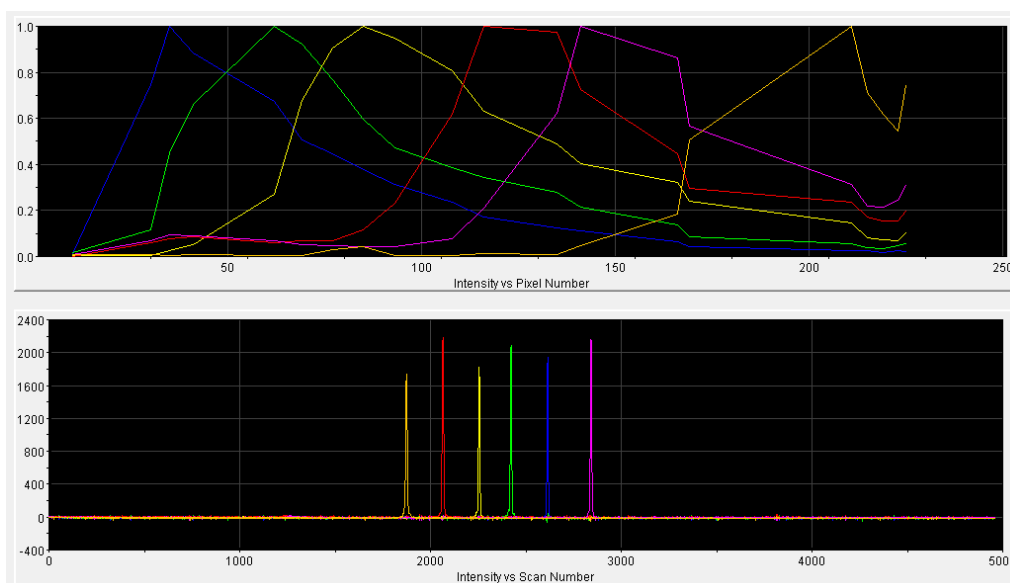
Prepare samples for electrophoresis (3500 Series and 3130 Series instruments)

This procedure applies to the 3500 Series and 3130 Series instruments.

Prepare the samples for electrophoresis immediately before loading.

1. Pipet the required volumes of components into an appropriately sized polypropylene tube:

Reagent	Volume per reaction
GeneScan™ 600 LIZ™ Size Standard v2.0	0.4 µL
Hi-Di™ Formamide	9.6 µL

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

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

2. Vortex the tube, then briefly centrifuge.

3. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, add:

   • 10 µL of the formamide/size standard mixture
   • 1 µL of PCR product or Allelic Ladder

    Note: For blank wells, add 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 at 95°C for 3 minutes.

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.

GeneMapper™ *ID-X* Software v1.4 or later analyzes 4-dye, 5-dye, and 6-dye data and is required to correctly analyze data that is generated using the GlobalFiler™ and GlobalFiler™ IQC kits. After electrophoresis, the data collection software stores information for each sample in a .fsa or .hid file. The GeneMapper™ *ID-X* Software v1.4 or later allows you to analyze and interpret the data from the .fsa or .hid files.

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 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: Set up GeneMapper™ *ID-X* Software

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

"Check panel, bin, and stutter file versions on your computer" on page 36

▼

"(If needed) Download newer versions of panel, bin, and stutter files" on page 37

▼

"Import panels, bins, and marker stutter" on page 37

▼

"(Optional) Define custom table or plot settings" on page 41

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

Note: GeneMapper™ *ID-X* Software v1.5.2 contains the latest panel, bin, and stutter files for the GlobalFiler™ kit. It does not contain files for the GlobalFiler™ IQC kit.

1. Start the GeneMapper™ *ID-X* Software , then log 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, then open the **Panels** folder, then check the version of panel, bin, and stutter files installed.

5. Check for newer versions of the files as described in the next procedure.

Chapter 4 Analyze data with GeneMapper™ *ID-X* Software
Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)

4

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

Note: GeneMapper™ *ID-X* Software v1.5.2 contains the latest panel, bin, and stutter files for the GlobalFiler™ kit. It does not contain files for the GlobalFiler™ IQC kit.

1. Go to **www.thermofisher.com/GMIDXsoftware**.

2. If the file versions listed are newer than the versions on your computer, download the appropriate file for the kit: **GlobalFiler Analysis files** or **GlobalFiler IQC Analysis files**.

   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.

Import panels, bins, and marker stutter

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

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

2. Select **Tools ▸ Panel Manager**.

3. Find, then open the folder containing 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, then open the **GlobalFiler Analysis files** or **GlobalFiler IQC Analysis files** folder that you unzipped in the previous procedure.

4. Select **GlobalFiler_Panel_v1.txt** or **GlobalFiler-IQC_Panel_v1.txt**, then click **Import**.

   Note: Importing this file creates a new folder in the navigation pane of the **Panel Manager**, **GlobalFiler_Panel_v1** or **GlobalFiler-IQC_Panel_v1**. This folder contains the panel and associated markers.

Chapter 4 Analyze data with GeneMapper™ *ID-X* Software
Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)

4
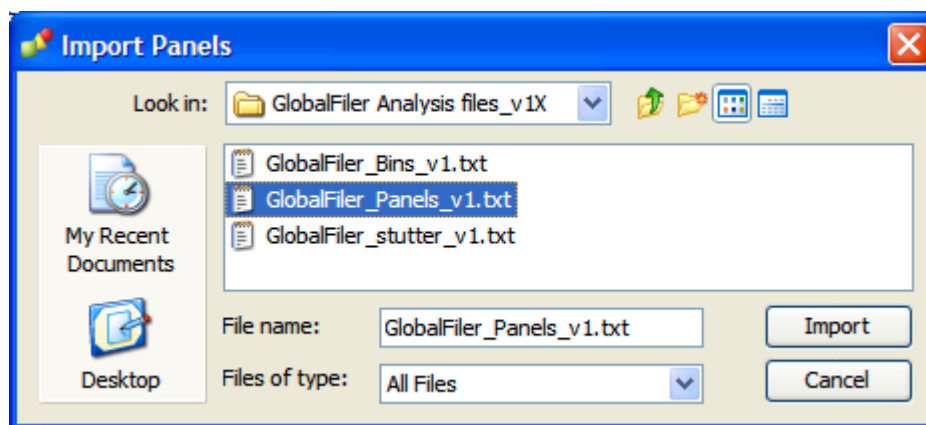
5. Import the bins file:

    a. Select the **GlobalFiler_Panel_v1** or **GlobalFiler-IQC_Panel_v1** folder in the navigation pane.
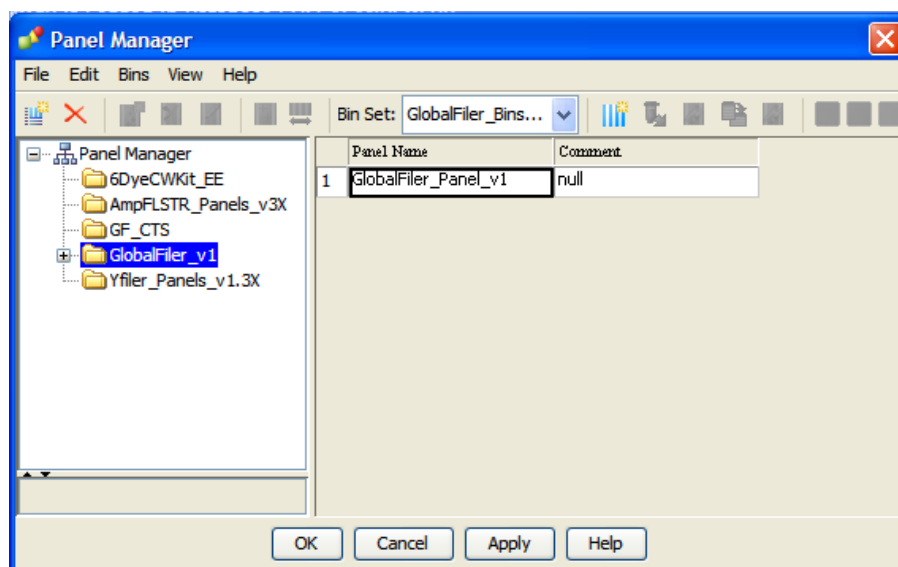
    b. Select **File ▸ Import Bin Set** to open the **Import Bin Set** dialog box.

    c. Navigate to, then open the **GlobalFiler Analysis files** or **GlobalFiler IQC Analysis files** folder.

Chapter 4 Analyze data with GeneMapper™ *ID-X* Software
Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)

4

d. Select **GlobalFiler_Bins_v1.txt** or **GlobalFiler-IQC_Bins_v1.txt**, then click **Import**.

> **Note:** Importing this file associates the bin set with the panels in the **GlobalFiler_Panel_v1** or **GlobalFiler-IQC_Panel_v1** folder.
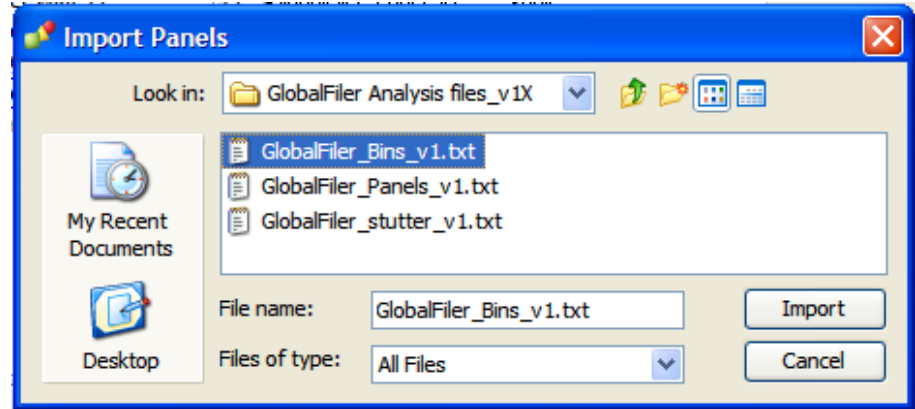
6. *(Optional)* View the imported panels and bins in the navigation pane: Double-click the **GlobalFiler_Panel_v1** or **GlobalFiler-IQC_Panel_v1** folder.

The panel information is displayed in the right pane and the markers are displayed below it.
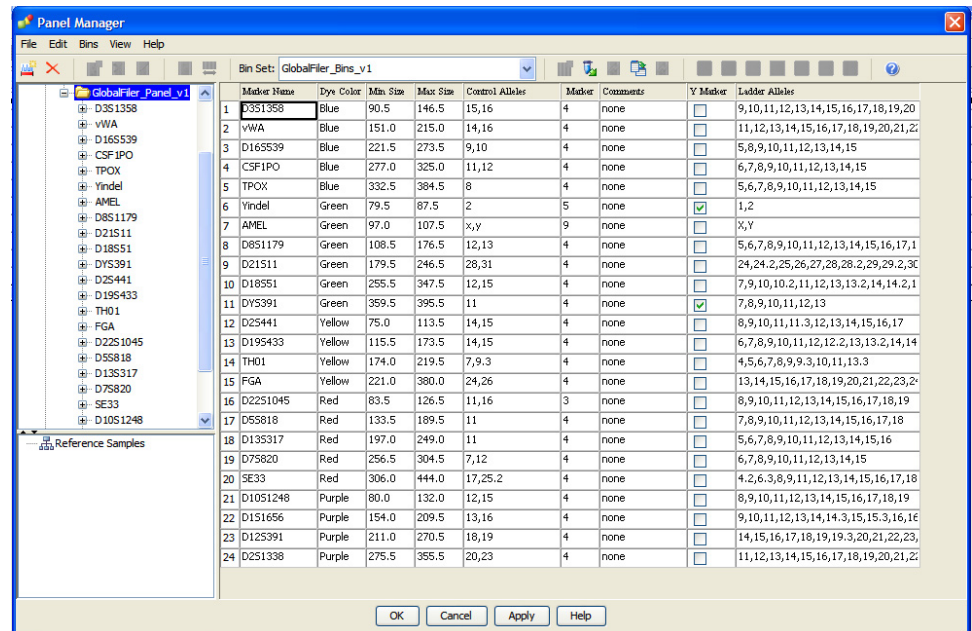
7. Import the stutter file:

a. Select the **GlobalFiler_Panel_v1** or **GlobalFiler-IQC_Panel_v1** folder in the navigation panel.

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

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Chapter 4 Analyze data with GeneMapper™ *ID-X* Software
Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)

c. Navigate to, then open the **GlobalFiler Analysis files** or **GlobalFiler IQC Analysis files** folder.

d. Select **GlobalFiler_stutter_v1.txt** or **GlobalFiler-IQC_stutter_v1.txt**, then click **Import**.

> **Note:** Importing this file associates the marker stutter ratio with the bin set in the **GlobalFiler_Panel_v1** or **GlobalFiler-IQC_Panel_v1** folder and overwrites any existing stutter ratios associated with the panels and bins in that folder.
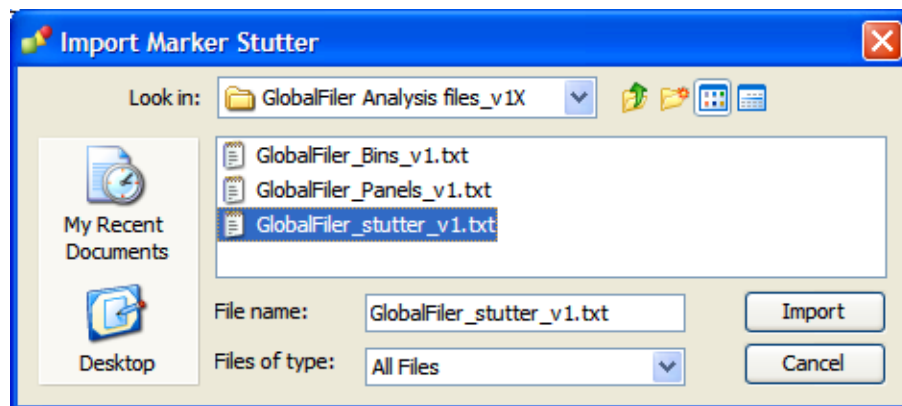
8. View the imported marker stutters in the navigation pane:

a. Double-click the **GlobalFiler_Panel_v1** or **GlobalFiler-IQC_Panel_v1** folder to display the folder.

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

c. Double-click a marker to display the **Stutter Ratio & Distance** view for the marker in the right pane.
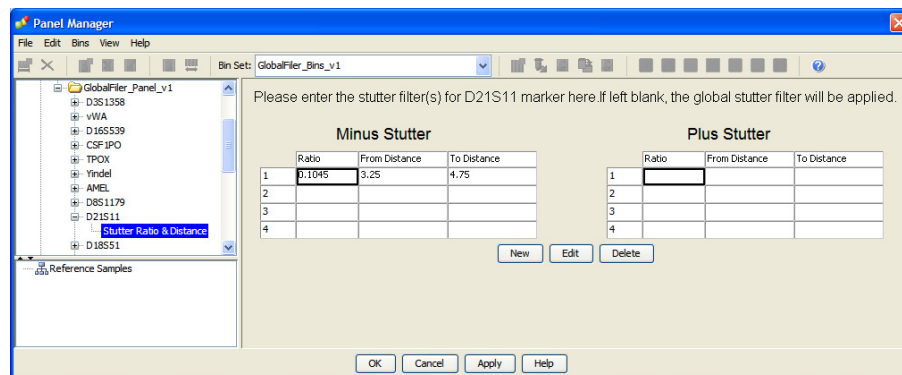
9. Click **Apply**, then click **OK** to add the panel, bin set, and marker stutter to the GeneMapper™ *ID-X* Software database.

> **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 Getting Started Guide— Basic Features*.

Create an analysis method

Create an analysis method

IMPORTANT! Analysis methods are version-specific, so you must create an analysis method for each version of the software. For example, an analysis method that is created in GeneMapper™ *ID-X* Software version 1.6 is not compatible with analysis methods that are created in earlier versions of software.

IMPORTANT! Because the GlobalFiler™ and GlobalFiler™ IQC kits use different panels and bins, you must create an analysis method for each kit. All settings other than panels and bins are the same in both methods.

1. Select **Tools ▸ GeneMapper® ID-X Manager** to open the **GeneMapper ID-X Manager**.
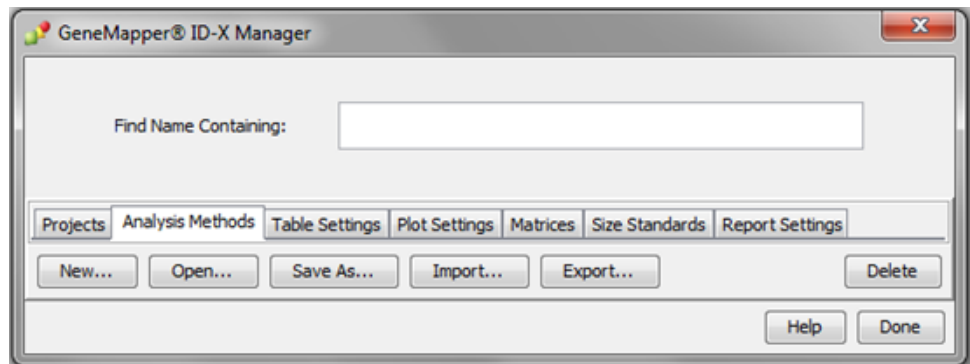
2. Click the **Analysis Methods** tab, then click **New** to open the **Analysis Method Editor** with the **General** tab selected.
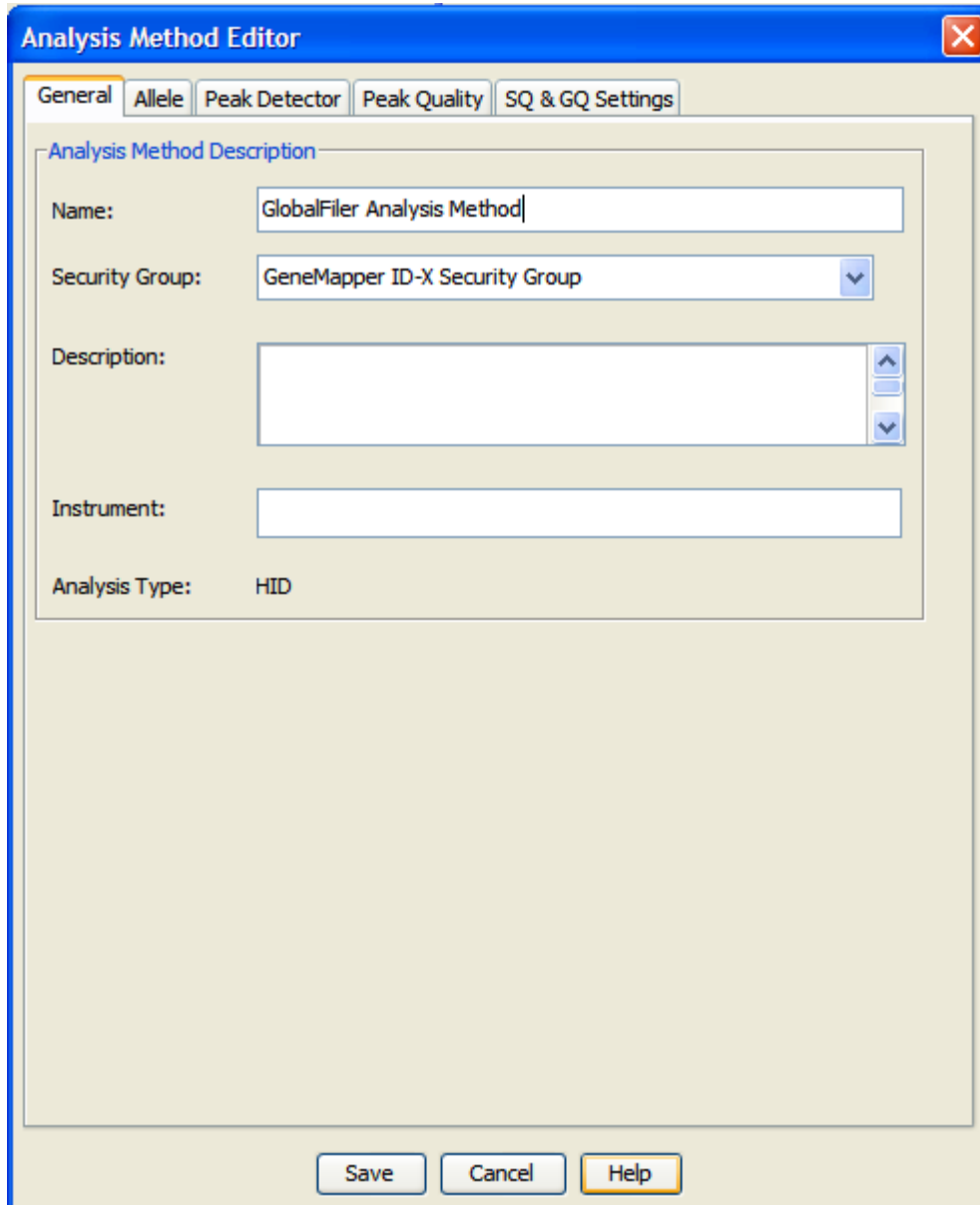
3. Enter the settings shown in the figures on the following pages.

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

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

Analysis method settings

General tab settings

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

Allele tab settings
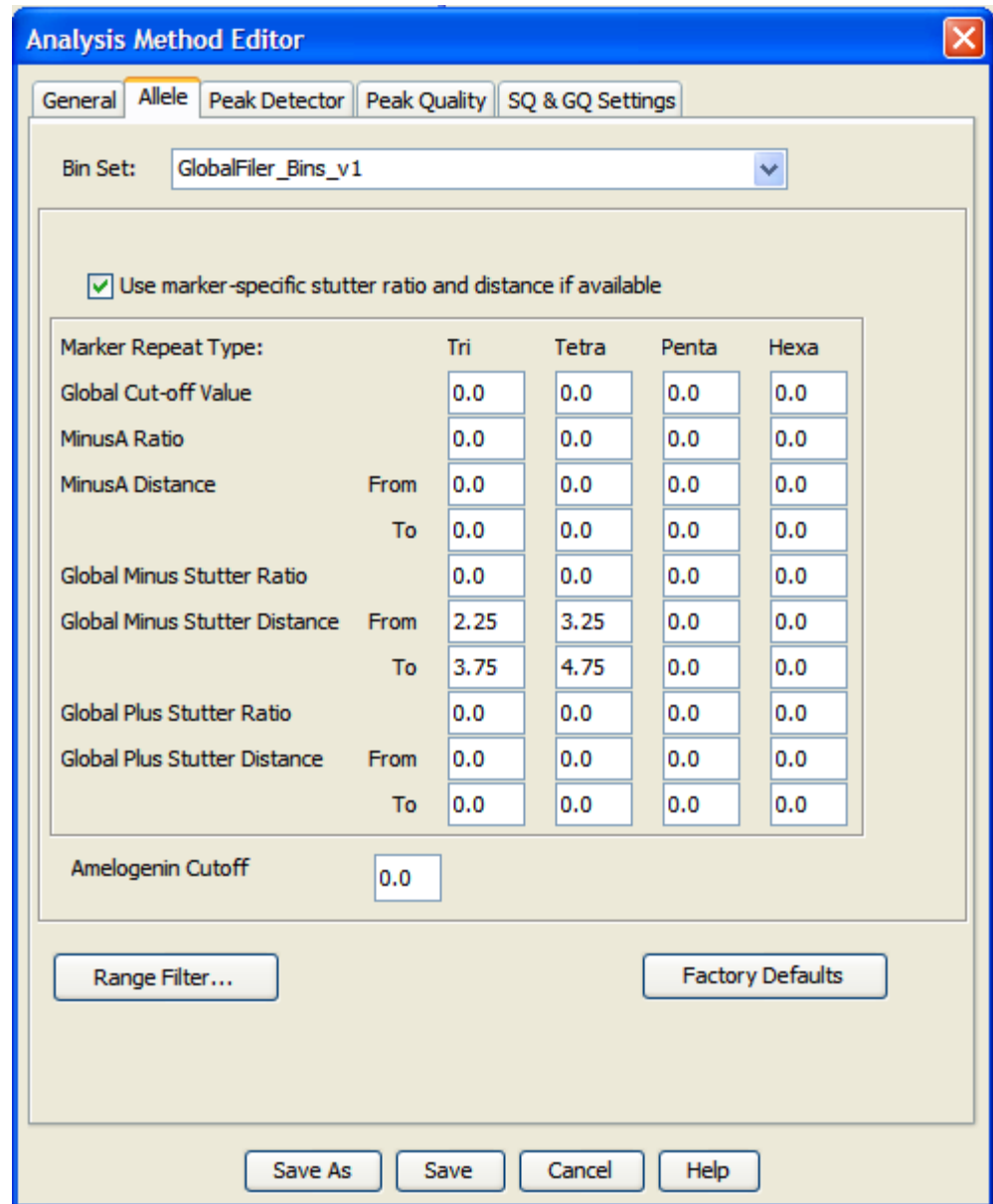
The following settings were used during developmental validation of the kit:

- In the **Bin Set** field, select the **GlobalFiler_Bins_v1** or **GlobalFiler-IQC_Bins_v1** bin set.
- GeneMapper™ *ID-X* Software v1.0.1 or later allows you to specify 4 types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- Specify the appropriate filter settings. To apply the stutter ratios contained in the **GlobalFiler_stutter_v1.txt** or **GlobalFiler-IQC_stutter_v1.txt**, select the **Use marker specific stutter ratio if available** checkbox (selected by default).

Perform appropriate internal validation studies to determine the appropriate filter setting to use.

Peak Detector tab settings

IMPORTANT! GlobalFiler™ kit data from 3130- and 3500-series instruments has been validated with the **3rd Order Least Squares** and the **Local Southern** size calling methods.

GlobalFiler™ IQC kit data from 3130- and 3500-series instruments has been validated with the **Local Southern** size calling method.

Analysis Method Editor

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

Peak Detection Algorithm: Advanced

Ranges

Analysis
Full Range
Start Pt: 0
Stop Pt: 10000

Sizing
All Sizes
Start Size: 60
Stop Size: 500

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

Factory Defaults

Save As | Save | Cancel | Help

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

Fields include:

- **Peak amplitude thresholds**—The software uses these parameters to specify the minimum peak height, to limit the number of detected peaks. Although GeneMapper™ *ID-X* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.

- **Peak Window Size and Baseline Window**—Using settings other than those shown in the preceding figure can affect peak resolution.

IMPORTANT! You must manually set the **Baseline Window** and **Peak Window Size** to 33 and 13 points respectively when you create the analysis method. If the data was collected on a 3500 instrument, make sure these settings match the settings in the **QC Protocol** that you used on the 3500 instrument..

- **Size calling method**—The GlobalFiler™ and GlobalFiler™ IQC kits have been validated using both the **3rd Order Least Squares** sizing method (80–460 base pairs) and the **Local Southern** sizing method (60–460 base pairs). Both sizing methods produced 100% concordance and generated acceptable data. Select alternative sizing methods only after performing the appropriate internal validation studies.

- **Size calling method**—The GlobalFiler™ kit has been validated using both the **3rd Order Least Squares** sizing method (80–460 base pairs) and the **Local Southern** sizing method (60–460 base pairs). Both sizing methods produced 100% concordance and generated acceptable data.

  The GlobalFiler™ IQC kit has been validated using the **Local Southern** sizing method (60–460 base pairs).

  Select alternative sizing methods only after performing the appropriate internal validation studies.

- **Normalization**—The normalization function in the GeneMapper™ *ID-X* Software is enabled by default for use with data generated on the 3500 Series Genetic Analyzers.

Peak Quality tab settings

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

SQ & GQ Settings tab settings

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

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 higher 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.

Create a size standard definition file if needed

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 that is provided with GeneMapper™ *ID-X* Software and used with the Local Southern 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 48.

Create a size standard definition file

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

2. Click the **Size Standards** tab, then click **New**.
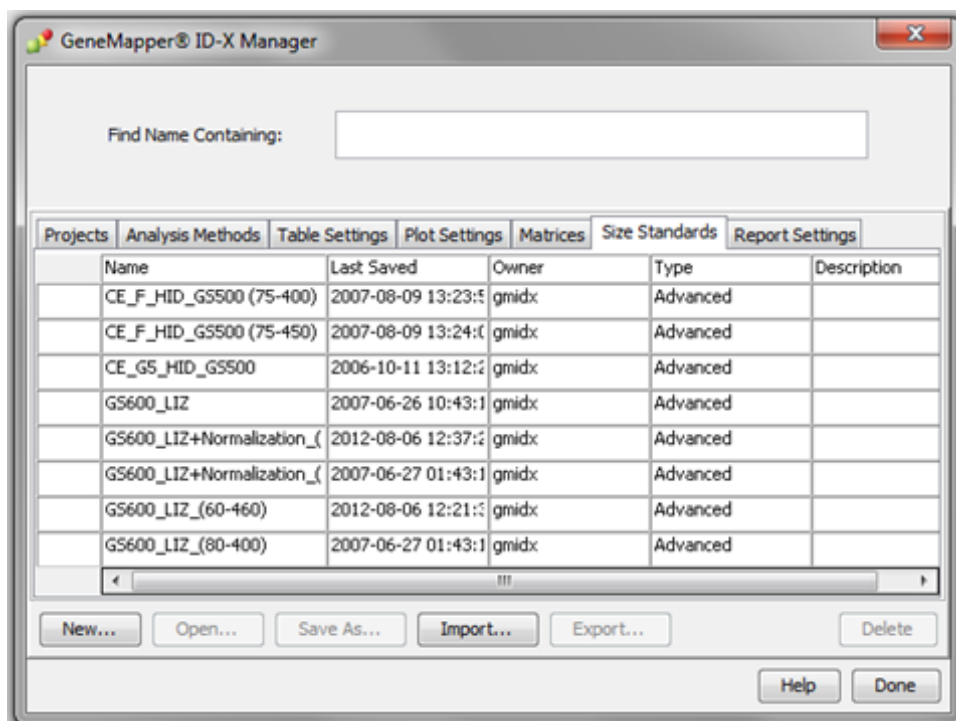
3. Specify settings in the **Size Standard Editor**:
   a. Enter a name as shown in the following figure or enter a new name.

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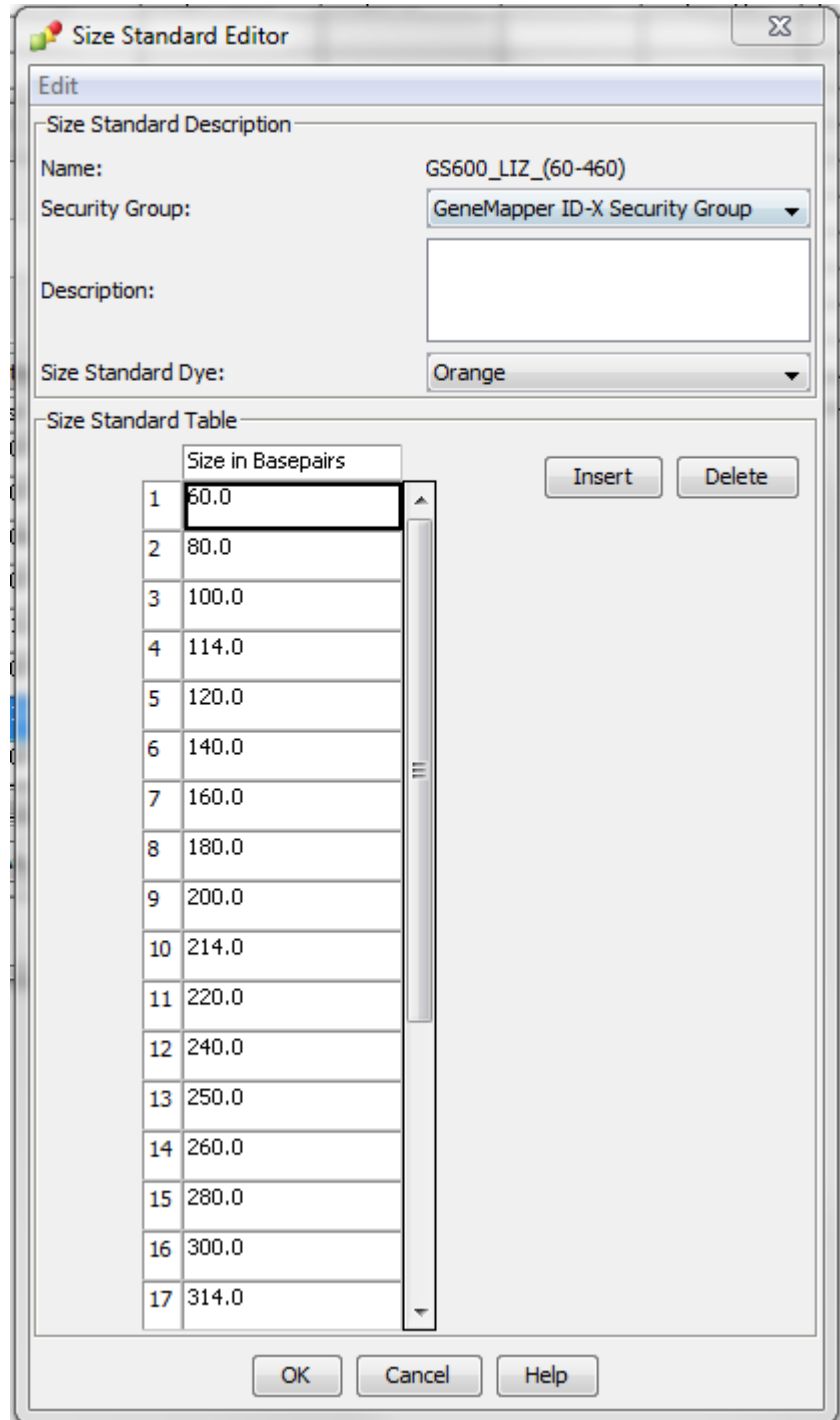
Analyze and edit sample files with GeneMapper™ *ID-X* Software

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.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	Select **GlobalFiler Analysis Method_v1** or **GlobalFiler-IQC Analysis Method_v1** (or the name of the analysis method you created).
Panel	Select **GlobalFiler_Panels_v1** or **GlobalFiler-IQC_Panels_v1**.
Size Standard	Use a size range of 60–460 bp for **Local Southern** size calling method or a size range of 80–460 bp for **3rd Order Least Squares** size-calling method.[1]

[1] The GlobalFiler™ kit was originally validated with the GeneScan™ 600 LIZ™ Size Standard v2.0. If you use a different size standard, perform the appropriate internal validation studies to support the use of this size standard with the GlobalFiler™ or GlobalFiler™ IQC kit.

3. Click **Analyze**, enter a name for the project (in the **Save Project** dialog box), then click **OK** to start analysis.

   - The status bar displays the progress of analysis as a completion bar.
   - 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 184 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 2 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 (as shown in Figure 4 on page 16).
In the allelic ladder, two peaks are present for the IQCS and IQCL markers with allele designation 1 and 2 (see Figure 2 on page 14). 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, greater than 2 ng) some suppression of the IQC peaks may also be seen.

Table 2 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 5
Ski-slope	• IQCL peak height significantly decreased relative to the IQCS peak • IQCL peak not present	Indication of inhibition	See Figure 6 and Figure 7
Ski-slope	Balanced	Indication of degradation	See "Ski slope profile with balanced IQC peaks (degradation)" on page 55
No peaks	Balanced	No DNA or very little sample DNA	See Figure 9
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 10

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
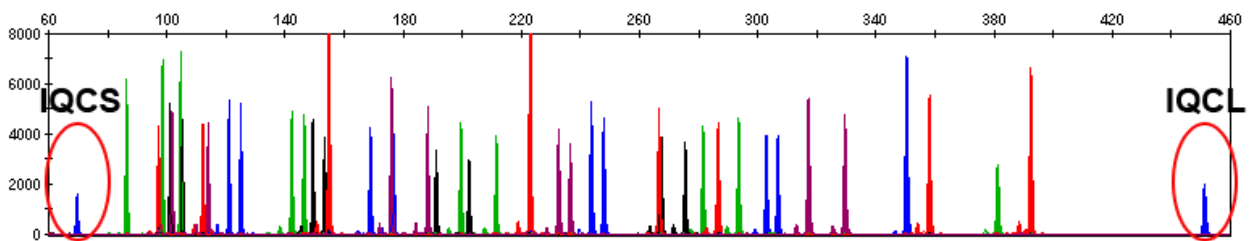
Figure 5 Balanced profile with 1.0 ng DNA (Y-axis scale to 0–8,000 RFU)

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

Figure 6 shows a ski slope pattern of sample peaks with a significantly lower IQCL peak height relative to the IQCS peak height. 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.

Figure 7 shows the complete absence of an IQCL peak, indicating a high level of inhibition.
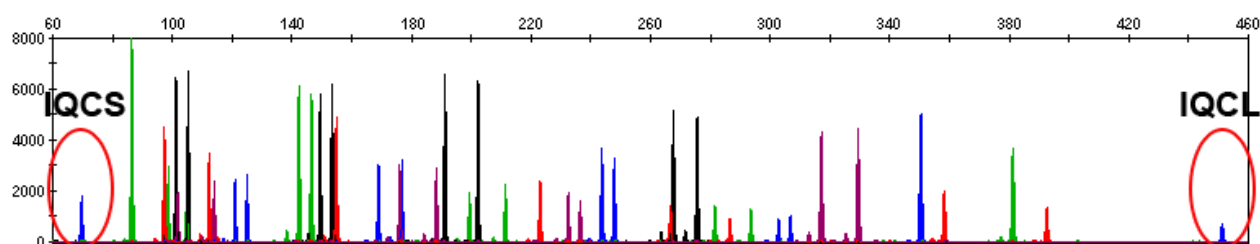
Figure 6   IQC peaks with 150 ng/µL humic acid (Y-axis scale 0–8,000 RFU)
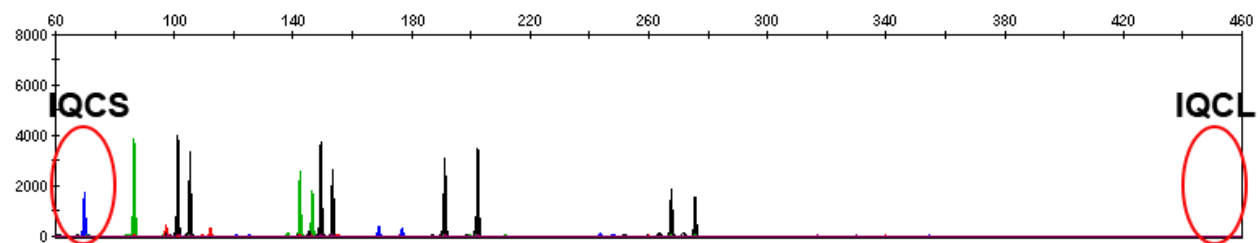
Figure 7   IQC peaks with 250 ng/µL humic acid (Y-axis scale 0–8,000 RFU)

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

Figure 8 shows that the IQCL peak height does not decrease in the presence of degraded sample at low, medium, and high levels of degradation.
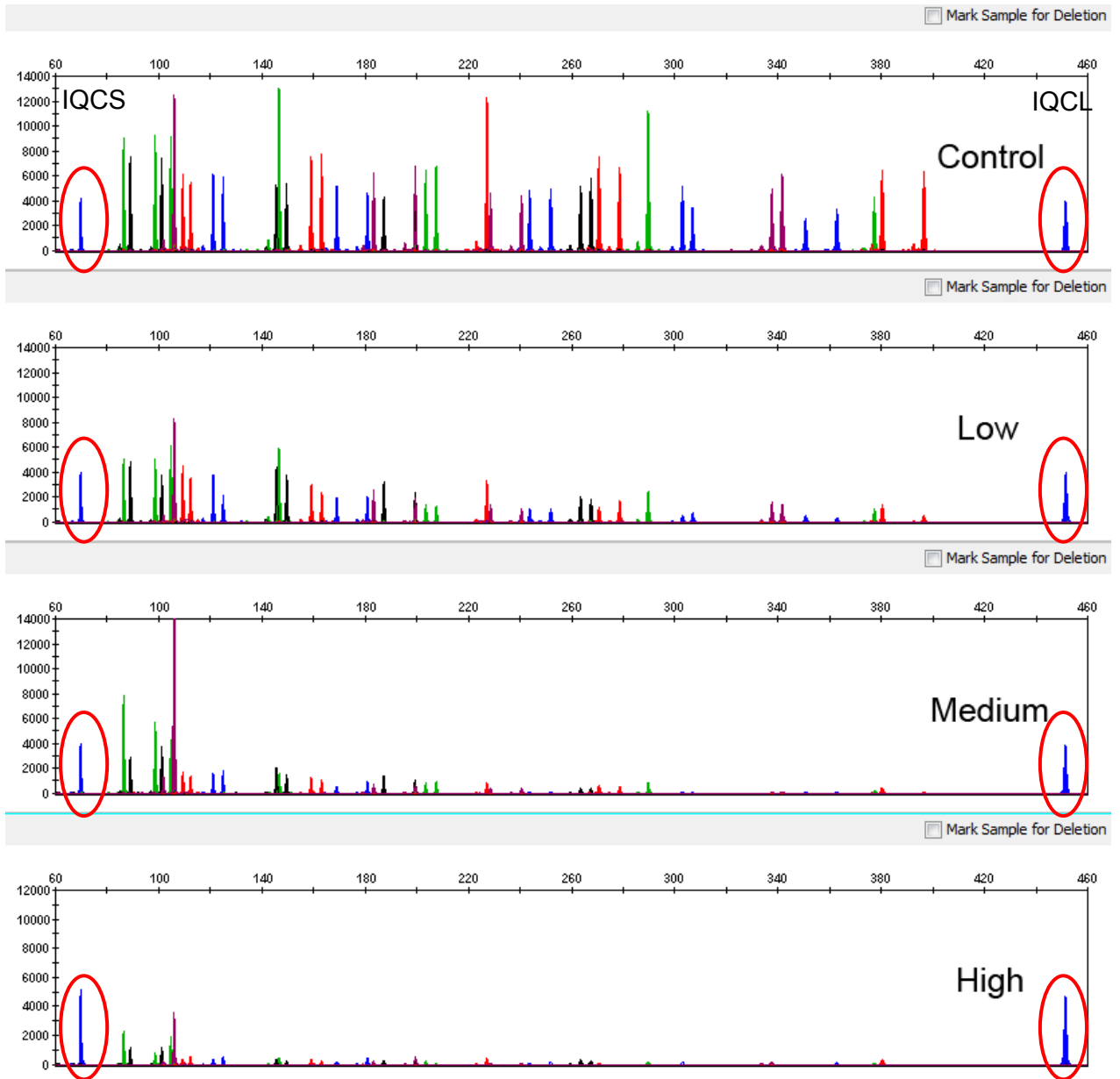
Figure 8 IQC peaks with degraded DNA at 0.5 ng 30 cycles (control Y-axis scale 0–14,000 RFU; low, medium, and high Y-axis scale 0–12,000 RFU;)

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.
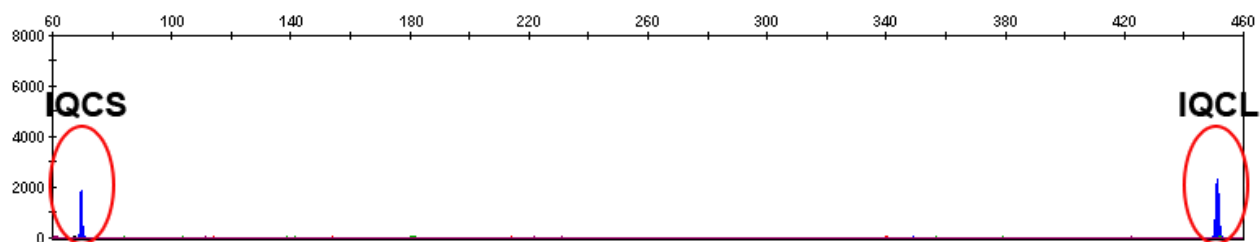
Figure 9 IQCS and IQCL peaks with 0 ng DNA (Y-axis scale 0–8,000 RFU)

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

Figure 10 shows the decreased IQCS peak and the missing IQCL peak in the presence of high levels of inhibitor.
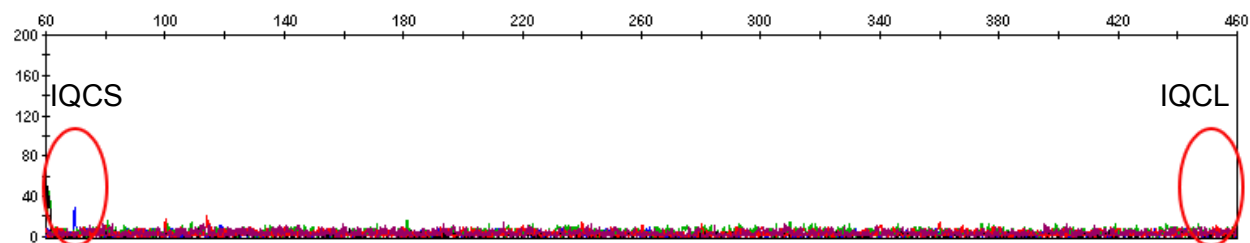
Figure 10 IQC peaks with very high hematin level (Y-axis scale 0–200 RFU)

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 of the kit and established that the concentration of each component was within the range where data indicated that the amplification met the required performance criteria for specificity, sensitivity, and reproducibility. For example, 1 ng of DNA Control 007 was amplified in the presence of varying concentrations of magnesium chloride, and the results were analyzed on an Applied Biosystems™ 3500xL Genetic Analyzer (Figure 11). The performance of the multiplex is most robust within ±20% of the optimal magnesium chloride concentration.
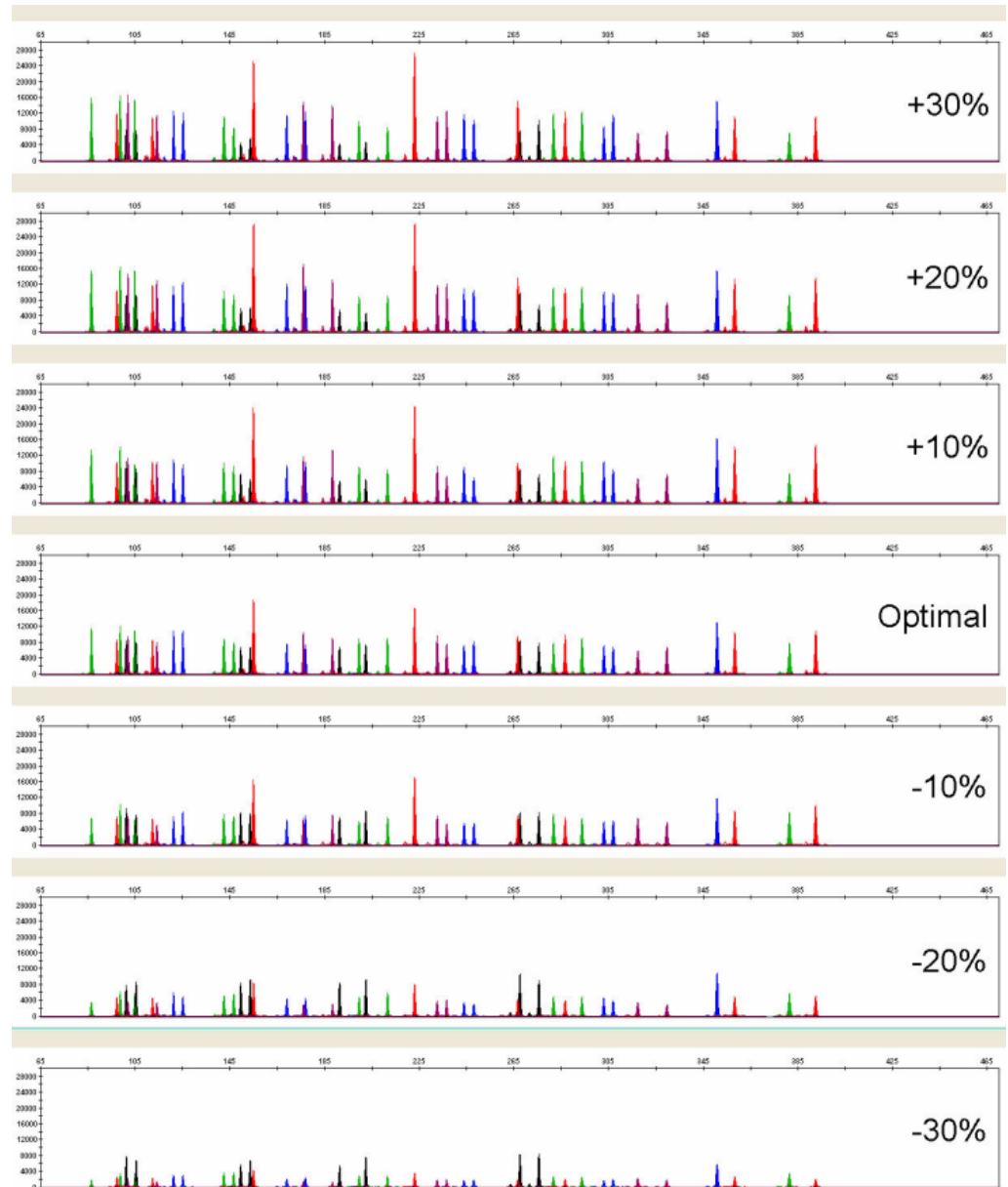
Figure 11 DNA Control 007 (1.0 ng) amplified with the GlobalFiler™ kit in the presence of varying concentrations of magnesium chloride and analyzed on an Applied Biosystems™ 3500xL Genetic Analyzer (Y-axis scale 0 to 30,000 RFU).

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 12). The PCR products were analyzed using an Applied Biosystems™ 3500xL Genetic Analyzer.

Of the tested annealing temperatures, 57 to 61°C produced robust profiles. At both 55 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.
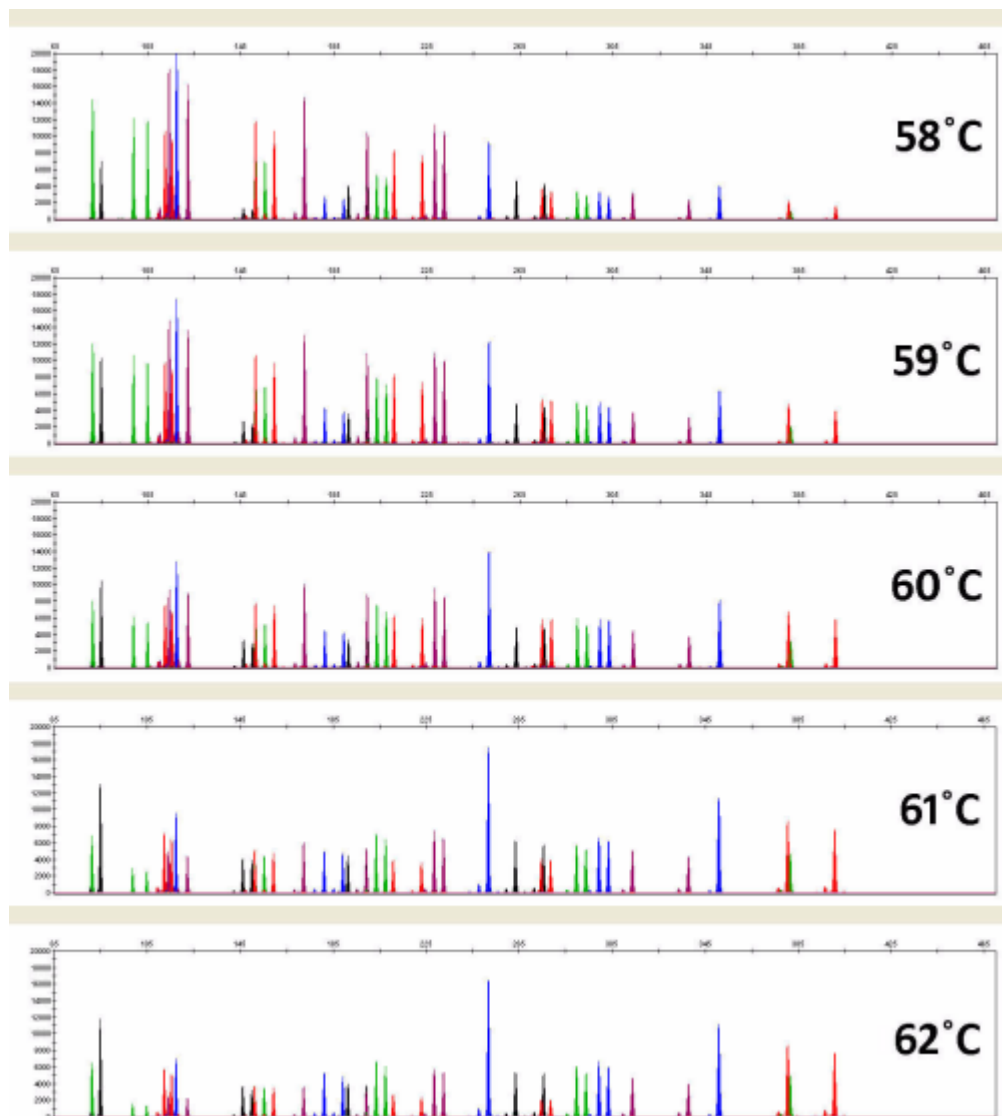
Figure 12 Electropherograms obtained from amplification of a 1.0 ng of DNA Control 007 at annealing temperatures of 55, 57, 59, 61, and 63°C, analyzed on an Applied Biosystems™ (Y-axis scale 0 to 16,000 RFU).

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 13).

We recommend using 29 cycles to optimize signal peak height and minimize artifactual or other undesirable peaks.
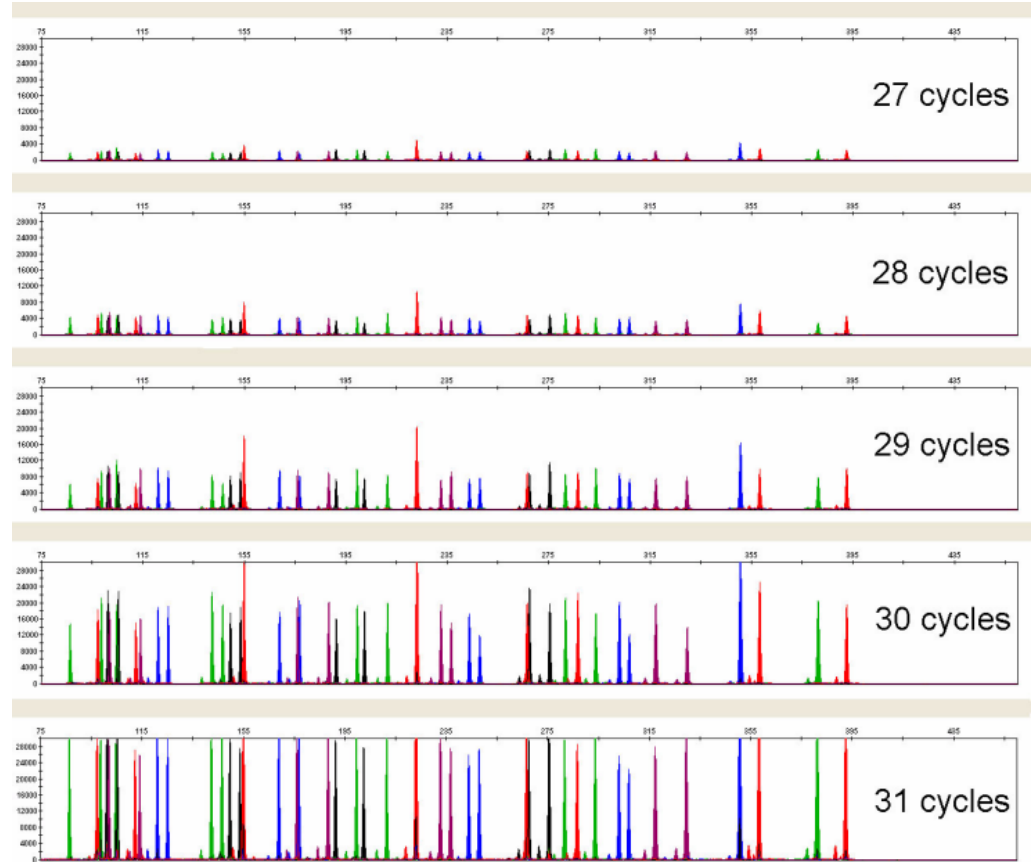
Figure 13 Representative GlobalFiler™ kit profiles obtained from amplification of 1.0 ng of DNA Control 007 using 27, 28, 29, 30, and 31 cycles, analyzed on anApplied Biosystems™ 3500xL Genetic Analyzer (Y-axis scale 0 to 30,000 RFU).

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

Laser-induced fluorescence detection of length polymorphism at short tandem repeat loci is not a novel methodology (Holt *et al.*, 2000; and Wallin *et al.*, 2002). However, accuracy and reproducibility of profiles have been determined from various sample types.

Figure 14, Figure 15, and Figure 16 show the size differences that are typically observed between sample alleles and allelic ladder alleles on the Applied Biosystems™ 3130 *xl*, 3500, and 3500xL Genetic Analyzers with POP-4™ Polymer. The X-axis in the

following figures represents the nominal nucleotide sizes for the GlobalFiler™ Allelic Ladder. The dashed lines parallel to the X-axis represent the ±0.25-nt windows. The y-axis represents the deviation of each sample allele size from the corresponding Allelic Ladder allele size. All sample alleles are within ±0.5 nt from a corresponding allele in the Allelic Ladder, irrespective of the capillary electrophoresis platforms.
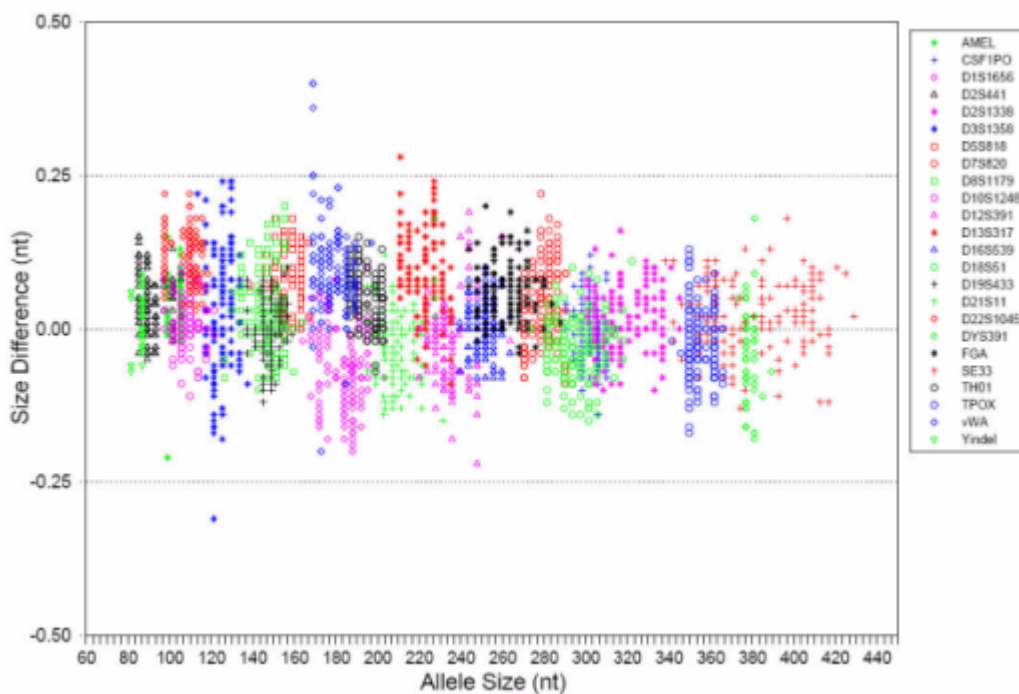
Figure 14 Allele Size vs. Allelic Ladder Sizing for 84 samples analyzed on a 3130 *xl* Genetic Analyzer . Size and ladder sizing for the GlobalFiler™ kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.
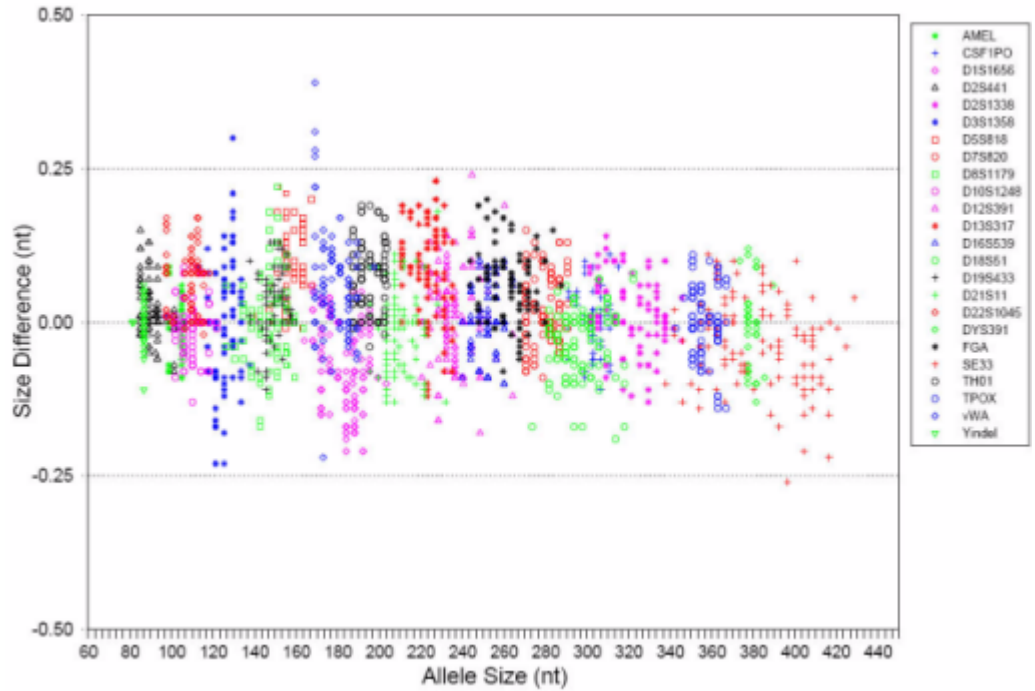
Figure 15 Allele Size vs. Allelic Ladder Sizing for 84 samples analyzed on a 3500 Genetic Analyzer. Size and ladder sizing for the GlobalFiler™ kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.
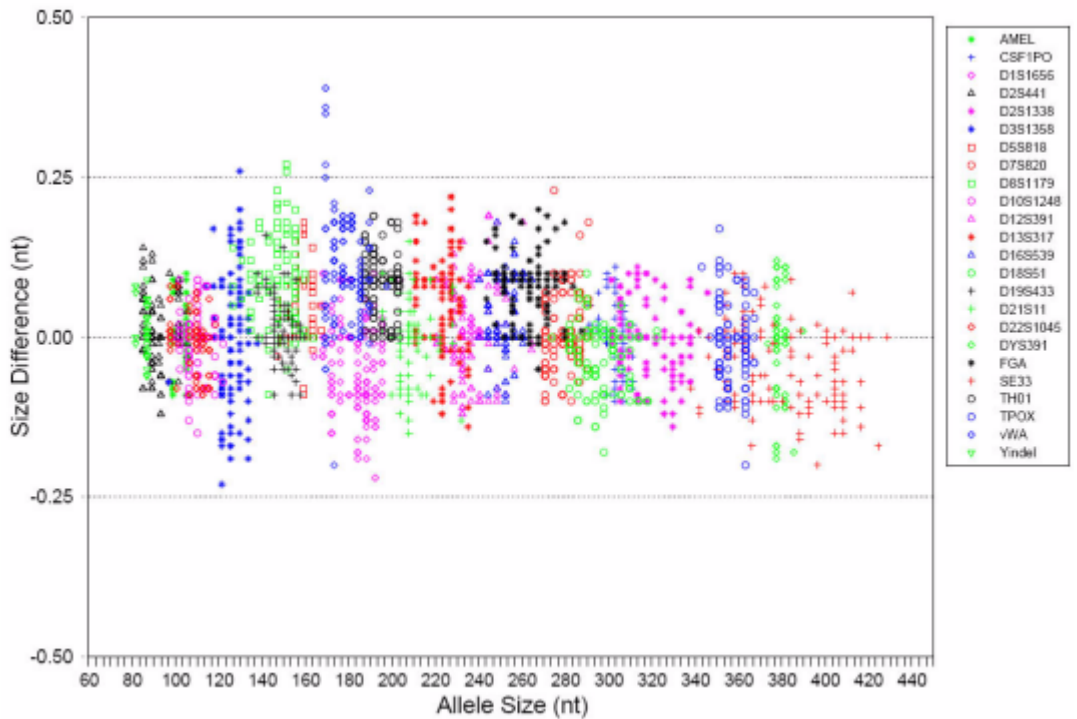
Figure 16 Allele Size vs. Allelic Ladder Sizing for 84 samples analyzed on a 3500xL Genetic Analyzer. Size and ladder sizing for the GlobalFiler™ kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

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-nt "window" around the size obtained for each allele in the allelic ladder. A ±0.5-nt window allows for the detection and correct assignment of alleles. Any sample allele that sizes outside the specified window could be either:

- 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 instrument.

Precision observation

Table 3 lists typical precision results obtained from multiple runs of the GlobalFiler™ Allelic Ladder using the GeneScan™ 600 LIZ™ Size Standard v2.0 . The results were obtained within a set of injections on a single capillary array. The number of repeated injections for each genetic analyzer platform is shown in the following table:

CE platform	Capillaries	# 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 3). 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-nt 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 figures in "Accuracy observation" on page 61 illustrate the tight clustering of allele sizes obtained, where the standard deviation in sizing is typically less than 0.15 nt. The instance of a sample allele sizing outside the ±0.5-nt window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 nt or less (Smith, 1995).

For sample alleles that do not size within a ±0.5-nt window, the PCR product must be rerun to distinguish between a true off–ladder allele versus measurement error of a sample allele that corresponds 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 ensure accurate genotyping, follow the guidelines in "Allelic ladder requirements for data analysis" on page 35 and use allelic ladders obtained from the same run as samples to analyze the samples.

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

Table 3   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
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

Allele	3130 xl		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
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
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

Allele	3130 xl		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
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
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

Allele	3130 xl		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
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
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

Allele	3130 xl		3500		3500xL	
	Mean	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**
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
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

Allele	3130 xl		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
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
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

Allele	3130 xl		3500		3500xL	
	Mean	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**
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
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

Allele	3130 xl		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
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
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

Allele	3130 xl		3500		3500xL	
	Mean	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**
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
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

Allele	3130 xl		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
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
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

Allele	3130 xl		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
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
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

Allele	3130 xl		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
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
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
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

Allele	3130 xl		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
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 (see DNA Advisory Board (DAB) Standard 8.1.2.2).

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 observations

Peak heights were measured for amplified samples at the loci 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 display a lower level of stutter relative to the longer alleles within each locus.
- Each allele within 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 three 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.

Figure 17 through Figure 23 show the stutter observed in the population study. All data were generated on the 3500xL Genetic Analyzer.

Additional marker-specific plus stutter observed in the population study with the GlobalFiler™ kit is listed in Table 4. Examples of non-standard stutter peaks at two loci are shown in "Example of non-standard stutter peaks observed at the D22S1045 and SE33 loci" on page 85.

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 this data are listed in "Stutter percentage filter settings that are provided with the GeneMapper™ ID-X Software" on page 87.
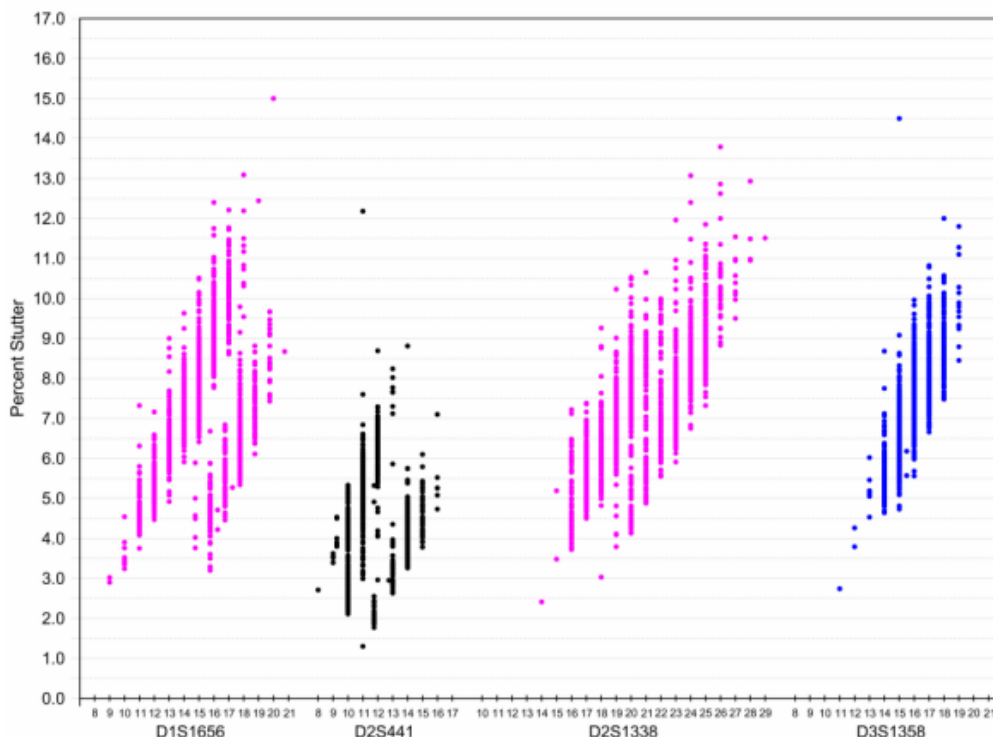
Figure 17 Stutter percentages for D1S1656, D2S441, D2S1338, and D3S1358 loci (Blue=FAM™ dye, black=NED™ dye, purple=SID™ dye)
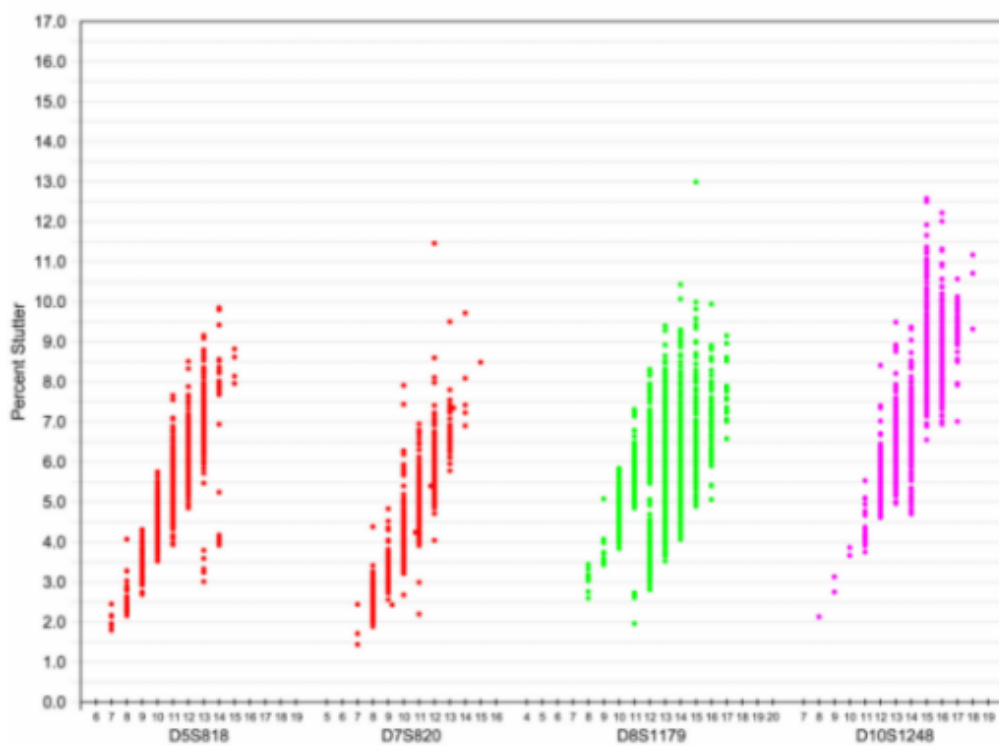
Figure 18 Stutter percentages for D5S818, D7S820, D8S1179, and D10S1248 (Green=VIC™ dye, red=TAZ™ dye, purple=SID™ dye)
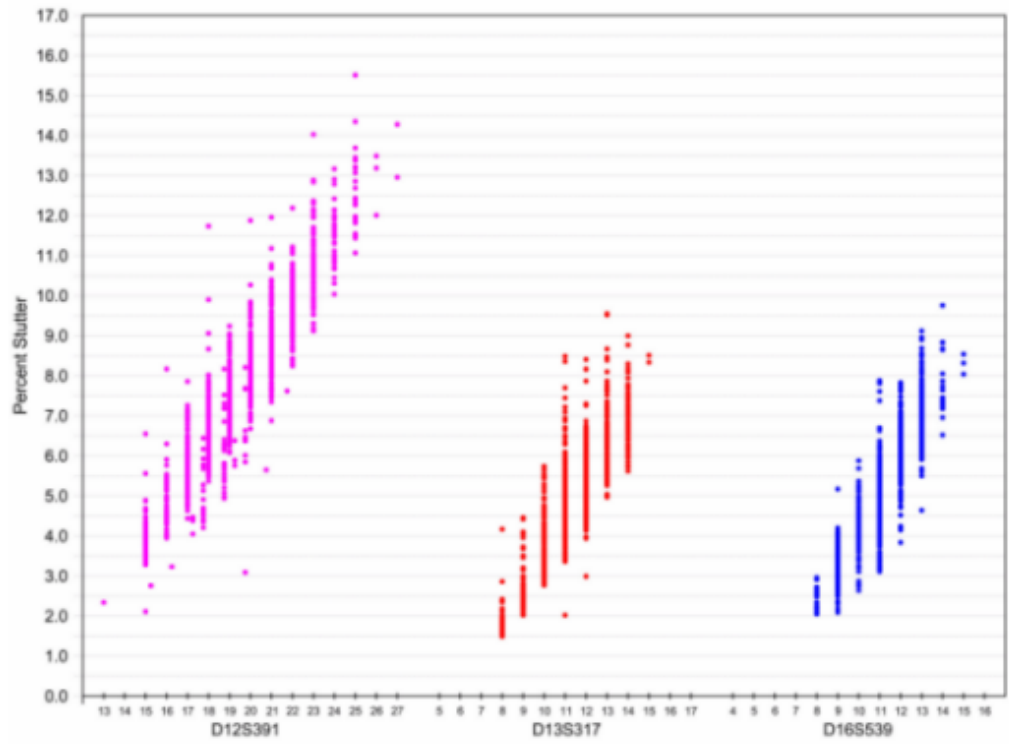
Figure 19 Stutter percentages for D12S391, D13S317, and D16S539 loci (Blue=FAM™ dye, red=TAZ™ dye, purple=SID™ dye)
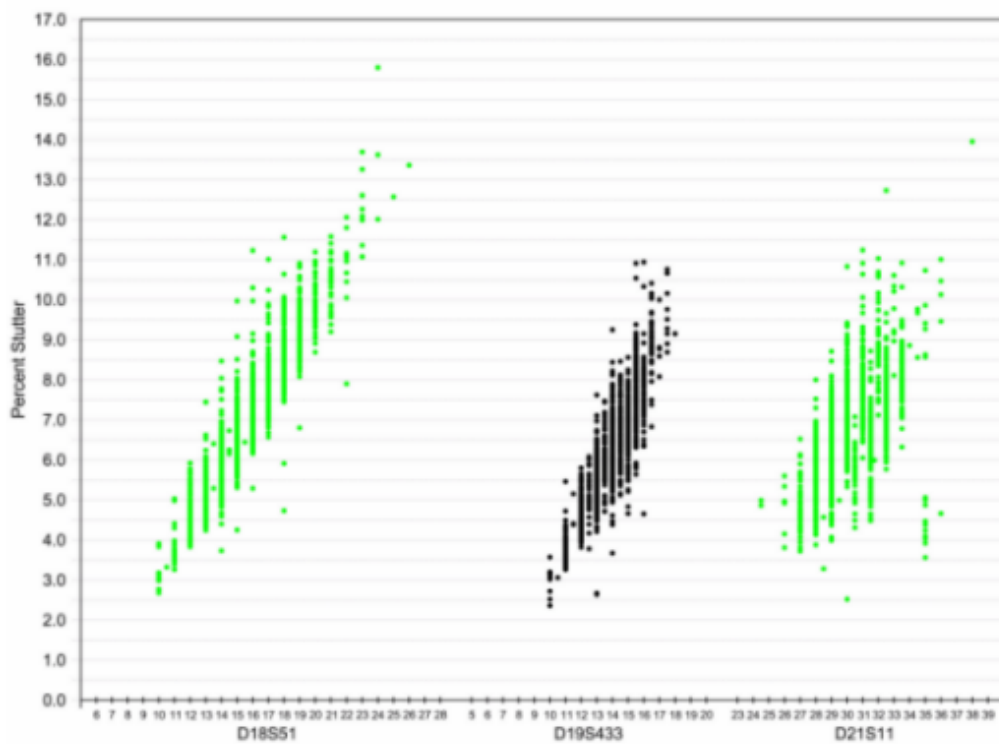
Figure 20 Stutter percentages for D18S51, D19S433, and D21S11 loci (Green=VIC™ dye, black=NED™ dye)
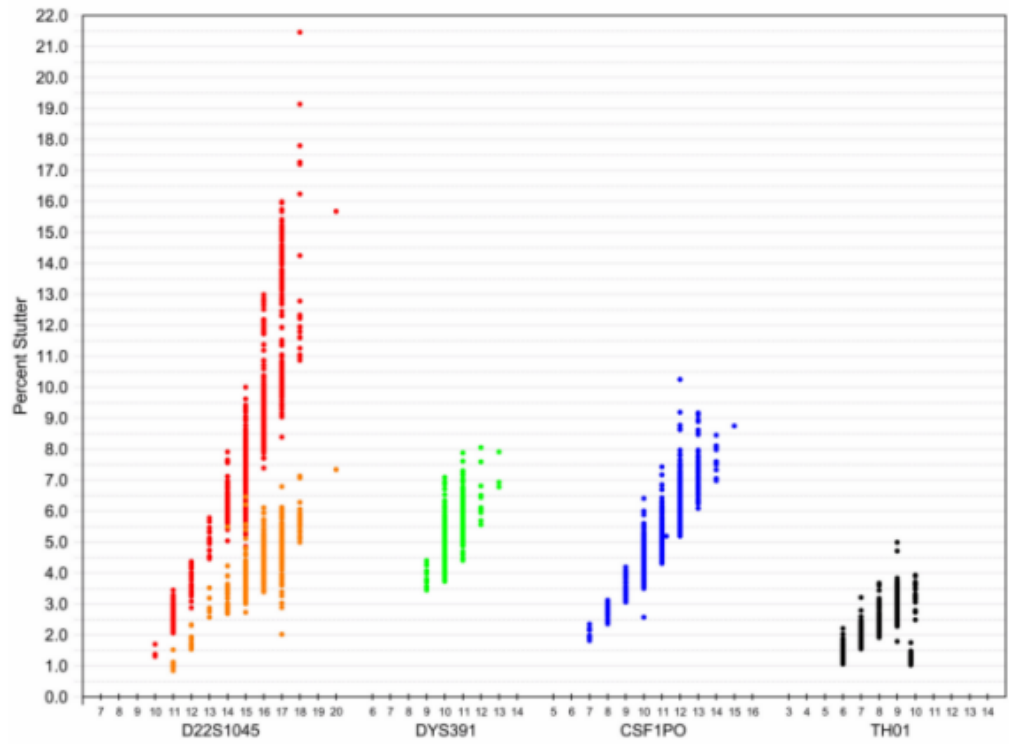
Figure 21 Stutter percentages for D22S1045, DYS391, CSF1PO, and TH01 loci (Blue=FAM™ dye, green=VIC™ dye, black=NED™ dye, red=TAZ™ dye). Red and orange data points associated with D22S1045 locus indicate minus and plus stutter, respectively.
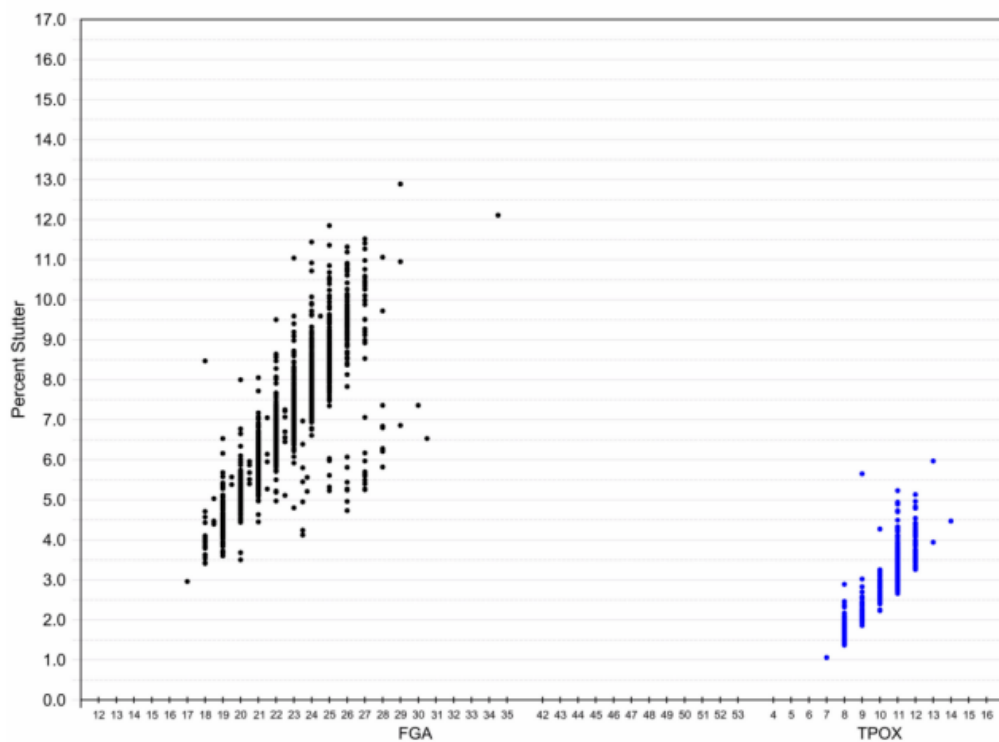
Figure 22 Stutter percentages for FGA and TPOX loci (Blue=FAM™ dye, black=NED™ dye)
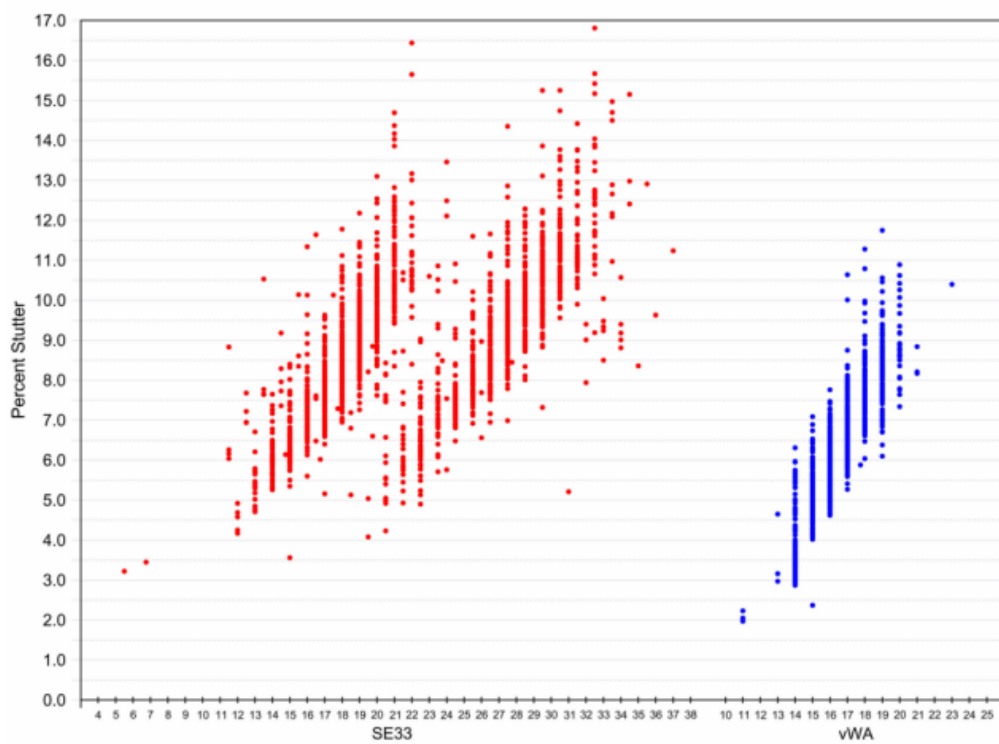
Figure 23 Stutter percentages for SE33 and vWA loci (Blue=FAM™ dye, red=TAZ™ dye)

Table 4 Marker-specific plus stutter observed in the population study with the GlobalFiler™ kit. Data produced on a 3500xL Genetic Analyzer using a 150 RFU threshold cutoff. Markers that showed negligible plus stutter are omitted.

Loci	Observations	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
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 and SE33 loci

STR loci such as D1S1656 (Figure 24) and SE33 (Figure 25) include more complex nucleotide sequences including regions of dinucleotide repeats which 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 contain a minus 2–nt stutter filter for SE33 and D1S1656 to prevent these peaks from being called in normal profiles (see Table 5).
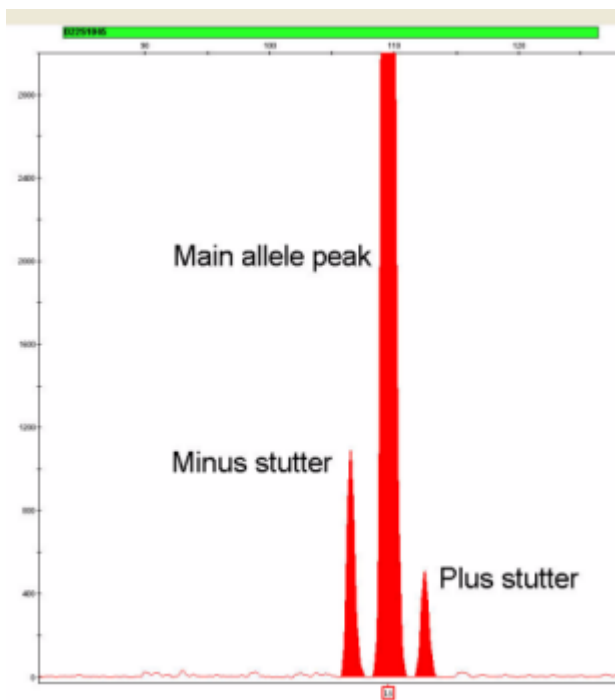
Figure 24 GlobalFiler™ kit electropherogram showing plus stutter associated with the D22S1045 STR locus. Data produced on a 3500xL Genetic Analyzer.
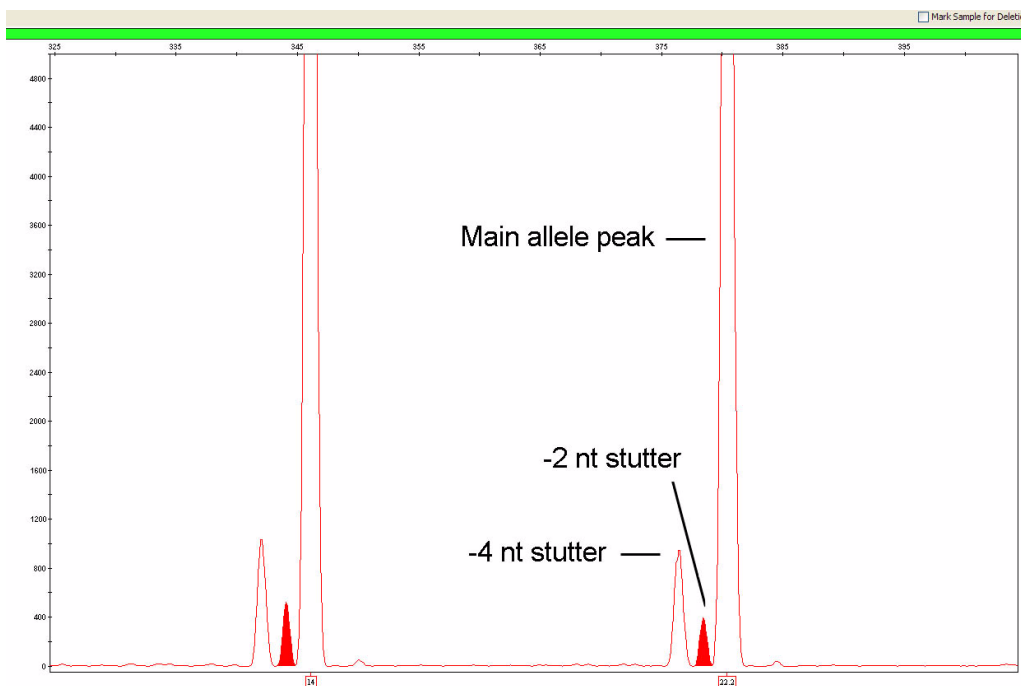
Figure 25 Example of a –2 nt reproducible artifact at the SE33 locus. Data produced on a 3500xL Genetic Analyzer.

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

The settings in Table 5 were derived using the data that is shown earlier in this section. 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. Always perform internal validation studies to determine the appropriate values to use for your applications.

Table 5 Stutter percentage filter settings provided with the GeneMapper™ *ID-X* Software

Locus [1]	% 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 nt)	2.45
D21S11	10.45
D22S1045	16.26
D22S1045 (+3 nt)	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

Locus [1]	% Stutter
SE33	14.49
SE33 (–2 nt)	3.97
TH01	4.45
TPOX	5.55
vWA	10.73

[1]   These percentages are used as stutter filters in **GlobalFiler_stutter_v1.txt** or **GlobalFiler-IQC_stutter_v1.txt**

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 observations

The efficiency of +A addition is related to the particular sequence of the DNA at the 3′ end of the PCR product.

The GlobalFiler™ 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 at The new, highly robust PCR chemistry allows complete +A addition with a short final incubation at 60°C for 10 minutes..

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products. Figure 26 shows examples of incomplete and normal +A addition. 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.
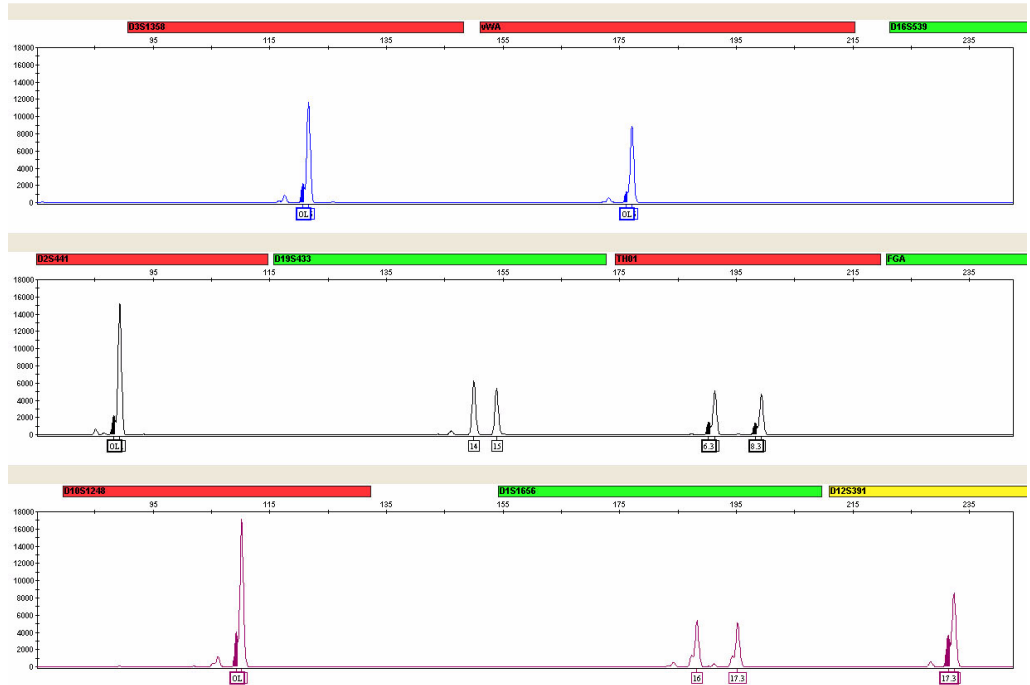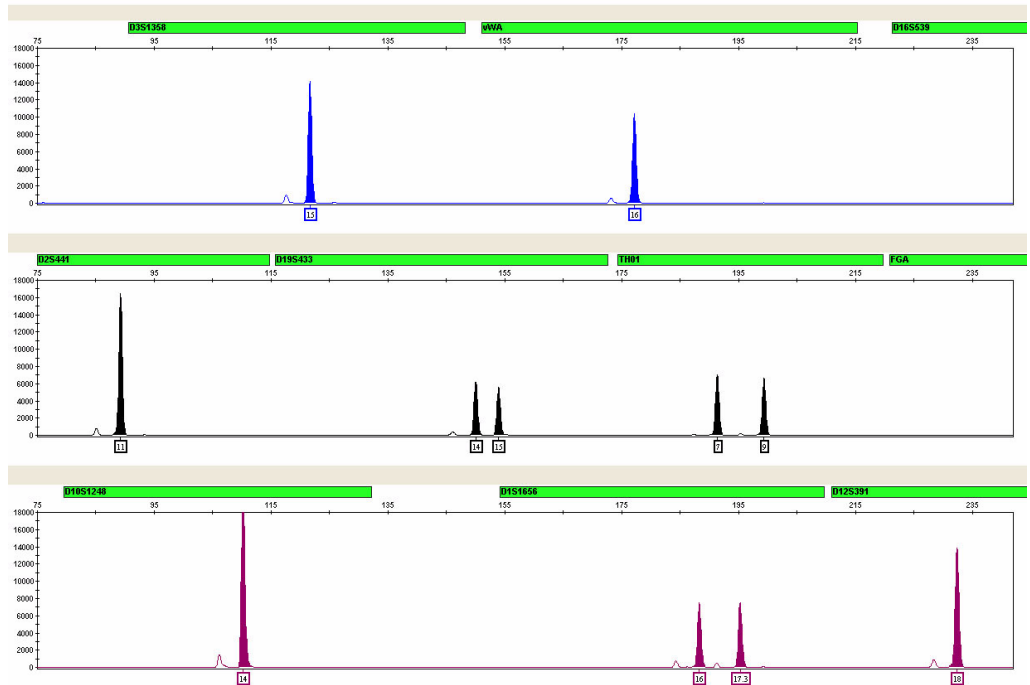
Figure 26 Omitting the final extension step results in shoulders on main allele peaks due to incomplete A nucleotide addition. Examples shown are the smaller amplicons of FAM™, NED™, and SID™ dye channel data from a 3500xL Genetic Analyzer using the GlobalFiler™ kit.

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 recommended concentration. Amplification of excess input DNA can also result in off-scale data.

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 nt for commonly used analytical thresholds. Figure 27 shows the low baseline-level fluorescence that is observed in a typical negative control PCR.

However, it is important to consider noise and other amplification-related artifacts when interpreting data.
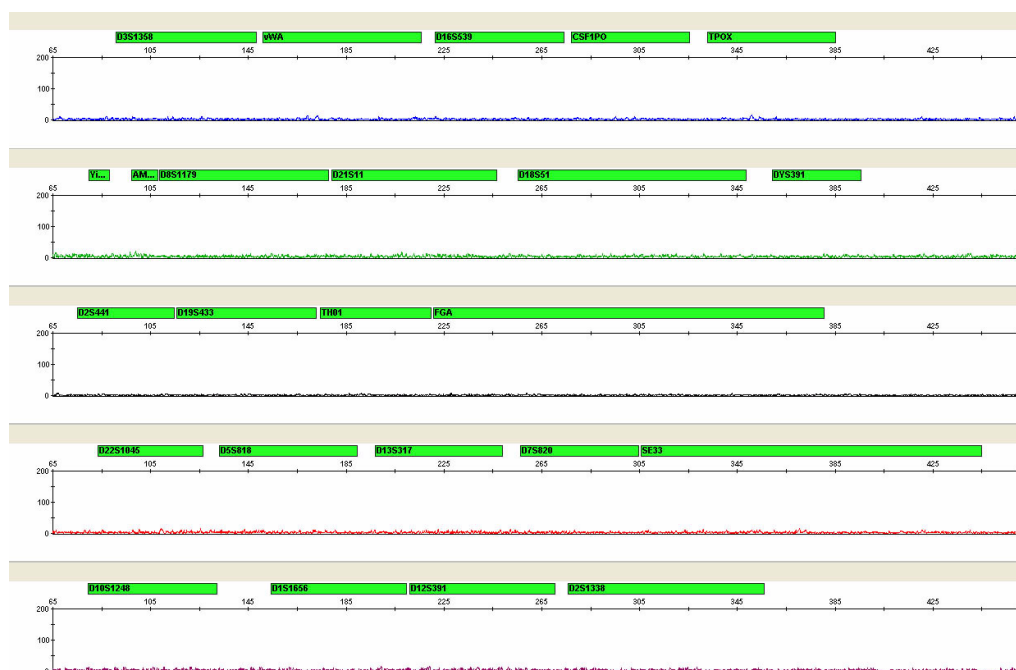
Figure 27 Examples of fluorescence background in data produced on a 3500xL Genetic Analyzer (Y-axis scale 0 to 200 RFU).

Extra peaks: 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 28):

- 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
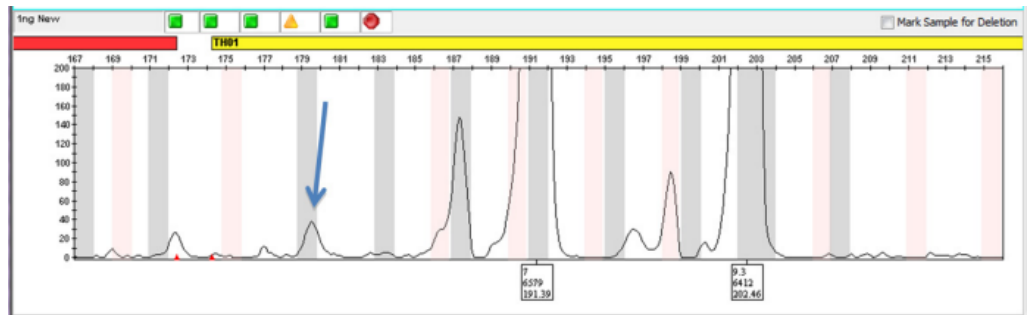
Figure 28   N-12 artifact (see arrow) associated with TH01 allele 7

TPOX locus artifact

A reproducible artifact intrinsic to the GlobalFiler™ kit assay has been identified at the TPOX locus (Figure 29):

- 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
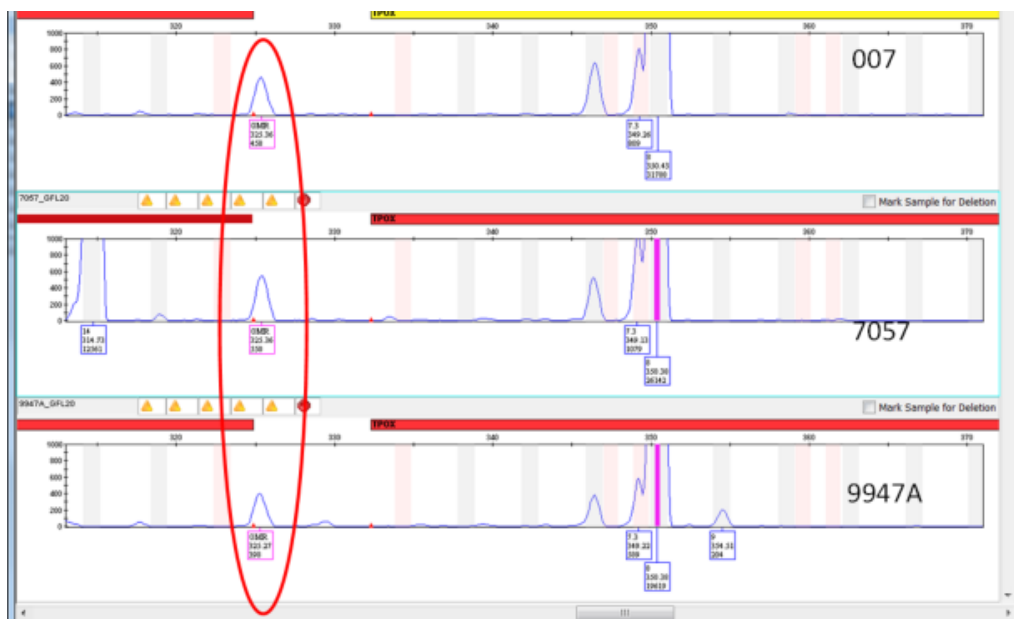
Figure 29 FAM™ dye artifacts in GlobalFiler™ kit profiles (size = 325 bp), three DNA samples at N-24 nucleotides from the TPOX peak depicted, 1 ng of DNA/30 cycles.

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 30):

- 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
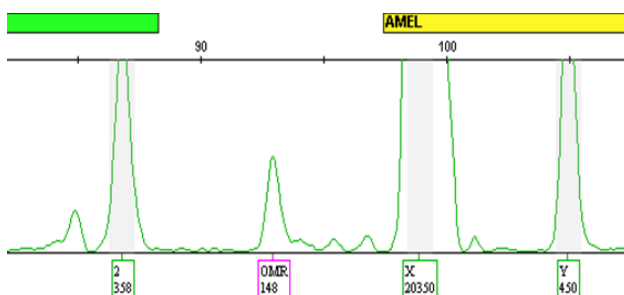
Figure 30 Artifact between the Amelogenin and Yindel loci (OMR=Outside Marker Range flag)

D21 locus artifact

A reproducible artifact that is intrinsic to the GlobalFiler™ kit assay has been identified at the D21 locus (Figure 31):

- 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.
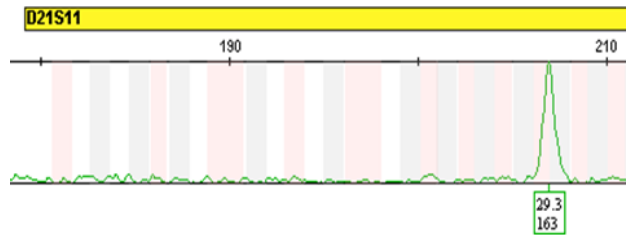
Figure 31 Artifact at the D21 locus

vWA locus artifact

A reproducible artifact that is intrinsic to the GlobalFiler™ kit assay has been identified at the vWA locus (Figure 32):

- 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.
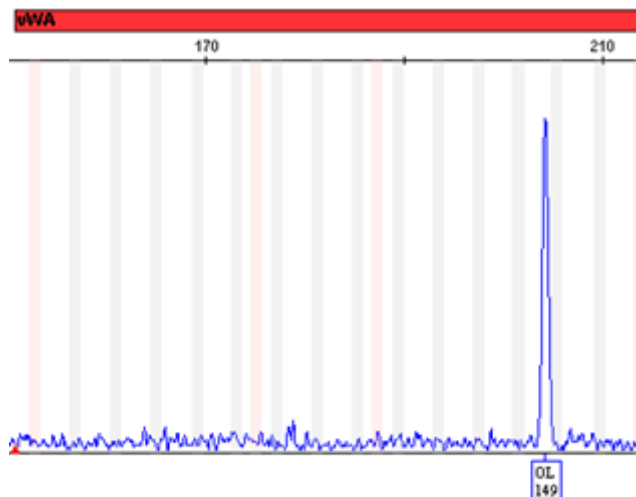
Figure 32 Artifact at the vWA locus

D5 locus artifact

A reproducible artifact that is intrinsic to the GlobalFiler™ kit assay has been identified at the D5 locus (Figure 33):

- 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
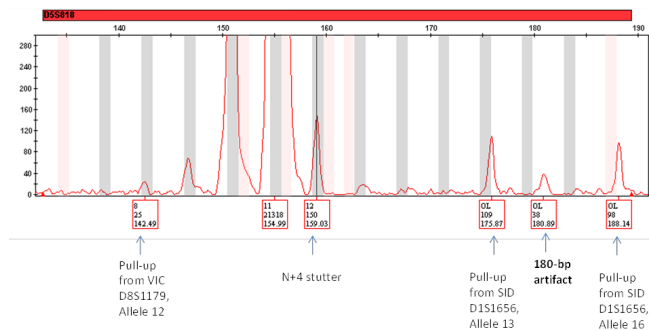
Figure 33 Artifact at D5 locus

D3 locus artifact

A reproducible artifact that is intrinsic to the GlobalFiler™ kit assay has been identified at the D3 locus (Figure 34):

- 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
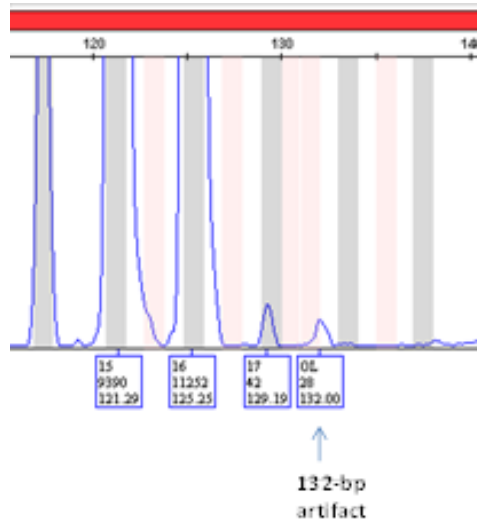
Figure 34 Artifact at D3 locus

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-nt and 105-nt 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

Two sets of STR loci in the GlobalFiler™ kit are located on the same chromosomes. vWA and D12S391 are located approximately 6.3 million bp apart on the p arm of chromosome 12; D2S1338 and D2S441 are located approximately 150 million bp apart on opposite arms of chromosome 2. Linkage disequilibrium analysis was conducted on the genotype results from 1,034 individuals of three ethnic groups (350 African American, 349 Caucasian, and 335 Hispanic). STR locus genotype results from the population study were analyzed using the Linkage Disequilibrium module of GenePop software version 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008). See Table 6 for results.

The relatively high probability values indicate that there is no statistically significant linkage disequilibrium found between the pairs of loci that are located on the same chromosome.

An independent analysis of data from the same collection of population samples (Budowle, *et al.*, 2010) also concluded that the 15 STR loci that are shared between the NGM™ and NGM SElect™ kits were independent at the population level (note that the SE33 locus was not part of this analysis). Therefore, to calculate the rarity of a profile for comparison to single-source and mixture samples, the frequencies of all loci including vWA and D12S391 could be multiplied. However, the 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.

Table 6 GenePop software LD Result (p-value for pairwise analysis of loci)

Locus	Chromosome map position[1]	Chromosome Nuclear Coordinates[1] (million bp)	African-American (n = 350)	Caucasian (n = 350)	Hispanic (n = 293)
vWA	12p13.31	5.9	0.86	0.29	0.27
D12S391	12p13.2	12.2			
D2S441	2p14	68	0.11	0.32	0.19
D2S1338	2q35	218			

[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) greater than 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 ensure 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 GlobalFiler™ kit 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 GlobalFiler™ kit with any of these non-human species.

Figure 35 shows example electropherogram results from the species specificity tests. The chimpanzee and gorilla DNA samples produced partial profiles in the 70–400 nucleotide 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.
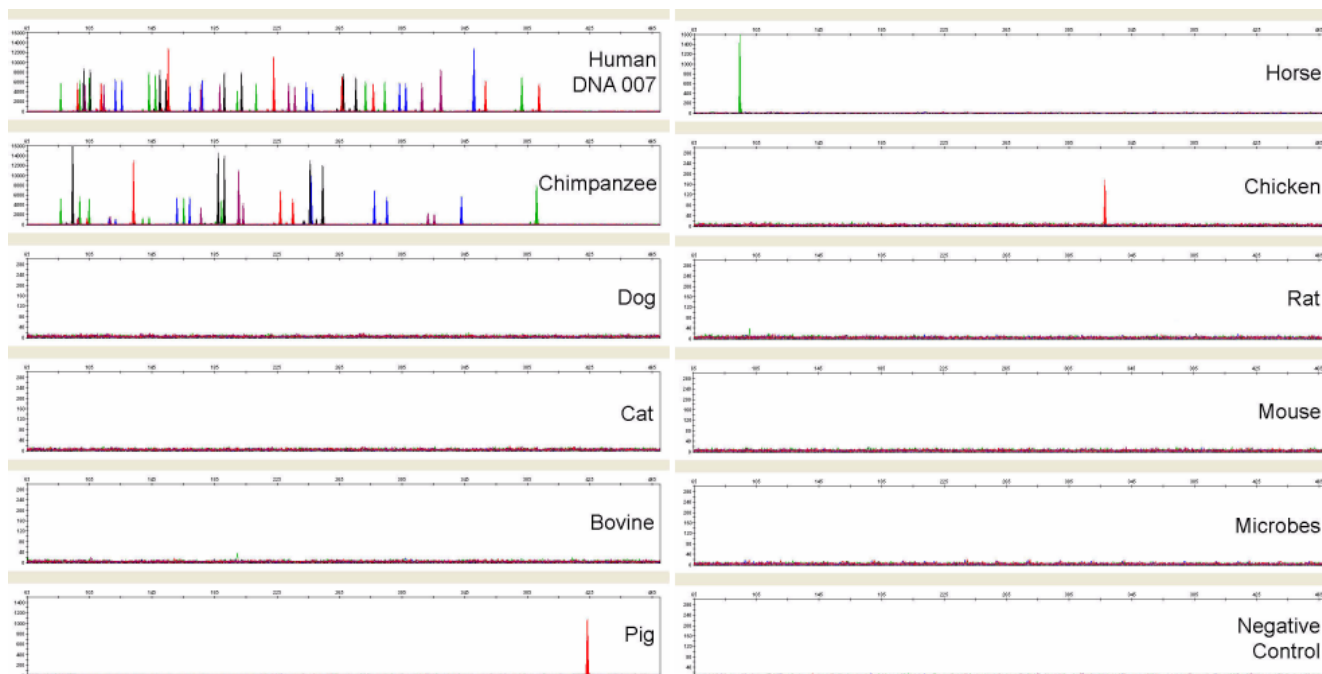
Figure 35 Representative electropherograms for some species tested in a species specificity study. Data produced on a 3500xL Genetic Analyzer.

Among the non-primate species, most produced no peaks over a threshold of 175 RFU. Horse yielded reproducible VIC™ dye peaks at 94 bp (less than 1,800 RFU) due to Amelogenin cross-reactivity. Pig yielded reproducible TAZ™ dye peaks at 424 bp (less than 1,200 RFU). Chicken yielded reproducible TAZ™ dye peak at 328 bp (less than 200 RFU). Individual replicate PCRs of dog, mouse, and chicken yielded single, small (less than 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.

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:

• Fluorescence intensity that exceeds the linear dynamic range for detection by the instrument ("off-scale" data).

Off-scale data is a problem because:

– Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause the corresponding stutter peak to appear higher in relative intensity, thus increasing the calculated percent stutter.

– Multicomponent analysis of off-scale data is not accurate. This inaccuracy results in poor spectral separation ("pull-up").

• Incomplete +A nucleotide addition.

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

If too little DNA is added to the PCR reaction, the total number of allele copies added to the PCR is extremely low and unbalanced amplification of the alleles may occur because of stochastic fluctuation.

Sensitivity observation

The recommended amount of input DNA for the GlobalFiler™ kit is 1.0 ng for 29 cycles of amplification based on quantification using chemistry, such as in the Quantifiler™ Duo DNA Quantification Kit or the Quantifiler™ Human DNA Quantification Kit. Perform studies to determine the optimum input DNA amount according to the quantification kit you use.

If the sample contains degraded or inhibited DNA, amplification of a higher concentration of DNA may be beneficial. In Figure 36, the DNA Control 007 was serially diluted from 1.0–0.031 ng. Full profiles (43 PCR products) were consistently obtained at 0.125 ng, but occasional partial profiles resulted at lower concentrations.
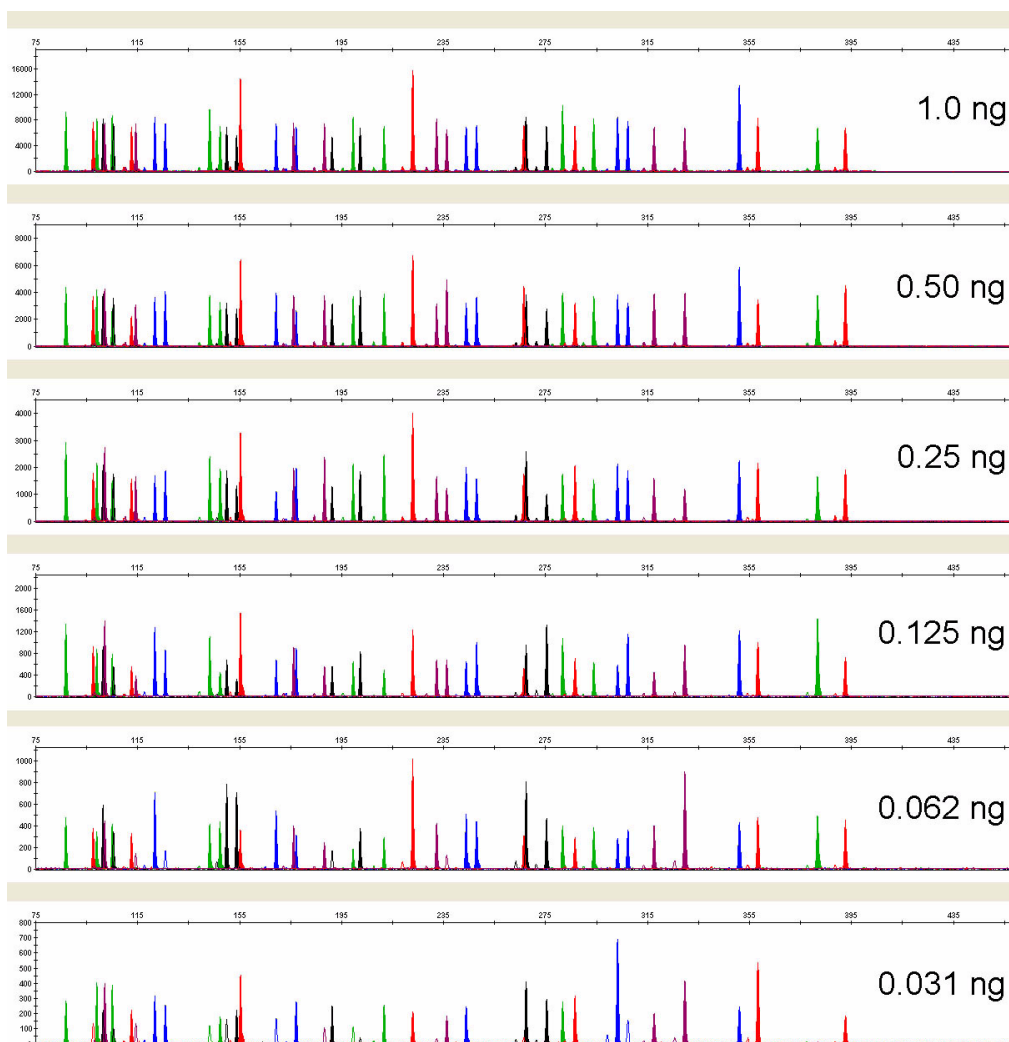
Figure 36 Electropherograms for amplifications using 1, 0.50, 0.25, 0.125, 0.062, and 0.031 ng of DNA Control 007. Electrophoresis was performed on an 3500xL Genetic Analyzer. Note that as the DNA input is serially diluted by 2-fold, the Y-axis scale is also magnified by 2-fold for the smaller input 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)

Degraded DNA

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

prepared to examine the potential for differential 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. Amplification of 1.0 ng of degraded DNA using the GlobalFiler™ kit was performed. As the DNA became progressively degraded, the loci failed to amplify robustly in order of decreasing size. Preferential amplification was not observed.
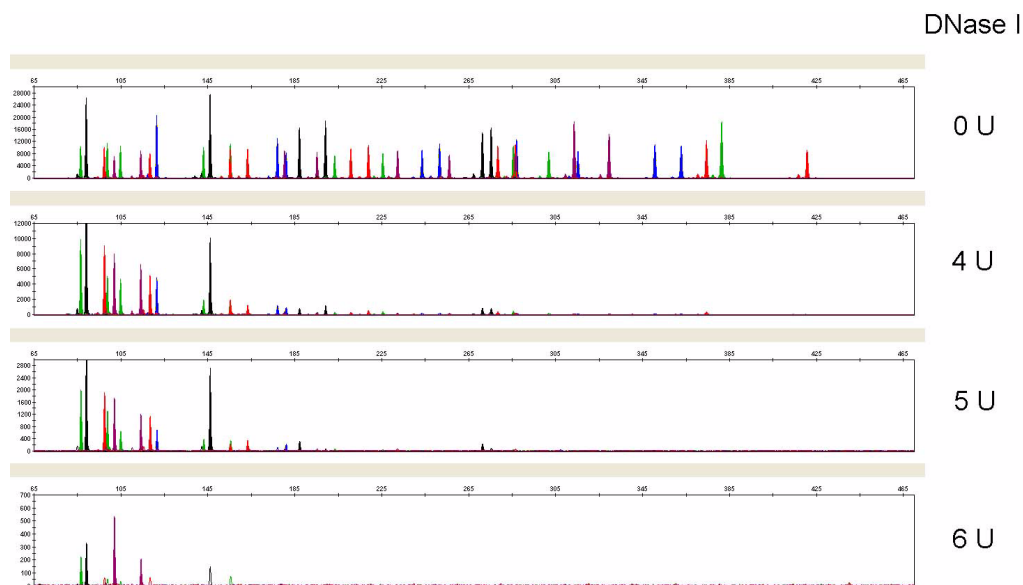
Figure 37 Amplification of A3121 DNA samples sonicated and incubated with increasing doses of DNase I. Panels 1, 2, 3, and 4 correspond to 0, 4, 5, and 6 units of DNase I. Note that the Y-axis scale is magnified for more degraded samples, which generate lower peak heights.

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 38). The concentrations of hematin used were 0, 200, 300, and 400 μM (Table 7).
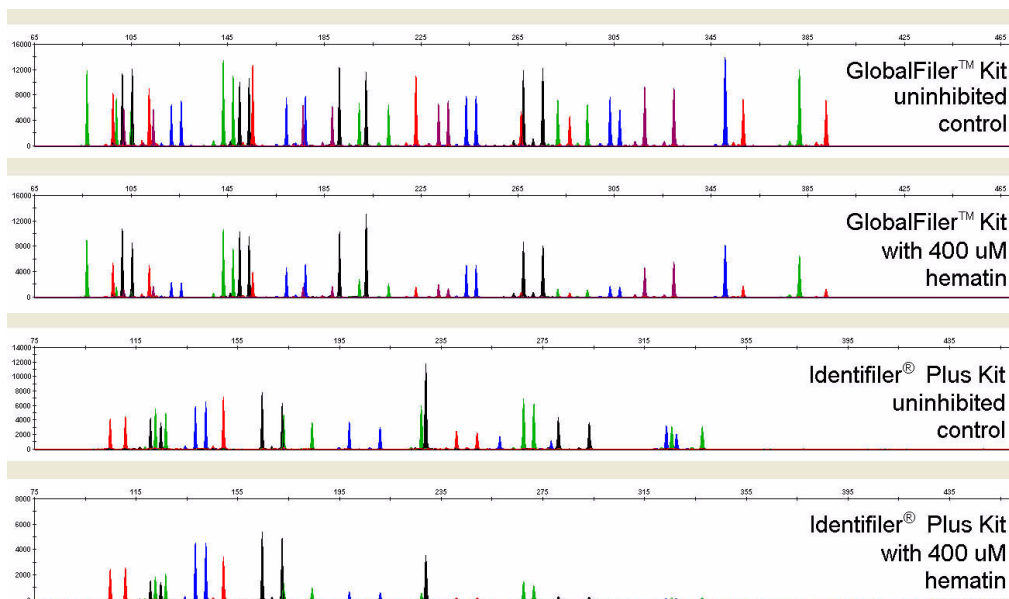
Figure 38 Electropherograms for the GlobalFiler™ kit and the Identifiler™ Plus kit show the improved performance of the GlobalFiler™ kit in the presence of hematin compared with previous AmpFℓSTR™ kits. The same set of inhibited samples was analyzed with the GlobalFiler™ kit and the Identifiler™ Plus kit for 29 cycles of amplification.

Table 7 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 may 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 39). The concentrations of humic acid tested were 0, 70, 140, and 210 ng/µL (Table 8).
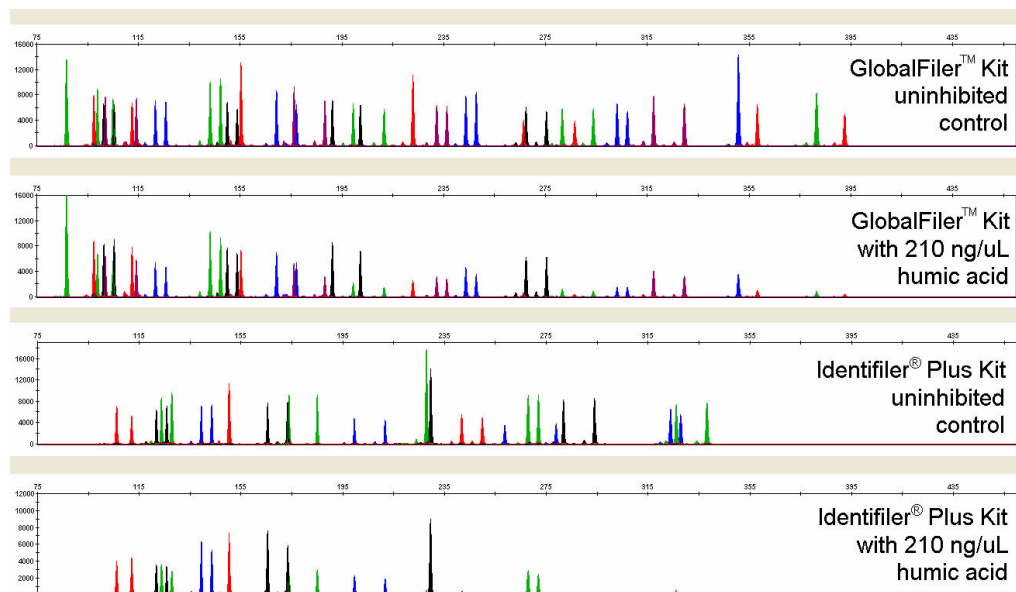
Figure 39 Electropherograms for the GlobalFiler™ kit and the Identifiler™ Plus kit show the improved performance of the GlobalFiler™ kit in the presence of humic acid compared with previous AmpFℓSTR™ kits. The same set of inhibited samples was analyzed with the GlobalFiler™ kit and the Identifiler™ Plus kit for 29 cycles of amplification.

Table 8 GlobalFiler™ kit performance in simulated humic inhibition (N = 3).

Humic acid concentration	Number of alleles detected[1]
0 µm	43, 43, 43
70 µm	43, 43, 43
140 µm	43, 43, 43
210 µm	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 may contain DNA from more than one individual. 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.

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 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, reamplify 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 40. The population samples used are listed in "Population samples used in these studies" on page 108.
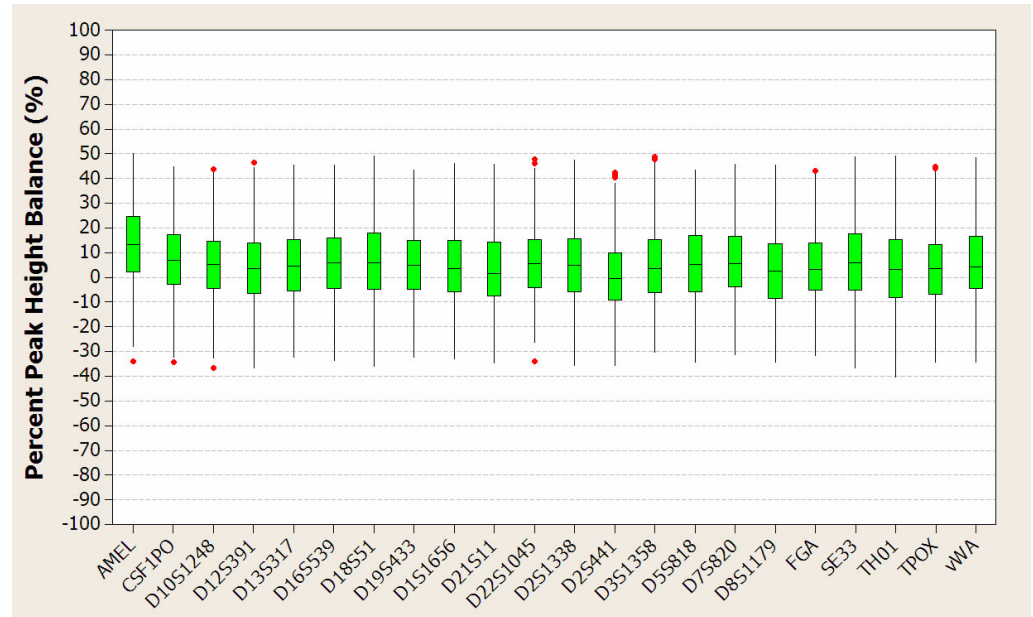
Figure 40 Heterozygote ratios for 1 ng of input DNA. The distribution of intra-locus peak height ratios are expressed as plus and minus percent, by locus. Green boxes show the middle 50% or interquartile range (IQR). Box halves below and above median show the second and third quartile, respectively. "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 containing DNA from two sources can comprise (at a single locus) any of the seven genotype combinations (see below).

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

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

Note: Peak detection is a complex process that involves the STR chemistry, capillary electrophoresis conditions, and the data analysis software. Contact HID Support for a Technical Note with additional information on detecting peaks in electropherograms.

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 an Applied Biosystems™ 3500xL Genetic Analyzer.

The results of the mixed DNA samples are shown in Figure 41 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 9.
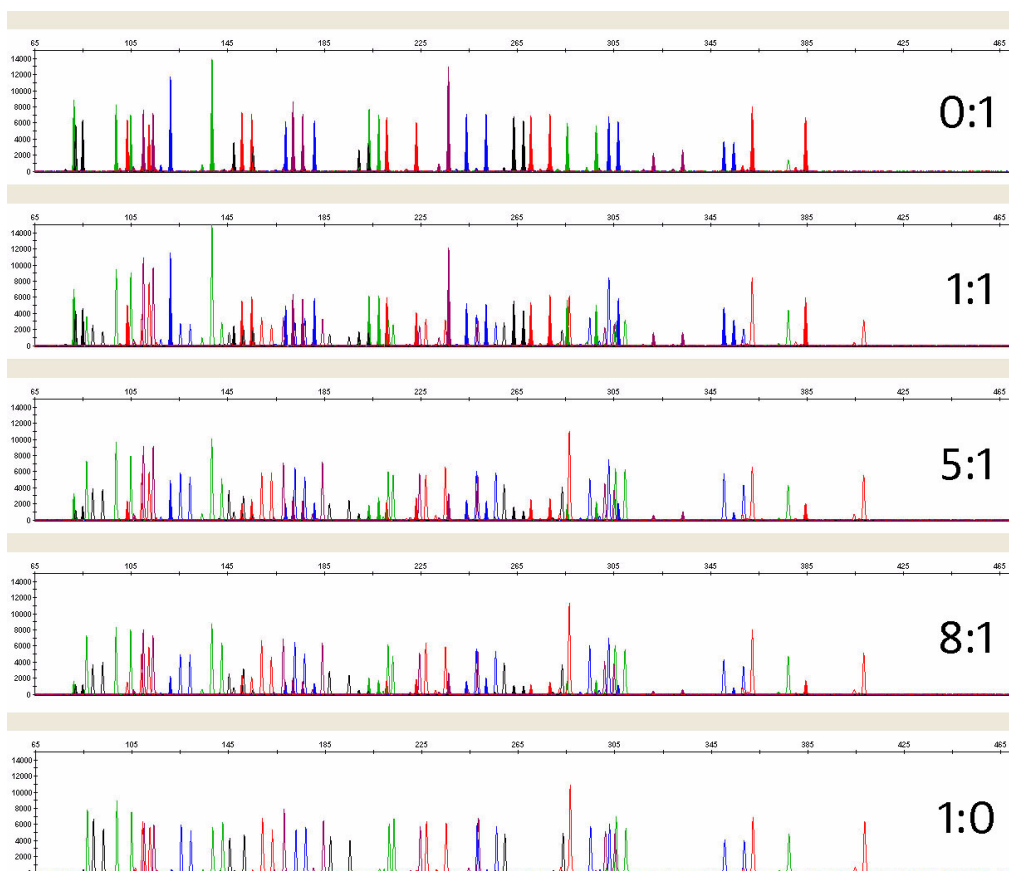
Figure 41 Amplification of DNA mixtures at various ratios. Panels show electropherograms for (top to bottom) 0:1 (minor contributor only), 1:1 mixture, 5:1 mixture, 8:1 mixture, and 1:0 (major contributor only).

Table 9   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
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)

Population data overview

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

- Different from the genotype of 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.

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 Kit User Guide* (Pub. No. 4458841) and the *AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit User Guide* (Pub. No. 4440211).

Population samples used in these studies

The GlobalFiler™ kit was used to generate the population data provided in this section. Whole blood samples, provided by the Interstate Blood Bank (Memphis, Tennessee) and Boca Biolistics (Coconut Creek, Florida), 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 using 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 **www.cstl.nist.gov/div831/strbase**).

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 definition

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

Probability of identity observation

Table 10 shows the Autosomal STR allele frequencies at GlobalFiler™ kit loci by population group.

Table 11 shows the Y-specific allele frequencies by population group for GlobalFiler™ kit DYS391 and Y indel loci. The Y-specific allele frequencies were not included in the probability of identity calculation.

Table 12 shows the Probability of identity (P_I) values of the GlobalFiler™ kit loci individually and combined.

Table 10 Autosomal allele frequencies by population group for GlobalFiler™ kit STR loci. (*=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
13	22.88	36.93	29.45	25.95

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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
24	0.61*	0.98*	3.64	1.9
25	0.61*	0.33*	1.9	1.36

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Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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
14	1.36	1.31*	1.75	1.22
15	*	*	0.15*	0.27*
16	*	*	*	*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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*
26	*	0.33*	*	*
27	*	*	*	*
28	*	*	*	*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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	*	*	*	*
19.2	*	*	*	*
D1S1656				
8	*	*	*	*
9	0.15*	*	*	0.14*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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	*	*	*	*
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

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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	*	*	*	*
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

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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
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*	*	*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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
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	*	*	*	*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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
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

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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	*	*	*	*
19	*	*	*	*
20	*	*	*	*
FGA				
12.2	*	*	*	*
13	*	*	*	*
14	*	*	*	*
15	*	*	*	*
16	*	0.33*	0.15*	*
16.1	0.30*	*	*	*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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	*	*	*	*
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	*	*	*	*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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*	*
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*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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*
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

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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
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*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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
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 11 Y-specific frequencies by population group for GlobalFiler™ kit DYS391 and Y indel loci. (*=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 12 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

Locus	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
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 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 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 2.17×10^{-27} for African American, 2.26×10^{-25} for Asian, 5.27×10^{-27} for Caucasian, and 5.0×10^{-27} for Hispanics.

Table 13 Allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), Match probability (MP), and p-value of STR loci

Marker	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

Marker	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 following table shows the Probability of paternity exclusion (PE) values of the GlobalFiler™ kit STR loci individually and combined.

The 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 GlobalFiler™ kit STR loci (Chakraborty, Stivers, and Zhong, 1996).

Table 14 Probability of paternity exclusion values for the GlobalFiler™ kit STR loci

Locus	African American (n = 330)	Asian (n = 153)	Caucasian (n = 343)	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 mix, 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 Mix	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 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	Control DNA 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 Control 007 DNA 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 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 42). 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.

Figure 42 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 43). 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.
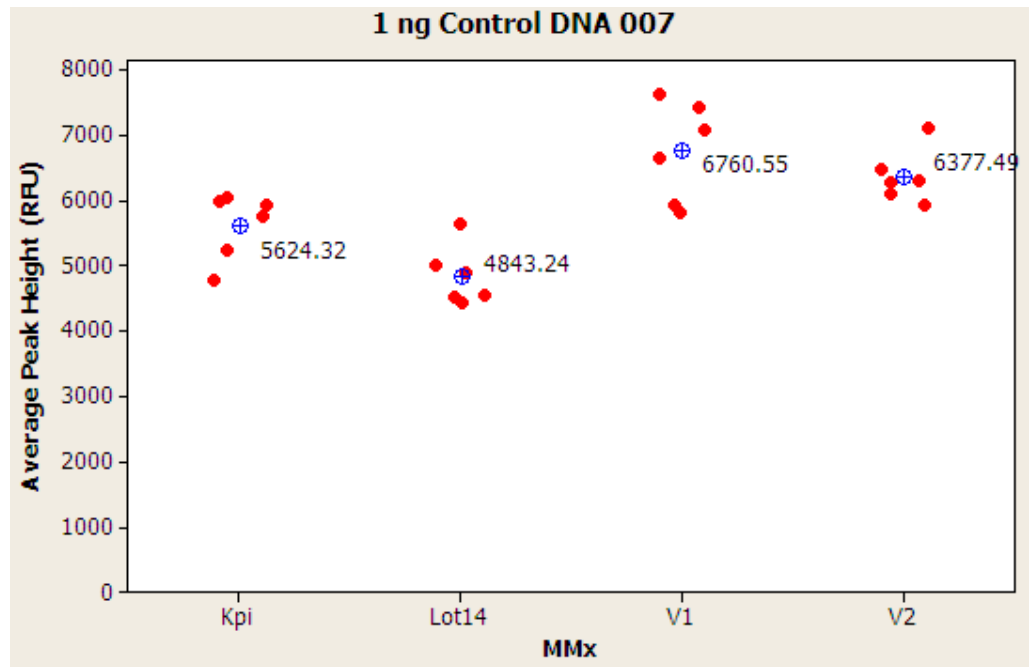

Figure 43 Average peak heights in RFU for the 1-ng replicates from the sensitivity study generated using the V1, V2, Kpi, and Lot 14 master mixes.

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 percentage filter settings that are provided with the GeneMapper™ ID-X Software" on page 87.

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 44).
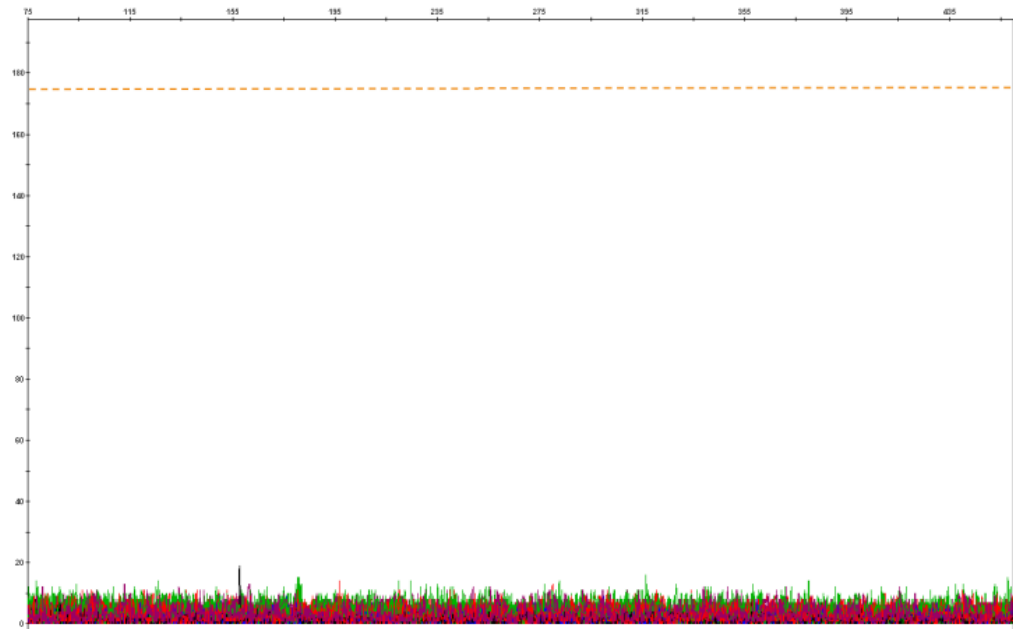

Figure 44 NTC overlay of 6 replicates from the V1 master mix. The Y-scale was set at 200 RFU; the orange line represents the analytical threshold of 175 RFU. The baseline noise was only ~20 RFU. Comparable results were obtained for the V2 and Kpi master mixes.

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 45).
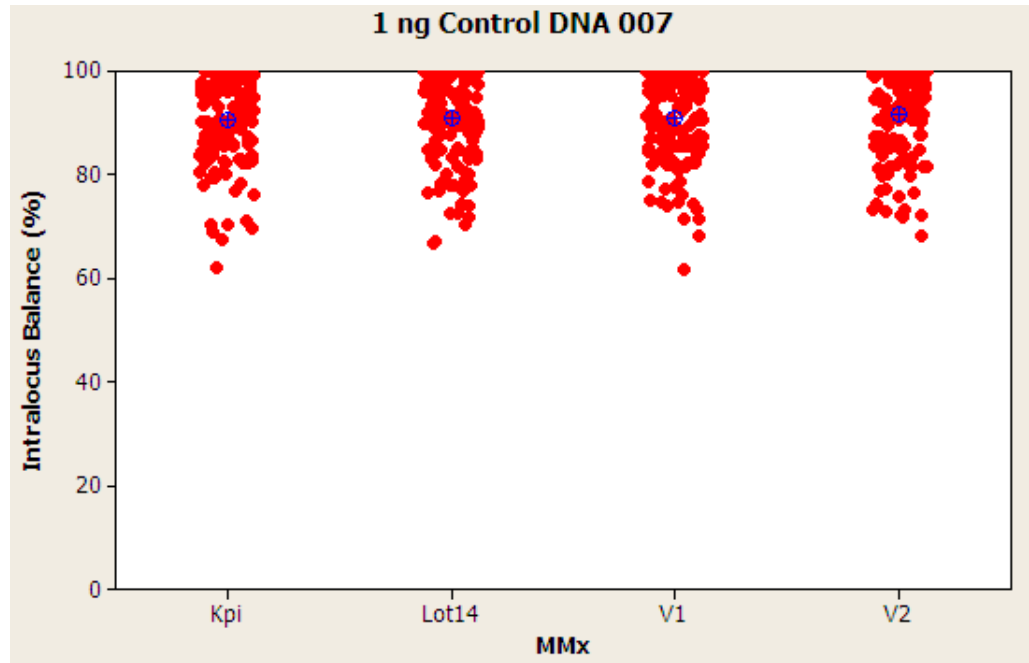

Figure 45 Average intralocus balance percentage for heterozygous loci for the 1-ng replicates from the sensitivity study generated using the Kpi, Lot 14, V1 and V2 master mixes.

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 46). 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.
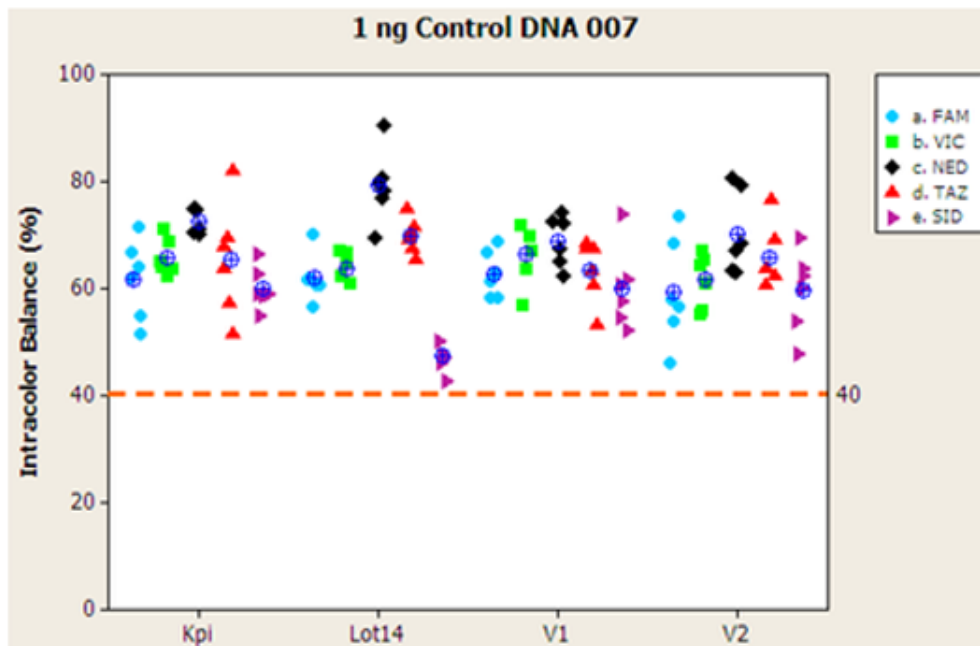
Figure 46 Average intracolor balance percentage plotted by dye channel for the 1 ng replicates from the sensitivity study generated using the, Kpi, Lot 14, V1 and V2 master mixes.

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 47). 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.
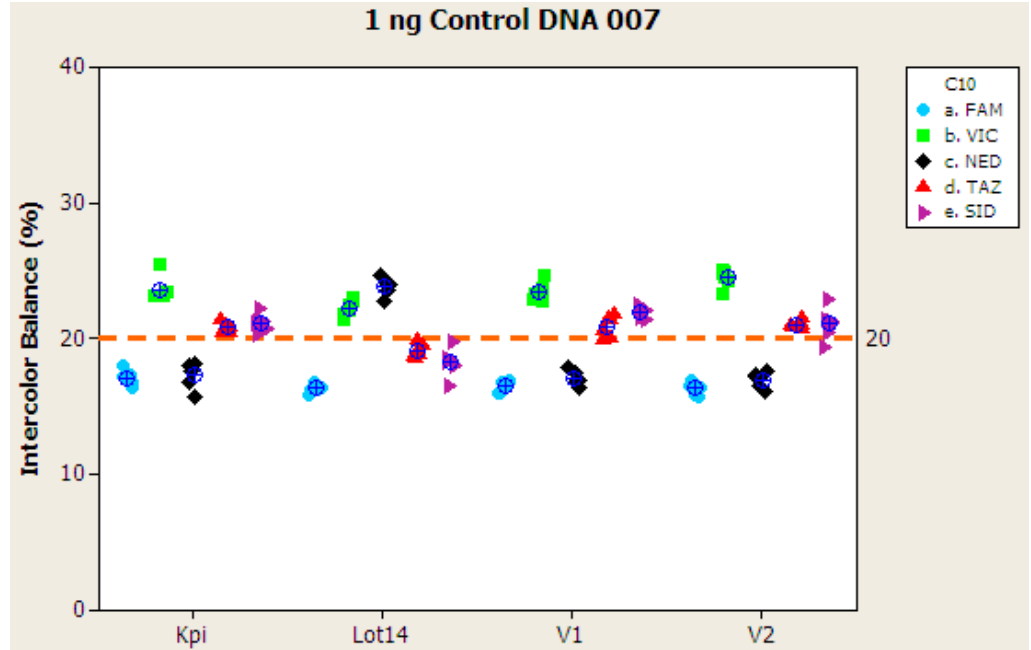
Figure 47 Average intercolor balance percentage plotted by dye channel for the 1 ng replicates from the sensitivity study generated using the Kpi, Lot 14, V1 and V2 master mixes.

Stability study results

Inhibitor study

Comparable allele recoveries were obtained for the freshly prepared V1, V2, and Kpi master mixes (Figure 48 and Figure 49). 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 48 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 49 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 50). 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 50 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.

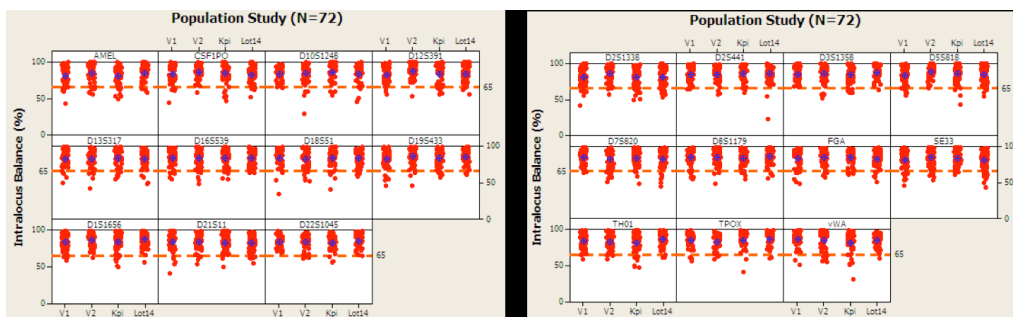All 22 heterozygous loci yielded intralocus balances >65% for all master mixes.

Figure 51 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 51). 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.
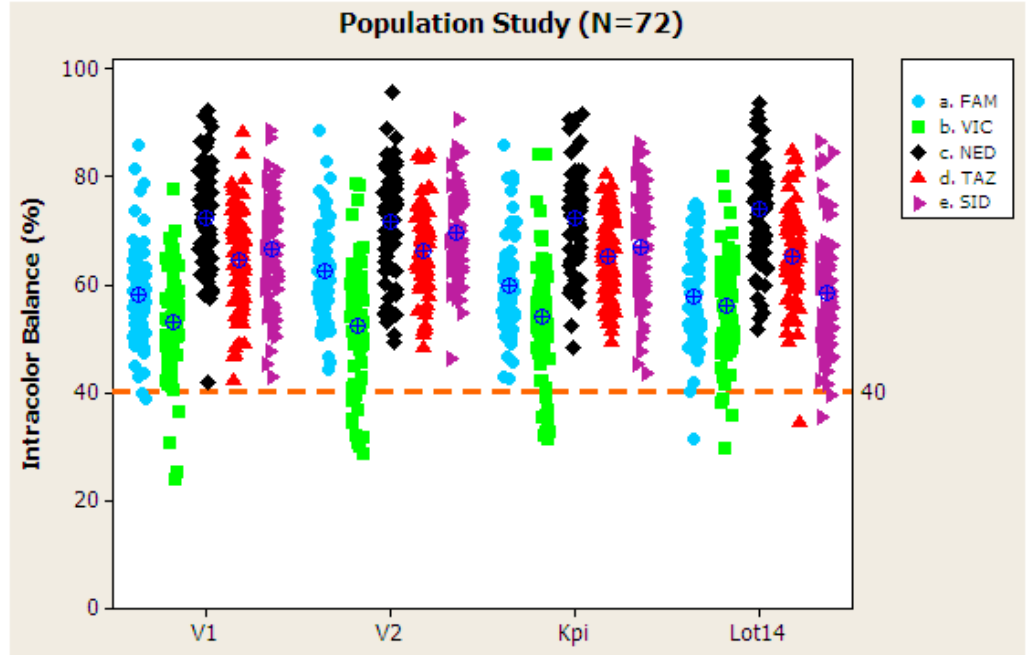

Figure 52 Average intracolor balance (%) plotted by dye channel for the 72 genomic DNA samples generated using the V1, V2, Kpi, and Lot 14 master mixes.

Mixture study results

All 27/27 non-overlapping alleles from the 007 minor contributor were recovered in all mixture samples amplified using all 4 master mix formulations (Figure 53, Figure 54, and Figure 55). The lowest minor contributor represented approximately 111 pg of the minor contributor template DNA added.

Ratio	Major [DNA]	Minor [DNA]	Minor 007 DNA Non-Overlapping Allele Recovery (full = 27 alleles)			
			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 53 Recovery of the minor contributor non-overlapping alleles in Raji:007 DNA mixtures. The maximum number of recoverable non-overlapping alleles is 27.
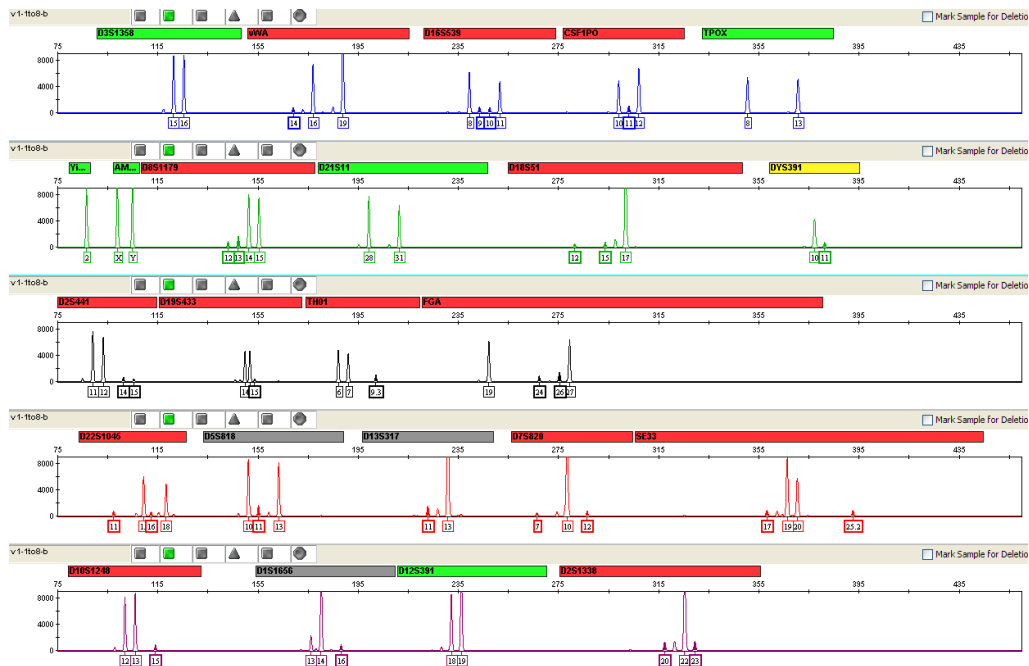
Figure 54 Electropherogram depicting the recovery of all non-overlapping minor alleles in the 8:1 Raji:007 DNA mixture with the V1 master mix formulation. The minor alleles are highlighted.
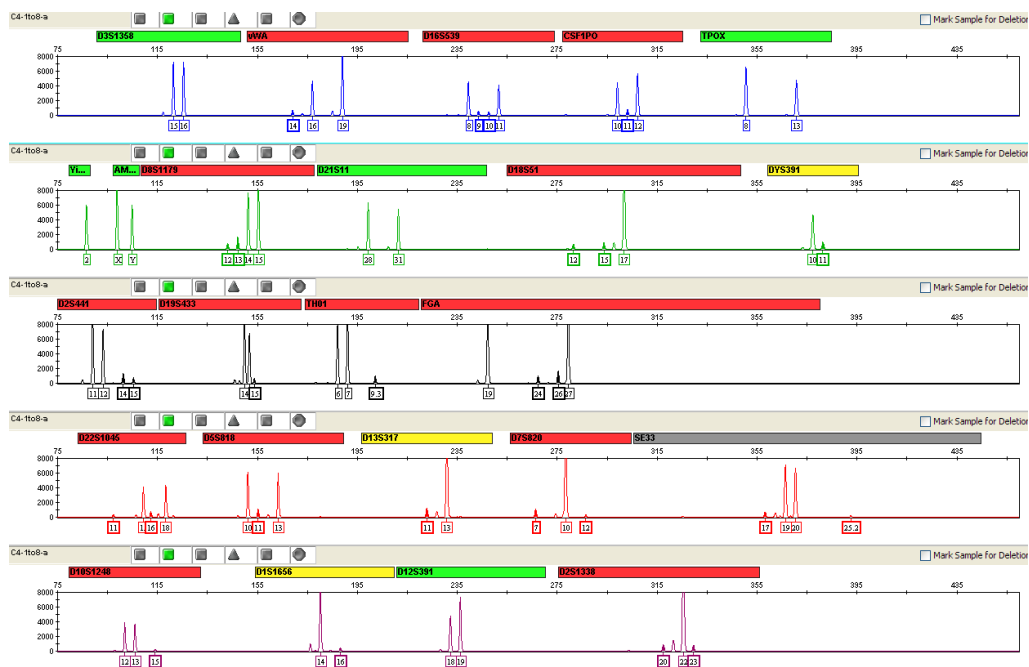

Figure 55 Electropherogram depicting the recovery of all non-overlapping minor alleles in the 8:1 Raji:007 DNA mixture with the Lot 14 master mix formulation. The minor alleles are highlighted.

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 56 and Figure 57 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 15   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
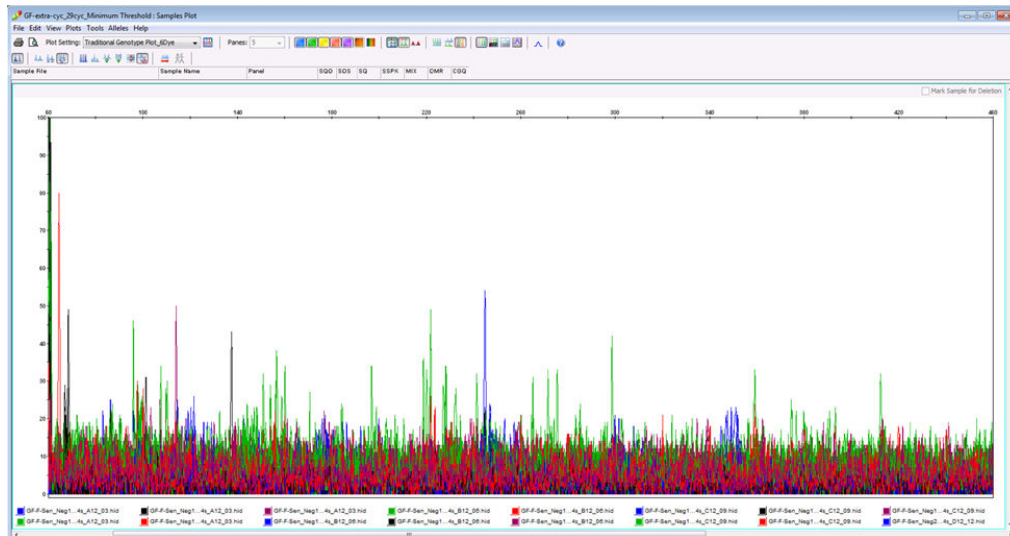
Figure 56 All negative samples at 29 cycles overlaid in all dyes except for LIZ™dye (Y-axis scale 0 to 100 RFU)
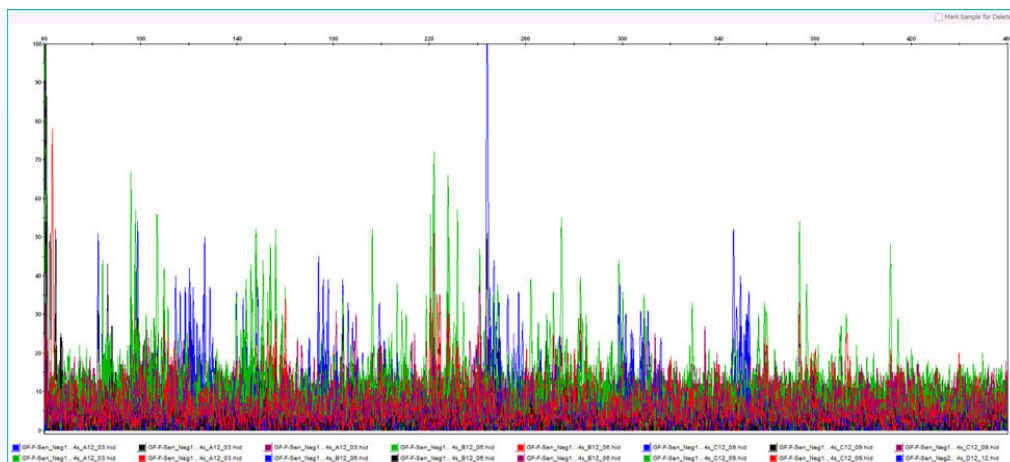

Figure 57 All negative samples at 30 cycles overlaid in all dyes except for LIZ™dye (Y-axis scale 0 to 100 RFU)

Sensitivity study results

The results in Table 16 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 16 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 58). 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.
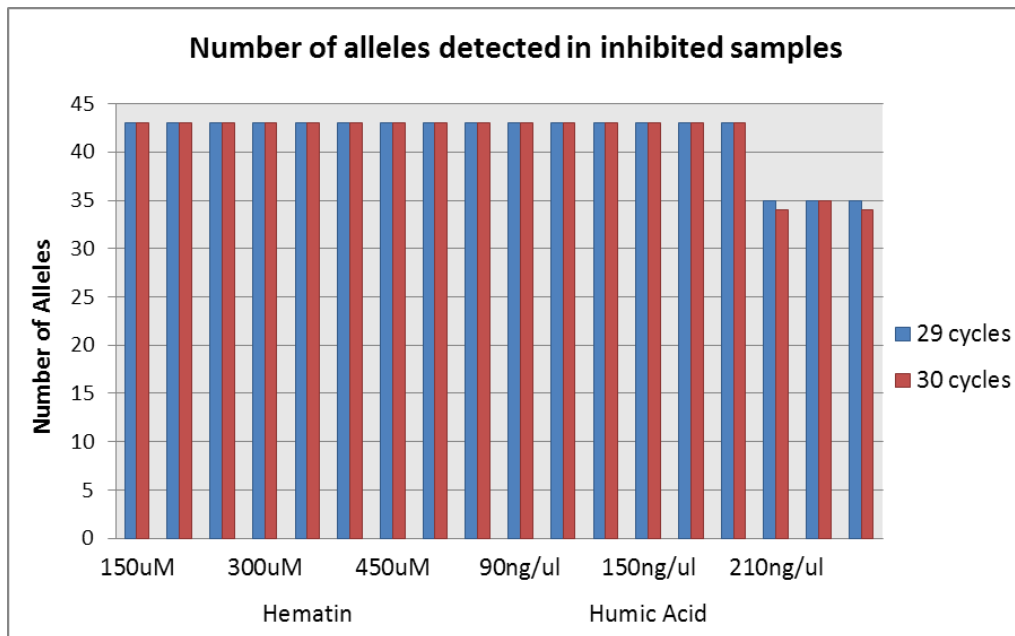
Figure 58   Number of allele calls for inhibited samples—29 vs 30 cycles

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 59).

In the 1 ng medium-degraded sample, off-scale peaks, pull-up peaks, elevated and additional stutter peaks, and other artifacts were observed.
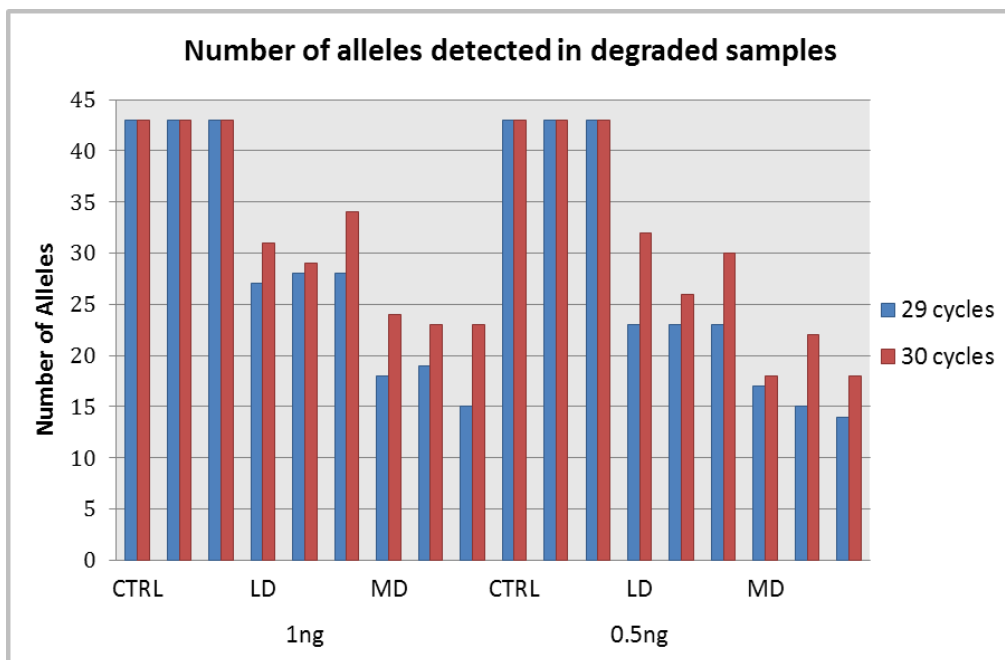
Figure 59   Number of allele calls for degraded samples—29 vs 30 cycles. CTRL=007 control, LD=low degradation, MD=medium degradation,

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 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 3130xL Genetic Analyzer was also used are noted below.

Test case	Description
Sensitivity	Control DNA 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	Control DNA 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.
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 degradaded fractions) were amplified at 0.5 ng input for 30 PCR cycles with the GlobalFiler™ IQC and GlobalFiler™ kits.

Test case	Description
PCR component and PCR cycle analysis	• Four replicates of Control DNA 007 were amplified at 1 ng for 29 PCR cycles with varying GlobalFiler™ IQC and GlobalFiler™ primer mix and master mix concentrations. • Four replicates of Control DNA 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 intrument. The experiment was repeated on the 3130xl 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 3130xl instrument.
Mixture study	Two-contributor DNA mixtures were prepared with male Control DNA 007 and human female 9947a DNA 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)	Control DNA 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 and GlobalFiler™ kits with a dilution series of human male control 007 genomic DNA. Dilutions were made in two-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.

Figure 60 shows example GlobalFiler™ IQC kit electropherograms for some of the Control DNA 007 dilutions (GlobalFiler™ kit profiles are not shown but gave very similar results). Both kits gave full profiles of 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 ($\alpha = 0.05$) showed that the allele counts for replicate PCR reactions between the two kits were statistically equivalent for all dilutions. Figure 61 shows a peak height comparison between the GlobalFiler™ IQC and GlobalFiler™ kits for 1ng samples, by locus.
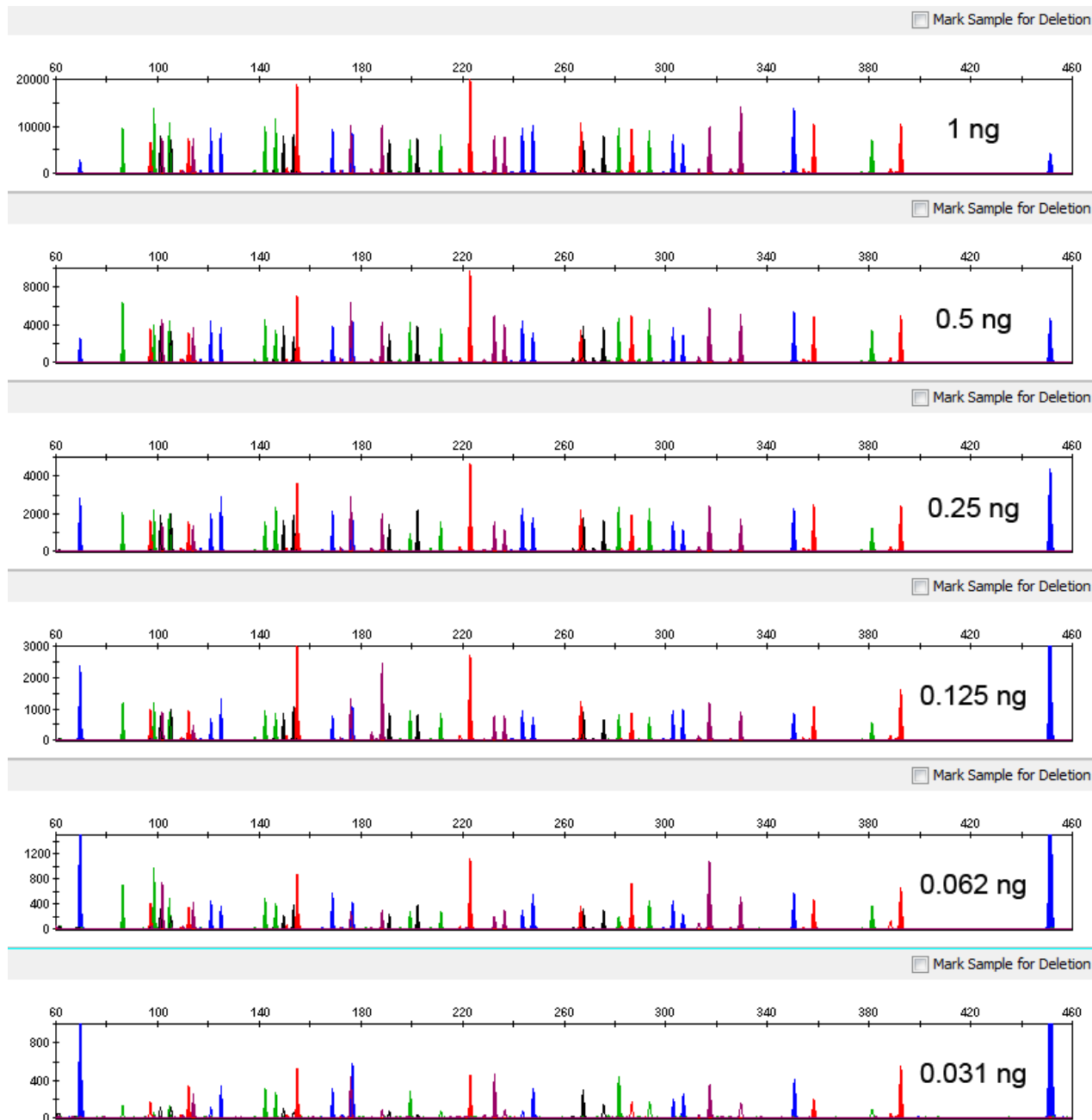

Figure 60   Example electropherograms are shown for GlobalFiler™ IQC kit reactions of a two-fold dilution series of control 007 human male genomic DNA. The y-axis was adjusted to provide better visibility of lower-concentration dilutions.
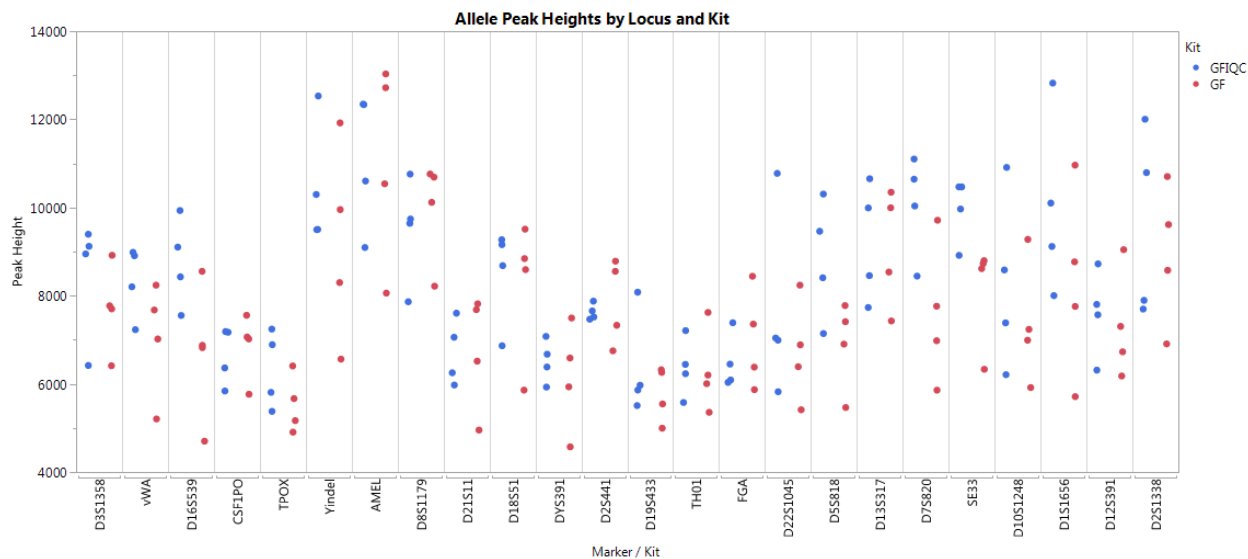
Figure 61 Mean heterozygote peak heights are shown for replicate PCRs of the 1ng 007 DNA dilution, plotted by locus (GlobalFiler™ IQC data = blue data points, GlobalFiler™ = red data points).

Two other important performance metrics were extracted from results for the 1 ng 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, $\alpha = 0.05$).

- **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 and GlobalFiler™ kits (two-tailed t-tests, $\alpha = 0.05$).

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 and GlobalFiler™ kits, samples were prepared with 1.0 ng of human male control DNA 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.

Figure 62 and Figure 63 show example electropherograms with humic acid and hematin. 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: Figure 64 on page 156 and Figure 65 on page 157 show electropherograms and allele count information for degraded samples.

Figure 62 GlobalFiler™ IQC and GlobalFiler™ kits with humic acid. GlobalFiler™ IQC electropherograms (left) with the IQCS and IQCL peaks (red ovals). Y-axis scale = 12,000 RFU.

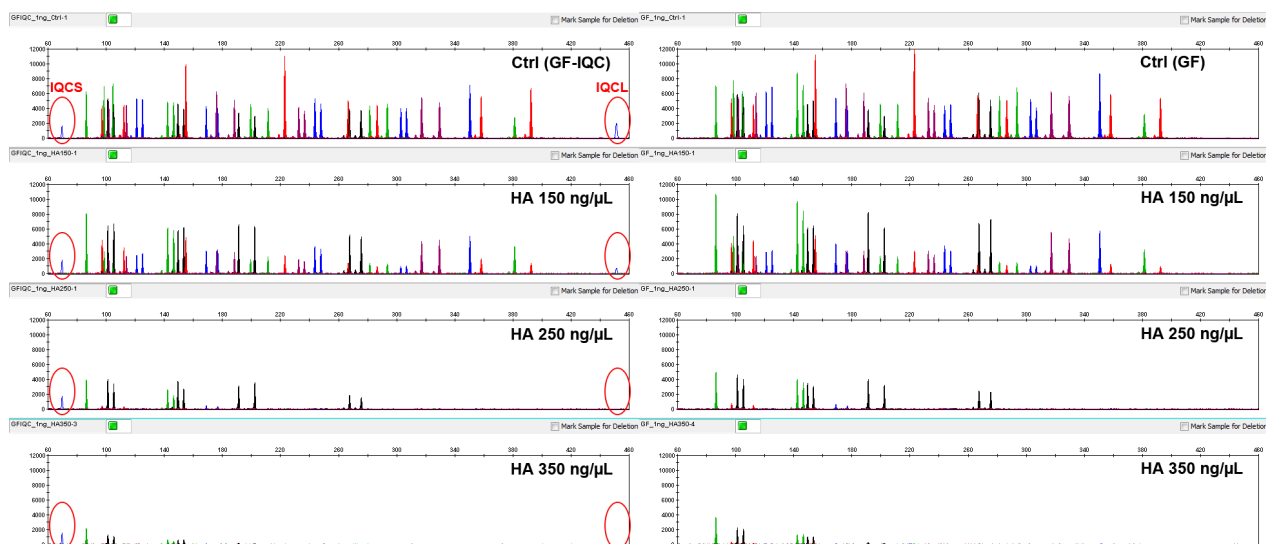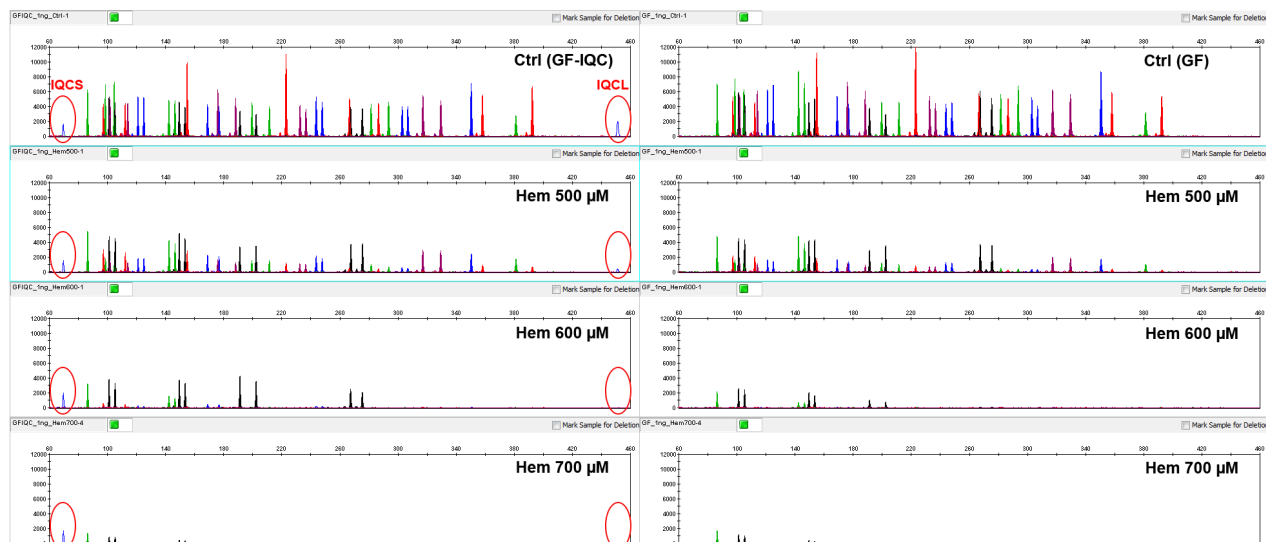**Figure 63** GlobalFiler™ IQC and GlobalFiler™ kits with hematin. GlobalFiler™ IQC electropherograms (left) with the IQCS and IQCL peaks (red ovals). Y-axis scale = 12,000 RFU.

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 64 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 62 on page 154 and Figure 63 on page 154 show electropherograms for inhibited samples.
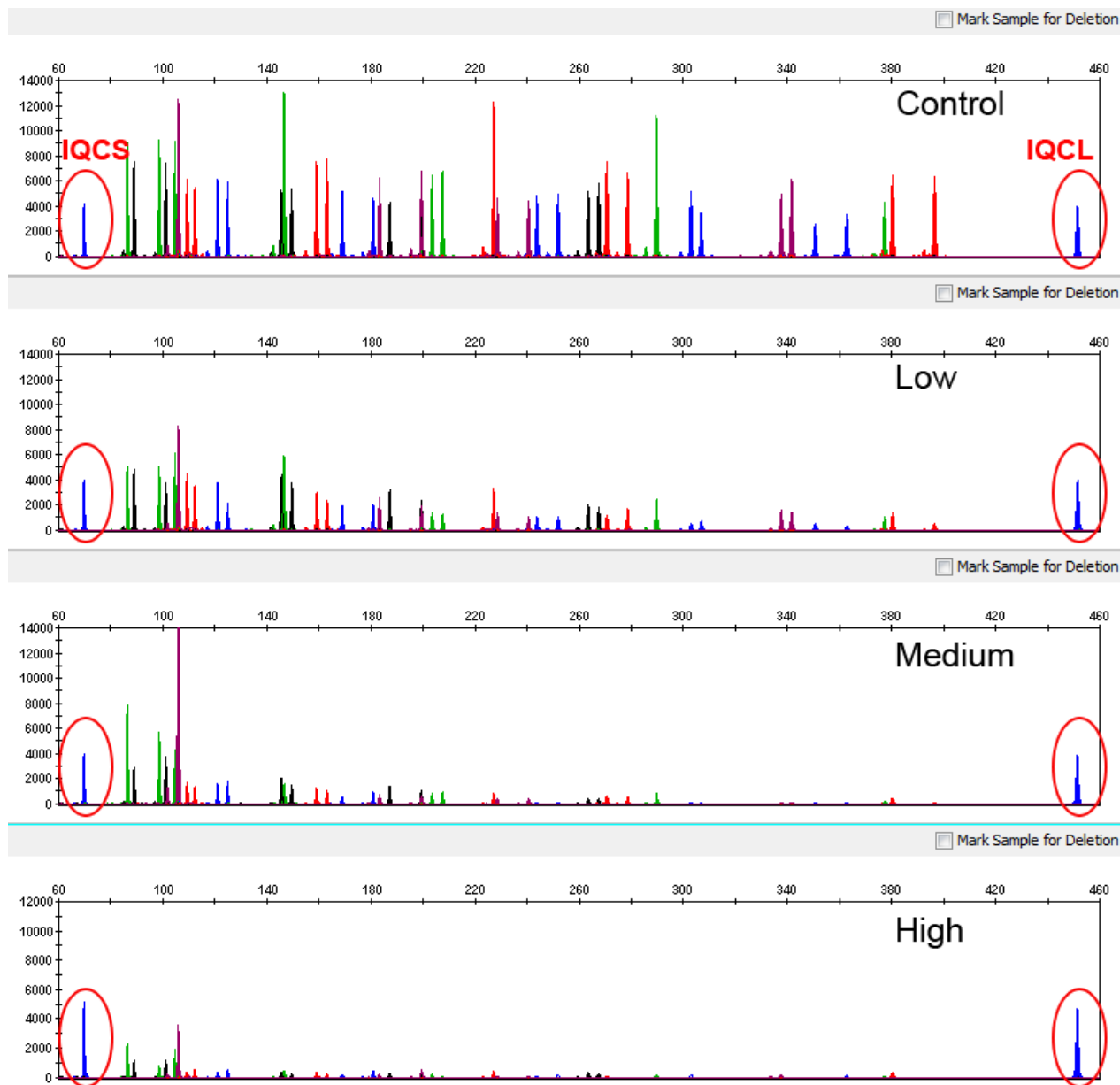
Figure 64 GlobalFiler™ IQC kit example electropherograms for degraded DNA

Figure 65 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, $\alpha = 0.05$).
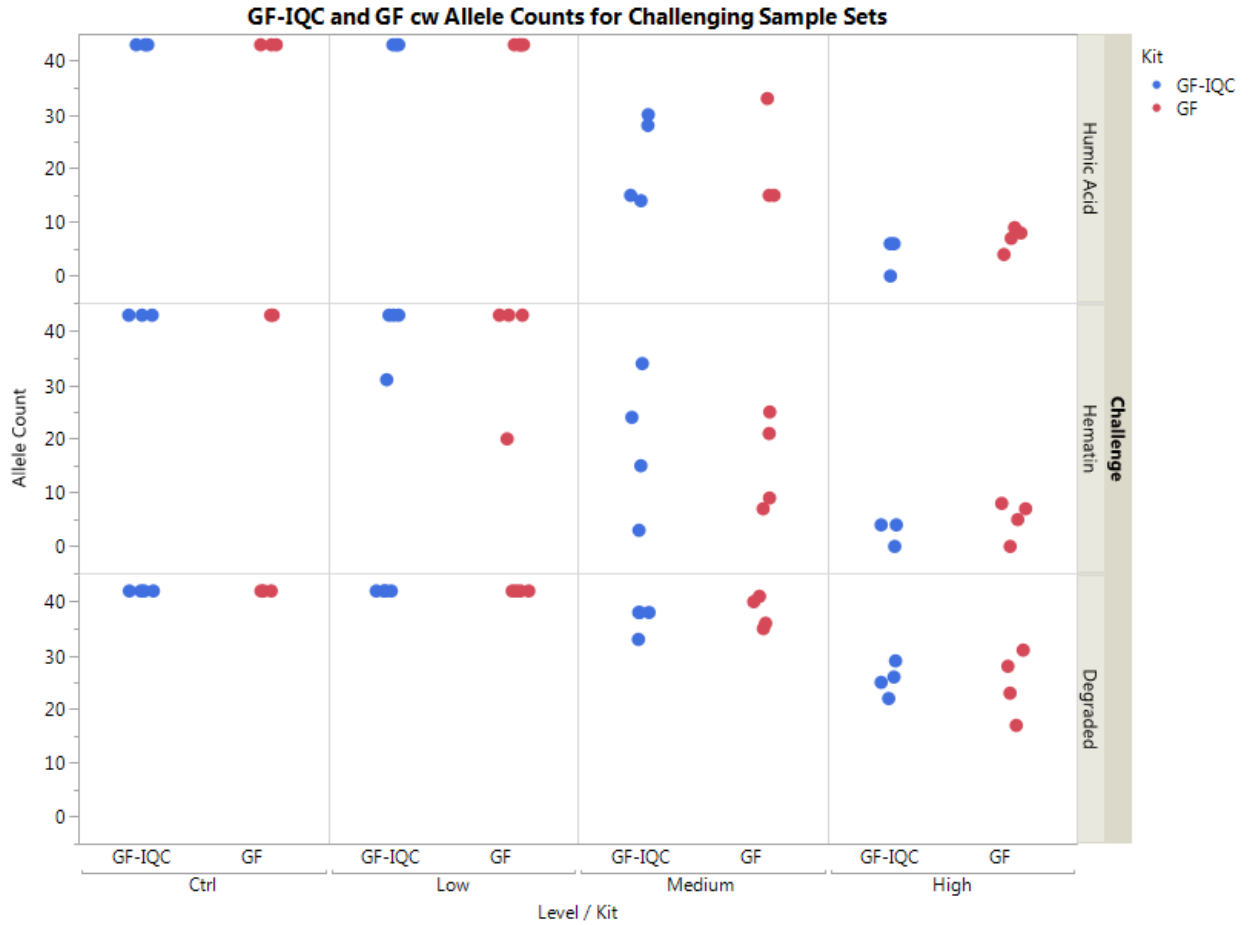
Figure 65 GlobalFiler™ IQC kit (blue) and GlobalFiler™ kit (red) allele counts for inhibited and degraded DNA samples

Figure 66 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.
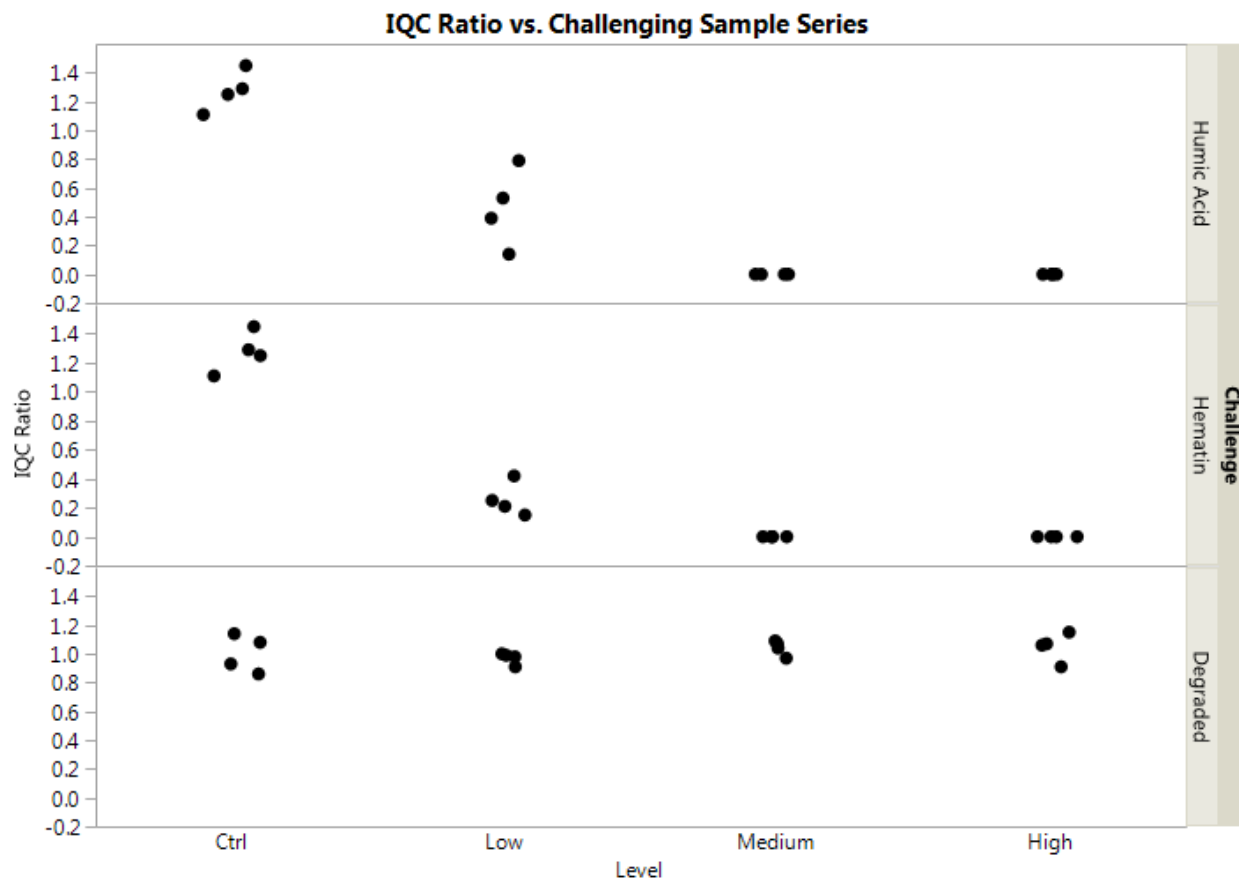
Figure 66 IQC Ratios (IQCL Peak Ht ÷ IQCS Peak Ht) for GlobalFiler™ IQC kit

PCR component concentration analysis results

Because the master mix formulation is identical between the GlobalFiler™ IQC and GlobalFiler™ kits, 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 mix and master mix added to GlobalFiler™ IQC kit reactions.

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

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

As Figure 67 and Figure 68 show, the ILB and ICB were largely insensitive to variations in primer mix or master mix amount. However, the average allele peak heights and the IQC Ratio were higher or lower in response to higher or lower amounts of primer mix or master mix. Importantly, both the GlobalFiler™ IQC and GlobalFiler™ kits showed similar responses to the variations in amount of each component.

Note: The GlobalFiler™ kit does not contain the IQC markers. Therefore the top right area of the figures are blank.
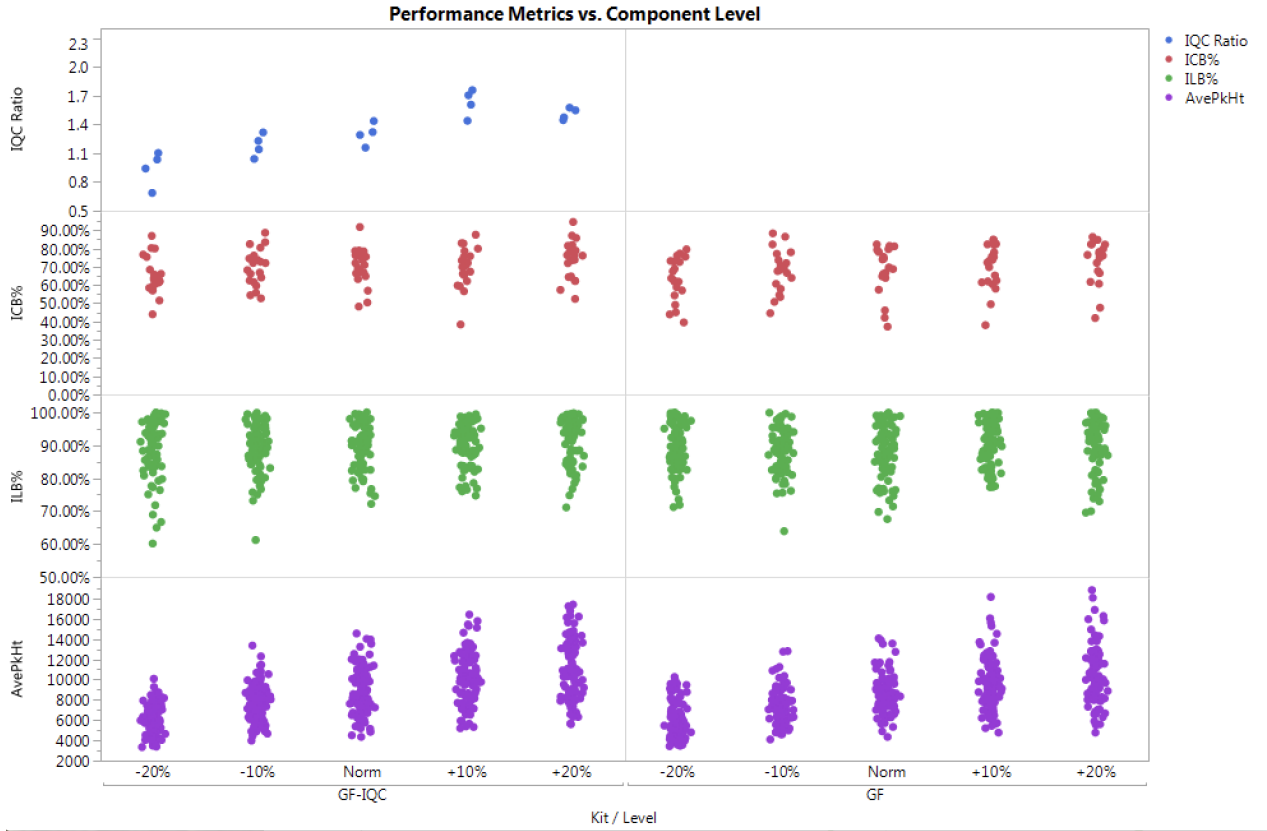
Figure 67 Primer mix amount variation for GlobalFiler™ IQC (left) and GlobalFiler™ (right) kits. Input is ±20% of standard 2.5 µL input per 25 µL reaction (with standard master mix amount) with 1.0 ng of control DNA 007.

Figure 68 Master mix amount variation for GlobalFiler™ IQC (left) and GlobalFiler™ (right) kits. Input is ± 20% of standard 7.5 µL input per 25 µL reaction (with standard primer mix amount) with 1.0 ng of Control DNA 007.

PCR cycle number analysis results

For this experiment, replicate PCR reactions were prepared with 1 ng of Control DNA 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 69).

GlobalFiler™ IQC and GlobalFiler™ kits are optimized for 29 or 30 cycles with either 1.0 ng or 0.5 ng of human genomic DNA input, respectively. At higher cycle numbers, full profiles were obtained. However, off-scale peaks were observed for 30 and 31 cycles, and especially at 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).
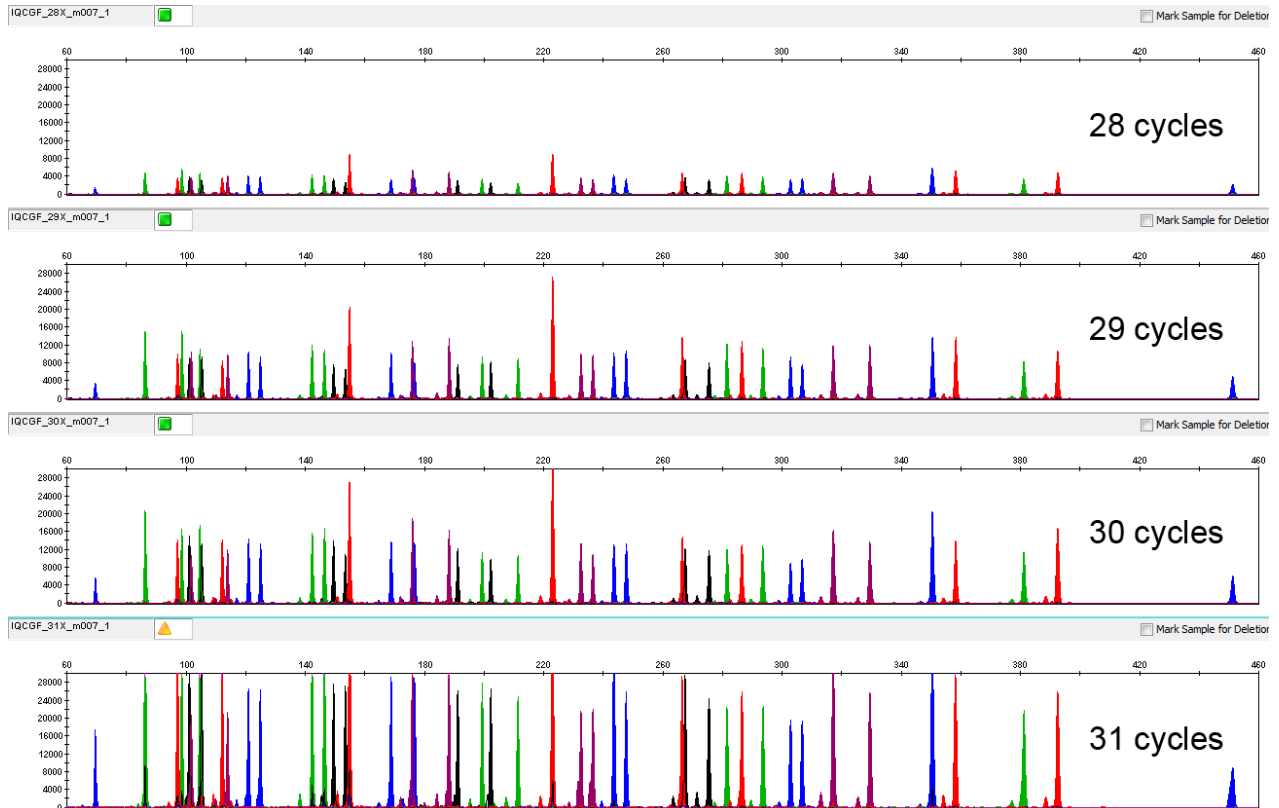
Figure 69 Representative GlobalFiler™ IQC kit electropherograms obtained from amplification of 1.0 ng of DNA Control 007 using 28, 29, 30, and 31 cycles (y-axis scale 0 to 30,000 RFU)

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 3130xl genetic analyzers. Within each injection (24 wells on the 3500xL and 16 wells on the 3130xl), 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 nt for the IQC peaks.

Locus	3500xL		3130xl	
	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 and GlobalFiler™ kits provide the required specificity for detecting human alleles. Species specificity testing was performed to 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 with both the GlobalFiler™ IQC and GlobalFiler™ 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 70.
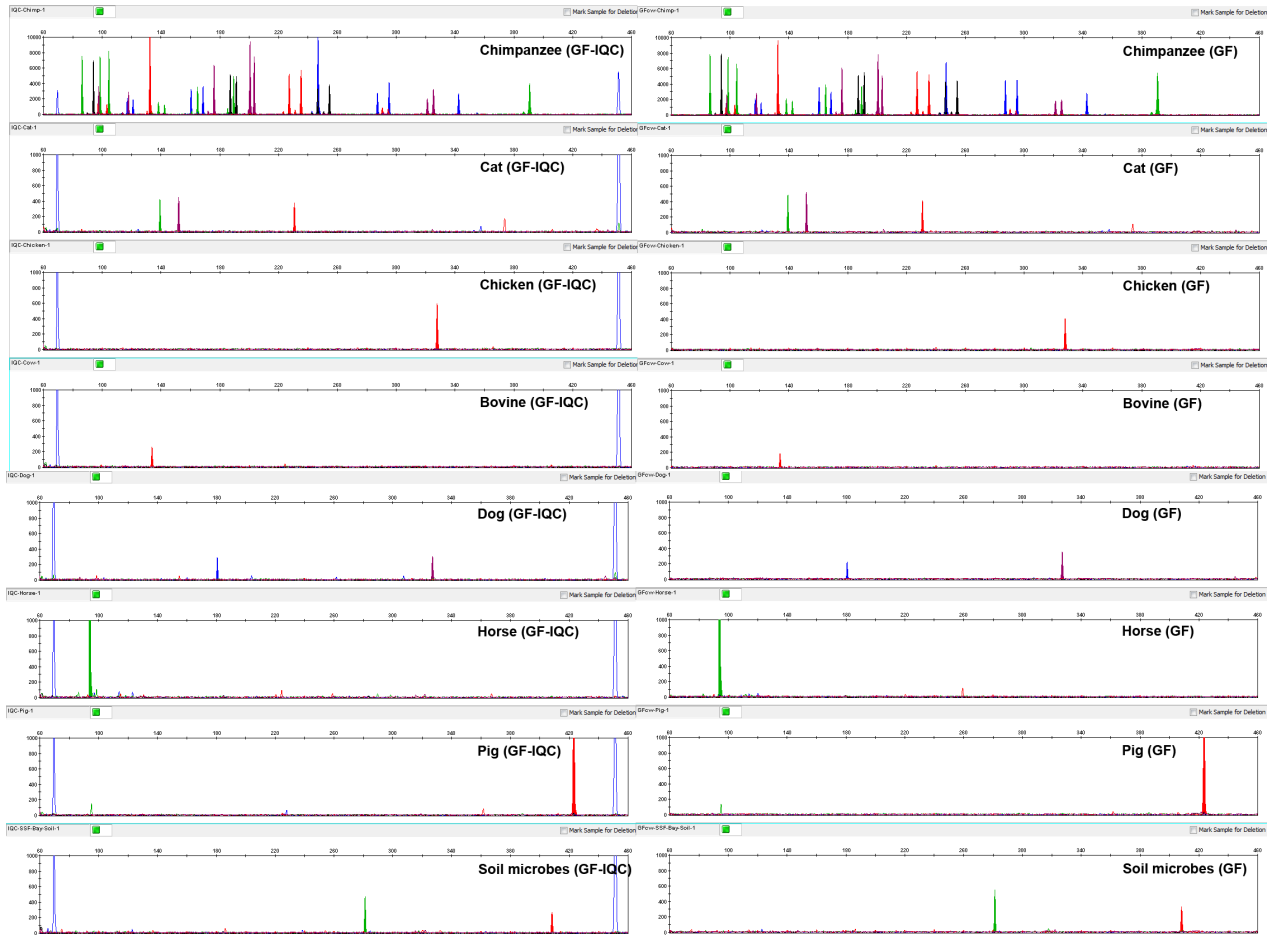
Figure 70 Example electropherograms for nonhuman species that produced reproducible cross-reactive peaks with the GlobalFiler™ IQC (left) and GlobalFiler™ (right) kits. The y-axis for chimpanzee DNA profiles (top) is 10,000 RFU; the y-axis for all other profiles is 1,000 RFU. The blue (FAM™ dye-labeled) peaks at the low- and high-MW ends of the GlobalFiler™ IQC profiles are the IQCS and IQCL peaks, respectively.

Table 17 list the reproducible peaks that were observed in non-primate, nonhuman species.

Table 17 Reproducible peaks for nonhuman species with GlobalFiler™ IQC and GlobalFiler™ kits

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

Chapter 9 Performance verification with the GlobalFiler™ IQC kit
Genotype concordance with population samples and NIST SRM-2391c

9

Species	Dye Channel	Size	GlobalFiler™ IQC kit	GlobalFiler™ kit
Dog	B	181	314	255
	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 with Data Collection software v3.1 (DC3.1), a 3500xL with DC4, and a 3130xl with v4 software. The run files were analyzed with GeneMapper™ *ID-X* Software v1.5.2 (3500xL DC3.1 and 3130xl runs), and GeneMapper™ *ID-X* Software v1.6 (for the 3500xL DC4 run).

Note: On the 3130xL 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 the 3130xl 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	3130xl
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 Control DNA 007 and human female 9947a DNA. 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).

Figure 71 shows an example electropherogram of the 1:7 sample (125 pg 007 DNA and 875 pg 9947a DNA) run with the GlobalFiler™ IQC kit.

Table 18 shows the DNA mixture composition of each sample and resulting allele counts of each contributor. Only alleles that were unique to each contributor (the allele was neither 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.
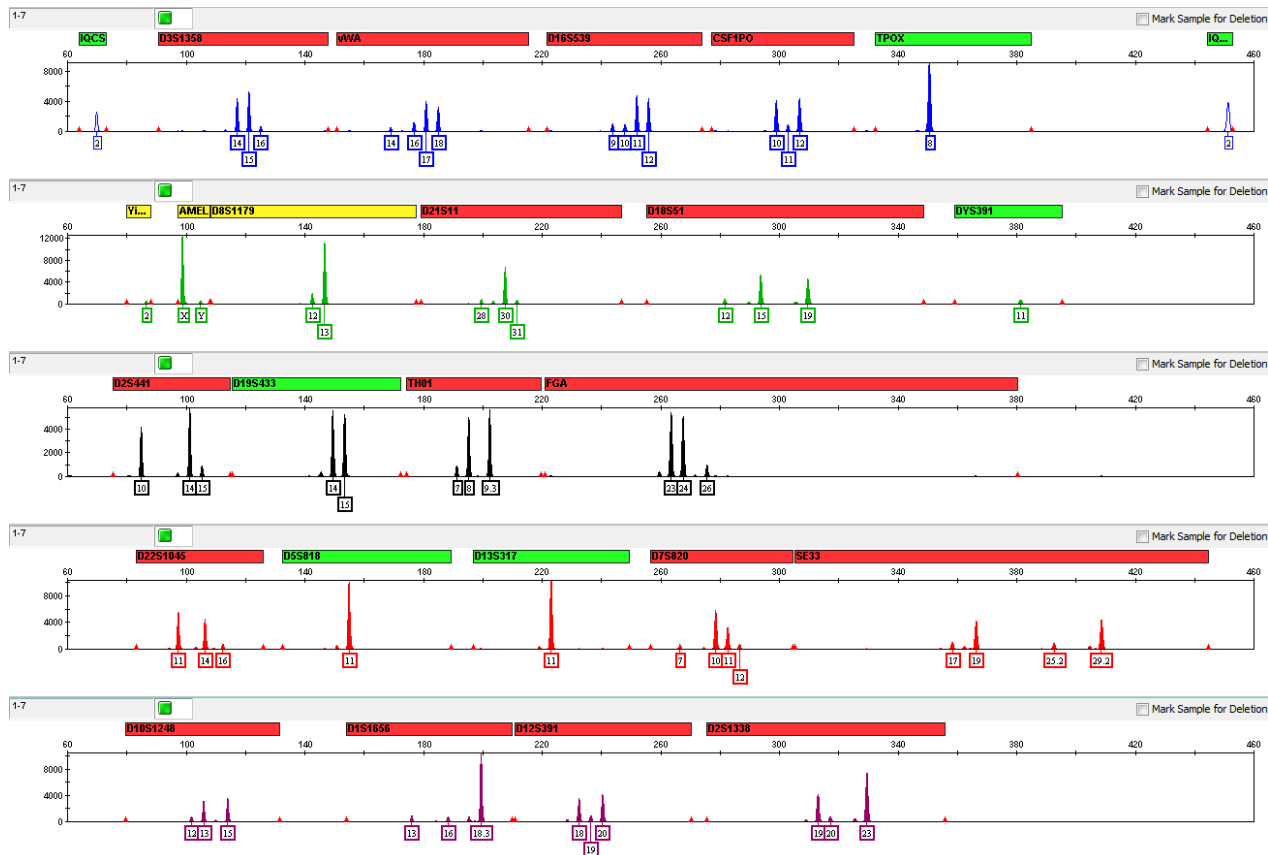
Figure 71 1:7 male:female mixture contained 125 pg of the 007 minor contributor (male) and 875 pg of the 9947a major contributor (female)

Table 18 DNA composition and mean allele counts per replicate GlobalFiler IQC reaction (full profiles of unique alleles for 007 and 9947a DNAs were 19 and 14, respectively)

Sample	Amt of Control DNA 007/rxn	Amt of 9947a/rxn	007 mean unique alleles per rep	9947a mean unique alleles per rep
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 and GlobalFiler™ kits, Control DNA 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 Control 007 DNA 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.

Figure 72 shows the results of the Control DNA 007 stutter experiment with stutter calculations at all STR loci except TH01 (TH01 stutter product was often below the 175 RFU detection threshold and were not included in the study).

The average difference in stutter for all loci combined was +1.65% between GlobalFiler™ and GlobalFiler™ IQC kits. 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 (DYS 391) 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™ stutter filter values can be used for the GlobalFiler™ IQC kit.
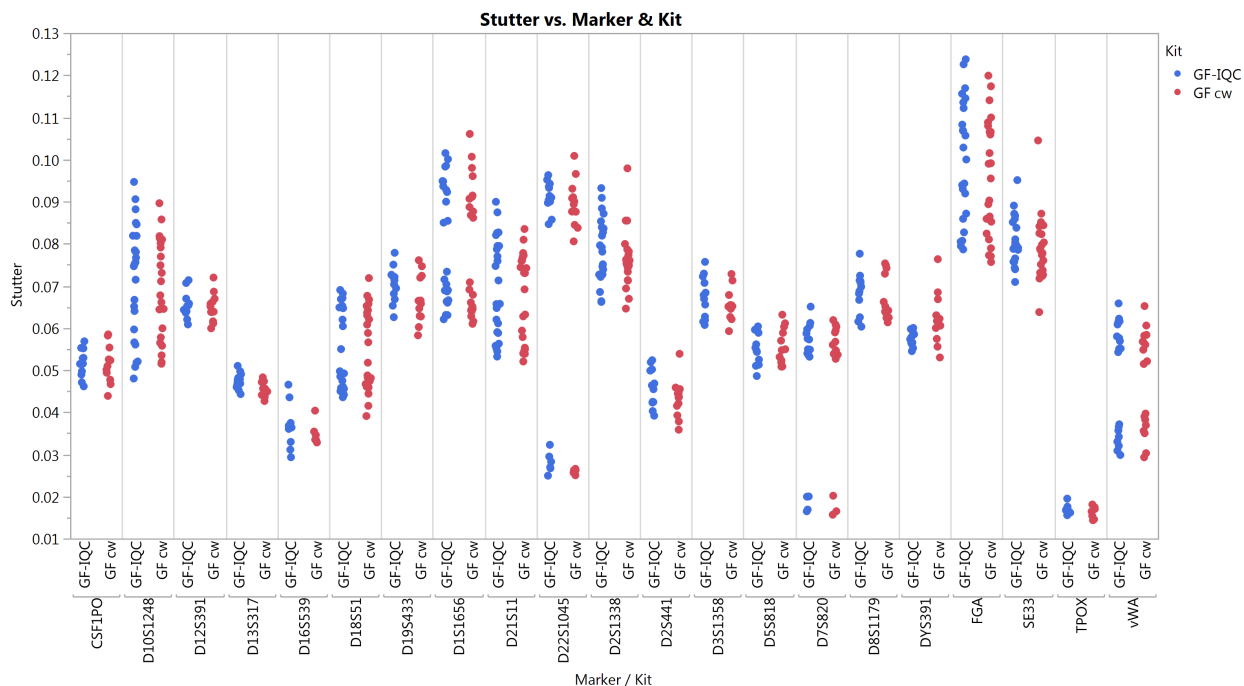
Figure 72 Stutter measured for GlobalFiler™ IQC (blue) and GlobalFiler™ (red) kits for all unobstructed main-allele/stutter-peak pairs of Control 007 genomic DNA

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 3130xl 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 73 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.
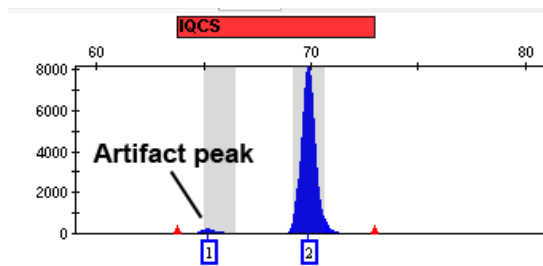
Figure 73 IQCS artifact peak that appears when a sample was amplified for 31 cycles

- An artifact that can appear in 3130xl 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 3130xl oven at 60°C for ≥30 minutes before a run. Figure 74 shows an example of this artifact.
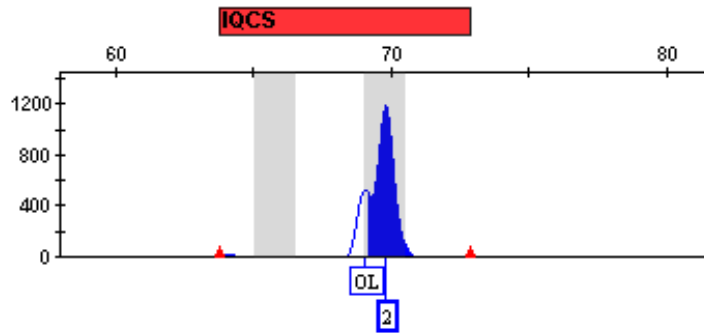
Figure 74 IQCS artifact peak that appears in 3130 sample data only if the oven is not sufficiently warmed up before a run

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

Test case	Expected result	Status
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]
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 164.

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 both the DNA Control 007 and the DNA 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 at 95°C for 1 minute.
	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 and that a compression pad was used with the GeneAmp™ PCR System 9700. (A compression pad should not be used with other validated thermal cyclers.)
	The thermal cycler malfunctioned.	See the thermal cycler user manual and check the instrument calibration.
	Incorrect thermal cycler conditions were used.	Use correct thermal cycler conditions.
	A MicroAmp™ base was used with a tray/retainer set and tubes in GeneAmp™ PCR System 9700.	Remove the MicroAmp™ base.
	The tubes or plate were not seated tightly in the thermal cycler during amplification.	Push the tubes or plate firmly into the block after first cycle.
	The wrong PCR reaction tubes or plate were used.	Use MicroAmp™ Reaction Tubes with Caps or the MicroAmp™ Optical 96-well Reaction Plate for the GeneAmp™ PCR System 9700 or Veriti™ Thermal Cycler.
	Insufficient PCR product was electrokinetically injected.	Use correct genetic analyzer settings.
	Degraded formamide was used.	Check the storage of formamide. Do not thaw and refreeze multiple times. Try Hi-Di™ Formamide.
Positive signal from DNA Control 007 but partial or no signal from DNA 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 DNA 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 necessary 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, reamplify 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 nt position) occured.	Include the final extension step of 60°C for 10 minutes in the PCR.
		See Chapter 6, "Experiments and results"
	The signal exceeds the dynamic range of the instrument and is causing signal "pull-up" into adjacent channels.	Check that you are using the recommended number of PCR cycles. Repeat PCR amplification using reduced input DNA amount, or interpret the off-scale data according to your laboratory procedure.
		3130 instruments: Depending on instrument sensitivity, DNA input amounts ≥1 ng amplified at 29 cycles can demonstrate off-scale peak heights for homozygous peaks. For more information, go to **thermofisher.com**, then search for the technical note *Evaluation of a decreased cycle number (28 cycles) and decreased injection time (5 seconds) when the GlobalFiler™ PCR Amplification Kit is run on a 3130 xl Genetic Analyzer*, or contact your local Human Identification representative.
	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.

Observation	Possible cause	Recommended action
More than two alleles present at a locus *(continued)*	The double-stranded DNA was not completely denatured.	Use the recommended amount of Hi-Di™ Formamide and heat the sample plate at 95°C for 3 minutes.
Poor peak height balance	Incorrect thermal cycler conditions were used.	Use correct thermal cycler conditions.
Some but not all loci visible on electropherogram of DNA Test Samples	The PCR reaction volume you used is lower 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 not accurate.	Ensure that DNA quantification is accurate.
		3130 instruments: Depending on instrument sensitivity, DNA input amounts ≥1 ng amplified at 29 cycles can demonstrate off-scale peak heights for homozygous peaks. For more information, go to **thermofisher.com**, then search for the technical note *Evaluation of a decreased cycle number (28 cycles) and decreased injection time (5 seconds) when the GlobalFiler™ PCR Amplification Kit is run on a 3130 xl Genetic Analyzer*, or contact your local Human Identification representative.

B Materials required but not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

STR kit materials

Item	Source
GlobalFiler™ PCR Amplification Kit, 200-reaction	4476135
GlobalFiler™ PCR Amplification Kit, 1,000-reaction	4482815
GlobalFiler™ IQC PCR Amplification Kit, 200-reaction	A43565

Sample preparation required materials

Item	Source
GeneScan™ 600 LIZ™ Size Standard v2.0, 2 × 200 µL **IMPORTANT!** Do not use GeneScan™ 350 ROX™, GeneScan™ 500 ROX™, or GeneScan™ 500 LIZ™ Size Standards with this kit.	4408399
Low-TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0)	Teknova T0223 or see "Prepare low-TE buffer" on page 23.
Hi-Di™ Formamide, 25-mL	4311320

Thermal cycler required materials

ProFlex™ PCR System

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

Veriti™ Thermal Cycler Thermal Cycler

Item	Source
Veriti™ 96-Well Thermal Cycler	4479071
(*Optional*) Tabletop centrifuge with 96-Well Plate Adapters	MLS

GeneAmp™ PCR System 9700

Item	Source
GeneAmp™ PCR System 9700, 96-Well Silver	N8050001
GeneAmp™ PCR System 9700, 96-Well Gold-Plated	4314878
Silver 96-Well Sample Block	N8050251
Gold-Plated 96-Well Block	4314443

Genetic analyzer required materials

3500 Series Genetic Analyzer

Item	Source
3500/3500xL Data Collection Software v4 (RUO)	A40059, A40376[1]
3500/3500xL Data Collection Software v3.1 (RUO)	4405186, 4405187[1]
3500/3500xL Data Collection Software v3 (RUO)	A26287
3500/3500xL Data Collection Software v2 (RUO)	4475183[1]
HID Updater 3500 Data Collection Software v2	4480670
Anode buffer container (ABC)	4393927
Cathode buffer container (CBC)	4408256
POP-4™ Polymer (960 samples) for 3500/3500xL Genetic Analyzers	4393710
POP-4™ Polymer (384 samples) for 3500/3500xL Genetic Analyzers	4393715
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
Conditioning reagent	4393718
8-Capillary array, 36 cm for 3500 Genetic Analyzers	4404683
24-Capillary array, 36 cm for 3500xL Genetic Analyzers	4404687
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers	4410231

Item	Source
8-Strip Septa for 3500/3500xL Genetic Analyzers	4410701
96-Well Septa for 3500/3500xL Genetic Analyzers	4412614
Septa Cathode Buffer Container, 3500 series	4410715

[1] Contact your Thermo Fisher Scientific HID representative.

3130 Series Genetic Analyzer

Item	Source
3130 Data Collection Software v4	4475105
3130*xl* Data Collection Software-v4	4475126
3130/3730 Data Collection Software-v4 6-Dye Module v1	4480670
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3130 *xl* Genetic Analyzer Capillary Array, 36-cm	4315931
POP-4™ Polymer for 3130/3130 *xl* Genetic Analyzers	4352755
Running Buffer, 10✕	402824
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
MicroAmp™ Optical 96-Well Reaction Plate	N8010560

Analysis software required materials

GeneMapper™ *ID-X* Software

Item	Source
GeneMapper™ *ID-X* Software v1.6 Full Installation	A39975
GeneMapper™ *ID-X* Software v1.6 Client Installation	A39976
GeneMapper™ *ID-X* Software v1.5 Full Installation	A27884
GeneMapper™ *ID-X* Software v1.5 Client Installation	A27886
GeneMapper™ *ID-X* Software v1.4 Full Installation	4479707
GeneMapper™ *ID-X* Software v1.4 Client Installation	4479711

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-Cap Strips	4323032
MicroAmp™ 96-Well Tray/Retainer Set	403081
MicroAmp™ 96-Well Base	N8010531
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Optical 96-Well Reaction Plate	N8010560

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

[1] Major laboratory supplier

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
- Pipettors

- Tube decapper, autoclavable
- 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.

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, see the "Documentation and Support" section in this document.

Chemical safety

⚠ **WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. 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.

⚠ **WARNING! HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

⚠ **WARNING! 4L Reagent and Waste Bottle Safety.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
>   **https://www.cdc.gov/labs/pdf/ CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf**
> - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
>   **www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf**

Documentation and support

Related documentation

Document title	Pub. No.
STR kits	
GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits—PCR Amplification and CE Quick Reference	4477593
GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits—PCR Setup Quick Reference	4477594
Quantification kits	
Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide	4485355
Quantifiler™ Human DNA Quantification Kit and Y Human Male DNA Quantification Kit User Guide	4344790
Quantifiler™ Duo DNA Quantification Kit User Guide	4391294
Thermal cyclers	
ProFlex™ PCR System User Guide	MAN0007697
Veriti™ Thermal Cycler User Guide	4375799
GeneAmp™ PCR System 9700 Base Module User Manual	4303481
3500 Series Genetic Analyzer	
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v1 User Guide	4401661
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v2 User Guide	4476988
HID Updater 3500 Data Collection Software v2.0 User Bulletin	NA
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3 User Guide	100025036
3500 Series Data Collection Software v3 User Bulletin: New Features and HID Validation Summary	MAN0010812
3500 Series Data Collection Software v3.1 User Bulletin: New Features and HID Validation Summary	MAN0014110
3500 Series Data Collection Software 4 User Bulletin: New Features and Developmental Validation	100075298
3130 Series Genetic Analyzer	
3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide	4352716
3130/3130xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin	4363787

Document title	Pub. No.
3130/3130xl Genetic Analyzers Getting Started Guide	4352715
3130/3130xl Genetic Analyzers Quick Reference Card	4362825
3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide	4359472
GeneMapper™ *ID-X* Software all versions	
GeneMapper™ ID-X Software Bin Overlap User Bulletin	100029546
GeneMapper™ *ID-X* Software v1.0	
GeneMapper™ ID-X Software v1.0 Getting Started Guide— Basic Features	4375574
GeneMapper™ ID-X Software v1.0 Quick Reference— Basic Features	4375670
GeneMapper™ ID-X Software v1.0 Installation Guide	4476603
GeneMapper™ ID-X Software v1.0 Administrator Guide	4376327
GeneMapper™ ID-X Software v1.0 Reference Guide	4375671
GeneMapper™ *ID-X* Software v1.1	
GeneMapper™ ID-X Software v1.1 Getting Started Guide— Mixture Analysis Tool	4396773
GeneMapper™ *ID-X* Software v1.2	
GeneMapper™ ID-X Software v1.2 Verification Experiments and Installation Procedures User Bulletin	4462639
GeneMapper™ ID-X Software v1.2 Quick Reference— Mixture Analysis Tool	4426482
GeneMapper™ ID-X Software v1.2 Reference Guide	4426481
GeneMapper™ *ID-X* Software v1.3	
GeneMapper™ ID-X Software v1.3 Verification Experiments and Installation Procedures User Bulletin	4470483
GeneMapper™ *ID-X* Software v1.4	
GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin	4477684
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
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

Document title	Pub. No.
GeneMapper™ ID-X Software v1.5 Reference Guide	100031707
GeneMapper™ *ID-X* Software v1.6	
GeneMapper™ ID-X Software v1.6 New Features and Software Verification User Bulletin	100073905

Customer and technical support

For support:

- **In North America**—Send an email to **HIDTechSupport@thermofisher.com**, or call **888-821-4443 option 1**.

- **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/or its affiliate(s) 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 any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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